www.ivbm2020.org

ABSTRACT BOOK

IVBM 2020
The 21st International Vascular Biology Meeting
September 9-12, 2020 | Conrad Hotel, Seoul, Korea

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) & the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

**September 9 (Wed) ~ October 10 (Sat), 2020 | Virtual Meeting (On demand)**

**September 9 (Wed) ~ 12 (Sat), 2020 | IVBM 2020 LIVE for Korean**

Organized by KSoLA The Korean Society of Lipid and Atherosclerosis

KVBM Korean Society for Vascular Biology and Medicine

IVBM 2020

The 21st International Vascular Biology Meeting

September 9-12, 2020 | Conrad Hotel, Seoul, Korea

# The 21st International Vascular Biology Meeting

## September 9 (Wed) ~ October 10 (Sat), 2020

C O N T E N T S

# WELCOME MESSAGE

Dear Colleagues,

The members of the IVBM 2020 Organizing Committee are honored and delighted to welcome you to the 21st International Vascular Biology Meeting held in Seoul, Korea. We are truly excited to have our colleagues participating in this meeting from around the world. We believe this experience of working together with the many faces of this society will help bring about meaningful change in the field of Vascular Biology.

With the start of this year bringing in one of the most pernicious challenges this century has ever seen, we want to commend the resilience and determination shown by the speakers, presenters, and exhibitors in seeing this meeting through to success.

We know we are not alone in the sad feelings that this year's meeting could not take place in the same manner it has since its inception over 40 years ago, where we come together to hear from each other's passionate work and reconnect with fellow colleagues.

Although the circumstances of the world may have dictated the terms of this year's gathering, it also opened a door of opportunity for you to demonstrate your awe-inspiring determination in proceeding with this meeting, no matter what challenges you faced. By adapting to a virtual delivery of the meeting, we have enabled ourselves to continue this longstanding tradition of sharing and growing.

It makes us proud to know we stand alongside a group of professionals who are so steadfast in their resolve to push the limits of what we know in vascular biology and who are this persevering in the face of the relentless challenges.

It is our sincere wish that many friends take part in this meeting to share their meaningful work with fellow scholars through exchange of scientific experience and knowledge.

Apart from the hard work and dedication that has been put in by the many people planning this meeting, we wish to thank the sponsors and many more including the speakers, presenters and exhibitors without whose support this great meeting would not be possible.

Finally, I thank you all for participating and wish you the very best with this meeting.

Sincerely and with best wishes,

Sincerely

**Hyo-Soo Kim, M.D., Ph.D, FAHA**
Co-President
Organizing Committee of IVBM 2020

**Gou Young Koh, M.D., Ph.D.**
Co-President
Organizing Committee of IVBM 2020

# WELCOME MESSAGE

Dear Colleagues,

I wish to first congratulate the organizing committee for successfully organizing and hosting the 21st International Vascular Biology Meeting in Seoul, Korea. The incredible preparation that has gone into planning this meeting, especially amidst these challenging times, has certainly yielded a remarkable result and I strongly commend them for their decisiveness in providing participants with a virtual attendance option.

I am very pleased and thankful to be working alongside of IVBM, which has, for decades, provided the forum for those involved in the field of vascular biology, lipid and atherosclerosis, to exchange the results of their research and learn from each other's knowledge and experience and ultimately, expand the roles of vascular biology and its applications in the 21st century.

KSoLA, since it's conception, has been providing significant research and information on lipids and atherosclerosis to both health professionals and to the public. Working in conjunction with IVBM, we can multiply our impact of our mission in preventing and curing atherosclerosis and improving public awareness of the seriousness of atherosclerosis and its risk factors.

I would like to personally thank the committee members, including the organizing committee presidents, the chairpersons, speakers, and all participants in their efforts to prepare this meeting.

We hope that IVBM 2020 is held in success and I look forward to your active participation.

Thank you,

**Joong-Yeol Park MD, PhD**

Chairman, Board of Directors
The Korean Society of Lipid & Atherosclerosis (KSoLA)

Secretary General
Organizing Committee of IVBM 2020

# WELCOME MESSAGE

Dear Colleagues,

On behalf of the KVBM, it is my great honor and pleasure to welcome you to the International Vascular Biology Meeting in Seoul, Korea. This year marks the 21st occasion of this great and long-running meeting and I am delighted to have our colleagues from around the world, in attendance at this biennial event.

While the challenges of this global pandemic have hindered the plans to proceed with the meeting as we have seen it to be in years past, I am yet inspired by the resilience and perseverance shown by the organizing committee and all of the organizing committee members and leaderships of the KVBM and the KSoLA.

I am excited to participate in this virtual meeting as I believe we can draw from this experience, a great deal of learning both from the great body of work that is to be presented by the speakers, but also with what it means to connect people across the world using the technological means available to us.

The KVBM is always eager to synergize with the mission and purpose of IVBM as it has been making efforts to revitalize vascular biology research in Korea since 2000. By aligning with the associated societies of EVBO, NAVBO, JVBMO, and AAVBM and working collaboratively with them, we can expand our area of knowledge and advance our understanding of new science and techniques.

In addition to the organizing committee and scientific committee, we express our deepest appreciation to all the corporate sponsors, speakers and exhibitors whose efforts have made this meeting possible.

We look forward to this year's IVBM 2020 and to providing each other with more ideas and resources that will drive our research toward a successful future.

Sincerely

**Goo Taeg Oh, DVM, PhD**

President
Korean society for Vascular Biology Medicine (KVBM)

Chair, Scientific Committee
Organizing Committee of IVBM 2020

# IVBM 2020 OVERVIEW

| | | |
|---|---|---|
| Date | On demand | September 9(Wed) ~ 12(Sat), 2020 |
| | Virtual | September 9(Wed) ~October 10(Sat), 2020 |
| Venue | Conrad Hotel, Seoul, Korea | |
| Organized by | IVBM 2020 Organizing Committee | |
| Host | KSoLA The Korean Society of Lipid and Atherosclerosis (KSoLA) | |
| | KVBM Korean Society of Vascular Biology Medicine (KVBM) | |
| Co-host | Institute for Basic Science(IBS), National Creative Initiatives Center for Immune and Vascular Cell Network, Ewha Womans University, National Research Foundation of Korea, Kangwon Institute of Inclusive Technology [KIIT], Inha University of Medicine Hypoxia-related Disease Research Center, Healthy Aging Korean Medical Research Center, Smart-aging Convergence Research Center | |
| Associated societies and institutions | EVBO, NAVBO, JVBMO, and AAVBM | |
| Supported by | SEOUL METROPOLITAN GOVERNMENT | |

IVBM 2020
The 21st International Vascular Biology Meeting
September 9-12, 2020 | Conrad Hotel, Seoul, Korea

The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# ORGANIZING COMMITTEE

| | | |
|---|---|---|
| Co-Presidents | Hyo-Soo Kim | Seoul National University |
| | Gou Young Koh | KAIST / IBS |
| Secretaries General | Joong-Yeol Park | University of Ulsan |
| | Young-Guen Kwon | Yonsei University |
| **Scientific Committee** | | |
| Chair | Goo Taeg Oh | Ewha Womans University |
| Vice-Chair | Cheol-Young Park | Sungkyunkwan University |
| Members | Kyung-Soo Kim | CHA University |
| | Injune Kim | KAIST |
| | Jaetaek Kim | Chung-Ang University |
| | Jaeho Kim | Pusan National University |
| | Jeong Seon Kim | National Cancer Center |
| | Pilhan Kim | KAIST |
| | Hun-Jun Park | The Catholic University of Korea |
| | G-One Ahn | Seoul National University |
| | Han-Mo Yang | Seoul National University |
| | Hyojee Joung | Seoul National University |
| | Suk-Won Jin | GIST |
| | Daehee Hwang | DGIST |
| **Steering Committee** | | |
| Chair | Woo Je Lee | University of Ulsan |
| Vice-Chair | Sung Wan Chun | Soonchunhyang University |
| Members | Yoo-Wook Kwon | Seoul National University |
| | Jeong Hun Kim | Seoul National University |
| | Jeong-Ki Min | KRIBB |
| | Jung-Hwan Park | Hanyang University |
| | Sunsik Bae | Pusan National University |
| | Eun Jung Rhee | Sungkyunkwan University |
| | Jin Han | Inje University |
| | Sangmo Hong | Hanyang University |
| | Soon Jun Hong | Korea University |
| **Public Relations Committee** | | |
| Chair | Kyung Woo Park | Seoul National University |
| Vice-Chair | Yoo-Wook Kwon | Seoul National University |
| Members | G-One Ahn | Seoul National University |

# INTERNATIONAL ADVISORY COMMITTEE

| | | |
|---|---|---|
| Ralf H. Adams | Max-Planck Institute for Molecular Biomedicine | Germany |
| Masanori Aikawa | Harvard Medical School | USA |
| Kari K. Alitalo | University of Helsinki | Finland |
| Hellmut G. Augustin | Heidelberg University | Germany |
| Victoria Bautch | The University of North Carolina at Chapel Hill | USA |
| Christer Betsholtz | Uppsala University | Sweden |
| Joyce Bischoff | Boston Children's Hospital/Harvard Medical School | USA |
| Matthias Blüher | University Hospital Leipzig | Germany |
| Alberico L. Catapano | University of Milan | Italy |
| Lena Claesson-Welsh | Uppsala University | Sweden |
| Myron Cybulsky | University of Toronto and Toronto General Hospital Research Institute | Canada |
| Elisabetta Dejana | Uppsala University | Sweden |
| Michael Detmar | Institute of Pharmaceutical Sciences | Switzerland |
| Jozef Dulak | Jagiellonian University | Poland |
| Anne Eichmann | Yale University | USA |
| Barbara Garmy-Susini | Research Director Inserm | France |
| Holger Gerhardt | Max-Delbrueck-Center for Molecular Medicine | Germany |
| Natasha Harvey | University of South Australia | Australia |
| Yulong He | Soochow University | China |
| Timothy Hla | Harvard Medical School, Boston Children's Hospital | USA |
| Benjamin Hogan | University of Melbourne | Australia |
| Luisa Iruela-Arispe | Northwestern University | USA |
| Sazzli Kasim | University Technology Mara | Malaysia |
| Eli Keshet | The Hebrew University of Jerusalem | Israel |
| Jan Kitajewski | University of Illinois at Chicago | USA |
| Terje Larsen | The Arctic University of Norway | Norway |
| Yi-Heng Li | National Cheng Kung University | Taiwan |

| | | |
|---|---|---|
| André Marette | Laval University | Canada |
| Donald M. McDonald | University of California San Francisco | USA |
| Naoki Mochizuki | National Cerebral & Cardiovascular Center Research Institute | Japan |
| Ryuichi Morishita | Osaka University School of Medicine | Japan |
| Richard O'Brien | University of Melbourne | Australia |
| Gary Owens | University of Virginia, Cardiovascular Research Center | USA |
| Susan E. Quaggin | Northwestern Universtiy | USA |
| Anna M. Randi | Imperial College London | UK |
| Raul D. Santos | University of São Paulo | Brazil |
| Simone Schoenwaelder | University of Sidney | Australia |
| Martin A. Schwartz | Yale University | USA |
| William C. Sessa | Yale University | USA |
| Michael Simons | Yale University | USA |
| Didier Stainier | Max Planck Institute for Heart and Lung Research | Germany |
| Gary Sweeney | York University | Canada |
| Nobuyuki Takakura | Osaka University | Japan |
| Lale Tokgözoglu | Hacettepe University | Turkey |
| Dietmar Vestweber | Max Planck Institute of Molecular Biomedicine | Germany |
| Miikka Vikkula | Institut de Duve | Belgium |
| Kenneth Walsh | University of Virginia | USA |
| Rong Wang | University of California, San Francisco | USA |
| Tetsuro Watabe | Tokyo Medical and Dental University | Japan |
| Hua-Lin Wu | National Cheng Kung University | Taiwan |
| Shizuya Yamashita | Rinku General Medical Center | Japan |
| Seppo Ylä-Herttuala | University of Eastern Finland | Finland |
| Masayuki Yoshida | Tokyo Medical and Dental University | Japan |
| Bin Zhou | Chinese Academy of Sciences | China |

# SCIENTIFIC PROGRAM

## Plenary Lecture

**PLENARY LECTURE 1** | September 9(Wed) 36

### Endothelial Heterogeneity in Health and Disease

Donald M. McDonald *University of California, San Francisco, USA*

**PLENARY LECTURE 2** | September 9(Wed) 37

### Guidance of Organ-Specific Vascular Barrier Formation

Anne Eichmann *Yale University, USA*

**PLENARY LECTURE 3** | September 9(Wed) 38

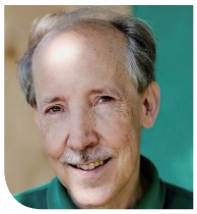
### Organ-Specific and Functional Specialization of Vascular Cells

Ralf H. Adams *Max Planck Institute for Molecular Biomedicine , Germany*

**PLENARY LECTURE 4** | September 9(Wed) 39

### CAP1 Binds to Resistin or PCSK9, Standing at the Nodal Point of Disease Clusters Such As Metabolic Syndrome, Fatty Liver, and Hypercholesterolemia and Cancer

Hyo-Soo Kim *Seoul National University, Korea*



**PLENARY LECTURE 5** | September 10(Thu) 40

### Translating Lymphangiogenesis Mechanisms to Therapeutics

Kari K. Alitalo *University of Helsinki, Finland*

**PLENARY LECTURE 6** | September 10(Thu) 41

### Cerebral Cavernous Malformation: From Mechanism to Therapy

Mark L. Kahn *University of Pennsylvania , USA*

# SCIENTIFIC PROGRAM

**PLENARY LECTURE 7** | September 10(Thu) 42

**"Tek Tok" - Discovery of Vascular Targets to Treat Ocular and Kidney Diseases**

Susan Quaggin *Northwestern University, USA*

**PLENARY LECTURE 8** | September 11(Fri) 43

**Normalizing the Tumor Microenvironment to Improve Cancer Immunotherapy: Bench to Bedside**

Rakesh K. Jain *Harvard Medical School, USA*

**PLENARY LECTURE 9** | September 11(Fri) 44

**Pulmonary Hypertension and Marfan Syndrome**

Issei Komuro *The University of Tokyo, Japan*

**PLENARY LECTURE 10** | September 11(Fri) 45

**Clonal Hematopoiesis at the Crossroads of Aging, Cancer and Cardiovascular Disease**

Peter Libby *Harvard Medical School, USA*

**PLENARY LECTURE 11** | September 11(Fri) 46

**CV Risk Reduction with Diabetes Medications: In Search of the Holy Grail**

Silvio Inzucchi *Yale University, USA*

**PLENARY LECTURE 12** | September 12(Sat) 47

**Heart Failure: Are We Winning?**

John J. V. McMurray *University of Glasgow, UK*

**PLENARY LECTURE 13** | September 12(Sat) 48

**The Intersection of Infection, Inflammation and Vascular Occlusion**

Jane E. Freedman *University of Massachusetts Medical School, USA*

# SCIENTIFIC PROGRAM

## Symposium

### Symposium 1 — September 9(Wed)

#### Angiogenesis and Vascular Remodeling 52

SMAD6 Regulates Vascular Flow Responses

Victoria Bautch *(UNC-Chapel Hill, USA)*

Endodermal-Mesoderman Transition for Vascular Development

Naoki Mochizuki *(National Cerebral & Cardiovascular Center Research Institute, Japan)*

Principles and Regulation of Endothelial Cell Dynamics in Vascular Remodeling

Holger Gerhardt *(Max-Delbrück-Centrum, Germany)*

Bone Morphogenetic Protein Signaling in Vascular Development and Homeostasis

Suk-Won Jin *(GIST, Korea)*

VEGF-Mediated Activation of ERK and PI3K Balances Endothelial Cell Migration and Proliferation During Angiogenesis

**(Short Talk)** Arndt Siekmann *(University of Pennsylvania, USA)*

Depletion of Endothelial TGF-β Signaling Induces Tumor Angiogenesis and Metastasis

**(Short Talk)** Fumiko Itoh *(Tokyo University of Pharmacy and Life Science, Japan)*

### Symposium 2 — September 9(Wed)

#### Specification and Differentiation of Endothelial Lineage 58

Biological Robustness: Genetic Compensation and Transcriptional Adaptation

Didier Stainier *(Max Planck Institute for Heart and Lung Research, Germany)*

Novel Blood and Lymphatic Endothelial Cell Origins

Christiana Ruhrberg *(University College London, UK)*

Cell Heterogeneity and Disease

Michael Simons *(Yale University, USA)*

Endothelial Cell Specification: Role of Cell Cycle State

Karen Hirschi *(University of Virginia, USA)*

Dynamics in Sprouting Endothelial Cells by Hippo-YAP/TAZ Pathway

You Mie Lee *(Kyungpook National University, Korea)*

Endothelial-Derived TGF-β/Activin Signals Activated by Inflammation-Related Cytokines During Endothelial Mesenchymal Transition Induce Epithelial Mesenchymal Transition

**(Short Talk)** Yasuhiro Yoshimatsu *(Niigata University, Japan)*

# SCIENTIFIC PROGRAM

## Symposium 3 — September 9(Wed)

### Blood-CNS-Barrier 64

Blood-Brain Barrier Dysfunction Predicts Cognitive Decline in Alzheimer's Disease: Effect of the APOE4 Gene

Berislav V. Zlokovic *(Keck School of Medicine of USC, USA)*

Endothelial Cell Clonal Expansion in the Development of Cerebral Cavernous Malformations

Elisabetta Dejana *(Uppsala University and FIRC Inst Mol Oncol, Italy)*

How does the BBB Regulate Brain Function and Behavior?

Richard Daneman *(University of California, San Diego, USA)*

Hypertension-Induced Vascular Disruption in the CNS

Injune Kim *(KAIST, Korea)*

Blood-Retinal Barrier Revisited: Choroidal Vasculature Regulates Retinal Homeostasis

Junyeop Lee *(University of Ulsan, Korea)*

Tau Deposition Is Associated With Intracranial Calcification in the P301L Mouse Model of Human Tauopathy

**(Short Talk)** Jan Klohs *(University of Zurich , Switzerland)*

## Symposium 4 — September 9(Wed)

### Stem Cells and Vascular Niche 70

Development and Aging of Endothelial Stem Cells

Nobuyuki Takakura *(Osaka University, Japan)*

Human Stem Cells & Genomics for Disease Modeling

Joseph C. Wu *(Stanford University, USA)*

Clinical Application of Human iPS Cell-Derived Regenerated Cardiomyocytes for the Treatment of Severe Congestive Heart Failure

Keiichi Fukuda *(Keio University, Japan)*

Vascular Regeneration with Stem Cells, Reprogrammed Cells and Engineering

Young-Sup Yoon *(Emory University, USA)*

Therapy for Peripheral Artery Disease using Human Induced Pluripotent Stem Cell-derived Endothelial Cells and Smooth Muscle Cells

Jae Ho Kim *(Pusan National University, Korea)*

An Endogenous Transmembrane Protein Controls Distinct mTORC2 Functions in Normal and Leukemic Hematopoiesis in the Bone Marrow

**(Short Talk)** Dongjun Lee *(Pusan National University, Korea)*

# SCIENTIFIC PROGRAM

## Symposium 5 — September 9(Wed)

### Lymphangiogenesis 76

Pathogenic Mechanisms of Lymphatic Malformations

Taija Mäkinen *(Uppsala University, Sweden)*

Meningeal Lymphatics in AD

Jonathan Kipnis *(Washington University in St. Louis, USA)*

Dissecting the Transcriptional Control of Lymphatic Endothelial Cell Identity

Natasha Harvey *(University of South Australia, Australia)*

Fluid Flow-Triggered Activation of Lymphatic Expansion

Young Kwon Hong *(University of Southern California, USA)*

Neural-Crest Derived and Endothelial Foxc2 Expression Is Required for Proper Morphogenesis of the Schlemm's Canal

**(Short Talk)** Tsutomu Kume *(Northwestern University, USA)*

Immune Regulation and Lymphatic Vessel Development in Severe Preeclampsia

**(Short Talk)** Yong-Sun Maeng *(Yonsei University, Korea)*

## Symposium 6 — September 9(Wed)

### Vascular Signaling 82

VEGFR2 Signaling in Vascular Permeability and Vessel Leakage

Lena Claesson-Welsh *(Uppsala University, Sweden)*

Somatic Mosaicism, Clonal Hematopoiesis and Vascular Diseases

Kenneth Walsh *(University of Virginia, USA)*

Endocytic Adaptor Protein Epsin Is a Gatekeeper of Quiescent Endothelium

Hong Chen *(Harvard Medical School, USA)*

Therapeutic Implication and Action Mechanism of Endothelial Dysfunction Blocker

Young-Guen Kwon *(Yonsei University, Korea)*

Vascular Intraluminal Pressure Load Inhibits Directed Endothelial Cell Migration in Angiogenesis

**(Short Talk)** Koichi Nishiyama *(Kumamoto University, Japan)*

Tyrosine Phosphorylation of eNOS Regulates Endothelial Function and Endothelial Redox Homeostasis

**(Short Talk)** Mauro Siragusa *(Goethe University Frankfurt, Germany)*

# SCIENTIFIC PROGRAM

## Symposium 7 — September 9(Wed)

### Neurovascular Interaction 88

Neuro-Vascular Interactions in the Brain

Chenghua Gu *(Harvard Medical School, USA)*

The Neurovascular Interface

Amparo Acker-Palmer *(Johann Wolfgang Goethe-University, Germany)*

Constitutively Active Notch4 in Endothelial Cells Initiates Arteriovenous Malformation via a Nitric Oxide Synthase-Mediated Mechanism

Rong Wang *(University of California, San Francisco, USA)*

Deciphering the Role of Genome Organization in Development and Disease

Rajan Jain *(University of Pennsylvania, USA)*

Neurovascular Changes in Alzheimer's Disease

Yong Jeong *(KAIST, Korea)*

Vascular Endothelial Cells Contribute to the Scavenging Meningeal Macrophage Population in Embryonic and Postnatal Development

**(Short Talk)** Neil Bower *(University of Queensland, Australia)*

## Symposium 8 — September 9(Wed)

### Cardiovascular Disease 94

Stress-Induced Premature Aging Mediated by Mitochondrial Hibernation Promotes Atherosclerosis

Jun-ichi Abe *(University of Texas MD Anderson Cancer Center, USA)*

Genetic Fate Mapping of Cardiomyocyte and Endothelial Cell Proliferation in Adult Mouse

Bin Zhou *(Chinese Academy of Sciences, China)*

Therapeutic Angiogenesis by Autologous Adipose-Derived Mesenchymal Stem Cells

Toyoaki Murohara *(Nagoya University, Japan)*

In Vivo Genome Editing for Angiogenesis-Related Blindness

Jeong Hun Kim *(Seoul National University, Korea)*

Therapeutic Targeting of Tumor Angiogenesis Using Endothelial Cell Fate Mechanisms

Jan Kitajewski *(University of Illinois at Chicago , USA)*

Cardiovascular Disease Risk Factors Reprogram Cardiac Endothelial Cell Transcriptome and Promote EndMT Features

**(Short Talk)** Riikka Kivelä *(University of Helsinki, Finland)*

# SCIENTIFIC PROGRAM

## Symposium 9 — September 10(Thu)

### Endothelial Regeneration 100

Adaptable and Hemodynamic Endothelial Cells for Physiological Vascularization of Organoids and Tumoroids

Shahin Rafii *(Weill Cornell Medical , USA)*

Endothelial Heterogeneity: a COVID-19 Update

Peter Carmeliet *(VIB-KU Leuven Center for Cancer Biology, Belgium)*

Vascular Niche for Skeletal Muscle Stem Cell: Application for Muscular Dystrophy Therapy

Atsushi Asakura *(University of Minnesota, USA)*

Promoting Healthy Againg by VEGF-Based Vascular Manipulations

Eli Keshet *(The Hebrew University of Jerusalem, Israel)*

Re-defining Early Endothelial Progenitor Cells for Ischemic Cardiovascular Repair

Sang-Mo Kwon *(Pusan National University, Korea)*

Mir-130a Potentiates the Reparative Properties of Human Endothelial Colony Forming Cells Facing Hypoxia via VEGFR2 and STAT3

**(Short Talk)** Reinhold Medina *(Queen's University Belfast, UK)*

## Symposium 10 — September 10(Thu)

### Organotypic Vasculature 106

Novel Regulators and Functions of Special Lymphatic Vessels

Gou Young Koh *(KAIST / IBS, Korea)*

Mechanisms of Lymphatic Vascular Specialization

Tatiana Petrova *(University of Lausanne, Switzerland)*

Mechanisms Ensuring Endothelial Junction Integrity Beyond VE-Cadherin

Dietmar Vestweber *(Max Planck Institute for Molecular Biomedicine, Germany)*

Metabolic Control of Endothelial Growth and Differentiation

Michael Potente *(Max Planck Institute for Heart and Lung Research, Germany)*

Mechanical Loading of Intraluminal Pressure Regulates Angiogenesis in Wound Healing

**(Short Talk)** Shigetomo Fukuhara *(Nippon Medical School, Japan)*

The Role of C-Kit in Brown Adipose Tissue

**(Short Talk)** Hyuek-Jong Lee *(IBS, Korea)*

# SCIENTIFIC PROGRAM

## Symposium 11 — September 10(Thu)

### Tumor Vasculature 112

Vascular Control of Tumor Progression and Metastasis

Hellmut G. Augustin *(Heidelberg University, Germany)*

Microenvironmental Regulation of Tumor Response and Resistance to Anti-Angiogenic Immunotherapy

Michele De Palma *(EPFL, Switzerland)*

Endothelial-to-Mesenchymal Transition Compromises Vascular Integrity to Induce Myc-Mediated Metabolic Reprogramming in Kidney Fibrosis

Raghu Kalluri *(MD Anderson Cancer Center, USA)*

Ontogeny and Function of Intratumoral High-Endothelial Venules

Gabriele Bergers *(VIB-KU Leuven, Center for Cancer Biology, Belgium)*

KAI1 (CD82) in Pericytes Suppresses Angiogenesis by Inducing Leukemia Inhibitory Factor (Lif) As Well as by Direct Binding and Quenching VEGF/PDGF

Yoo-Wook Kwon *(Seoul National University, Korea)*

Vascular Niche Signals via Ceruloplasmin-Iron Ion Metabolism Promote Glioma Resistance to Anticancer Drugs

**(Short Talk)** Fumitaka Muramatsu *(Osaka University, Japan)*

## Symposium 12 — September 10(Thu)

### Mechanotransduction and ECM 118

GPCRs in Fluid Sheer Stress Mechanotransduction

Martin A Schwartz *(Yale University, USA)*

Regulation of Endothelial Phenotype by Different Flow Patterns

Stefan Offermanns *(Max Planck Institute for Heart and Lung Research, Germany)*

Disturbed Flow Reprograms Endothelial Cells by Altering Transcriptomic and Epigenomic Landscapes in Mouse Carotid Artery in Vivo as Revealed by Single-Cell RNAseq and ATACseq Studies

Hanjoong Jo *(Emory University, USA)*

Regulation of Angiogenic Sprouting by the Extracellular Matrix

Britta Trappmann *(Max Planck Institute for Molecular Biomedicine, Germany)*

PAR-1 Is a Novel Mechano-Sensor Transducing Laminar Flow-Mediated Endothelial Signaling

Chang-Hoon Woo *(Yeungnam University, Korea)*

Impaired SMAD 1/5 Mechanotransduction and Inflammation Converge on Connexin37(Cx37) Enabling Arteriovenous Malformations

**(Short Talk)** Elizabeth Jones *(KU Leuven, Belgium)*

# SCIENTIFIC PROGRAM

## Symposium 13 — September 10(Thu)

### Vascular Cell Biology 1 ..... 124

The RNA Helicase Ddx21 Controls Developmental Lymphangiogenesis by Balancing Endothelial Cell Ribosome Biogenesis and p53-p22 Signalling

Benjamin Hogan *(The University of Melbourne, Australia)*

Organotypic Specialization of Lymphatic Vessels

Friedemann Kiefer *(University of Münster, Germany)*

Endothelial Lipid Metabolism

William C. Sessa *(Yale University, USA)*

[EVBO Award Lecture] Inside Out: PI3Ks Taking Control of Vessel Growth

Mariona Graupera *(IDIBELL, Spain)*

Axon Guidance Molecules Contribute to the Cerebrovascular Recovery Following Ischemic Damage

Won-Jong Oh *(KBRI, Korea)*

aPKC Is a Key Regulator of Endothelial Proliferation by Modulating C-Myc via FoxO1 DNA Binding Ability

**(Short Talk)** Masanori Nakayama *(Max Planck Institute for Heart and Lung Research, Germany)*

## Symposium 14 — September 10(Thu)

### Translational Research in Vascular Biology ..... 130

Endotheliopathy in COVID19: Yale Experience

Hyung Joon Chun *(Yale University, USA)*

Cell-to-Cell Communication Regulating Angiogenesis and Lymphangiogenesis

Yoshiaki Kubota *(Keio University, Japan)*

Single Cell Transcriptomic Characterization of Smooth Muscle Cell Transitions

Thomas Quertermous *(Stanford University, USA)*

Endothelial microRNAs and Pulmonary Arterial Hypertension

Beata Wojciak-Stothard *(Imperial College London, UK)*

The Renin-Angiotensin-Aldosterone System (RAAS) Is One of the Effectors by Which Vascular Endothelial Growth Factor (VEGF)/Anti-VEGF Controls the Endothelial Cell Barrier

**(Short Talk)** Andrius Kazlauskas *(University of Illinois at Chicago, USA)*

Fursultiamine Alleviates Choroidal Neovascularization by Suppressing Inflammation and Metabolic Reprogramming

**(Short Talk)** Dong Ho Park *(Kyungpook National University, Korea)*

# SCIENTIFIC PROGRAM

## Symposium 15 — September 10(Thu)

### Immune Cells and Cardiovascular Diseases 1 ..... 136

Interactions Between Endothelial and Intimal Myeloid Cells in Homeostasis and the Initiation of Atherosclerosis

Myron Cybulsky *(University of Toronto, Canada)*

Systems Approach to Target Discovery for Vascular Disease: A Focus on Macrophage Activation

Masanori Aikawa *(Harvard Medical School, USA)*

Dietary Protein and Amino Acid-Mediated mTOR Signaling in Atherosclerosis

Babak Razani *(Washington University, USA)*

Atheroprotective Roles of Myeloid Cells in the Pathogenesis of Atherosclerosis

Goo Taeg Oh *(Ewha Womans University, Korea)*

LncRNA-Mediated Control of Vascular Senescence and Atherosclerosis

**(Short Talk)** Mark Feinberg *(Harvard Medical School, USA)*

Exploring Human Atherosclerosis With Single-Cell Resolution

**(Short Talk)** Ljubica Matic *(Karolinska Institute, Sweden)*

## Symposium 16 — September 11(Fri)

### Balanced Carbohydrate Diet for Cardiovascular Health ..... 142

Carbohydrate Intakes, Dietary Patterns, and Risk of Cardiovascular Diseases in Asian Populations

Rob Martinus van Dam *(National University of Singapore, Singapore)*

Introduction of KSoLA Dietary Guideline and KDRI for Carbohydrate/Sugar Intakes

YoonJu Song *(The Catholic University of Korea, Korea)*

Rice and Cardiovascular Health: Focus on Amylose Content

Koutatsu Maruyama *(Ehime University, Japan)*

Dietary Guideline of Acceptable Macronutrient Distribution Range and Recommended Food Sources for Carbohydrate

SuJin Song *(Hannam University, Korea)*

Regulating Glucose Delivery to the Body and Physiological Importance

Byung-Hoo Lee *(Gachon University, Korea)*

**(Short Talk)** Balanced Carbohydrate Diet: Focus on Intakes and Food Sources of Protein and Fat

Kyungho Ha *(Jeju University, Korea)*

# SCIENTIFIC PROGRAM

## Symposium 17 — September 11(Fri)

### Recent Cardiovascular Clinical Trial Outcomes on Cardiometabolic Drugs 148

Ezetimibe

Anselm Gitt *(Klinikum Ludwigshafen, Germany)*

PCSK9 Inhibitors in Familial Hypercholesterolemia A Revolution in Treatment

Frederick J. Raal *(University of the Witwatersrand, South Africa)*

Incretin-Based Therapeutics: DPP-4 Inhibitor and GLP-1RA

Mansoor Husain *(University Health Network, Canada)*

SGLT-2 Inhibitors

Matthias Blüher *(University Hospital Leipzig, Germany)*

Past, Present, and Future of NOACs in Patients with Atrial Fibrillation

So-Ryoung Lee *(Seoul National University, Korea)*

Comparison of the Effects of High-dose Statin alone versus Intermediate-does Statin combined with Ezetimibe on Major Adverse Cardiovascular Events in Patients with Acute Myocardial Infarction: A Nationwide Cohort Study

**(Short Talk)** Kihyun Kim *(Gangneung Asan Hospital, Korea)*

## Symposium 18 — September 11(Fri)

### Cellular Heterogeneity 154

Pericyte-Specific Vascular Expression of SARS-CoV-2 Receptor ACE2 – Implications for Microvascular Inflammation and Hypercoagulopathy in COVID-19

Christer Betsholtz *(Uppsala University, Sweden)*

Targeting the Endothelial Cell Barrier to Prevent Tumor Cell Metastasis

Luisa Iruela-Arispe *(Northwestern University, USA)*

GPCR Regulation of Gene Expression During Vascular Development and Organotypic Specialization

Timothy Hla *(Harvard Medical School, USA)*

Transcription Networks and Endothelial Heterogeneity

Anna M. Randi *(Imperial College London, UK)*

Vascular Precision Biology and Mapping the Human Body at Single Cell Resolution

**(Short Talk)** Zorina Galis *(National Institutes of Health , USA)*

Real-Time Intravital Characterization of Non-Classical Monocytes Mediating Resistance to Anti-Angiogenic Treatment in Cancers

**(Short Talk)** Keehoon Jung *(Seoul National University, Korea)*

# SCIENTIFIC PROGRAM

## Symposium 19 — September 11(Fri)

### Are All Calcifications the Same? ..... 160

New Insight of Vascular Calcification; Metabolic Reprogramming

In-kyu Lee *(Kyungpook National University, Korea)*

CAC Score to Guide CV Risk Assessment When Decision to "Statin" is Uncertain

Erin D. Michos *(Johns Hopkins University, USA)*

Intracranial Arterial Calcification

Kwang-Yeol Park *(Chung-Ang University, Korea)*

Pathophysiologic Aspect of Calcification in CKD

Makoto Kuro-o *(Jichi Medical University, Japan)*

Clinical Implication of Vascular Calcification in Patients With Diabetes

Eun-Jung Rhee *(Sungkyunkwan University, Korea)*

Zingerone Attenuates Pi-Induced Vascular Calcification via AMPK-Mediated TIMP4 Expression

**(Short Talk)** Won-Gu Jang *(Daegu University, Korea)*

## Symposium 20 — September 11(Fri)

### Familial Hypercholesterolemia: The Discordance Between Its Prevalence and Diagnostic Rate ..... 166

Cardiovascular Risk Heterogeneity in FH: How to Identify the Severe Forms?

Raul D. Santos *(University of Sao Paulo, Brazil)*

Pharmacotherapy of FH; Present and Future

Sang-Hyun Kim *(Seoul National University, Korea)*

Discordance on FH Care: Lessons From the "Ten Countries Study"

Brian Tomlinson *(Macau University of Science & Technology, Macau)*

Genetic Characteristics and Response to LLT in FH

Sang-Hak Lee *(Yonsei University, Korea)*

Cardiovascular Risk and Effect of Lipid-Lowering Therapy in Koreans With Phenotype of Familial Hypercholesterolemia

**(Short Talk)** Chan Joo Lee *(Yonsei University, Korea)*

Characteristics of Korean Patients With Familial Hypercholesterolemia: KFH Registry Data 2020

**(Short Talk)** Hyoeun Kim *(Yonsei University, Korea)*

# SCIENTIFIC PROGRAM

## Symposium 21 — September 11(Fri)

### Immune Cells and Cardiovascular Diseases 2 ..... 172

Functional Diversity and Modulation of Macrophages in Organ Crosstalk and Cardiovascular Disease

Ichiro Manabe *(Chiba University, Japan)*

Single Cell Perspective of Resident Cardiac Macrophages in Health and Disease

Slava Epelman *(University Health Network, Canada)*

Phagocyte Regulation of Cardiac and Vascular Inflammation

Edward B. Thorp *(Northwestern University, USA)*

Single Cell Landscapes of Hyperlipidemic Cardiovascular Diseases

Jae-Hoon Choi *(Hanyang University, Korea)*

CGRP and Its Receptor, RAMP1 Regulate Lymphangiogenesis in the Pathological Conditions

**(Short Talk)** Masataka Majima *(Kitasato University, Japan)*

Reduction in Lung Vascular Leakage in Acute Respiratory Distress Syndrome (ARDS) by Tie2 Activation or VE-PTP Inhibition

**(Short Talk)** Peter Baluk *(University of California, San Francisco, USA)*

## Symposium 22 — September 12(Sat)

### Vascular Cell Biology 2 ..... 178

Immunometabolic Fabric in Atherosclerosis

Laurent Yvan-Charvet *(INSERM, France)*

Endothelial Cell Regulation of Macrophage Plasticity

Asrar Malik *(University of Illinois at Chicago, USA)*

Longitudinal Intravital Cellular-Level Imaging of Vascular System

Pilhan Kim *(IVIM Technology / KAIST, Korea)*

Vascular Control of Metastatic Colonization

**(Short Talk)** Mahak Singhal *(German Cancer Research Center, Germany)*

The Transcription Factor SRF Regulates Pericyte Migration During Retinal Angiogenesis

**(Short Talk)** Michael Orlich *(Tuebingen University, Germany)*

# SCIENTIFIC PROGRAM

## Symposium 23 — September 12(Sat)

### Bioactive Extracellular Vesicles 183

Macrophage Exosomes: New Mediators to Control Hematopoiesis, Monocyte Activation & Atherosclerosis

Robert L. Raffai *(University of California, San Francisco, USA)*

Bacterial Exosomes as Next-Generation Cancer Immunotherapy

Yong Song Gho *(POSTECH, Korea)*

Circulating Extracellular Vesicles a Potential Prognostic Marker of Atherosclerosis-Related Lymphatic Dysfunction

Catherine Martel *(Université de Montréal, Canada)*

Engineered Exosomes for Intracellular Biologics: Principles and Application of EXPLOR Technology

Chulhee Choi *(KAIST, Korea)*

Tip Cell Invasive Behaviour Is Controlled by Myosin IIA-Dependent Inhibition of Arp2/3 Activity

**(Short Talk)** Claudio Franco *(Instituto de Medicina Molecular, Portugal)*

## Symposium 24 — September 12(Sat)

### Vascular Development & Maintenance 188

Customized Vasculatures – Lessons From Organoids and Embryos

Ondine Cleaver *(UT Southwestern Medical Center, USA)*

Identifying Novel Modifier Genes Related to Naturally-Occurring Heterogeneity of Lymphatics Within a Diverse Genetic Population

Kathleen M. Caron *(University of North Carolina at Chapel Hill, USA)*

Hypoxia-Inducible Transcription Factors Regulate Brain Capillary Development

Wiebke Herzog *(University of Münster, Germany)*

Negative Regulation of VEGFC Transcription by Sox7 in Blood Vessels Is a Key Determinant to Lymphatic Morphogenes

**(Short Talk)** Mathias Francois *(The University of Sydney, Australia)*

Transcriptional Regulation and Physiological Significance of ALK1 Signal Target Genes in Embryonic Vascular Endothelial Cells

**(Short Talk)** Osamu Nakagawa *(National Cerebral and Cardiovascular Center Research Institute, Japan)*

# SCIENTIFIC PROGRAM

## Symposium 25 — September 12(Sat)

### Mitochondria in Cardiovascular Diseases 193

Regulation of Cardiac Robustness by Reactive Sulfide Species

Motohiro Nishida *(Kyushu University, Japan)*

Role of Mitochondrial Fusion During Cardiac Developments and Heart Energy Metabolism

Arnaud Mourier *(IBGC UMR 5095, France)*

Circulating Amylin Dysregulation Triggers Microvascular Dysfunction

Florin Despa *(University of Kentucky, USA)*

Mitochondria and Cardiac Differentiation

Sung Woo Cho *(Inje University, Korea)*

TIMP3 Is a Potent Regulator of Cancer-Associated Fibroblasts-Mediated Angiogenesis in Invasive Breast Cancer

**(Short Talk)** Kyu-Tae Kang *(Duksung Women's University, Korea)*

## Symposium 26 — September 12(Sat)

### Novel Role of HDL – Clinical Perspectives 198

HDL and Glucose Metabolism

Alicia Jenkins *(The University of Sydney, Australia)*

International Lipid Expert Panel (ILEP) 2020 Guidelines on the Definition and Management With Statin Intolerance

Maciej Banach *(Medical University of Lodz, Poland)*

HDL and Cognitive Decline in Older People With Diabetes Mellitus

Timothy Kwok *(The Chinese University of Hong Kong, Hong Kong)*

High-Density Lipoprotein Cholesterol and the Risk of Myocardial Infarction, Stroke, and Cause-Specific Mortality

Yeoree Yang *(The Catholic University of Korea, Korea)*

Altered Lipid Droplet Metabolism in the Endothelium Causes Vascular Dysfunction

**(Short Talk)** Boa Kim *(University of Pennsylvania, USA)*

## Symposium 27 — September 12(Sat)

### Precision Medicine in Cardiovascular Diseases 203

Integrative Functional Genomics for Regulatory Mechanisms of Coronary Artery Disease

Thomas Quertermous *(Stanford University, UK)*

# SCIENTIFIC PROGRAM

Genomic Approaches to Identify Novel Therapeutic Targets for Cardiovascular Disease

Nathan Stitziel *(Washington University , USA)*

Using Proteomics and Metabolomics for a Precision Medicine Approach in Cardiovascular Disease

Lars Lind *(Uppsala University, Sweden)*

Korea National Bio Big Data Project

Hyun-Young Park *(Korea National Institute of Health, Korea)*

Novel Scoring System to Predict Coronary Artery Calcification

**(Short Talk)** Hyo-Wook Gil *(Soonchunhyang University Cheonan Hospital, Korea)*

## Symposium 28 — September 12(Sat)

### Vascular Inflammation ..... 208

An Update on Anti-Inflammatory Therapy for Atherosclerosis: CANTOS and Beyond.

Peter Libby *(Harvard Medical School, USA)*

Non-Canonical Functions of Nuclear microRNAs in Endothelial Protection

Christian Weber *(Ludwig-Maximilians-Universität (LMU) München, Germany)*

Mechanisms Meditating Platelet-Neutrophil Interactions in Thromboinflammation: Role of Platelet PDI

Jaehyung Cho *(Washington University, USA)*

Endothelial Phenotype Regulation by ECM

**(Short Talk)** Sanguk Yun *(Inje University, Korea)*

Endothelial Sharpin Regulates Vascular Leakage in Inflammation

**(Short Talk)** Anne Pink *(University of Helsinki, Finland)*

## Symposium 29 — September 12(Sat)

### Metabolic Signaling in Cardiovascular System ..... 213

Defective D-Lactate Metabolism Induce Methylglyoxal Accumulation and Cause Cardiomyopathy

Chan Bae Park *(Ajou University, Korea)*

Rebalancing Metabolism to Improve Cardiac Function in Diabetes: "Walking the Tightrope" Between Glucose and Fatty Acid Metabolism

Lisa Heather *(University of Oxford , UK)*

Endothelial Cells Control Muscle Regeneration Through Angiocrine Lactate

Katrien De Bock *(ETH Zurich, Switzerland)*

# SCIENTIFIC PROGRAM

Loss of Dscr-1 Accelerates Hypercholesterolemia Leading to Corneal Neovascularization and Opacity

**(Short Talk)** Masashi Muramatsu *(Kumamoto University, Japan)*

Angiopoietin-2–Integrin α5β1 Signaling Enhances Vascular Fatty Acid Transport and Prevents Ectopic Lipid–Induced Insulin Resistance

**(Short Talk)** Hosung Bae *(IBS, Korea)*

## Symposium 30 — September 12(Sat)

### Clinical Implication and Application of Arterial Stiffness ..... 218

Contribution of Arterial Stiffness to Cardiovascular Disease

Teemu Niiranen *(Finnish Institute for Health and Welfare, Finland)*

Left Ventricle and Arterial System Interaction in Heart Failure With Preserved Ejection Fraction

Chi Young Shim *(Severance Cardiovascular Hospital, Korea)*

Effects of Systemic Arterial Pulsatile Function on Cerebral Small Vessel Disease

Naoki Saji *(National Center for Geriatrics and Gerontology, Japan)*

The Role of Aortic Stiffness in Orthostatic Hypotension

Hack-Lyoung Kim *(Seoul National University, Korea)*

The Role of Arterial Stiffness in Chronic Kidney Disease

Hyo Jin Kim *(Pusan National University, Korea)*

Overexpression of the Amyloid-β Precursor Protein Exerts a Direct Corticosterone-Dependent Effect On in Vivo Arterial Stiffness Without Affecting Ex Vivo Arterial Wall Biomechanics

**(Short Talk)** Jhana O. Hendrickx *(University of Antwerp, Belgium)*

## Symposium 31 — September 12(Sat)

### Gut Microbiota and Atherosclerosis ..... 224

The Gut Microbiota in Atherosclerosis and Atherothrombosis

Christoph Reinhardt *(Johannes Gutenberg-University Mainz, Germany)*

Dietary Sucrose Induces Metabolic Inflammation and Atherosclerotic Cardiovascular Diseases More Than Dietary Fat in Mice: Role of the Gut Microbiota

André Marette *(Laval University, Canada)*

Probiotics and Targeted Therapeutics in Atherosclerosis

Sae Hun Kim *(Korea University, Korea)*

Single Cell Genomics of Beige Adipogenesis in Germ Free Mouse

Je Kyung Seong *(Seoul National University, Korea)*

# SCIENTIFIC PROGRAM

Current Clinical Trials With Gut Microbiota for Prevention of Atherosclerosis

Sang Hoon Kim *(CHA University School of Medicine, Korea)*

Effects of Oral Porphyromanas Gingivalis Infection on Intestinal Microbiota and Atherosclerosis

**(Short Talk)** Inyeong Kim *(Ewaha Womans University, Korea)*

## Symposium 32 — September 12(Sat)

### Lipid Guideline: What's the Difference Between Old and New? ..... 230

Comparison of Recent Guideline Statement

Alberico L. Catapano *(University of Milan, Italy)*

Nutritional Therapy with Polyunsaturated Fatty Acids: Results from Clinical Trials

Philip C. Calder *(University of Southampton, UK)*

JAS Guidelines for the Prevention of Cardiovascular Disease

Hidenori Arai *(National Center for Geriatrics and Gerontology, Japan)*

Korean Guidelines for the Management of Dyslipidemia

In-Kyung Jeong *(Kyung Hee University, Korea)*

Statin Therapy in HIGH Risk Patients With NORMal Coronary Arteries: HIGH-NORM Study

**(Short Talk)** Kyeong-Hyeon Chun *(Yonsei University, Korea)*

Assessing Validity for Extreme Risk Group of Cardiovascular Disease: A Nationwide Population-Based Study

**(Short Talk)** Kyung-Soo Kim *(CHA University School of Medicine, Korea)*

## Symposium 33 — September 12(Sat)

### Cardiovascular Health and Dietary Components: From Molecular Modification to Sensory Behaviour ..... 236

Nutritional Therapy with Polyunsaturated Fatty Acids: Results from Clinical Trials

Philip Calder *(University of Southampton, UK)*

Telomere, Cardiovascular Aging, and Lifestyle Modification

Inkyung Baik *(Kookmin University, Korea)*

The Role of Taste Receptors in CVD Risk

Emma Feeney *(University College Dublin, Ireland)*

Diverse Biological Functions of Ectopic Taste Receptors

Sung-Joon Lee *(Korea University, Korea)*

# SCIENTIFIC PROGRAM

Role of Taste Receptor in Health Behaviours Outcome: Focusing on Epidemiological Evidence

Jeong-Hwa Choi *(Keimyung University, Korea)*

Real-World Evidence of Cardiovascular Effects of Calcium Supplementation: A Nationwide Cohort Study

**(Short Talk)** Kyoung Jin Kim *(Yonsei University, Korea)*

## IVBM Joint Session

### AAVBM — September 10(Thu)

### Symposium for Asia-Australia Vascular Biology Meeting 244

Effects of Stroke Beyond the Brain

Connie Wong *(Monash University, Australia)*

Vascular Specification and Homeostasis in Development

Yulong He *(Soochow University, China)*

Lymphatic Vasculature and Cholesterol: A Love Affair?

Veronique Angeli *(National University of Singapore, Singapore)*

Tumor Vasculature as a Target for Anticancer Radiotherapy

G-One Ahn *(Seoul National University, Korea)*

Regulatory T Cell (Treg) Control of Cardiovascular Regeneration

**(Short Talk)** Kathy Lui *(The Chinese University of Hong Kong, Hong Kong)*

VEGF-Mediated Unique Epigenetic Modifications Initiated Accurate Angiogenesis via Bivalent Marked Gene-Set Activation in Endothelium

**(Short Talk)** Takashi Minami *(Kumamoto University, Japan)*

### JK Joint Session 1 250

Involvement of Vital NETosis in the Progression of High Fat Diet-Induced Atherogenic Vascular Inflammation

Masayuki Yoshida *(Tokyo Medical and Dental University, Japan)*

Role of Mitochondrial UQCRB in Tumor Angiogenesis

Ho Jeong Kwon *(Yonsei University, Korea)*

# SCIENTIFIC PROGRAM

Targeting Senescent Cells for the Treatment of Life-Style-Related Disease

Tohru Minamino *(Juntendo University, Japan)*

Heat Shock Protein 70 as a Potential Therapeutic Target for Diabetic Erectile Dysfunction

Ji-Kan Ryu *(Inha University, Korea)*

Identification of the Master Regulators of Aortic Valve Calcification

**(Short Talk)** Tomohisa Sakaue *(Ehime University , Japan)*

YAP and TAZ Regulate Lymphatic Valve Development

**(Short Talk)** Boksik Cha *(DGMIF, Korea)*

## JK Joint Session 2 ..... 256

Fibroblast Growth Factor 12, a New Regulator of Vascular Remodeling in Pulmonary Arterial Hypertension

Wonhee Suh (*Chung-Ang University, Korea)*

New Insights Into Drug-Resistance: Abnormality in Tumor Endothelial Cells

Kyoko Hida *(Hokkaido University, Japan)*

Roles of TGF-β Family Signals During Formation and Maintenance of Blood and Lymphatic Vascular Systems

Tetsuro Watabe *(Tokyo Medical and Dental University, Japan)*

Epigenetic Regulation of Vascular Calcification

Hyun Kook *(Chonnam National University, Korea)*

Analysis of the Angiopoietin-1 Expressing Macrophage in Mouse Wound Healing Model

**(Short Talk)** Seiji Yamamoto *(University of Toyama, Japan)*

Role of AIM2 Inflammasome in Post-Stroke Cognitive Impairment

**(Short Talk)** Hwa Kyoung Shin *(Pusan National University, Korea)*

## ICoLA Joint Session

### KSoLA-SHVM — September 10(Thu)

### Novel Metabolic Mediators in the Heart and Vasculature ..... 264

Clinical Aspects of Genetic Architecture of the Circulating Very Long-Chain Ceramides (24:0 and 22:0)

Linda R. Peterson *(Washington University, USA)*

# SCIENTIFIC PROGRAM

Novel Sphingolipid Mediators of Cardiovascular Pathology

L. Ashley Cowart *(Virginia Commonwealth University, USA)*

Oxidative Stress Related With Ca2+ Overload in Phosphate-Induced Vascular Calcification

Kyu-Sang Park *(Yonsei University, Korea)*

Metabolic Impairment of Akt1 Signaling and Consequences in Angiogenesis

Sunsik Bae *(Pusan National University, Korea)*

## KSoLA-APSAVD — September 10(Thu)

### Various Researches Related to the Field of Lipid and Atherosclerosis 268

Lipidomics and Mitochondrial Dysfunction in Cardiometabolic Disease

Gary Sweeney *(York University, Canada)*

The Role of Lp(a) in Premature Vascular Disease

Richard O'Brien *(University of Melbourne, Australia)*

Cardiovascular Fate of Metabolically Healthy Obese Population in Asia

Yu Mi Kang *(Yale New Haven Health, USA)*

Association of Arachidonic Acid-Derived Lipid Mediators With Subsequent Onset of Acute Myocardial Infarction in Patients With Coronary Artery Disease

Chin-Chou Huang *(Taipei Veterans General Hospital, Taiwan)*

Role of Renin-Angiotensin System Inhibitor in Hypertensive Patients with Cardiometabolic Disorder

Sang Min Park *(Eulji University Medical Center, Korea)*

Lipid Abnormalities Among Young Myocardial Infarctions

Sazzli Kasim *(University Technology Mara, Malaysia)*

## KSoLA-JAS — September 11(Fri)

### Lipid Management in Diabetes Mellitus 274

Diabetic Dyslipidemia and Coronary Heart Disease

Shinji Koba *(Showa University, Japan)*

Lipid Management in Diabetes Mellitus Secondary Prevention

Katsumi Miyauchi *(Juntendo University, Japan)*

Impact of Gender on Cardiovascular Disease in Diabetes

Hyun Min Kim *(Chung-Ang University, Korea)*

# SCIENTIFIC PROGRAM

Approach to Patients With Diabetes and Hypertriglyceridemia Whose LDL Cholesterol Is at Goal

Sung Hee Choi *(Seoul National University, Korea)*

## Special Session — September 11(Fri)

### What You Need to Know About COVID-19 ..... 278

Spillover Infections of Coronavirus From One Health Perspective

Daesub Song *(Korea University, Korea)*

COVID-19 and Cardiovascular Disease

Kyung Woo Park *(Seoul National University, Korea)*

COVID-19 and DM

Jun Sung Moon *(Yeungnam University, Korea)*

Treatment Strategy for COVID-19: Vaccine and Antiviral Drug

Pyoeng Gyun Choe *(Seoul National University , Korea)*

## KSoLA-CSATVB — September 11(Fri)

### Novel Mediators in Lipid Metabolism ..... 282

The Effect of Altered Sphingolipid Acyl Chain Length on ER Stress and Fatty Acid Synthesis

Joo-Won Park *(Ewha Womans University, Korea)*

Energy Sensing Regulates Immunometabolism in Atherosclerosis

Morgan Fullerton *(University of Ottawa, Canada)*

Roles of ELOVL5 in Lipid Metabolism

Young-Ah Moon *(Inha University, Korea)*

Numeric and Pathologic Significance of Smooth Muscle Cell Foam Cells in Atherosclerosis

Gordon Francis *(The University of British Columbia, Canada)*

Clonal Hematopoiesis as a Novel Risk Factor for Cardio-Metabolic Disorders

Changhee Jung *(University of Ulsan, Korea)*

## Publication Committee Session — September 11(Fri)

### Top Articles of 2020 in the JLA ..... 287

Persistent Organic Pollutants (POPs), Lipids and Atherosclerosis

Monica Lind *(Uppsala University, Sweden)*

# SCIENTIFIC PROGRAM

Association Between Serum Bilirubin and the Progression of Carotid Atherosclerosis in Type 2 Diabetes

Inkuk Lee *(Yonsei University, Korea)*

Associations of Dietary Intake With Cardiovascular Disease, Blood Pressure, and Lipid Profile in the Korean Population: A Systematic Review and Meta-Analysis

Tung Hoang *(National Cancer Center, Korea)*

Hepatic Expression of Serine Palmitoyltransferase Subunit SPTLC2 Reduces Lipid Droplets in Liver by Activation of VLDL Secretion

Tae-Sik Park *(Gachon University, Korea)*

Preoperative Cessation of Both Dual Anti-Platelet Agents Is Safe in Patients Receiving Percutaneous Coronary Intervention After 1-Year

Sang-Ho Jo *(Hallym University, Korea)*

## Breakfast Symposium

### Breakfast Symposium 01 — September 9(Wed)

**Sponsored by MSD** ..... 290

Strategic Cardiovascular Risk Reduction in Diabetes – What You Need to Focus

Jun Hwa Hong *(Eulji University, Korea)*

### Breakfast Symposium 02 — September 9(Wed)

**Sponsored by SERVIER** ..... 295

The Benefits of Early Initiation of Sulfonylurea and CV Safety of Gliclazide MR for Type 2 Diabetes With CV Risk

So Hun Kim *(Inha University Hospital, Korea)*

### Breakfast Symposium 03 — September 11(Fri)

**Sponsored by Pfizer Upjohn** ..... 296

Why Is It Imperative to Consider Maximum Tolerable Statin as a First Treatment Option for ASCVD Patients?

Hyun-Jae Kang *(Seoul National University Hospital, Korea)*

# SCIENTIFIC PROGRAM

## Breakfast Symposium 04 — September 11(Fri)

**Sponsored by Boehringer-ingelheim** ..... 297

EMPA-REG OUTCOME: Does Glucose Lowering Have Anything to Do with the CV Benefits of this Glucose Lowering Drug?

Silvio Inzucchi *(Yale University, USA)*

## Breakfast Symposium 05 — September 11(Fri)

**Sponsored by Takeda** ..... 298

The Power of One Pill, NesinaAct for T2DM Patients With Cardiovascular Disease Risk

Changhee Jung *(University of Ulsan, Korea)*

## Breakfast Symposium 06 — September 12(Sat)

**Sponsored by Sanofi** ..... 299

Lipid Management for ACS Patients: Resetting the Goals and Optimizing the Treatment

Alberico L. Catapano *(University of Milan, Italy)*

## Breakfast Symposium 07 — September 12(Sat)

**Sponsored by Hanmi** ..... 300

Ezetimibe/Rosuvastatin Combination for CVD Prevention

Chan Joo Lee *(Yonsei University, Korea)*

## Breakfast Symposium 08 — September 12(Sat)

**Sponsored by Chong Kun Dang** ..... 301

Comparison of the Efficacy, Safe, and Cost-Effectiveness of Atorvastatin 10mg and 20mg in High-Risk Asian Patients with Hypercholesterolemia, The PEARL Study: A Randomized Controlled Multicenter Trial

Ji Bak Kim *(Gimpo Woori Hospital, Korea)*

# SCIENTIFIC PROGRAM

## Luncheon Symposium

### Luncheon Symposium 01 — September 10(Thu)

**Sponsored by Thermo Fisher Scientific** ..... 304

3D Imaging and Analysis Platform for Angiogenesis

Taewoo Kim *(Thermo Fisher Scientific Solutions, Korea)*

### Luncheon Symposium 03 — September 10(Thu)

**Sponsored by IVIM Technology** ..... 305

IVIM Technology's IntraVital Microscopy (IVM): In Vivo Live Cell Imaging

Pilhan Kim *(IVIM Technology / KAIST, Korea)*

### Luncheon Symposium 04 — September 10(Thu)

**Sponsored by Daewoong** ..... 306

Time in Range International Consensus and the Application in Clinical Practice in Korea

Sang Soo Kim *(Pusan National University, Korea)*

### Luncheon Symposium 05 — September 10(Thu)

**Sponsored by Yuhan** ..... 307

Complications and Management in Diabetes Patients With Coronay Heart Disease

Jae-hyuk Lee *(Myongji Hospital, Korea)*

### Luncheon Symposium 06 — September 10(Thu)

**Sponsored by JW Pharmaceutical** ..... 308

Additional Benefits of Anagliptin Beyond Glycemic Control

Jeong Hyun Park *(Inje Univeristy, Korea)*

# SCIENTIFIC PROGRAM

## Luncheon Symposium 07 — September 11(Fri)

**Sponsored by Amgen** ..... 309

The Importance of Earlier and Lower LDL-C Reduction With Evolocumab in ASCVD Patients

Jeehoon Kang *(Seoul National University Hospital, Korea)*

## Luncheon Symposium 08 — September 11(Fri)

**Sponsored by MSD** ..... 310

How Can We Manage LDL-Cholesterol? – From the Past to the Future

Anselm Gitt *(Klinikum Ludwigshafen, Germany)*

## Luncheon Symposium 09 — September 11(Fri)

**Sponsored by AstraZeneca** ..... 311

See the Unseen; SGLT2 Inhibitors' Potential Beyond Glucose Control

Soo Lim *(Seoul National University Bundang Hospital, Korea)*

## Luncheon Symposium 10 — September 12(Sat)

**Sponsored by Daewoong** ..... 312

Dyslipidemia Management in High-Risk Patients: "Rosuvastatin+Ezetimibe" Combination Therapy

Weon Kim *(Kyung Hee University, Korea)*

## Luncheon Symposium 11 — September 12(Sat)

**Sponsored by Yuhan** ..... 313

The Benefits of Intensive Lipid Lowering Therapy

Jong-Young Lee *(Sungkyunkwan University, Korea)*

## Luncheon Symposium 12 — September 12(Sat)

**Sponsored by JW Pharmaceutical** ..... 314

All Statins Are the Same in Outcome and Safety for Asian?

Hyuk-Sang Kwon *(The Catholic University of Korea, Korea)*

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# Plenary Lecture

September 9 (Wed) ~ October 10 (Sat), 2020 I Virtual Meeting (On demand)
September 9 (Wed) ~ 12 (Sat), 2020 I IVBM 2020 LIVE for Korean

KSoLA
The Korean Society of Lipid and Atherosclerosis

KVBM
Korean Society for Vascular Biology and Medicine

# Endothelial Heterogeneity in Health and Disease

**Donald M. McDonald**[1*]

[1] Cardiovascular Research Institute & Department of Anatomy, University of California San Francisco (UCSF), USA

*donald.mcdonald@ucsf.edu*

My lecture will review the contributions we have made to understanding endothelial cell heterogeneity in the vasculature in health and disease. The story began years ago with the development of methods for elucidating endothelial cell diversity in the microcirculation under normal conditions and then in inflammation and cancer, where permeability is increased. The work revealed striking disease-dependent changes in endothelial cells. In inflammation, focal intercellular gaps rapidly and reversibly form in venules. When inflammation is sustained, the phenotype of capillary endothelial cells can acquire the features of venules. By contrast, endothelial cells in tumor blood vessels are highly abnormal, regardless of the tumor type, and lack the features of arterioles, capillaries, or venules. Endothelial cells of tumor vessels are especially leaky because of intercellular separations much larger than the focal gaps that form in inflammation. The abnormalities in tumor blood vessels are reduced by inhibition of VEGF signaling, through the process of vascular normalization, which can increase blood flow and improve drug delivery. However, the distinctive abnormalities of endothelial cells in tumors can also enable homing of therapeutics to tumors. The finding that cationic liposomes are internalized selectively by tumor vascular endothelial cells has been used for drug delivery. This property also led to recognition that oncolytic vaccinia viruses injected intravenously selectively infect endothelial cells of tumors before they infect tumor cells. Viruses that thereby target tumors evoke anti-tumor immune responses that lead to tumor cell killing and amplify effects of immune checkpoint inhibitors. With these and many other advances in the understanding of vascular diversity and plasticity, endothelial cell heterogeneity is now recognized as not only an essential feature of normal vascular physiology but also a property of diseased tissues that can be exploited therapeutically.

Plenary Lecture 02

# Guidance of Organ-Specific Vascular Barrier Formation

**Anne Eichmann**[1*]

[1] Internal Medicine (Cardiology), Yale University, USA

*anne.eichmann@yale.edu*

Tissue homeostasis requires coordinated barrier function in blood and lymphatic vessels. Opening of junctions between endothelial cells (ECs) lining blood vessels leads to tissue fluid accumulation that is drained by lymphatic vessels. A pathological increase in blood vessel permeability or lack or malfunction of lymphatic vessels leads to edema and associated defects in macromolecule and immune cell clearance. Unbalanced barrier function between blood and lymphatic vessels contributes to neurodegeneration, chronic inflammation, and cardiovascular disease. We sought to gain mechanistic understanding into coordination of barrier function between blood and lymphatic vessels, how this process is altered in disease models and how it can be manipulated for therapeutic purposes. We have focused on two critical barriers with diametrically opposing functions, the blood-brain barrier (BBB) and the lymphatic capillary barrier (LCB). ECs of the BBB form very tight junctions that restrict paracellular access to the brain. In contrast, open junctions of the LCB ensure uptake of extravasated fluid, macromolecules and immune cells, as well as lipid in the gut. We have identified novel effectors of BBB and LCB junctions and their role in formation of tissue specific EC barriers, with perspectives for research and treatment of neurovascular and cardiovascular disease.

# Organ-Specific and Functional Specialization of Vascular Cells

**Ralf H. Adams**[1*]

[1] Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Germany

*ralf.adams@mpi-muenster.mpg.de*

Blood vessels in the skeletal system control bone formation and provide niches for hematopoietic stem cells (HSCs). Insight into the architecture and function of the vasculature in the skeletal system was previously limited by the heavily calcified and matrix-rich properties of bone. We have managed to overcome many of these limitations with the help of improved protocols for the processing, immunostaining and live imaging of bone (Kusumbe et al., Nat. Protoc. 2015; Bixel et al., Cell Rep. 2017). We found that vascular growth in bone involves a specialized form of angiogenesis that is distinct from other organs. Notch signaling promotes vascular growth in postnatal long bone, which is the opposite of the well-established function of this pathway in endothelial cells (ECs) of other organs. In addition, Notch controls the release of angiocrine signals from the bone endothelium and thereby controls immature perivascular mesenchymal cells and osteoprogenitors. We also discovered that endothelial hypoxia-inducible factor 1α promotes angiogenesis in the postnatal skeletal system, leads to the amplification of osteoprogenitor cells and thereby improves bone formation (Kusumbe et al., Nature 2014; Ramasamy et al., Nature 2014; Langen et al., Nat. Cell Biol. 2017; Sivaraj et al. eLife 2020). Aging is associated with a loss of bone mass and reduced HSC functionality. Our work has uncovered extensive age-related changes in the bone vasculature, which involve alterations in arteries, blood flow, perivascular mesenchymal cells, and capillary EC subpopulations. These changes are associated with a strong reduction in endothelial Notch signaling and, remarkably, EC-specific reactivation of the Notch pathway in aged mice triggers increases in bone formation and HSC niches (Kusumbe et al., Nature 2016; Ramasamy et al., Nat. Commun. 2016). EC subpopulations are also critical for regeneration following lethal irradiation and bone marrow transplantation (Chen et al., Cell Stem Cell 2019).

Plenary Lecture 04

# CAP1 Binds to Resistin or PCSK9, Standing at the Nodal Point of Disease Clusters Such As Metabolic Syndrome, Fatty Liver, and Hypercholesterolemia and Cancer

**Hyo-Soo Kim**[1*]

[1] Strategic Center of Cell & Bio Therapy, Cardiovascular Center, Seoul National University Hospital, Seoul, Korea

*usahyosoo@gmail.com*

Resistin is an adipose-secreted cytokine first identified as a mediator of insulin resistance in obese mice. In human, however, peripheral blood mononuclear cells and macrophages are the primary source of resistin. We demonstrated that resistin is a causal factor to aggravate atherosclerosis by stimulating monocytes and inducing vascular inflammation (JACC 2011).

We identified adenylyl cyclase-associated protein 1 (CAP1) as a novel functional receptor for human resistin and clarified its intracellular signaling pathway to modulate inflammatory action of monocytes (Cell Metabolism 2014). We found that human resistin directly binds to CAP1 in human monocytes to mediate up-regulation of intracellular cAMP concentration, PKA activity and NF-kB related transcription of inflammatory cytokines such as IL-6, TNFα. Adenovirus-mediated over-expression of CAP1 in monocytes resulted in the increased cAMP, PKA and NF-kB activities by resistin, and these CAP1-over-expressed monocytes aggravated adipose tissue inflammation in humanized resistin mouse obesity model. In contrast, suppression of CAP1 expression by small-interfering RNA abrogated the resistin-mediated activity of monocytes both in vitro and in vivo. Taken together, CAP1 serves as a functional receptor for human resistin and modulates production of inflammatory cytokines in monocytes through cAMP dependent signaling pathway.

To further substantiate the role of CAP1 in metabolism in vivo, we made TALEN-mediated CAP1 knock-out mouse. CAP1 homogenous knock-out mouse was lethal. In TALEN-mediated CAP1 heterogenous knock-out mouse (CAP1+/−mouse), we confirmed the significant reduction of the expression levels of endogenous CAP1 compared with WT controls (CAP1+/+ mouse). Up to 16 weeks of age, however, the CAP1+/−mice and those of age- and sex-matched control mice were not different grossly. But when they were fed with high fat diet, CAP1+/− mice showed better profiles of lipid and glucose metabolism (low LDL-C & more sensitive to insulin). The viable heterozygous CAP1 knock-out mice had higher protein levels of LDLR in the liver and lower LDL-C levels in the plasma, than the control mice. We noticed that globular C-terminus of CAP1 is structurally similar to the C-terminal cysteine-rich domain (CRD) of PCSK9. We investigated the role of CAP1 in PCSK9-mediated lysosomal degradation of LDLR and plasma LDL cholesterol (LDL-C) levels. The direct binding between PCSK9 and CAP1 was confirmed by immunoprecipitation assay, far-western blot, biomolecular fluorescence complementation and surface plasmon resonance assay. Fine mapping revealed that the CRD of PCSK9 binds with the Src homology 3 binding domain (SH3BD) of CAP1. Mechanistic analysis revealed that PCSK9-induced endocytosis and lysosomal degradation of LDLR were mediated by caveolin but not by clathrin, which were dependent on binding between CAP1 and caveolin-1 (European Heart J 2019). We identified CAP1 as a new binding partner of PCSK9 and a key mediator of caveolae-dependent endocytosis and lysosomal degradation of LDLR.

# Translating Lymphangiogenesis Mechanisms to Therapeutics

**Kari K. Alitalo**[1*]

[1] Research Programs Unit, Wihuri Research Institute, University of Helsinki, Finland

*kari.alitalo@helsinki.fi*

Lymphangiogenesis and lymphatic vasculature research has gained increased momentum during the past several years. This has resulted in an amazing amount of new discoveries about lymphatic vessel development, and plasticity and functions of lymphatic endothelium, capillaries, collecting vessels and lymph nodes in fluid transport, immunosurveillance and various organ-specific functions. Vascular endothelial growth factor C (VEGF-C) is arguably the most important growth factor for the lymphatic vascular system and the formation of the VEGF-C/ADAMTS3/CCBE1 multiprotein complex on the surface of the lymphatic endothelial cells is the rate-limiting step for VEGF-C activation. – Consequently, VEGF-C treatment of secondary lymphedema after breast cancer surgery has already proceeded to a phase 2 clinical trial whose results should be published in the near future. - In primary lymphedema, germline mutations have been identified most commonly in the VEGF-C receptor VEGFR-3 gene (FLT4), but also in over 20 genes that encode proteins acting around the VEGFR-3 signaling. Yet, even today, for the majority of hereditary lymphedema conditions, causative genes have not been identified. Thus, there are still ample opportunities to identify novel interventional targets, of which I will present one example. It is clear that lymphatic vessels participate also in pathophysiological conditions interfering with interstitial and ocular fluid pressure, immune and adipose tissue and metabolic regulation, and in anticancer immunity, tumor metastasis, neurodegenerative, neuroinflammatory and inflammatory bowel diseases. In many of these, the emerging in-depth mechanistic insights into the lymphangiogenic process have encouraged further development therapeutic interventions and organ-specific regenerative strategies.

Plenary Lecture 06

# Cerebral Cavernous Malformation: From Mechanism to Therapy

**Mark L. Kahn**[1*]

[1] Medicine/Cardiovascular Institute, University of Pennsylvania, USA

*Markkahn@pennmedicine.upenn.edu*

Cerebral cavernous malformations (CCMs) are common vascular malformations that often cause stroke and seizures in younger individuals due to associated hemorrhage. In the past decade tremendous progress has been made in understanding the molecular mechanisms that underlie this disease, and we are on the cusp of introducing new medical therapies based on these new mechanistic insights. Biochemical and genetic studies in model organisms have identified the core molecular mechanism of CCM disease as loss of negative regulation of MEKK3, a MAPK that drives expression of KLF2 and KLF4 transcription factors in endothelial cells. Studies to identify the upstream activators of MEKK3-KLF2/4 signaling have unexpectedly revealed a direct gut-brain axis for CCM disease in which gram negative bacteria in the gut drive the disease through release of lipopolysaccharide that enters the bloodstream and activates brain endothelial TLR4 receptors. Studies in mice and humans have shown that individuals with higher levels of gram negative bacteria are at higher risk for the disease, and that impairment of the gut barrier also accelerates disease progression. Recent studies to address the downstream effector mechanisms for CCM formation have revealed ADAMTS5 cleavage of versican and cell non-autonomous effects of versican proteolytic products as an important driver. These insights are likely to lead to new therapies, such as glucocorticoids that in mice potently suppress CCM formation by action at both brain endothelial cells and gut epithelial cells. These studies provide deep insight into a single vascular malformation and serve as a paradigm for investigation and treatment of other vascular diseases.

# 'TEK-TOK' – Discovery of Vascular Targets to Treat Ocular Diseases

**Susan Quaggin[1*], Benjamin Thomson[1], Tuncer Onay[1]**

[1] Feinberg School of Medicine, Northwestern University, USA

*quaggin@northwestern.edu*

Primary congenital glaucoma (PCG) is a particularly severe form of glaucoma that results in vision loss in children. It is characterized by developmental defects in Schlemm›s canal (SC) and the trabecular meshwork (TM), which comprise the conventional aqueous humor outflow pathway of the eye leading to raised intraocular pressure. Recently, loss of function mutations in genes encoding components of the Angiopoietin-Tie2/TEK (Angpt/Tie2) vascular signaling pathway as well as variants in a gene encoding a vascular-associated matrix protein have been identified in children with PCG. In addition, variants in the ANGPT1 gene have been linked to primary open angle glaucoma in adults. We propose a model whereby cellular crosstalk between trabecular meshwork cells expressing Angiopoietin-1 and SC endothelial cells expressing Tie2/TEK is required for development and maintenance of the outflow pathway. Single-cell transcriptomic analysis of normal and glaucomatous Angpt1 deficient mouse eyes allowed us to identify distinct TM and SC cell populations and additional candidate glaucoma genes. Furthermore, activation of the Angpt/Tie2 pathway in a mouse model of PCG, is able to promote SC development in glaucomatous eyes. Our data highlight the central role of Angiopoietin-Tie2/TEK signaling and TM-SC crosstalk in intraocular pressure homeostasis and provide new candidates for SC-targeted glaucoma therapy.

Plenary Lecture 08

# Normalizing the Tumor Microenvironment to Improve Cancer Immunotherapy: Bench to Bedside

**Rakesh K. Jain**[1*]

[1] Massachusetts General Hospital, Harvard Medical School, USA

*jain@steele.mgh.harvard.edu*

Immunotherapy has revolutionized cancer treatment. However, less 15% of the patients currently benefit from immunotherapy. Our hypothesis is that the abnormal microenvironment of tumors compromises the efficacy of all therapies, including immunotherapy. Our laboratory has shown that the blood and lymphatic vasculature, fibroblasts, immune cells and the extracellular matrix associated with tumors are abnormal, and these different entities collaborate together to create a hostile biochemical and physical tumor microenvironment characterized by hypoxia, low pH and high interstitial fluid pressure and solid stress. These abnormalities can fuel tumor progression and metastasis, induce immunosuppression, impair delivery of therapeutic molecules and cells to and within tumors, and compromise their efficacy once they accrue in tumors. Our hypothesis is that "normalizing" the tumor microenvironment can improve cancer treatment. I'll discuss two "normalizing" strategies developed in our laboratory –"vascular normalization" and "stroma normalization"– to overcome these barriers and improve delivery and efficacy of immunotherapeutics, and how we have taken these strategies from bench to bedside.

RK Jain. Normalization of the tumor vasculature: An emerging concept in anti-angiogenic therapy of cancer. Science 307: 58 (2005)

M Pinter and RK Jain. Targeting the renin-angiotensin system to improve cancer treatment: Implications for immunotherapy. Science Translational Medicine 9: eaan5616 (2017)

D Fukumura, et al. Enhancing Cancer Immunotherapy Using Antiangiogenics: Opportunities and Challenges. Nature Rev Clinical Oncology 15:325 (2018)

JE Murphy JE et al. Total Neoadjuvant Therapy with FOLFIRINOX in Combination with Losartan Followed by Chemoradiotherapy for Locally Advanced Pancreatic Cancer: A Phase II Clinical Trial. JAMA Oncology 5:1020 (2019)

VP Chauhan, et al. Reprogramming the microenvironment with tumor-selective angiotensin blockers enhances cancer immunotherapy. PNAS 116: 10674 (2019)

L Munn and RK Jain. Vascular regulation of anti-tumor immunity. Science 365: 544 (2019)

F Mpekris, et al. Combining microenvironment normalization strategies to improve cancer immunotherapy. PNAS. 117: 3728 (2020)

# Pulmonary Hypertension and Marfan Syndrome

**Issei Komuro**[1*]

[1] Cardiovascular Medicine, The University of Tokyo, Japan

*komuro-tky@umin.ac.jp*

Pulmonary hypertension (PH), characterized by progressive occlusion or narrowing of the pulmonary artery, is a disease of very bad prognosis. There is abnormal proliferation of endothelial cells and smooth muscle cells of the pulmonary artery, but its mechanisms and the role of vascular endothelial growth factor (VEGF) remain unknown. We have established novel 3D visualization method using tissue-clearing method. We used 3 kinds of PH murine models; two mild PH models and ove severe PH model. 3D-image analysis revealed that vessels are stretched at peripheral lung in mild PH models but not in severe PH model. PGC1α expression was upregulated only in mild PH models. Endothelial cell-specific ablation of Pgc-1α (eKO) decreases pulmonary vessel density with reduced Vegfa expression and exacerbates hypoxia-induced PH, suggesting that PGC1⟨-induced VEGF induces angiogenesis and ameliorates PH. Marfan syndrome (MFS) is a genetic disease with various abnormalities including aortic aneurysm and dissection. Fiburillin-1 mutation causes MFS and activation TGF-® seems to be involved in aortic abnormalities, however, it is still elusive how TGF-® is activated and what else factors are involved. We have found that xanthine oxidase (XO) is upregulated in aorta of MFS. Endothelial cell specific deletion of XO ameliorated enlargement of aorta. Aortic wall thickening with degeneration of elastic fibers and deposition of proteoglycan were attenuated and activation of FAK, p38 MAPK, smad2 and ERK1/2 was suppressed in knockout MFS mice, suggesting that XO plays a critical role in aortic abnormalities of MFS. Mechanical stresses in vitro and in vivo upregulated XO and activated FAK and p38 MAPK. Inhibition of p38MAPK inhibited mechanical stress-induced upregulation of XO. These results suggest that enhanced responses to mechanical stresses upregulates XO and increased reactive oxygen species induced aortic abnormalities in MFS.

Plenary Lecture 10

# Clonal Hematopoiesis at the Crossroads of Aging, Cancer, and Cardiovascular Disease

**Peter Libby**[1*]

[1] Cardiovascular Medicine, Brigham and Women's Hospital - Harvard Medical School, USA

*PLIBBY@BWH.HARVARD.EDU*

Age or exposure to environmental mutagens can lead to mutations in hematopoietic stem cells in the bone marrow in leukemia driver genes. Such mutational events license expansion of the mutated leukocyte lineage such that a clone of mutant cells appears in peripheral blood, but does not cause a hematologic malignancy. The development of leukemia generally requires the acquisition of 2-3 successive mutations within the same clone. Clonal hematopoiesis (CH) occurs when a single dominant stem cell lineage expands and comprises > 4% of the population of mature leukocytes (measured in terms of variant allele frequency, or VAF). CH affects more than 10% of septuagenarians. Most people with clonal hematopoiesis have no symptoms and have a modest but increased risk of developing acute leukemia (~ 0.5-1% per year.) Surprisingly, CH links strongly and consistently with overall cardiovascular mortality. Individuals with CH have a several fold increase in major adverse cardiovascular events. The relationship between CH and cardiovascular disease is independent of traditional atherosclerosis risk factors such as age, cholesterol concentration, blood pressure, diabetes, or smoking. CH associates significantly with atherosclerosis, acute coronary syndromes, stroke, and ischemic cardiomyopathy and survival following percutaneous aortic valve replacement. Multiple observational studies support the relationship between CHIP and cardiovascular events, and experimental investigations support a causal relationship between CH and cardiovascular disease. Moreover, an Interleukin (IL)-1β and NLRP3 inflammasome-dependent mechanism contributes to accelerated atherosclerosis in CH. Thus, CH represents a newly recognized, common, strong, and independent cardiovascular risk factor, and a novel link between inflammation and atherosclerosis.

# CV Risk Reduction With Diabetes Medications: In Search of the Holy Grail

**Silvio Inzucchi**[1*]

[1] Endocrinology, Yale School of Medicine, USA

*silvio.inzucchi@yale.edu*

Cardiovascular (CV) disease is the leading cause of mortality in patients with type 2 diabetes (T2D.) Yet, controlling blood glucose itself has had little to no impact on reducing the risk of these atherosclerotic complications. Over the years, individual glucose lowering drug categories have also been tested for their CV benefits. Insulin, sulfonylureas, and DPP-4 inhibitors appear to be neutral for major adverse CV events (MACE.) Metformin may be beneficial but the database is far from robust. TZDs have a complex history in this regard. The only widely used agent of this class, pioglitazone, reduces atherosclerotic events but increases heart failure. Two newer medication categories, the SGLT2 inhibitors and the GLP-1 receptor agonists have more recently been demonstrated to reduce MACE, particularly in those with established macrovascular disease. With the SGLT2 inhibitors, the benefits include reducing heart failure (HF) hospitalizations and the progression of chronic kidney disease (CKD.) In addition, these advantages extend to non-diabetic individuals with HF with reduced ejection fraction as well as CKD, respectively. These two evidence-based categories are now prioritized in various diabetes treatment guidelines for their specific benefits in high risk T2D patients. I will review the link between diabetes and CVD, the current T2D therapeutic landscape, the major CV outcome trials of older and newer glucose lowering agents, and the latest therapeutic algorithms for using these medications in patients with T2D.

Plenary Lecture 12

# Heart Failure: Are We Winning?

**John J. V. McMurray**[1*]

[1] BHF Cardiovascular Research Centre, University of Glasgow & Queen Elizabeth University Hospital, Glasgow, Scotland, UK

*john.mcmurray@glasgow.ac.uk*

The focus of my presentation is on heart failure with reduced ejection fraction (HFrEF). I will review the exciting developments in pharmacological therapy that we have seen over the past 5-6 years and especially in the last year. Specifically, I will concentrate on sacubitril/valsartan (an ARNI), dapagliflozin (a SGLT2 inhibitor) and vericiguat (a soluble guanylate cyclase stimulator) and the key trials demonstrating the value of these new therapies i.e. PARADIGM-HF, DAPA-HF and VICTORIA, respectively. I will describe the evidence that shows that the two of these that reduce mortality (sacubitril/valsartan and dapagliflozin) have additive benefit and should be used in combination. I will show new data demonstrating the substantial gains in event-free survival obtained with combination therapy. Finally, I will look ahead at what else is in the therapeutic pipeline, specifically mentioning the GALACTIC-HF trial, using the cardiac-specific myosin activator omecamtiv mecarbil, which will report by the end of 2020.

# The Intersection of Infection, Inflammation and Vascular Occlusion

**Jane E. Freedman**[1*]

[1] Medicine, University of Massachusetts Medical School, USA

*jane.Freedman@umassmed.edu*

Heart and vascular diseases are the leading cause of death in many developed countries and acute ischemia is well known to be due to occlusive events. However, recently, increased mortality due to thrombosis has been noted during infection, influenza and pneumonia. Normal hemostasis may be overwhelmed by pathological factors, leading to uncontrolled clot formation and vessel occlusion in either the arteries or veins. Platelets are the central mediators of both normal arterial hemostasis and pathological thrombosis. Normally, platelets and immune cells can form a physical barrier of confinement to prevent dissemination of pathogens and stimulate the immune system, but platelets may also become activated and occlude vessels. The mechanisms by which the immune system and platelets communicate is being appreciated as complex, diverse, and necessary to maintain normal immunity while avoiding thrombosis. It requires activation of specific membrane receptors that appear dependent upon the select pathogen as well as platelet release of cytokines, chemokines, and antimicrobial peptides, all leading to sequestration and destruction of bacterial and viral pathogens. Harnessing our growing understanding of these complex cell-like structures in the setting of infection will allow for better treatment while avoiding vascular occlusive diseases.

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# Symposium

September 9 (Wed) ~ October 10 (Sat), 2020 I Virtual Meeting (On demand)
September 9 (Wed) ~ 12 (Sat), 2020 I IVBM 2020 LIVE for Korean

KSoLA
The Korean Society of Lipid and Atherosclerosis

KVBM
Korean Society for Vascular Biology and Medicine

# SMAD6 Regulates Vascular Flow Responses

**Victoria Bautch[1,2*], Dana L Ruter[1], Ziqing Liu[1], Kimlynn Ngo[1], Shaka X[1], Allison Marvin[1], Elise Kidder[1], Danielle B Buglak[1]**

[1] McAllister Heart Institute, UNC-Chapel Hill, USA
[2] Dept of Biology, UNC-Chapel Hill, USA

*bautch@unc.edu*

Blood vessel formation requires endothelial cell responses to proangiogenic signals, such as VEGF-A and BMP, to form and expand vessel networks via sprouting angiogenesis. Once networks form, increased blood flow sets up mechanical cues via fluid shear stress, and blood vessels then switch to vascular homeostasis characterized by endothelial cell quiescence and alignment in the direction of flow. In larger arteries, significant laminar shear stress contributes to maintenance of vascular homeostasis throughout life, and loss of this state is associated with hemorrhage and atherosclerosis. We investigated how a negative regulator of BMP signaling, SMAD6, functions in vascular homeostasis. We show that genetic loss of SMAD6 leads to vascular hemorrhage and early lethality. Under laminar flow, SMAD6 is required for endothelial cell flow-mediated responses leading to maintenance of barrier function and vascular homeostasis, downstream of the mechanosensor Notch1. SMAD6 functions downstream of ligand-induced Notch signaling and transcription regulation, and mechanistically, full-length SMAD6 protein is needed to rescue Notch loss-induced flow misalignment. Endothelial cells depleted for SMAD6 were "activated" under flow, with upregulation of proliferation-associated genes and down regulation of junction-associated genes. The vascular protocadherin PCDH12 was upregulated by SMAD6 and required for proper flow-mediated endothelial cell alignment, placing it downstream of SMAD6. Thus, SMAD6 is a required transducer of flow-mediated signaling inputs downstream of Notch1 and upstream of PCDH12, as vessels transition from an angiogenic phenotype to maintenance of a homeostatic phenotype.

Symposium 01-2

# Endodermal-Mesoderman Transition for Vascular Development

**Hiroyuki Ishikawa[1], Hiroyuki Nakajima[1], Didier Stainier[2], Naoki Mochizuki[1*]**

[1] Dept. of Cell Biology, National Cerebral & Cardiovascular Center Research Institute, Japan
[2] Dept. of Genetics, Max Planck Institute for Heart and Lung Research, Germany

*mochizuki@ncvc.go.jp*

Blood vessel-constituting cells including endothelial cells and smooth muscle cells orginate from mesodermal cells. Endothelial cells of main blood vessels, doral aorta (DA) and posterior cardinal vein (PCV), of zebra fish are thought to come from posterior lateral plate mesoderm. However, the exact origin of endothelial cells in the tail is not unclear. We here demonstrate that sox32-positve endodermal cells beside lateral plate mesodermal cells become venous endothelial cells of PCV but not DA.

The depletion of sox32 resulted in impairment of PCV development. These sox32-positive cells expressed islet-1 (isl1) and became etv2-positive endothelial cells and required npas4l to give rise to endothelial cells. Sox32-positive cells outside of Kupffer's vesicle differentiated into venous endothelial cells of the tail and expressed isl1. We further tracked isl1 promoter-activated cells by crossing Tg(isl1:Cre) driver fish crossed with Tg(fli1:loxp-mCherry-loxp-EGFP) fish. Isl1-positive cells were found exclusively in the posterior part of PCV. Npas4l (cloche) mutant fish exhibited less PCV cells. Therefore, we conclude that sox32-positive endodermal cells contribute to the formation of PCV.

# Principles and Regulation of Endothelial Cell Dynamics in Vascular Remodelling

**Holger Gerhardt**[1,2,3,4*]

[1] Integrative Vascular Biology, Max-Delbrück-Centrum für Molekulare Medizin (MDC), Germany
[2] Vascular Biomedicine, Berlin Institute of Health, Germany
[3] Partner site Berlin, German Center for Cardiovascular Research (DZHK), Germany
[4] Center for Cancer Biology, VIB, KU Leuven, Belgium

*Holger.Gerhardt@mdc-berlin.de*

Formation, expansion and functional adaptation of vascular networks are critical for development and physiology in vertebrates. How endothelial cells orchestrate their behavior to form the shape and size of individual vessels and establish the hierarchical branching pattern of functional networks remains poorly understood. Using a combination of in vivo cell biology experimentation and generative computational modelling, our lab uncovered a series of fundamental principles of endothelial cell behavior driving the first steps of branching and lumen formation, as well as subsequent remodeling to achieve functional patterning. The surprising dynamics of endothelial rearrangements in already perfused vessels suggest that vessel adaptations rely on differential migration of cells to reshape vessels in response to changing flow conditions. I will discuss concepts and mechanisms of how physical forces and signaling pathways jointly regulate functional endothelial rearrangements to first establish symmetry in primitive vascular networks, and then break this symmetry to drive formation of arteries, veins and an adequately branched capillary plexus. Emerging insights into endothelial heterogeneity in these processes will be discussed.

Symposium 01-4

# Bone Morphogenetic Protein Signaling in Vascular Development and Homeostasis

**Suk-Won Jin**[1*]

[1] Life Sciences, GIST, Korea

*sukwonjin@gist.ac.kr*

Bone Morphogenetic Protein (BMP) signaling has been shown to provide critical regulatory input during vascular development. In particular, we and others have previously shown that BMP signaling is critical for modulating angiogenesis from venous endothelial cells. However, function of individual BMP receptors has not been fully elucidated in endothelial cells due to the innate complexity of signaling cascade. To delineate unique function of each BMP receptors, we analyzed the phenotype of endothelial specific indocile knockout mice. We find that Activin A Type I Receptor (ALK2/ACVR1) but not ALK3/BMPRIA receptor mediates pro-angiogenic BMP signaling in venous ECs during angiogenesis. Our findings suggest that the presence of multiple receptors with spatiotemporally distinct expression patterns in conjunction with the promiscuity of the BMP ligand-receptor interaction enables BMP signaling to create subtype-specific signaling outcomes, and provide a conceptual framework for developing novel therapies targeting angiogenesis.

# VEGF-Mediated Activation of ERK and PI3K Balances Endothelial Cell Migration and Proliferation During Angiogenesis

**Arndt Siekmann[1,2*], Martin Lange[2], Nils Ohnesorge[2]**

[1] Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, USA
[2] Laboratory for Cardiovascular Patterning, Max Planck Institute for Molecular Biomedicine, Germany

*arndt.siekmann@pennmedicine.upenn.edu*

Vascular Endothelial Growth Factor (VEGF) is a key signalling pathway controlling blood vessel sprouting via its function in regulating endothelial cell migration, proliferation, artery formation and vascular permeability. However, it is still not clear how the activation of individual signalling pathways downstream of VEGF ligand-receptor interaction is being controlled in order to allow for ordered angiogenesis.

We have generated mutants for the two VEGFA homologs in zebrafish, VEGFAA and VEGFAB and analyzed their vascular phenotypes. In addition we performed gain and loss of function experiments for VEGFAB, PI3 kinase and p21 and analyzed the effects of these perturbations on embryonic vascular development.

As expected, mutants for VEGFAA showed severe vascular defects. Surprisingly, we observed only subtle angiogenesis defects in VEGFAB mutants despite a reduction in endothelial cell numbers. Using in vivo imaging in combination with loss and gain of function studies in zebrafish embryos, we found that VEGFAB specifically affected endothelial cell proliferation. Analysis of VEGF downstream signalling pathway activation in VEGFAB mutants revealed defects in Phosphoinositide 3-kinase (PI3K) signalling, while Extracellular signal-regulated kinase (ERK) signalling was unaffected. Blocking PI3K signalling resulted in similar vascular defects as we observed in VEGFAB mutants. Finally, we identified the negative cell cycle regulator cdkn1a (p21) as a transcriptional target for VEGFAB signalling. Accordingly, knockdown of cdkn1a was capable of rescuing endothelial cell numbers in VEGFAB mutants.

Together, our results uncover an unanticipated selectivity of PI3K activation downstream of VEGFAB signalling and shed light on how this activity contributes to proper angiogenesis via controlling endothelial cell proliferation.

Symposium 01-6 Short Talk

# Depletion of Endothelial TGF-β Signaling Induces Tumor Angiogenesis and Metastasis

**Fumiko Itoh[1*], Takahiro Takagi[1], Yuki Saito[1], Kako Hanada[1], Marcus Fruttiger[2], Susumu Itoh[3]**

[1] Laboratory of Cardiovascular Medicine, Tokyo University of Pharmacy and Life Science, Japan
[2] National Institute of Medical Research,, MRC, London,, UK
[3] Laboratory of Biochemistry, Showa Pharmaceutical University, Japan

*mame.fumiko@gmail.com*

At the late stage of tumorigenesis, cancer cells produce a number of TGF-β which promotes tumor proliferation and metastasis. Although angiogenesis plays a key role in the growth of cancer cells, it remains veiled how abundant TGF-β is involved in tumor angiogenesis.

In the current study, we have generated tamoxifen-inducible knockout mice of TGF-β type II receptor (TβRII) specifically in endothelial cells by crossing TβRII-floxed (TβRIIfl/fl) mice with the platelet derived growth factor (Pdgfb)-iCreERT2 mice; TβRIIfl/fl; Pdgfb-iCreERT2 (TβRIIΔiBEC). The mice were transplanted with Lewis lung carcinoma (LLC) cells and administrated tamoxifen to induce gene deficiency, and we analyzed the effect of tumor angiogenesis and metastasis were verified.

depletion of endothelial TGF-β type II receptor (TβRII) in the postnatal mouse significantly potentiated tumor angiogenesis in vivo. However, the loss of TβRII inhibited the maturation process of the blood formation to form leaky blood vessels. Consequently, hypoxia was accelerated in the tumor tissues. These changes in the tumor microenvironment led to the increase of circulated tumor cells, whereas spontaneous pulmonary metastasis in mice lacking endothelial TβRII decreased compared to wild-type mice. Similarly, three weeks after tamoxifen administration, experimental metastases were tested, and knockout mice also suppressed lung metastases of intravenously injected LLC.

In primary tumors endothelial TGF-β signals promotes maturation of blood vessels and inhibits tumor invasion, whereas in metastatic organs vascular endothelial cells were promoted adhesion of cancer cells by TGF-β signals. These results revealed part of the complex role of TGF-β signaling in vascular endothelial cells in vivo.

# Biological Robustness: Genetic Compensation and Transcriptional Adaptation

**Kenny Mattonet[1], Sven Reischauer[1], Didier Stainier[1*]**

[1] Developmental Genetics, Max Planck Institute for Heart and Lung Research, Germany

*Didier.Stainier@mpi-bn.mpg.de*

The zebrafish Cloche/Npas4l transcription factor is necessary and sufficient for endothelial cell differentiation and it functions by regulating the expression of tal1, etsrp and lmo2, which themselves encode transcription factors. However, the precise role of Cloche/Npas4l and its downstream effectors in endothelial cell differentiation remains unclear. To this end, we generated a knock-in reporter in the npas4l locus to track the fate of npas4l-expressing cells in wild-type and mutant embryos. We find that in cloche/npas4l mutants endothelial progenitors fail to migrate to the midline and that this defect can be rescued by tal1 global overexpression. A similar migration defect is observed in tal1 mutants whereas in etsrp mutants, endothelial progenitors migrate to the midline but fail to differentiate, instead turning on blood genes. Altogether, these data suggest that Npas4l promotes endothelial cell differentiation by getting them to the midline via tal1 expression and driving their differentiation via etsrp expression.

Symposium 02-2

# Novel Blood and Lymphatic Endothelial Cell Origins

**Christiana Ruhrberg**[1*]

[1] UCL Institute of Ophthalmology, University College London, UK

*c.ruhrberg@ucl.ac.uk*

Elucidating the cellular mechanisms by which blood and lymphatic vasculature forms will increase our understanding of normal lymphatic vascular growth and thereby underpin the design of novel therapies to ameliorate ischemic diseases or lymphedema and associated inflammation. We have recently shown that erythro-myeloid progenitors (EMPs) originating in the embryonic yolk sac contribute endothelial cells to blood vessels in developing organs, and that the EMP-derived endothelial cells persist into adulthood. We now show that this novel lineage contributes to vascular repair, and that EMP-like progenitors also give rise to endothelial cells in the lymphatic vasculature of several developing organs.

# Cell Heterogeneity and Disease

**Michael Simons**[1*]

[1] Medicine & Cell Biology, Yale School of Medicine, USA

*michael.simons@yale.edu*

Cell fate transitions have emerged as key events driving pathogenesis of a number of diseases, from atherosclerosis and aneurysm development to tissue fibrosis and pulmonary hypertension. Singe cell transcriptomics and AI/machine learning-based analysis of these data sets are paving the way for new understanding of normal biology and disease pathogenesis. The lecture will focus on application of these techniques to endothelial and smooth muscle cell fate transitions.

Symposium 02-4

# Regulation of Endothelial Cell Specialization

**Karen Hirschi**[1,2*]

[1] Alumni Professor of Cell Biology, University of Virginia School of Medicine, USA
[2] Adjunct Professor of Medicine and Genetics, Yale University School of Medicine, USA

*kkh4yy@virginia.edu*

Formation and maturation of a functional blood vascular system is required for the development and maintenance of all tissues in the body. During the process of blood vessel development, primordial endothelial cells are formed and become specified toward arterial or venous fates to generate a circulatory network that provides nutrients and oxygen to, and removes metabolic waste from, all tissues. Specification of arterial and venous endothelial cells occurs in conjunction with suppression of endothelial cell cycle progression, and endothelial cell hyperproliferation is associated with potentially lethal arterial-venous malformations. However, the mechanistic role that cell cycle state plays in arterial-venous specification is unknown. In our current studies of retinal vascular development in Fucci2aR reporter mice, we found that venous and arterial endothelial cells are in distinct cell cycle states during development and in adulthood. That is, venous endothelial cells reside in early G1 state, while arterial endothelial cells reside in late G1 state. We are trying to understand the molecular role of endothelial cell cycle state in arterial-venous network formation.

# Dynamics in Sprouting Endothelial Cells by Hippo-YAP/TAZ Pathway

**You Mie Lee**[1*]

[1] Vessel-Organ Interaction Research Center (VOICE, MRC), College of Pharmacy, Kyungpook National University, Korea

*lym@knu.ac.kr*

During active angiogenesis, dynamic changes in endothelial cells shapes and behaviors are observed. Hippo-YAP/TAZ pathway play a critical role in organ size regulation, regeneration and self-renewal. Recent accumulating evidences indicate the involvement of Hippo- YAP/TAZ pathway in angiogenic processes in multiple model systems. CCN1 (CYR61) is a target of Hippo pathways and stimulates active angiogenesis in tumor and some pathological conditions. In addition, RUNX3 is a tumor suppressor which inhibits YAP/TAZ pathway and has an anti-angiogenic function via inhibiting VEGF expression and enhancing HIF-1α degradation. First, we suggest here that CCN1 is a key regulator of endothelial tip cell activity in angiogenesis. Microvessel networks and directional vascular cell migration patterns were deformed in ccn1-knockdown zebrafish embryos. CCN1 activated VEGFR2 and downstream MAPK/PI3K signalling pathways, YAP/TAZ, as well as Rho effector mDia1 to enhance tip cell activity and CCN1 itself. VEGFR2 interacted with integrin αvβ3 through CCN1. Integrin αvβ3 inhibitor repressed tip cell number and sprouting in postnatal retinas from endothelial cell-specific Ccn1 transgenic mice, and allograft tumours in Ccn1 transgenic mice showed hyperactive vascular sprouting. Cancer patients with high CCN1 expression have poor survival outcomes and positive correlation with ITGAV and ITGB3 and high YAP/WWTR1. Second, we demonstrate retina angiogenic phenotypes in Runx3-EC specific KO mice. In conclusion, our data underscore the positive feedback regulation of tip cells by CCN1 through integrin αvβ3/VEGFR2 and increased YAP/TAZ activity, and Runx3 is a negative regulator of YAP/TAZ signaling in endothelial cells, suggesting a promising therapeutic intervention for pathological angiogenesis.

Symposium 02-6 Short Talk

# Endothelial-Derived TGF-β/Activin Signals Activated by Inflammation-Related Cytokines During Endothelial Mesenchymal Transition Induce Epithelial Mesenchymal Transition

**Yasuhiro Yoshimatsu[1,2*], Ikumi Wakabayashi[2], Shiori Kimuro[2], Naoya Takahashi[2], Kazuki Takahashi[2], Miho Kobayashi[2], Katarzyna A. Podyma-Inoue[2], Kohei Miyazono[3], Tetsuro Watabe[2]**

[1] Division of Pharmacology, Graduate School of Medical and Dental Sciences, Niigata University, Japan
[2] Department of Biochemistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Japan
[3] Department of Molecular Pathology, Graduate School of Medicine, The University of Tokyo, Japan

*yyoshi85@med.niigata-u.ac.jp*

To elucidate the molecular mechanisms underlying transforming growth factor-β (TGF-β)-induced endothelial-to-mesenchymal transition (EndMT) enhanced by inflammation-related cytokines such as tumor necrosis factor-α (TNF-α), and the roles of the secretion of endothelial-derived TGF-β/Activin ligands during EndMT and in cancer cell malignancy.

Multiple types of endothelial cells were treated with TGF-β and TNF-α for 72 hours and were subjected to quantitative RT-PCR and immunofluorescence analyses for various markers including ones of endothelial and mesenchymal cells. Culture supernatants from endothelial cells which undergo EndMT were subjected to TGF-β family signal reporter assay to examine whether the supernatants have the activity of TGF-β/Activin signals, and were also added to HSC-4 human oral squamous carcinoma cells to examine whether the cells undergo epithelial-to-mesenchymal transition (EMT) as an indicator for cancer cell malignancy.

Multiple types of endothelial cells underwent EndMT in response to TGF-β and TNF-α, which was accompanied by increased and decreased expression of mesenchymal and endothelial markers, respectively. In addition, treatment of endothelial cells with TGF-β and TNF-α exhibited sustained activation of TGF-β family signals, which was presumably induced by elevated expression of TGF-β type I receptor, TGF-β2, and Activin A, suggesting that TNF-α enhanced TGF-β-induced EndMT by augmenting TGF-β/Activin signals. Furthermore, HSC-4 cancer cells underwent the EMT in response to humoral factors produced by TGF-β and TNF-α-cultured endothelial cells.

These results suggest that TNF-α enhances the TGF-β-dependent EndMT, which contributes to cancer progression by augmenting TGF-β/Activin signals, and may provide a unique molecular basis for the pathological roles of endothelial-derived TGF-β/Activin signals in cancer progression.

# Blood-Brain Barrier Dysfunction Predicts Cognitive Decline in Alzheimer's Disease: Effect of the APOE4 Gene

**Berislav V. Zlokovic**[1*]

[1] Zilkha Neurogenetic Institute, Dept of Physiology and Neuroscience, Keck School of Medicine of USC, USA

*zlokovic@usc.edu*

The growing evidence suggests that vascular dysfunction contributes to cognitive impairment and Alzheimer's disease (AD) by ways that may not be related to classical amyloid-β (Aβ) and tau AD pathology. First, I will review recent evidence suggesting that breakdown in the blood-brain barrier (BBB) is an early biomarker of human cognitive dysfunction, including the early clinical stages of AD. I will describe our novel neuroimaging MRI technique in the living human brain showing that BBB breakdown in the hippocampus, a center for learning and memory, occurs early in the disease process, and is associated with elevated levels in the cerebrospinal fluid (CSF) of the BBB pericyte injury biomarker soluble sPDGFRβ which is independent of CSF Aβ or tau status, i.e., whether they were positive for Aβ or tau suggesting early stage AD, or negative for Aβ or tau. Next, I will focus on the E4 variant of apolipoprotein E (APOE4), the main susceptibility gene for AD. I will show that individuals bearing APOE4 (with the ε3/ε4 or ε4/ε4) are distinguished from those without APOE4 (ε3/ε3 homozygotes) by breakdown of the BBB in the hippocampus and medial temporal lobe, which is apparent in cognitively unimpaired APOE4 carriers, and more severe in those with cognitive impairment, but is not related to Aβ or tau pathology measured in CSF or PET. I will show that high baseline levels of sPDGFRβ in the CSF predict future cognitive decline in APOE4 carriers but not in non-carriers, even after controlling for Aβ and tau status, and that pericyte injury correlated with increased activity of the proinflammatory BBB-degrading cyclophilin A-matrix metalloproteinase-9 pathway in CSF. I will conclude that breakdown of the BBB contributes to early human cognitive dysfunction and APOE4-associated cognitive decline independently of AD pathology, and might be a therapeutic target in APOE4 carriers.

Symposium 03-2

# Endothelial Cell Clonal Expansion in the Development of Cerebral Cavernous Malformations

**Elisabetta Dejana**[1*]

[1] Dept Immunology, Genetics and Pathology Uppsala University and FIRC Inst Mol Oncol Milan, Italy

*elisabetta.dejana@ifom.eu*

Cerebral cavernous malformation (CCM) is a neurovascular disease characterized by enlarged and multiple lumen cerebral vessels that often lead to hemorrhages, seizures and strong headache. CCM occurs in both sporadic and familial forms. Loss-of-function mutations in anyone of three genes, namely CCM1 (also known as KRIT1), CCM2 (OSM) and CCM3 (PDCD10) result in induction of CCM lesions. Mouse model studies of CCM disease show that Endothelial-to-Mesenchymal transition (End-MT) of resident progenitor cells (Malinverno et al Nature Comm 2019) is the trigger for the development and progression of vascular CCM malformations. We combined different techniques to define the functional characteristics of endothelial cells responsible for the formation of cavernomas and to comprehensively characterize endothelial cells in both normal conditions and after deletion of CCM3 at early postnatal stage. These data provide important insights into the plasticity of the endothelial system of the brain in physiological and pathological conditions.

# How does the BBB Regulate Brain Function and Behavior?

**Richard Daneman**[1*]

[1] Pharmacology and Neurosciences, University of California at San Diego, USA

*rdaneman@ucsd.edu*

Vascular endothelial cells in the central nervous system (CNS) form a barrier that restricts the movement of molecules and ions between the blood and the brain. This blood-brain barrier (BBB) is crucial to ensure proper neuronal function and protect the CNS from injury and disease. Although the properties of the BBB are manifested in the endothelial cells, transplantation studies have demonstrated that the BBB is not intrinsic to the endothelial cells, but is induced by interactions with the neural cells. We are interested in identifying how the BBB interacts with the neural circuitry to regulate brain function and behavior, addressing the following questions: How does neural activity dynamically regulate the BBB? How do changes to the BBB regulate brain function and behavior? Are there regional specializations of the BBB that are important for local circuit function? Here we use a genomic, genetic and molecular approach to elucidate these questions. We have found that neural activity robustly alters the gene expression of CNS endothelial cells, regulating key BBB properties including efflux transport. We have further identified roles for vascular metabolism in regulating behavior, and have identified significant regional heterogeneity of the BBB.

Symposium 03-4

# Hypertension-Induced Vascular Disruption in the CNS

**Injune Kim**[1*]

[1] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Korea

*injunek@kaist.ac.kr*

Hypertension is a major risk factor of various vascular and degenerative diseases in the central nervous system such as brain and retina. Although disruption of blood-brain barrier (BBB) and blood-retinal barrier (BRB) is speculated as a major mechanism, genetic contributors, the location, and underlying pathophysiology of hypertension-induced vascular disruption remain unknown.

We found that hypertension induced arterial BRB disruption in the superficial layer of the retina and vision-threatening retinal edema by downregulating Dll4 in retinal arterial endothelial cells (ECs) and subsequently increasing caveolar-mediated transcytosis.

In addition, we found that an endothelial transcription factor Sox17 protects BBB in the thalamus, one of the areas most frequently affected by hypertensive intracerebral hemorrhage (ICH), against hypertension. *Sox17* deficiency promoted hypertension-driven BBB disruption primarily in the thalamus via destabilized tight junctions and increased transcytosis in ECs, leading to non-hemorrhagic leakage and increased activity of theta brainwaves without detectable edema, inflammation, amyloid-b accumulation, or tauopathy in the brain. Prolonged hypertension induced persistent fluid leakage and further cerebral microbleeds in the thalamus. Interestingly, hypertension upregulated p16INK4a, a cardinal molecule of senescence, in *Sox17*-deficient thalamic vessels and *p16ink4a* co-deletion in ECs suppressed vascular leakage and cerebral microbleeds. Importantly, incidence of cerebral IgG leakage and microbleeds was higher in hypertension patients with low Sox17 levels than ones with normal Sox17 levels, implicating low Sox17 expression in hypertensive BBB disruption. Together, *Sox17* deficiency promotes hypertension-induced non-hemorrhagic leakage impairing brain function and subsequent cerebral microbleeds potentially preceding hypertensive ICH via premature endothelial senescence.

Symposium 03-5

# Blood-Retinal Barrier Revisited: Choroidal Vasculature Regulates Retinal Homeostasis

**Soo-Jin Kim[1], Sang-A Kim[2], Yeong A Choi[2], Yurim Kim[2], Junyeop Lee[1*]**

[1] Department of Ophthalmology, Asan Medical Center, College of Medicine, University of Ulsan, Korea
[2] Department of Ophthalmology, College of Medicine, Yeungnam University, Korea

*j.lee@amc.seoul.kr*

The blood-retinal barrier (BRB) has two components: an inner and outer barrier. One of the major elements of outer BRB is the choroid, a highly vascularized tissue of the eye. Choriocapillaris locates at the innermost choroidal layer and provides oxygen and nourishment to the retina, which is crucial for maintaining visual function. We are investigating the unique endothelial polarity of choriocapillaris that is fenestrated or pericyte-covered in the opposite direction. We generated tamoxifen-inducible endothelial-specific conditional mice lacking plasmalemmal vesicle-associated protein (PLVAP), a primary gene for endothelial fenestration and caveolae. This presentation will cover our recent data on ultrastructural analysis and intravital imaging demonstrating the importance of PLVAP and its related signal pathways in the choroid-retinal interaction. In addition, we evaluated the regional distributions and functional aspects of choroidal perivascular mural cells (PVMC) depending on the aging or diseases, and investigated physiological role of subtypes of PVMC using cell-specific fluorescent reporter and ablation genetic mice models. These data provide important insights into the outer BRB, especially, choroidal vasculature contributing to the pathology of vision-threatening retinal diseases including age-related macular degeneration and diabetic retinopathy.

Symposium 03-6 Short Talk

# Tau Deposition Is Associated With Intracranial Calcification in the P301L Mouse Model of Human Tauopathy

**Ruiqing Ni[1], Yvette Zarb[2], Gisela Kuhn[3], Ralph Müller[3], Yankey Yundung[1], Roger Nitsch[4], Luka Kulic[4], Annika Keller[2], Jan Klohs[1*]**

[1] Institute for Biomedical Engineering, University of Zurich, Switzerland
[2] Institute for Neuropathology, Universitätsspital Zurich, Switzerland
[3] Dept. Health Sciences and Technology, ETH Zurich, Switzerland
[4] Institute for Regenerative Medicine, University of Zurich, Switzerland

*klohs@biomed.ee.ethz.ch*

Brain calcifications are associated with ageing and are more prevalent in several neurodegenerative proteinopathies such as Alzheimer's disease, cerebral amyloid angiopathy, frontotemporal dementia, Parkinson's disease and Down syndrome, but their pathogenesis are yet poorly understood. Here we applied novel magnetic resonance imaging techniques, micro computed tomography to characterize the age-dependent occurrence of intracranial calcifications in the P301L mouse model of human tauopathy.

P301L mice (Thy1.2) of 3, 5, 9 and 18-25 months-of-age and age-matched non-transgenic littermates were assessed. Magnetic resonance imaging data were collected with a gradient recalled echo sequence. From the raw data, phase and susceptibility weighted images were computed. In addition, and micro computed tomography of ex vivo whole head samples was performed. Histochemistry and immunohistochemistry was used to investigate the nature of the imaging lesions in different brain regions of P301L mice.

Susceptibility weighted images c revealed regional hypointensities in the hippocampus, cortex, caudate nucleus and thalamus of P301L mice, which in corresponding phase images indicated diamagnetic lesions. Concomitantly, micro computed tomography detected hyperdense lesions. Occurrence of diamagnetic susceptibility lesions in the hippocampus, increased with age. Immunochemical staining of brain sections revealed bone protein-positive (osteocalcin) deposits. Furthermore, intra-neuronal and vessel-associated osteocalcin-containing nodules co-localized with phosphorylated-tau (AT8 and AT100) in the hippocampus. Protein-containing nodules were detected also in the thalamus in the absence of phosphorylated-tau deposition. In contrast, osteocalcin-containing nodules were vessel-associated, indicating ossified vessels, in the thalamus in absence of phosphorylated-tau.

Here, we report a new phenotype of intracranial calcification in transgenic P301L mice overexpressing 4 repeat tau, where we identified two types of calcifications (intra-neuronal and vessel-associated). The P301L mouse model may thus serve as a future model to study the pathogenesis of brain calcifications in tauopathies. Magnetic resonance imaging can sensitively detect intracranial calcifications with high sensitivity.

# Development and Aging of Endothelial Stem Cells

**Nobuyuki Takakura**[1*]

[1] Department of Signal Transduction, Research Institute for Microbial Diseases, Osaka University, Japan

*ntakaku@biken.osaka-u.ac.jp*

Existence of tissue specific stem cells has been elucidated in many organs, such as hematopoietic, neural, gut, epidermal stem cells and so on. In the cardiovascular system, although cardiac stem cell population was identified by using cell surface markers, vascular endothelial stem cells have not been designated by their cell surface markers. We previously reported that endothelial cells (ECs) with highly proliferative potential was fractioned in side population (SP) cells which express ABC transporter abundantly (Naito, EMBO J 2012). By comparing gene expression between SP cells and main population (MP) cells in ECs, we found that CD157, cell surface molecule, is expressed in stem cell property of ECs and CD157 positive ECs can generate new blood vessels effectively and sustainably in hindlimb ischemia model and reconstitute sinusoidal vascular unit in liver injury model. We isolated that CD157 and CD200 positive ECs gradually differentiate terminally into ECs negative for CD157 nor CD200 which do not show proliferative ability through ECs negative for CD157 and positive for CD200, i.e., CD157 and CD200 positive ECs exist in the pre-existing blood vessels at the top of hierarchy of ECs (Wakabayashi, Cell Stem Cell 2018). It has been recently identified that the number of ECs in our body gradually decrease with ageing and stem cell ageing may be responsible for that. In this session, we will discuss about recently identified TNF alpha /TAK1 signal for long-term survival of ECs (Naito, Dev Cell 2019) and how stemness of endothelial stem cells is affected by TNF alpha.

Symposium 04-2

# Human Stem Cells & Genomics for Disease Modeling

**Joseph C. Wu**[1*]

[1] Stanford Cardiovascular Institute, Stanford University, USA

*joewu@stanford.edu*

Heart disease is the most significant cause of morbidity and mortality in the industrialized world, accounting for nearly 25% of all deaths in the United States alone. While the use of human induced pluripotent stem cell (iPSCs) in regenerative medicine is a long-term goal, a growing body of studies has shown promising results in the fields of drug discovery, development, and toxicity screening. Specifically, recent technological advancement has enabled the generation of patient-specific and disease-specific human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) in vitro. These iPSC-CMs carry all the genetic information from the individuals from whom they are derived. Here I will discuss recent advances in this technology and how it may be used for elucidating mechanisms of rare inherited cardiovascular diseases, for drug discovery, and for precision medicine.

# Clinical Application of Human iPS Cell-Derived Regenerated Cardiomyocytes for the Treatment of Severe Congestive Heart Failure

**Keiichi Fukuda**[1*]

[1] Department of Cardiology, Keio University, Japan

*kfukuda@a2.keio.jp*

Although heart transplantation can drastically improve the survival, shortage of the donor heart is a serious problem. The regenerative medicine of the failing heart had been long awaited. To address this question, we used human HLA haplotype homo-iPS cells, which matches to approximately 20% of the Japanese population, to generate ventricular cardiomyocytes. We performed transcriptome of the metabolic enzymes and fluxome analysis using 13glucose and 13lactic acid on both ES/iPS cells and cardiomyocytes, and found that their metabolic pathways were completely different. Moreover, amino acid consumption analysis and metabolome analysis revealed that glutamine is another important energy source for the iPS cells. Based on these findings, we could purify the cardiomyocytes with more than 99% purity. The transplanted cardiomyocytes did not make teratoma formation in immuno-deficient NOG mice skin and heart. We transplanted the aggregate (spheroid) cardiomyocytes using our newly developed device. The transplanted cardiomyocytes could survive in the heart for the long period, showed physiological cell hypertrophy after transplantation, and could improve cardiac function due to myocardial infarction. We are now planning to examine the first in human clinical trial to transplant the human regenerated cardiomyocytes to the patients with HLA-6 class matched dilated cardiomyopathy in the near future.

Symposium 04-4

# Vascular Regeneration with Stem Cells, Reprogrammed Cells and Engineering

**Young-Sup Yoon**[1,2*]

[1] Medicine, Cardiology, Emory University, USA
[2] Yonsei Biomedical Science Institute, Yonsei University, Korea

*yyoon5@emory.edu*

We developed a fully defined, clinically-compatible cell culture system that can generate purified, functional, and therapeutically effective endothelial cells (ECs) from human pluripotent stem cells (hPSCs), which include human embryonic stem cells and induced pluripotent stem cells. We further encapsulated hPSC-ECs within the nanomatrix gel and transplanted them into experimental hindlimb ischemia. These encapsulated hPSC-ECs remained engrafted for more than 10 months in ischemic tissues, and when compared to bare hPSC-ECs, they exerted higher and prolonged neovascularization and showed better vascular regenerative capacity.

Direct conversion or reprogramming of human postnatal cells into ECs, bypassing stem or progenitor cell status, is crucial for cell therapy, and pathophysiological investigation but has remained largely unexplored. We thus sought to directly reprogram human postnatal dermal fibroblasts (HDFs) to ECs with vasculogenic and endothelial transcription factors (TFs) and determine their vascularizing and therapeutic potential. We found that ER71/ETV2 alone is able to directly reprogram human postnatal cells to functional, mature ECs, referred to as reprogrammed ECs (rECs). These rECs could be valuable for cell therapy, disease investigation, and exploration of the reprogramming process.

# Therapy for Peripheral Artery Disease using Human Induced Pluripotent Stem Cell-derived Endothelial Cells and Smooth Muscle Cells

**Jae Ho Kim**[1*]

[1] Department of Physiology, Pusan National University School of Medicine, Korea

*jhkimst@pusan.ac.kr*

Peripheral artery disease is a condition in which tissue necrosis occurs due to arterial occlusion, resulting in limb amputation in severe cases. Both endothelial cells (EC) and vascular smooth muscle cells (SMC) are needed for the regeneration of peripheral arteries in ischemic tissues. However, it is difficult to isolate and cultivate primary EC and SMC from patients for therapeutic angiogenesis. Induced pluripotent stem cells (iPSC) are regarded as useful stem cells due to their pluripotent differentiation potential. In this study, we explored the therapeutic efficacy of human iPSC-derived EC and iPSC-derived SMC in peripheral artery disease model. After the induction of mesodermal differentiation of iPSC, CD34+ progenitor cells were isolated by magnetic-activated cell sorting. Cultivation of the CD34+ progenitor cells in endothelial culture medium induced the expression of endothelial markers and phenotypes. Moreover, the CD34+ cells could be differentiated into SMC by cultivation in SMC culture medium. In a murine hindlimb ischemia model, co-transplantation of EC with SMC improved blood perfusion and increased the limb salvage rate in ischemic limbs compared to transplantation of either EC or SMC alone. Moreover, co-transplantation of EC and SMC stimulated angiogenesis and led to the formation of capillaries and arteries/arterioles in vivo. Conditioned medium derived from SMC stimulated the migration, proliferation, and tubulation of EC in vitro, and these effects were recapitulated by exosomes isolated from the SMC-conditioned medium. Together, these results suggest that iPSC-derived SMC enhance the therapeutic efficacy of iPSC-derived EC in peripheral artery disease via an exosome-mediated paracrine mechanism.

Symposium 04-6 Short Talk

# An Endogenous Transmembrane Protein Controls Distinct mTORC2 Functions in Normal and Leukemic Hematopoiesis in the Bone Marrow

**Dongjun Lee**[1*]

[1] Department of Convergence Medicine, Pusan National University School of Medicine, Korea

*lee.dongjun@pusan.ac.kr*

mTOR/AKT are the Ser/Thr kinases, central to cellular proliferation, survival, and metabolic responses to cytokine signals, activated in many human cancers. mTOR exists as two distinct multi-subunit protein complexes, mTORC1, and mTORC2. Activation of AKT in hematopoiesis leads to a myeloproliferative syndrome and eventual loss of hematopoietic stem cells. mTORC1 deletion leads to hematopoietic failure during the stress condition. Moreover, pharmacologically inhibition of mTOR resulted in anti-leukemia effects in a mouse model of acute myeloid leukemia (AML). Further, Acute lymphocytic leukemia (ALL) is more clearly driven by activation of the AKT/mTOR pathway. More than ~ 50% of the human T-ALL cells depend on ongoing NOTCH-initiated signals for their growth. And enforced NOTCH signaling is a potent inducer of T-ALL in the mouse model.

We identified Upstream-of-mTORC2 as a transmembrane molecule altered in leukemic cells that emerged from an animal with modifications in specific bone marrow stromal cells. Hypothesizing that the gene-altered in the malignant cells that emerge from this niche-induced oncogenesis model might reflect how an abnormal microenvironment leads to cancer.

We have used engineered mouse models to selectively deplete Upstream-of-mTORC2 from the differential subsets in the bone marrow. Conditional deletion of Upstream-of-mTORC2 effect perturbs normal and leukemic hematopoiesis in vivo.

These studies provide mechanistic insight into how a distinctive molecular inhibitor of the mTORC2 signaling pathways can be a possible contributor to the viable therapeutic strategy.

# Pathogenic Mechanisms of Lymphatic Malformations

**Taija Mäkinen**[1*]

[1] Uppsala University, Sweden

*taija.makinen@igp.uu.se*

Lymphatic malformations (LMs) are debilitating vascular anomalies presenting with large cysts (macrocystic) or lesions that infiltrate tissues (microcystic). Somatic mutations in the PIK3CA gene were recently identified as causative of LM, yet the cellular mechanisms underlying LM pathology are poorly understood. We found that the somatic PIK3CAH1047R mutation, resulting in constitutive activation of the p110α PI3K, underlies both macrocystic and microcystic LMs in human. Using a mouse model of PIK3CAH1047R-driven LM, we further found that both types of malformations arise due to lymphatic endothelial cell (LEC)-autonomous defects, with the developmental timing of p110α activation determining the LM subtype. Unexpectedly, our results showed that although the mutant cells had hyperactive PI3-kinase signalling, additional stimulation by vascular endothelial growth factor C (VEGF-C) was required for the malformations to grow. Combined inhibition of VEGF-C signaling and the PI3K downstream target mTOR using Rapamycin, but neither treatment alone, promoted the regression of experimental LM in mice. Our findings provide important implications for the treatment of microcystic LM in human and suggest that therapies targeting the key upstream pathway in combination with PI3K pathway inhibition may be relevant for other PIK3CA-driven pathologies.

Symposium 05-2

# Meningeal Lymphatics in AD

**Jonathan Kipnis**[1*]

[1] Neurology, Neuroscience and Neurosurgery, Washington University in St. Louis, USA

*kipnis@wustl.edu*

Immune cells and their derived molecules have major impact on brain function. Our results demonstrate that meningeal space, surrounding the brain, is the site where CNS-associated immune activity takes place. We discovered a presence of meningeal lymphatic vessels that drain CNS molecules and immune cells to the deep cervical lymph nodes and also regulate perfusion of the brain by CSF (glymphatic flow). This communication between the CNS and meninges is playing a key role in several neurological, primarily in Alzheimer's disease. Interestingly, the integrity of meningeal lymphatics also dictates the response to immunotherapy in AD. Meningeal lymphatics, overall, may serve as a novel therapeutic target for neurological disorders.

Symposium 05-3

# Dissecting the Transcriptional Control of Lymphatic Endothelial Cell Identity

**Natasha Harvey**[1*]

[1] Centre for Cancer Biology, University of South Australia and SA Pathology, Australia

*natasha.harvey@unisa.edu.au*

Lymphatic vessels are an integral component of the cardiovascular system. These specialised vessels play key roles in fluid homeostasis, dietary lipid absorption and the regulation of immune cell trafficking. We and others recently demonstrated that heterozygous germline mutations in the zinc finger transcription factor GATA2 underlie Emberger syndrome, a disorder characterised by lymphedema and predisposition to myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) (Kazenwadel et al., Blood, 2012, Ostergaard et al., Nat Gen, 2011). This discovery was the first to demonstrate an important role for GATA2 in the lymphatic vasculature. We subsequently determined that Gata2 is crucial for lymphatic vascular development by orchestrating the construction and maintenance of lymphatic vessel valves (Kazenwadel et al., J Clin Invest, 2015). Our current work is focussed on defining the transcriptional mechanisms by which GATA2 controls valve morphogenesis in the lymphatic vasculature. We have identified both GATA2-bound transcriptional regulatory elements and GATA2 target genes important for valve development, some of which are also mutated in human lymphoedema syndromes. These genes and their roles in lymphatic vessel morphogenesis will be discussed. Ultimately, understanding the genetic basis of lymphoedema will inform our knowledge of the cellular events and signalling pathways important for building functional lymphatic vessels, information that will underpin the design of novel, targeted therapeutics able to promote lymphatic vessel function and treat lymphoedema.

Symposium 05-4

# Fluid Flow-Triggered Activation of Lymphatic Expansion

**Dongwon Choi[1], Young Kwon Hong[1*]**

[1] Department of Surgery, University of Southern California, USA

*young.hong@usc.edu*

Lymphatic vessels function to drain interstitial fluid from a variety of tissues. Although shear stress generated by fluid flow is known to trigger lymphatic expansion and remodeling, the molecular basis underlying flow-induced lymphatic growth is unknown. Here, we aimed to identify a mechanotransduction pathway that translates laminar flow–induced shear stress to activation of lymphatic sprouting. Primary dermal blood vascular endothelial cells (BECs) and lymphatic endothelial cells (LECs) were exposed to low-rate steady laminar flow. Shear stress–induced molecular and cellular responses were defined and verified using various mutant mouse models. While low-rate laminar flow commonly induces the classic shear stress responses in BECs and LECs, only LECs display reduced Notch activity and increased sprouting capacity. In response to flow, the plasma membrane calcium channel ORAI1 mediates calcium influx in LECs and activates calmodulin to facilitate a physical interaction between Krüppel-like factor 2 (KLF2), the major regulator of shear responses, and PROX1, the master regulator of lymphatic development.

The PROX1/KLF2 complex upregulates the expression of DTX1 and DTX3L. DTX1 and DTX3L, functioning as a heterodimeric Notch E3 ligase, concertedly downregulate NOTCH1 activity and enhance lymphatic sprouting. Notably, overexpression of the calcium reporter GCaMP3 unexpectedly inhibited lymphatic sprouting, presumably by disturbing calcium signaling. Endothelial-specific knockouts of Orai1 and Klf2 also markedly impaired lymphatic sprouting. Moreover, Dtx3l loss of function led to defective lymphatic sprouting, while Dtx3l gain of function rescued impaired sprouting in Orai1 KO embryos. Together, data reveal a molecular mechanism underlying laminar flow–induced lymphatic sprouting

# Neural-Crest Derived and Endothelial Foxc2 Expression Is Required for Proper Morphogenesis of the Schlemm's Canal

**Pieter Norden[1], Lisa Beckmann[2], Zhen Cai[2], Xian Zhang[2], Hao Zhang[2], Tsutomu Kume[1*]**

[1] Medicine, Northwestern University School of Medicine, USA
[2] Biomedical Engineering, Northwestern University, USA

*t-kume@northwestern.edu*

The Schlemm's Canal (SC), located in the iridocorneal angle, is a unique vessel characterized by both venous and lymphatic identity that has a key role in intraocular pressure maintenance in physiological and pathological conditions in regulating aqueous humor outflow along with the trabecular meshwork. Mechanisms of paracrine signaling from the neighboring tissues and cells contributing to SC development are poorly understood. Our laboratory previously demonstrated that neural crest (NC)-derived periocular mesenchymal cells require expression of the Forkhead box (Fox) transcription factor Foxc2 for proper development of the anterior segment (Seo et al., Investig. Ophthalmol. Vis. Sci, 2017); however, it's role in contribution to the development of the SC has yet to be investigated and recent evidence has shown that FOXC2 variants possess a role as putative modifiers for the development of primary congenital glaucoma (Medina-Trilloet al., PLoS One, 2019).

Foxc2 was conditionally deleted from NC-derived cells by crossing Foxc2fl/fl mice with Wnt1-Cre; Foxc2fl/+ mice. Eyes of 7-8 week old mice were then imaged in vivo using a custom built visible light optical coherence tomography (vis-OCT) system which captured the entire 360° of the SC and its surrounding vessels (Zhang et al., Investi. Ophthalmol. Vis. Sci, 2020). Immunohistochemistry for characterization of SC morphology was also performed on flatmounted eyes. Similarly, vascular endothelial cell (EC)-specific Foxc2 mutant mice by treating VE-cadherin-CreERT2; Foxc2fl/flx mice with tamoxifen were generated and analyzed.

We found abnormal SC morphology, or the absence of SC, in NC-Foxc2-/- mutants compared to controls. Furthermore, NC-Foxc2-/- mutants exhibited reduced expression of the key lymphatic regulators PROX1 and VEGFR3 in the SC compared to controls, suggesting impaired SC cell identity. We also found elevated intraocular pressure in NC-Foxc2-/- mutant mice compared to controls. Moreover, EC-Foxc2-/- mutant mice had reduced SC area, accompanied by a significant decrease in Tie2 expression.

These results demonstrate that Foxc2 expression in the NC and EC lineages is required for proper development of the SC vasculature.

Symposium 05-6 Short Talk

# Immune Regulation and Lymphatic Vessel Development in Severe Preeclampsia

**Yong-Sun Maeng**[1*]

[1] Department of Obstetrics and Gynecology, Yonsei University College of Medicine, Korea

*mysdm70@gmail.com*

Lymphatic vasculature control lymphocyte trafficking and limit adaptive immune response. Unbalanced immune cells in the maternal-fetal interface may be one cause of severe preeclampsia (PE). Therefore, impaired lymphangiogenesis and immune regulation around placenta are theorized to contribute to the severe PE pathophysiology, yet the current research on this field is limited. Therefore, we aimed to investigate the association between lymphangiogenesis and immune regulation in severe PE.

We obtained fetal chorioamniotic membranes containing decidua, and the chorionic plate from pregnant women with PE (n=15) and gestational age matched controls (n=15). In the uterus of normal pregnant mice, LYVE1-positive lymphatic vessels were only present in the uterine wall. In human, LYVE1-positive lymphatic vessels were abundance in decidua and seemed like large tubular structure in normal. In contrast, in decidua of severe PE showed a low density and small diameter of LYVE1-positive lymphatic vessels.

Lymphatic endothelial cell (DLEC) isolated from decidua of PE pregnancy showed a marked reduction of migration, adhesion, morphologic differentiation, consistent with a decrease in lymphatic vessel sprouting in a three-dimensional lymphatic ring assay compare to normal. DLEC from PE pregnancy also showed decreased CCL21 expression and reduced attraction of dendritic cell to lymphatic vessel. In addition, DLEC of PE presented the decrease of Akt- eNOS- nitric oxide signaling pathway that inhibit cytotoxic T cell activation in decidua.

Collectively, our findings suggest that impaired function of lymphatic vessel in decidua during pregnancy may induce the defective immune tolerance and associate with severe PE

# VEGFR2 Signaling in Vascular Permeability and Vessel Leakage

**Lena Claesson-Welsh**[1*]

[1] Immunology, Genetics and Pathology, Uppsala University, Sweden

*lena.welsh@igp.uu.se*

Leakage from blood vessels into tissues is governed by mechanisms that control endothelial barrier function and vascular permeability to maintain homeostasis. Dysregulated endothelial permeability contributes to many pathological conditions and can influence disease morbidity and treatment by accumulation of edema, resulting in high interstitial pressure, which interferes with normal tissue function and eventually causes tissue damage. Vascular endothelial growth factor A (VEGFA), originally named vascular permeability factor, increases leakage of macromolecules through its main receptor on endothelial cells, VEGFR2. A wide range of drugs that block VEGFA or VEGFR2 are used to suppress leakage and edema, exemplified by antibodies and recombinant receptor fragments used to treat diabetic retinopathy, wet age-related macular degeneration, and several other eye diseases. VEGFR2 regulation of vascular permeability and leakage depends on activation of Src family kinases (SFKs) and Rho monomeric GTPases, which signal in pathways that regulate opening of discrete paracellular gaps. SFKs are cytoplasmic tyrosine kinases implicated in regulation of leakage by phosphorylation of the adherens junction molecule vascular endothelial (VE)-cadherin, leading to its internalization, gap formation and leakage. c-Src has been the focus of much research in this context, but the specific contributions of the closely related SFKs, Yes and Fyn, in regulation of endothelial junctions, remains to be determined. SKFs are constitutively active in postcapillary venules in a flow-dependent manner, as reflected by phosphorylation of the conserved tyrosine Y418. In this presentation, I will show how specific VEGFR2 phosphorylation sites regulate different endothelial responses, in particular vascular leakage. I will present different methodologies for analysis of vascular leakage in vivo. Finally, I will discuss novel data on the roles of c-Src and Yes in regulation of the vascular barrier.

Symposium 06-2

# Somatic Mosaicism, Clonal Hematopoiesis and Vascular Diseases

**Kenneth Walsh**[1*]

[1] Lockhart B. McGuire Professor of Internal Medicine Director, Hematovascular Biology Center (HBC), University of Virginia, USA

*kwgar@virginia.edu*

Somatic mutations inevitably accumulate during embryonic development and over the course of an individual's lifetime. Recent advances in DNA sequencing methodology reveal that somatic mutations are remarkably prevalent, indicating a degree of cell-to-cell heterogeneity that was previously unappreciated. When somatic mutations occur in "driver" genes, these cells can undergo a clonal expansion in physiologically normal tissues and cell populations. This process has been extensively studied in the hematopoietic system, and it is referred to as "clonal hematopoiesis". Depending on how it is defined and measured, robust levels of clonal hematopoiesis range from more than 10% in individuals past the age of 70 to more than 50% in individuals past the age of 85. Epidemiological studies indicate that clonal hematopoiesis is associated with increased mortality due in large part to an increase in cardiovascular disease risk. Our work supports the concept that clonal hematopoiesis is a causal risk factor for cardiovascular disease, and we have defined aspects of this new disease mechanism. This work has shown that expanding mutant clones, attributed to mutations in TET2, DNMT3A, JAK2V617F and other driver genes, increasing give rise to progeny leukocytes with altered phenotypic properties. While different mutant driver genes confer distinct phenotypes to their progeny leukocytes within the clone, a growing body of experimental evidence suggest that activation of IL-1β and/or IL-6 expression represents a common mechanistic feature shared by multiple forms of clonal hematopoiesis. Overall, these findings support the concept that clonal hematopoiesis represents a new mechanism of age-related disease development that shares features with hematologic malignancy. Further research in the area could provide a mechanistic framework for personalized anti-inflammatory therapies to treat individuals who carry specific somatic mutation clones within their hematopoietic cell populations.

# Endocytic Adaptor Protein Epsin Is a Gatekeeper of Quiescent Endothelium

**Hong Chen**[1*]

[1] Vascular Biology Program, Harvard Medical School / Boston Children Hospital, USA

*hong.chen@childrens.harvard.edu*

Impeding pathological angiogenesis associated with vascular disorders is paramount in treating disabling and deadly diseases such as blindness, diabetes and cancer. Epsins are a family of prominent endocytic adaptor proteins. We show that epsins, via their ubiquitin-interacting motifs (UIM), are critical for activated VEGFR2 internalization and degradation and VEGF signaling attenuation. Intriguingly, endocytosis of VEGFR2 via a different endocytic adaptor protein, Dab2, results in enhanced VEGF signaling. We show that epsins and Dab2 competitively interact with VEGFR2 via a mutually exclusive mechanism. Consequently, mice lacking epsins and Dab2 reduce heightened angiogenesis in epsin mutants and restore attenuated angiogenesis in Dab2 mutants. However, whether the antagonism of epsins and Dab2 is governed by upstream signals is poorly understood. Our latest study revealed that Sphingosine 1 Phosphate (S1P) enhances epsins while reduces Dab2 binding to VEGFR2 to potentiate VEGFR2 degradation, implicating that S1P may be one of long-sought-after upstream cues that triggers epsin-mediated downregulation of VEGF signaling. Given that VEGF signaling plays a central role in normal, as well as pathological angiogenesis, thus, our work to discover new molecules and pathways, in particular, upstream signals and genetic modifiers that reign epsins' activity in regulating VEGF signaling and pathological angiogenesis paves the way to develop new therapeutic approaches for the prevention and treatment of cardiovascular and other diseases.

Symposium 06-4

# Therapeutic Implication and Action Mechanism of Endothelial Dysfunction Blocker

**Young-Guen Kwon**[1*]

[1] Biochemistry, Yonsei University, Korea

*ygkwon@yonsei.ac.kr*

Vascular endothelial cells (ECs) play a key role in a physical barrier between the vessel wall and lumen and endothelial dysfunction (ED) precedes the development of human diseases, which involve vascular leakage, coagulation, and inflammation. We have developed a small molecule ED blocker, named CU06-1004, which inhibits apoptosis, hyperpermeability, and NF-kB activation of ECs. The therapeutic efficacy and action mechanism of CU06-1004 has been tested in ED-related disease models such as macular edema, ischemic/reperfusion injuries, inflammatory bowel disease, non-alcoholic steatohepatitis, and sepsis. Surprisingly, this molecule significantly alleviated disease activity indexes in all employed models with improved EC barrier function and reduced inflammatory scores. Moreover, CU06-1004 normalized tumor blood vessels and it enhanced therapeutic efficacy of anti-PD-1 antibody in MC-38 tumor model. Thus, we propose that primary protection of ECs is pivotal in controlling inflammation and CU06-1004 could be a promising candidate for treating ED-associated human diseases.

# Vascular Intraluminal Pressure Load Inhibits Directed Endothelial Cell Migration in Angiogenesis

**Koichi Nishiyama[1*], Shinya Yuge[2], Yuichiro Arima[1], Yasuyuki Hanada[1], Sanshiro Hanada[1], Ryuji Yokokawa[3], Takashi Miura[4], Shigetomo Fukuhara[2]**

[1] International Research Center for Medical Sciences, Kumamoto University, Japan
[2] Department of Molecular Pathophysiology, Nippon Medical School, Japan
[3] Department of Micro Engineering, Kyoto Univeristy, Japan
[4] Department of Anatomy and Cell Biology, Kyushu University, Japan

*nkanako@kumamoto-u.ac.jp*

Angiogenesis is a multicellular morphogenesis to increase vascular beds, in which endothelial cells (ECs) concertedly elongate the branch, whilst forming lumen structure with blood flow. We reported that directed EC migration drives angiogenic branch elongation (Development 2015, Cell Rep 2011). However, it is still well unknown whether or not blood flow-mediated vascular intraluminal pressure can affect the morphogenetic EC behaviors.

To dissect the issue, we established an on-chip reconstitution assay system, in which angiogenesis was induced in 3D way in a microfluidic device and further, vascular intraluminal pressure was reproduced by placing hydrostatic pressure on the reconstructed vascular lumen. Identified observations were also confirmed in vivo using zebrafish models.

Using this system, we identified that hydrostatic pressure load inhibits angiogenic branch elongation. Time-lapse imaging at cellular and subcellular levels and whole-mount immunostaining revealed that the pressure load stretched vascular endothelial wall, which abruptly caused failure of directed migration with anterior-posterior polarity loss in ECs. We further found that Arp2/3 complex failed to be formed locally in the leading front of ECs in the very acute phase of the load, followed by suppression of proper F-actin bundling and remodeling and lamellipodia formation. Finally, we examined whether or not similar phenomenon could be observed in vivo. Time-lapse imaging of injury-induced angiogenesis in zebrafish showed new blood vessels to emerge from the downstream side of blood flow, but never from the upstream side in which intraluminal pressure is supposed to be relatively high due to cardiac pumping pressure, supporting our ex vivo results.

These results indicate a novel function of vascular intraluminal pressure as an inhibitory signal of angiogenic directed EC migration. We are now investigating the underlying mechano-sensing and -transduction mechanisms of circumferential wall stretch in angiogenic ECs.

Symposium 06-6 Short Talk

# Tyrosine Phosphorylation of eNOS Regulates Endothelial Function and Endothelial Redox Homeostasis

**Mauro Siragusa[1,2*], Alberto Fernando Oliveira Justo[1], Janina Thöle[1,2], Sofia-Iris Bibli[1,2], Ilka Wittig[2,3], Juliana Heidler[2,3], Andreas Weigert[4], Pedro Felipe Malacarne[5], Anna Strano[1], Akshay Buch[6], Barbara Withers[6], Kevin G. Peters[6], Beate Fisslthaler[1,2], Ingrid Fleming[1,2]**

[1] Institute for Vascular Signalling, Centre for Molecular Medicine, Goethe University, Frankfurt am Main, Germany
[2] German Center for Cardiovascular Research (DZHK), Partner site RheinMain, Frankfurt am Main, Germany
[3] Functional Proteomics, SFB 815 Core Unit, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany
[4] Institute of Biochemistry I, Goethe University, Frankfurt am Main, Germany
[5] Institute for Cardiovascular Physiology, Faculty of Medicine, Goethe University, Frankfurt am Main, Germany
[6] Aerpio Pharmaceuticals, Inc., Cincinnati, Ohio, USA

*Siragusa@vrc.uni-frankfurt.de*

Decreased nitric oxide (NO) bioavailability is a hallmark of endothelial dysfunction and cardiovascular disease. This study investigated whether NO bioavailability and endothelial function may be improved by either blocking the phosphorylation of eNOS on Tyr657 or by enhancing the phosphorylation of eNOS on Tyr81.

Vascular reactivity studies were performed in wild-type, eNOS Tyr656Phe knock-in mice and diabetic mice. Mechanistic and functional studies were performed in human and murine endothelial cells.

The vascular endothelial protein tyrosine phosphatase (VE-PTP) formed a complex with eNOS and directly dephosphorylated Tyr81 in endothelial cells. In rings of murine aorta, the VE-PTP inhibitor AKB-9785 (AKB) induced a concentration-dependent relaxation and significantly enhanced the response to acetylcholine. In human endothelial cells, AKB increased the basal phosphorylation of Tyr81 as well as the increase induced by PIEZO1 activation (with Yoda1) and enhanced eNOS activity. Src inhibition and knockdown of the abelson-tyrosine protein kinase 1 significantly reduced the basal and Yoda1-induced tyrosine phosphorylation of eNOS and generation of NO. Vascular reactivity studies in vessels from diabetic mice demonstrated that diabetes-induced endothelial dysfunction was abrogated by VE-PTP inhibition. In line with these findings, chronic VE-PTP inhibition in hypertensive diabetic patients significantly lowered systolic blood pressure. The Tyr656 to Phe mutation in mice rendered eNOS insensitive to inactivation by oxidative stress and enhanced NO-dependent vascular relaxation. Angiotensin II-induced hypertension and endothelial dysfunction were attenuated compared to wild-type mice and preserved endothelial function and reduced inflammation were linked to delayed atherosclerosis development and progression. Preserved NO bioavailability contributed to endothelial redox homeostasis through the S-nitrosation and inhibition of pyruvate kinase M2.

# Neuro-Vascular Interactions in the Brain

**Chenghua Gu[1*], Brian Chow[1], Vicente Nunez[1], Luke Kaplan[1], Hannah Zucker[1]**

[1] Neurobiology, Harvard Medical School, USA

*Chenghua_Gu@hms.harvard.edu*

Proper brain function depends on neurovascular coupling: neural activity rapidly increases local blood flow to meet moment-to-moment changes in regional brain energy demand. Neurovascular coupling is the basis for functional brain imaging, and its impairment is implicated in neurodegeneration1. The underlying molecular and cellular mechanisms of neurovascular coupling remain poorly understood. The conventional view is that neurons or astrocytes release vasodilatory factors that act directly on smooth muscle cells (SMC) to induce arterial dilation and increase local blood flow. Here, using two-photon microscopy to image neural activity and vascular dynamics simultaneously in the barrel cortex of awake mice under whisker stimulation, we found that arteriolar endothelial cells (aECs) play an active role in mediating neurovascular coupling. We found that aECs, unlike other vascular segments of ECs in the CNS, have abundant caveolae. Acute genetic perturbations that eliminated caveolae in aECs, but not in neighboring SMCs, impaired neurovascular coupling. Strikingly, caveolae function in aECs is independent of the eNOS-mediated nitric oxide (NO) pathway. Ablation of both caveolae and eNOS completely abolished neurovascular coupling, whereas each single mutant exhibited partial impairment, revealing that caveolae-mediated pathway in aECs is a major contributor to neurovascular coupling. Our findings indicate that vasodilation is largely due to ECs that actively relay signals from the CNS to SMCs via a caveolae-dependent pathway.

Symposium 07-2

# The Neurovascular Interface

**Amparo Acker-Palmer**[1*]

[1] Institute of Cell Biology and Neuroscience, Johann Wolfgang Goethe-University, Germany

*acker-palmer@bio.uni-frankfurt.de*

The development of the nervous and the vascular systems exhibit extensive similarities, both on the anatomical and the molecular level. Blood vessels and nerves are structurally similar and often aligned, following parallel routes. The brain is the most vascularized tissue in our body. In the past, we have discovered that the same molecular mechanisms are used to orchestrate the development of the nervous and the vascular system. It is now believed that blood vessels in the brain exert instructive functions that go beyond supplying nutrients and oxygen, for example supplying ligands that directly influence neuronal behavior by activating corresponding receptors and signaling pathways in neuronal cells. We are interested in elucidating the molecular pathways involved in the crosstalk between vessels and nerves and how this crosstalk signaling is integrated among the different cellular players (neurons, endothelial cells, astrocytes) at the neurovascular interface during CNS development.

# Constitutively Active Notch4 in Endothelial Cells Initiates Arteriovenous Malformation via a Nitric Oxide Synthase-Mediated Mechanism

**Rong Wang[1*], Lawrence Huang1*, Feng Cheng[1*], Weiwei Xiang[1], Kunal Raygor[1], Matthew A. Nystoriak2, Xitao Wang[1], Weiya Jiang[1], Arsalan Urrab Syed[2], Kevin S. Hou1, Jiayi Zhang[1], Livius V. d'Uscio[3], Jacek Zielonka[4], Katusic, Zvonimir[3], Manuel F. Navedo[2], Rong A. Wang[1]**

[1]Laboratory for Accelerated Vascular Research, Department of Surgery, University of California, San Francisco.
[2]Department of Pharmacology, University of California, Davis.
[3]Departments of Anesthesiology, Molecular Pharmacology, and Experimental Therapeutics, Mayo Clinic.
[4]Department of Biophysics, Free Radical Research Laboratory, Medical College of Wisconsin.
*equal contributions.

*Rong.Wang@ucsf.edu*

Arteriovenous (AV) malformations (AVMs) are characterized by a nidus of enlarged vessels that shunt blood directly from arteries to veins. Mechanisms underlying AVM pathogenesis are poorly understood, hindering therapeutic development. We have shown that endothelial expression of constitutively active Notch4 (Notch4*) initiates brain AVMs in mice through enlargement of capillary-like vessels without an increase in endothelial cell number or proliferation. Here, we hypothesized that Notch4* initiates AVM by disrupting endothelial nitric oxide synthase (eNOS) signaling, increasing vasodilation, hence permitting vessel enlargement and AV shunting. Supporting this, eNOS gene deletion or inhibition of nitric oxide synthase (NOS) with NG-nitro-L-arginine (L-NNA) decreased brain AV shunt diameter, severity of brain AVM-associated pathologies, and illness progression in mice expressing endothelial Notch4*. Arterioles in live mutant brains exhibited decreased arterial tone. Isolated Notch4* Pial arteries also exhibited decreased pressure-mediated arterial tone, which was abolished by L-NNA. NOS-dependent superoxide production was elevated in mutant brains at the initial stages of AV shunting formation. Administering the superoxide dismutase mimetic 4-Hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (Tempol) decreased brain AV shunt diameter, severity of brain AVM-associated pathologies, and illness progression in mice expressing endothelial Notch4*, mirroring the effects of NOS inhibition and eNOS deletion. Our data suggest that endothelial Notch4*-induced brain AVM involves an eNOS-dependent molecular mechanism.

Symposium 07-4

# Deciphering the Role of Genome Organization in Development and Disease

**Rajan Jain**[1*]

[1] Depts. of Medicine and Cell & Developmental Biology, University of Pennsylvania, USA

*jainr@pennmedicine.upenn.edu*

The nuclear lamina has emerged as an important scaffold to organize chromatin in multiple organisms and cell types. However, the functional importance of nuclear lamina-chromatin interactions in progressive lineage restriction remains elusive. I will discuss our efforts to understand the molecular mechanisms by which spatial positioning of the genome affects cellular identity. By manipulating a subset of Histone Deacetylase 3 bound at the nuclear lamina, our data demonstrate the physiologic relevance of spatial positioning during cardiomyocyte specification and differentiation. I will also present unpublished studies which have extended this work into a class of diseases termed laminopathies. Laminopathies are characterized by mutations in LMNA and related genes which result in a variety of human phenotypes, including dilated cardiomyopathy. By studying pathogenic LMNA variants in various tissues, our data suggest that lamina-chromatin interactions are cell-type specific and functionally serve to protect cellular identity. Taken together, our results suggest that availability of regions of the genome for activation by lineage-specific transcription factors is regulated in part through chromatin-nuclear lamina interactions. Inductive niche signals shape cellular identity, but it remains unclear how different cells can respond differently to the same cues. We propose that the competence of a progenitor cell to respond to inductive signals may depend upon the coordinated movement of responding gene loci away from the nuclear periphery.

# Neurovascular Changes in Alzheimer's Disease

**Yong Jeong**[1,2*]

[1] Department of Bio and Brain Engineering, KAIST, Korea
[2] KI for Heath Science and Technology, KAIST, Korea

*yong@kaist.ac.kr*

Along with molecule mechanism, neurovascular mechanism is profoundly engaged in the pathogenesis of various neurodegenerative diseases such as Alzheimer's disease (AD) and subcortical vascular dementia (SVaD). As it name is indicating, there are various pathological changes in vascular components and also neuronal and glial components in SVaD, however the lack of appropriate animal model hampered the understanding of it. In case of AD, vascular risk factors such as hypertension, diabetes, hyperlipidemia are known as risk factors, however the way of working on the pathogenesis is still unclear. In this talk, I will introduce models for SVaD and its behavioral and pathological characteristics. Further, the role of microvascular components in the regulation of cerebral blood flow. Also I will introduce the physiological and pathological changes in neurovascular system in Alzheimer's disease.

Symposium 07-6 Short Talk

# Vascular Endothelial Cells Contribute to the Scavenging Meningeal Macrophage Population in Embryonic and Postnatal Development

**Neil Bower[1], Maria Rondon Galeano[1,2], Elizabeth Mason[2], Kaustav Das Gupta[1], Kok Leong Chong[3], Renae Skoczylas[1], Matt Sweet[1], Vukovic Jana[3], Benjamin Hogan[1,2*]**

[1] Institute for Molecular Bioscience, University of Queensland, Australia
[2] Organogenesis and Cancer Program, Peter MacCallum Cancer Centre, Australia
[3] School of Biomedical Science, University of Queensland, Australia

*Ben.Hogan@petermac.org.au*

The brain is long considered to be immune-privileged and largely closed off from the immune system due to the function of the blood brain barrier and the impervious meningeal membranes. Nevertheless, the brain must be able to effect an efficient innate immune response and so harbors unique innate immune cells that include microglia and macrophages. Recent studies have demonstrated surprising heterogeneity in the immune lineages of the brain with many different innate immune subtypes profiled in physiological and pathological settings. How this diversity is generated in development and maintained in homeostasis remains to be fully understood. The meninges that surround the vertebrate brain harbor unique populations of macrophages of distinct morphology that scavenge large macromolecular wastes. In zebrafish at least one similar lineage derives from vasculature in a surprising process related to lymphangiogenesis. Here we probed the molecular nature, heterogeneity and developmental origins of meningeal scavenging macrophages in mice.

We used single cell sequencing combined with lineage tracing to identify the developmental origins of meningeal macrophages in mice.

Using an unbiased single cell sequencing approach to accurately profile 16000 scavenger cells of the mouse meninges, we find a meningeal macrophage subtype that has similarities to the zebrafish lineage. These cells are found mural to blood vessels and identify as a unique macrophage lineage that shares many characteristics with vascular derived scavenger cells from zebrafish. To determine if this meningeal macrophage lineage may have vascular origins, we used lineage tracing coupled with singe cell sequencing and traditional immunohistology. We find that mouse meningeal macrophages derive from heterogeneous origins that include a vascular endothelial origin in the embryonic and postnatal brain.

This work indicates a partially conserved vascular origin for scavenging macrophages in the vertebrate brain and underscores surprising heterogeneity in the immune system of the brain.

# Stress-Induced Premature Aging Mediated by Mitochoindrial Hibernation Promotes Atherosclerosis

**Jun-ichi Abe**[1*]

[1] Department of Cardiology, The University of Texas MD Anderson Cancer Center, USA

*JAbe@mdanderson.org*

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality in all cancer survivors approximately 5-10 years after their cancer diagnosis. It has been suggested that many cancer treatments, including radiation therapy, activate shared signaling events, promoting a premature aging and senescence-associated secretory phenotype (SASP) and leading to an increase in CVD, but exact regulatory mechanism remains largely unknown. Our goal in this study was to identify the shared signaling events activated by these cancer treatments.

To detect ERK5 inhibitors, we performed high-throughput screening with the MicroSource SPECTRUM Collection by using the ERK5 transcriptional activity reporter cell system. Bone marrow-derived macrophages (BMDMs) were isolated from control and ERK5 S496A knock-in mice, and SASP was determined by evaluating their efferocytotic ability, antioxidation-related molecule expression, telomere length, and inflammatory gene expression. We also detected mitochondrial (mt) function and reactive oxygen species (ROS) using Seahorse XF96 and MitoSOX Red. We assessed the involvement of p90RSK-ERK5 S496 phosphorylation and poly (ADP-ribose) polymerase (PARP) activation in cancer treatment-induced SASP and atherosclerosis by p90RSK- and PARP-specific inhibitors and in ERK5 S496A KI mice.

Four cancer therapy-related compounds (doxorubicin, ifosfamide, paclitaxel, and methotrexate) were selected by high-throughput screening. Ionizing radiation (IR) dose-dependently activated p90RSK and ERK5 S496 phosphorylation, which triggered all components of SASP in concert; IR induced mt hibernation by abolishing the ATP level but induced mtROS production and made macrophages more sensitive to ROS than vehicle-treated macrophages (priming). Priming was also detected in cancer patients after radiation therapy. Transient inhibition of PARP activity only at the time of IR eradicated IR-induced monocyte mt hibernation, priming, and acceleration of atherosclerosis. Lastly, the data obtained from ERK5 S496A KI mice revealed that ERK5 S496 phosphorylation has a crucial role in inducing SASP in macrophages and atherogenesis.

Four cancer therapy-related compounds (doxorubicin, ifosfamide, paclitaxel, and methotrexate) were selected by high-throughput screening. Ionizing radiation (IR) dose-dependently activated p90RSK and ERK5 S496 phosphorylation, which triggered all components of SASP in concert; IR induced mt hibernation by abolishing the ATP level but induced mtROS production and made macrophages more sensitive to ROS than vehicle-treated macrophages (priming). Priming was also detected in cancer patients after radiation therapy. Transient inhibition of PARP activity only at the time of IR eradicated IR-induced monocyte mt hibernation, priming, and acceleration of atherosclerosis. Lastly, the data obtained from ERK5 S496A KI mice revealed that ERK5 S496 phosphorylation has a crucial role in inducing SASP in macrophages and atherogenesis.

Symposium 08-2

# Genetic Fate Mapping of Cardiomyocyte and Endothelial Cell Proliferation in Adult Mouse

**Bin Zhou**[1*]

[1] Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences, China

*zhoubin@sibs.ac.cn*

Monitoring of cell proliferation provides essential information about cellular mechanisms of tissue homeostasis and the etiopathogenesis of many diseases. However, current methods could not monitor lineage specific cell proliferation for long term analysis. Here, we developed a new genetic system, ProTracer, for seamless recording of cell proliferation using Ki67- or Ccna2-based genetic fate mapping approach. By employing cardiomyocyte specific ProTracer, we found ~0.25% of cardiomyocytes have undergone cell-cycle activity each month in adult mouse heart. High resolution 3D image of cardiomyocyte proliferation for the entire adult heart revealed a previously unappreciated regional cardiomyocyte proliferation pattern during cardiac homeostasis and after stresses, such as myocardial infarction and pressure overload. Combining two-photon microscopy with ProTracer on mouse aorta, we found that ~1.5% of endothelial cells have undergone cell division each month in adult stage. Interestingly, oscillatory sheer stress significantly reduced the formation of new endothelial cells and also skewed the angel of endothelial cell division towards perpendicular orientation to the blood flow. Thus, our study introduces a genetic method that enables monitoring of previously unobtainable in vivo cell proliferation data, which could be applied in multiple areas of biology and medicine.

Symposium 08-3

# Therapeutic Angiogenesis by Autologous Adipose-Derived Mesenchymal Stem Cells

**Toyoaki Murohara**[1*]

[1] Cardiology, Nagoya University, Japan

*murohara@med.nagoya-u.ac.jp*

Clinical trials using proangiogenic cytokines or stem/progenitor cells have been performed in patients with severe ischemic disease, and this strategy is termed "therapeutic angiogenesis". We had previously demonstrated that implantation of autologous bone marrow mononuclear cells (BM-MNCs) significantly augmented angiogenesis and collateral vessel formation in animal models and human cases of critical limb ischemia (CLI). More recently, we examined whether implantation of adipose tissue-derived mesenchymal stem cells termed adipose tissue-derived regenerative cells (ADRCs) might augment neovascularization in response to tissue ischemia. ADRC released various angiogenic cytokines and ADRC implantation increased SDF-1 and VEGF expression from ischemic tissue in vivo, which mobilized endothelial progenitor cells. Direct local implantation of ADRCs significantly augmented angiogenesis in mouse and rabbit models of hindlimb ischemia. As a case of translational research, we applied this method to human CLI cases and we have so far experienced 15 cases of therapeutic angiogenesis. We are also trying to analyze the roles of various adipokine for the cardiovascular disease model with special focus onto angiogenesis.

Symposium 08-4

# In Vivo Genome Editing for Angiogenesis-Related Blindness

**Jeong Hun Kim**[1*]

[1] Ophthalmology, Seoul National University College of Medicine, Korea

*steph25@snu.ac.kr*

From the FDA approval of anti-VEGF aptamer to wet-type age-related macular degeneration (AMD) of choroidal neovascularization, anti-VEGF aptamer and antibody have been widely used against all kinds of vaso-proliferative retinopathy. Actually, current therapies directed at controlling vascular abnormalities in vaso-proliferative retinopathy target VEGF and can slow the progression of these diseases. While the general role of VEGF in development has been well described, the specific function of locally synthesized VEGF in the eye is incompletely understood. Recently, RNA-guided genome surgery using CRISPR-Cas9 nucleases has shown promise for the treatment of diverse genetic diseases. Yet, the potential of such nucleases for therapeutic applications in non-genetic diseases including AMD, diabetic retinopathy (DR) as well as retinopathy of prematurity (ROP) is largely unexplored. Those vision-threatening retinopathies such as AMD, DR, and ROP are leading causes of blindness in adults and children, which is associated with retinal over-expression of, rather than mutations in, the VEGFA gene.

Herein I would like to provide some my recent experimental results of therapeutic applications such as small peptide, small molecule as well as nanoparticles beyond anti-VEGF antibody. In addition, some results of in vivo genome editing in vision-threatening retinopathy would be provided.

# Therapeutic Targeting of Tumor Angiogenesis Using Endothelial Cell Fate Mechanisms

**Jan Kitajewski**[1*]

[1] University of Illinois Chicago, IL, USA

*kitaj@uic.edu*

As a key regulator of tumor angiogenesis, Notch signaling offers an attractive target for therapeutic intervention. Several current approaches for targeting the Notch pathway include gamma secretase inhibitors (GSIs), Notch antibodies, or Notch ligand antibodies. Unfortunately, with many of these approaches, especially with GSIs, severe toxicity has been observed. Based on the mammalian Notch1 extracellular domain, which contains 36 EGF-like repeats, we developed ligand-specific Notch1 inhibitors that we have named the Notch1 decoys. Using an assay developed to induce ligand-specific NOTCH activation by culturing cells on tissue culture plates tethered with recombinant Dll4 or Jagged1 ligands, a Notch1 decoy comprised of EGF-like repeats 1-13 fused to human Fc (N11-13Fc) specifically inhibited Dll4-mediated NOTCH activation, while a Notch1 decoy comprised of EGF-like repeats 10-24 (N110-24Fc) specifically inhibited Jagged1-mediated NOTCH activation. Interestingly, N11-13Fc decoy and N110-24Fc decoy exhibited differential effects on in vitro, retinal, and tumor angiogenesis indicating unique mechanisms of DLL4/NOTCH and JAG1/NOTCH signaling on angiogenesis. Inhibition of Dll4-mediated Notch signaling by N11-13 decoy in tumors led to paradoxical hypersprouting of a nonfunctional tumor vasculature, while inhibition of Jagged1-mediated Notch signaling by N110-24 decoy led to reduced angiogenic sprouting in tumors; however, both decoys were able to reduce tumor growth in several mouse tumor models. Based on the fact that human JAGGED1 (JAG1) is highly expressed in triple negative breast cancer (TNBC) cells, we explored the hypothesis that JAG1 promotes both tumor angiogenesis and metastatic spread. Using both Jag-specific N1 decoys and reduction of expression of JAG1 in TNBC we provide evidence that JAG1 on tumor cells promotes endothelial adhesion and transendothelial migration. Therefore, we conclude that ligand-specific Notch inhibition by the Notch1 decoys represents effective therapeutics to inhibit tumor angiogenesis and metastatic spread.

Symposium 08-6 Short Talk

# Cardiovascular Disease Risk Factors Reprogram Cardiac Endothelial Cell Transcriptome and Promote EndMT Features

**Karthik A Hemanthakumar[1,2], Shentong Fang[1,2], Mikko I Mäyränpää[3], Eero Mervaala[1], Riikka Kivelä[1,2*]**

[1] Faculty of Medicine, University of Helsinki, Finland
[2] Wihuri Research Institute, Helsinki, Finland
[3] Pathology, Helsinki University and Helsinki University Hospital, Finland

*riikka.kivela@helsinki.fi*

Aging, obesity, hypertension and physical inactivity are major risk factors for cardiovascular disease (CVD). A better understanding of the pathways that are affected by these risk factors is of great importance for the development of novel treatment strategies.

We aimed to investigate how endothelial cells (EC) in the heart respond and adapt to physiological (exercise training) and pathological (aging, obesity and pressure overload) stimuli. Furthermore, we sought to identify novel therapeutic targets in ECs to treat CVD.

Four different studies were conducted: exercise tr ained vs. sedentary mice, high-fat diet (HFD) -induced obese vs. lean mice, aged (18 mo) vs. young (2 mo) mice and transverse aortic constriction (TAC) vs. sham -operated mice. Cardiac ECs were isolated and sorted by FACS, and analyzed by RNA sequencing. Human ECs and heart samples were used in mechanistic and translational analyses.

Exercise training significantly increased, whereas aging, HFD and TAC decreased cardiac EC number and heart function. All the interventions had significant effect on the cardiac EC transcriptome. CVD risk factors upregulated pathways related to inflammation, vascular permeability, oxidative stress, collagen synthesis and cell aging, whereas exercise training downregulated many of the same pathways. Interestingly, both aging and obesity induced EndMT gene signature in cardiac ECs. We identified a collagen chaperone SerpinH1/HSP47 to be upregulated by aging and obesity and downregulated by exercise training. Overexpression of HSP47 in human ECs induced increased cell size and stress fibers and promoted endothelial-to-mesenchymal transition (EndMT), while its silencing inhibited collagen deposition and reduced EC growth and survival. Staining of human heart sections demonsrated that HSP47 is abundantly expressed throughout the heart in both fibroblasts and ECs.

Here we demonstrated that aging and obesity activated several adverse pathways and EndMT gene signature, while exercise training promoted opposite and protective changes in cardiac ECs.

# Adaptable and Hemodynamic Vasculogenic Endothelial Cells for Organogenesis and Tumorigenesis

**Shahin Rafii[1*], Brisa Palikuqi[1], Duc-Huy Nguyen[1], Ryan Schreiner[1], Paolo De Coppi[2], Sina Rabbany[1]**

[1] Medicine/Regenerative Medicine, Weill Cornell Medicine, USA
[2] Stem Cell and Regenerative Medicine Section, Institute of Child Health, University College London, UK

*srafii@med.cornell.edu*

Endothelial cells (ECs) adopt tissue-specific properties to instruct organ development1,2. This adaptability is lost in adult ECs and they fail to vascularize tissues in an organotypic manner. Here, we show that transient reactivation of embryonic-restricted ETS variant 2-transcription factor (ETV2)3 in mature human ECs in three-dimensional (3D) Laminin-Entactin-CollagenIV (L.E.C) matrix "Resets" these stringent ECs into amenable vascular ECs (R-VECs), forming perfusable and adaptable vascular plexi. ETV2 via chromatin remodeling induces tubulogenic pathways, including Rap1-activation4,5, promoting durable lumen formation. In 3D matrices, without the constraints of bioprinted scaffolds, R-VECs self-assemble into stable multi-layered vascular networks within large-volume sizable microfluidic chambers capable of transporting human blood. In vivo, implanted R-VEC self-organize into pericyte-coated vessels that functionally anastomose to host circulation and manifest long-lasting patterning, without malformations or angiomas. R-VECs, without the need for restrictive synthetic semipermeable membranes, directly interact with the cells within 3D cocultured organoids, establishing an Organ-On-VascularNet platform. R-VECs physiologically perfuse human pancreatic islets, vascularize decellularized intestines, and arborize normal and tumor organoids. Through scRNA-sequencing and epigenetic profiling, we demonstrate that R-VECs establish an adaptive vascular niche, differentially adjusting and conforming to tissue-specific organoids and tumoroids. Deciphering the cross-talk between R-VECs and parenchymal cells facilitates the identification of EC heterogeneity determinants and warrants metabolic, immunological and physiochemical studies and screens, setting the stage for therapeutic organ repair and tumor targeting.

Symposium 09-2

# Endothelial Heterogeneity: a COVID-19 Update

**Peter Carmeliet**[1*]

[1] Department of Oncology, VIB-KU Leuven Center for Cancer Biology, Belgium

*peter.carmeliet@kuleuven.vib.be*

On the basis of emerging evidence from patients with COVID-19, we postulate that endothelial cells are essential contributors to the initiation and propagation of severe COVID-19. Coronavirus disease 2019 (COVID-19), caused by the betacoronavirus SARS-CoV-2, is a worldwide challenge for health-care systems. The leading cause of mortality in patients with COVID-19 is hypoxic respiratory failure from acute respiratory distress syndrome (ARDS). To date, pulmonary endothelial cells (ECs) have been largely overlooked as a therapeutic target in COVID-19, yet emerging evidence suggests that these cells contribute to the initiation and propagation of ARDS by altering vessel barrier integrity, promoting a pro-coagulative state, inducing vascular inflammation (endotheliitis) and mediating inflammatory cell infiltration. Therefore, a better mechanistic understanding of the vasculature is of utmost importance. Here, we discuss current insights into endothelial cell biology in health and disease focusing on their heterogeneity between and within vascular beds, the divergent global and metabolic characteristics they display and that correlate with the specific functions of the particular endothelial cell subtypes, and discuss the link between endothelial cells, viral infection and inflammatory changes, proposing novel therapeutic strategies.

References:
G. Eelen, et al . Nature 561, 63-9 (2018)
J. Kalucka et al. Cell Metab 28 (2018)
S. Vandekeere et al. Cell Metab 28: 573-587 (2018)
B.W. Wong et al. Nature 542: 49-54 (2017)
S. Schoors et al. Nature 520: 192-7 (2015)
S. Schoors et al. Cell Metab 19: 37-48 (2014)
K. De Bock et al. Cell 154: 651-63 (2013)
K. De Bock et al. Cell Metab 18: 634-47 (2013)
L.A. Teuwen, et al. Nat Rev Immunol doi: 10.1038/s41577-020-0343-0
COI:No

Symposium 09-3

# Vascular Niche for Skeletal Muscle Stem Cell: Application for Muscular Dystrophy Therapy

**Atsushi Asakura**[1*]

[1] Stem Cell Institute, University of Minnesota Medical School, USA

*asakura@umn.edu*

Muscle satellite cells are a stem cell population responsible for skeletal muscle growth, repair and regeneration. Maintenance of the balance between satellite cell differentiation and self-renewal is required for muscle homeostasis. By utilizing fluorescent reporter mice, muscle tissue clearing and confocal/two-photon microscopy to investigate the specific niche for muscle satellite cells in 3-dimensions, we recently reported that the juxtavascular niche of satellite cells for stem cell maintenance via VEGF and Notch pathways (Verma, et al. Cell Stem Cell, 2018). In creased vascular density could induce increased number of satellite cells, which leads to a beneficial effect on Duchenne muscular dystrophy (DMD) model mice. Based on these results, we recently generated anti-FLT1 monoclonal antibodies, which blocked binding of VEGF and FLT1 (VEGFR1), a decoy receptor for VEGF. The histological and functional improvement of dystrophic muscle by FLT1-blockade provides a novel pharmacological strategy for the potential treatment of DMD (Verma et al., PLoS Genet, 2019).

Symposium 09-4

# Promoting Healthy Againg by VEGF-Based Vascular Manipulations

**Eli Keshet**[1*], **Myriam Grunewald**[1]

[1] Dept. Dev Biol & Cancer Research, Hebrew University -Hadassah Medical School, Israel

*elik@ekmd.huji.ac.il*

While cell-autonomous aging processes are well-characterized, the impact of failing organ support systems is not fully appreciated. Here we show that deteriorated vascular function due to age-related VEGF signaling insufficiency and resultant microvascular rarefaction is an hierarchically high driver of physiological aging at large. Securing adequate VEGF signaling via compensatory modest increase of circulatory VEGF prevented microvascular rarefaction, improved tissue perfusion, alleviated major cellular hallmarks of organ aging culminating in dramatic lifespan and healthspan extension. Healthier aging was indicated in maintenance of a young-like metabolism, energetics and body composition. Age-associated pathologies ameliorated included hepatic steatosis, sarcopenia, osteoporosis, inflammaging and increased tumor burden. The study thus suggests harnessing VEGF-mediated preservation of a young-like vascular homeostasis as a yet uncharted modality for holistic geroprotection and multifaceted healthspan increase.

# Re-defining Early Endothelial Progenitor Cells for Ischemic Cardiovascular Repair

**Sang-Mo Kwon**[1*]

[1] Department of Physiology, Pusan National University, Korea

*smkwon323@pusan.ac.kr*

Cardiovascular diseases (CVDs), including atherosclerosis, stroke, and myocardial infarction, is a major cause of death worldwide. In aspects of cell therapy against CVD, it is generally accepted that endothelial progenitor cells (EPCs) are potent neovascular modulators in ischemic tissues. In response to ischemic injury signals, EPCs located in a bone marrow niche migrate to injury sites and form new vessels by secreting various vasculogenic factors including VEGF, SDF-1, and FGF, as well as by directly differentiating into endothelial cells. Nonetheless, in ischemic tissues, most of engrafted EPCs do not survive under harsh ischemic conditions and nutrient depletion. Therefore, an understanding of diverse EPC-related cytoprotective mediators underlying EPC homeostasis in ischemic tissues may help to overcome current obstacles for EPC-mediated cell therapy for CVDs. Additionally, to enhance EPC's functional capacity at ischemic sites, full understanding of origin of EPCs and their lineage commitment should be required. Since 1997, multiple research groups provide us a pivotal clue that there are two types of EPCs, early EPC and late EPCs (outgrowth endothelial cells, OECs). Both early EPCs and late EPCs play a pivotal role in vascular regeneration in ischemic tissues; however, defining early EPCs and late EPCs has remained to be solved. In this presentation, I will update re-defining early EPCs for EPC-based cardiovascular repair including the development of xeno-free-based EPC therapeutics and propose promising therapeutic strategies for the treatment of CVDs.

Symposium 09-6 Short Talk

# Mir-130a Potentiates the Reparative Properties of Human Endothelial Colony Forming Cells Facing Hypoxia via VEGFR2 and STAT3

**Reinhold Medina[1*], Jasenka Guduric-Fuchs[1], Edoardo Pedrini[1], Judith Lechner[1], Sarah Chambers[1], Christina O'Neill[1], Alan Stitt[1]**

[1] Experimental Medicine, Queen's University Belfast, UK

*r.medina@qub.ac.uk*

Hypoxia controls reparative angiogenesis. MiRNAs are master regulators of gene expression in hypoxia and angiogenesis. However, we do not yet have a clear understanding of how hypoxia-induced miRNAs modulate vasoreparative processes. This study investigated the role of miR-130a in human endothelial progenitors under hypoxic conditions.

We used a well-characterized subtype of human endothelial progenitor known as primary endothelial colony forming cells (ECFCs). ECFCs were exposed to 1% oxygen to study their response to hypoxia in terms of miRNA profiling, cell functional assays, transcriptome changes, and effects on hypoxia-angiogenesis related protein signalling pathways. Loss or gain of function modulation of miR-130a were performed using miRNA mimics and LNA-miRNA inhibitors. NGS RNA sequencing was performed using the NextSeq500, data normalised, and differential expression studied using DeSeq2. For evaluating miRNA bioactivity, we generated a miR-130 3'UTR luminescence reporter ECFCs. In vitro assays included clonogenics, 3D Matrigel tube formation, 2D migration, and the Seahorse glycolysis assay. Ex vivo and in vivo models include the sprouting choroid explant model, the matrigel subcutaneous implant assay, and the oxygen induced retinopathy model.

Here, we identify miR-130a as a mediator of the hypoxic response in human primary endothelial colony forming cells (ECFCs), a well-characterized subtype of endothelial progenitor. Under hypoxic conditions, miR-130a overexpression enhances ECFC pro-angiogenic capacity in vitro and potentiates their vasoreparative properties in vivo. Mechanistically, miR-130a orchestrates upregulation of VEGFR2, activation of STAT3-dependent transcription, and accumulation of HIF1α via translational inhibition of DDX6.

miR-130a provides ECFCs with a therapeutic advantage under hypoxia via stimulation of the VEGFR2/STAT3/HIF1 axis.

# Novel Regulators and Functions of Special Lymphatic Vessels

**Gou Young Koh**[1*]

[1] Center for Vascular Research, KAIST / IBS, Korea

*gykoh@kaist.ac.kr*

Lymphatic vessel network constitutes one of two arms of the vertebrate cardiovascular system and play complementary roles in maintaining body homeostasis and is closely related to multiple intractable diseases. Lymphatic vessels are internally lined with lymphatic endothelial cells (LECs), which are derived distinct endothelial cell lineage by a specific transcriptional program. Well-known functions of lymphatic vessels are fluid transport and immunosurveillance. However, special functions and regulations of specialized lymphatic vessels are lately discovered. In this meeting, I will talk about Schlemm's canal (SC). SC is an endothelium-lined channel that encircles the cornea and has morphological, molecular, and functional similarities with lymphatic vessels. SC dysfunction or regression during chronic inflammation or aging reduces aqueous humor drainage and elevates intraocular pressure, ultimately leading to glaucoma. Angiopoietin-Tie2 system is critical for maintaining SC integrity. Each small intestinal villus contains a highly specialized lymphatic capillary called a lacteal, which is essential for dietary fat uptake and gut immune surveillance. Distinct subsets of villi fibroblasts regulating lacteal integrity through YAP/TAZ–induced VEGF-C secretion will be discussed. Finally, I will present our recent work for the basolateral meningeal lymphatic vessels as a novel, hot spot for drainage of cerebrospinal fluid, which has made a conceptual advance in the regulating CSF drainage.

Symposium 10-2

# Mechanisms of Lymphatic Vascular Specialization

**Tatiana Petrova**[1*]

[1] Department of Oncology, University of Lausanne, Switzerland

*Tatiana.petrova@unil.ch*

Vertebrates have two vascular systems, both of which are indispensable for life: blood vessels, which bring oxygen and nutrients to the tissues, and lymphatic vessels, which remove proteins and excess of fluid from the interstitial space and return them back to the blood circulation. Lymphatic vessels are also important regulators of the immune response, as they transport peripheral antigens and antigen-presenting cells to lymph nodes.

Lymphatic vasculature is hierarchically organized into capillaries, important for fluid uptake, and collecting lymphatic vessels, which transport lymph to lymph nodes. In addition, lymphatic vessels have important tissue-specific functions, such as transport of lipids in intestine and the delivery of antigens and cell trafficking within the lymph node. However, the phenotypic and functional diversity of lymphatic vessels is only beginning to be appreciated. In my presentation I will discuss our recent data on the mechanisms of renewal of adult lymphatic vasculature and the roles of mechanical forces in collecting lymphatic vessel maintenance and development of specialized vessels of lymph node.

# Mechanisms Ensuring Endothelial Junction Integrity Beyond VE-Cadherin

**Dietmar Vestweber**[1*]

[1] Vascular Cell Biology, Max Planck Institute for Molecular Biomedicine, Germany

*vestweb@mpi-muenster.mpg.de*

VE-cadherin is of dominant importance for the formation and stability of endothelial junctions, yet induced gene inactivation enhances vascular permeability in the lung and heart, but does not cause junction rupture as analyzed by electron microscopy. Similar effects were found for anti VE-cadherin adhesion blocking antibodies. In a search for other adhesion molecules which would provide physical integrity of endothelial junctions in vivo in the absence of VE-cadherin we examined JAM-A, PECAM-1 and ESAM. We found that gene inactivation of ESAM enhanced vascular permeability in the lung, but not in heart, skin and brain. In contrast, deletion of JAM-A or PECAM-1 did not affect barrier integrity in any of these organs. Blocking VE-cadherin with antibodies caused lethality in ESAM-/- mice within 30 min, but had no such effect in JAM-A-/-, PECAM-1-/- or WT mice. Likewise, induced gene inactivation of VE-cadherin caused rapid lethality only in the absence of ESAM. Ultrastructural analysis revealed that only combined interference with VE-cadherin and ESAM disrupted endothelial junctions, and caused massive blood coagulation in the lung. Thus, despite well documented roles of JAM-A and PECAM-1 for the regulation of endothelial junctions, only for ESAM we detected an essential role for baseline endothelial barrier integrity in a tissue specific way. In addition, we found that it is ESAM which prevents endothelial junction rupture in the lung when VE-cadherin is absent. Although VE-cadherin is assisted by other adhesion mechanisms in maintaining junction integrity, it is essential to interfere with the function of VE-cadherin in order to "open" endothelial junction during inflammatory processes. Latest results will be discussed which reveal how extravasating leukocytes achieve this by downregulation of VE-cadherin. Furthermore, it will be discussed why the covalent fusion of VE-cadherin and α-catenin leads to the stabilization of endothelial junctions.

Symposium 10-4

# Metabolic Control of Endothelial Growth and Differentiation

**Michael Potente**[1*]

[1] Angiogenesis & Metabolism Laboratory, Max Planck Institute for Heart and Lung Research, Germany

*michael.potente@mpi-bn.mpg.de*

Angiogenesis – the growth of new blood vessels from pre-existing vasculature – is traditionally viewed from the perspective of how endothelial cells coordinate migration and proliferation in response to growth factor stimulation. However, endothelial cells must also coordinate their metabolism and adapt metabolic fluxes to the rising energy and biomass demands of sprouting vessels. Recent studies have highlighted the importance of such metabolic regulation in the endothelium and uncovered core metabolic pathways and mechanisms of regulation that drive the angiogenic process. In the presentation, current principles of endothelial metabolic regulation will be discussed. A particular focus will be given to the role of metabolites as signaling molecules and their role in controlling vascular growth and differentiation.

# Mechanical Loading of Intraluminal Pressure Regulates Angiogenesis in Wound Healing

**Shinya Yuge[1], Koichi Nishiyama[2], Yuichiro Arima[2], Sanshiro Hanada[2], Yasuyuki Hanada[2], Tomohiro Ishii[1], Yuki Wakayama[3], Kazuya Tsujita[4], Ryuji Yokokawa[5], Takashi Miura[6], Naoki Mochizuki[3], Shigetomo Fukuhara[1*]**

[1] Department of Molecular Pathophysiology, Institute of Advanced Medical Sciences, Nippon Medical School, Japan
[2] The International Research Center for Medical Sciences, Kumamoto University, Japan
[3] Department of Cell Biology, National Cerebral and Cardiovascular Center Research Institute, Japan
[4] Department of Biochemistry and Molecular Biology, Kobe University Graduate School of Medicine, Japan
[5] Graduate School of Engineering and Faculty of Engineering, Kyoto University, Japan
[6] School of Medicine and Graduate School of Medical Sciences, Kyushu University, Japan

*s-fukuhara@nms.ac.jp*

Angiogenesis is regulated not only by chemical factors but also by mechanical cues acting on endothelial cells (ECs). Here, we investigated the mechanisms underlying mechanical regulation of angiogenesis during wound healing by exploiting a fluorescence-based live-imaging system for adult zebrafish.

We introduced wounds onto the flank of adult zebrafish in which fluorescent proteins label ECs and analyzed wound angiogenesis.

Cutaneous wounding immediately induced angiogenesis not only by promoting vessel sprouting but also by inducing elongation of the injured vessels. Interestingly, elongation of the injured vessels was actively induced from the downstream side of blood flow, whereas the vessels located at the upstream side only marginally elongated. Preferential elongation of the injured downstream vessels was also observed when intersegmental vessels of zebrafish larvae were severed by laser ablation. By investigating the cause of differential elongation of injured vessels, we found that blood flow-driven intraluminal pressure inhibits elongation of the injured upstream vessels during wound angiogenesis. Furthermore, we investigated how intraluminal pressure inhibits elongation of the injured upstream vessels. In the injured downstream vessels, actin-related protein (Arp2/3) complex, an actin nucleator, was recruited to the leading edge of ECs and induced formation of actin-rich protrusion and establishment of front-rear polarity to promote vessel elongation. In contrast, intraluminal pressure induced expansion of the injured upstream vessels, which resulted in mechanical stretching of ECs. Intraluminal pressure-induced stretching of ECs in the injured upstream vessels prevented leading edge localization of Arp2/3 complex, thereby inhibiting actin polymerization and front-rear polarization to restrict vessel elongation. These results indicate that during wound angiogenesis, mechanical loading of intraluminal pressure prevents elongation of the injured upstream vessels through inhibition of actin polymerization and disruption of front-rear polarity.

In conclusion, we successfully uncover a novel regulatory mechanism of wound angiogenesis by intraluminal pressure load on the blood vessels.

Symposium 10-6 Short Talk

# The Role of C-Kit in Brown Adipose Tissue

**Hyuek-Jong Lee**[1*]

[1] Center for Vascular Research, Institute for Basic Science, Korea

*hyuekjong.lee@gmail.com*

Brown adipose tissue is a specialized tissue for thermogenesis. Unlike other tyrosine kinase receptors such as insulin receptor and IGF receptor, the function of c-Kit receptor in brown adipose tissue (BAT) has not been uncovered well. The purpose of this study is to identify the function of c-Kit receptor in brown adipose tissue.

To find out the expression pattern of c-Kit and its ligand SCF in major metabolic organs, we took an advantage of c-Kit-cre-ERt2 mouse mixed with tdTomato and SCF-GFP mouse. To activate UCP1, mice were housed in cold chamber or treated with CL316,242. UCP1 deactivation was induced denervation of brown adipose tissue. For the analysis of c-Kit function in brown adipocytes, single cell analysis was performed.

c-Kit receptor was expressed on pancreatic endocrine cells and endothelia cells of liver, skeletal muscle and adipose tissues. Interestingly, a part of brown adipocytes, not white adipocytes, expressed c-Kit receptor. SCF expression was limited on endothelial cell in pancreas and skeletal muscle and adipose tissues. In addition, macrophage in adipose tissues is a source of SCF. UCP1 activation with cold exposure or CL316,242 treatment significantly decreased the expression of c-Kit in brown adipocytes. However, UCP1 inhibition with denervation increased c-Kit expression in brown adipocytes. Results from single cell analysis with brown adipocytes showed that the expressions of fatty acid synthase and transketolase were significantly increased in c-Kit positive brown adipocytes.

The expression of c-Kit receptor on brown adipocytes has negative correlation with UCP1 expression. However, c-kit receptor expression was correlated with lipid accumulation in brown adipocytes.

# Vascular Control of Tumor Progression and Metastasis

**Hellmut G. Augustin**[1,2,3*]

[1] Vascular Biology, European Center for Angioscience, Medical Faculty Mannheim, Heidelberg University, Germany
[2] Vascular Oncology and Metastasis, German Cancer Research Center (DKFZ), Heidelberg, Germany
[3] German Cancer Consortium, DKTK, Heidelberg, Germany

*augustin@angiogenese.de*

The molecular analysis of tumor vessel interactions during tumor progression and metastasis has primarily focused on the study of tumor cell-derived angiogenic and lymphangiogenic signals with the purpose to exploit such factors as therapeutic targets. Angiogenic factors activate endothelial cells in nearby blood and lymphatic capillaries to sprout towards the tumor. Tumor angiogenesis thereby not only nourishes the growing tumor, but access to the blood and lymphatic vasculatures enables cells from the primary tumor to enter the circulation to eventually form metastases at distant sites. The complex cellular interactions between tumor cells and endothelial cells have in this context mostly been studied from a tumor cell-centric perspective, i.e., the tumor cells send signals to which endothelial cells merely respond. Yet, the past decade has witnessed a fundamental change of paradigm with the discovery that the vascular endothelium does not just respond to exogenous cytokines, but exerts active 'angiocrine' gatekeeper roles controlling their microenvironment in an instructive manner. We have applied the concepts of angiocrine signaling towards the study of tumor progression and metastasis. Employing novel surgical preclinical metastasis models that better mimic tumor progression and the response to therapy as it occurs in humans, we have molecularly dissected vascular and lymphatic endothelial cells in progressing primary tumors as well as in the pre-metastatic and metastatic niches. These experiments were on the one hand aimed at establishing the systems map of endothelial transcriptomic changes during tumor progression and metastasis and on the other hand to identify and validate novel therapeutic targets. This presentation will review recent advances in the field of tumor microenvironment research and present novel angiocrine signaling mechanisms as promising targets of future mechanism-driven anti-metastatic therapy.

Symposium 11-2

# Microenvironmental Regulation of Tumor Response and Resistance to Anti-Angiogenic Immunotherapy

**Michele De Palma**[1*]

[1] ISREC, EPFL, Switzerland

*michele.depalma@epfl.ch*

Immune checkpoint blockade (ICB) can bolster the tumor-antagonizing functions of T cells and be potentially curative in some cancer types. Among other parameters, the lack of pre-existing tumor-infiltrating T cells (TILs) may predict a poor response to ICB. Increasing data indicate that tumor-associated angiogenic blood vessels limit TIL numbers and functionality. Two key pro-angiogenic factors, VEGFA and angiopoietin-2 (ANGPT2), sustain abnormal tumor angiogenesis, promote the expansion of immunosuppressive myeloid cells, and limit T-cell trafficking in tumors. We recently showed that combined blockade of VEGFA and ANGPT2 reprograms the tumor blood vessels to a form that is conducive to improved T-cell activation and trafficking, thereby enhancing tumor response to PD-1 ICB in mouse models of cancer. In my lecture I will discuss the applications, prospects and current limitations of anti-angiogenic immunotherapies in mouse models of breast and lung cancer.

# Endothelial-to-Mesenchymal Transition Compromises Vascular Integrity to Induce Myc-Mediated Metabolic Reprogramming in Kidney Fibrosis

**Raghu Kalluri**[1*]

[1] Department of Cancer Biology, The University of Texas MD Anderson Cancer Center, USA

*RKalluri@mdanderson.org*

Endothelial-to-mesenchymal transition (EndMT) is a cellular transdifferentiation program in which endothelial cells partially lose their endothelial identity and acquire mesenchymal-like features. Renal capillary endothelial cells can undergo EndMT in association with persistent damage of the renal parenchyma. The functional consequence(s) of EndMT in kidney fibrosis remains unexplored. Here, we studied the effect of Twist or Snail deficiency in endothelial cells on EndMT in kidney fibrosis. Conditional deletion of Twist1 (which encodes Twist) or Snai1 (which encodes Snail) in VE-cadherin+ or Tie1+ endothelial cells inhibited the emergence of EndMT and improved kidney fibrosis in two different kidney injury/fibrosis mouse models. Suppression of EndMT limited peritubular vascular leakage, reduced tissue hypoxia, and preserved tubular epithelial health and function. Hypoxia, which was exacerbated by EndMT, resulted in increased Myc abundance in tubular epithelial cells, enhanced glycolysis, and suppression of fatty acid oxidation. Pharmacological suppression or epithelial-specific genetic ablation of Myc in tubular epithelial cells ameliorated fibrosis and restored renal parenchymal function and metabolic homeostasis. Together, these findings demonstrate a functional role for EndMT in the response to kidney capillary endothelial injury and highlight the contribution of endothelial-epithelial cross-talk in the development of kidney fibrosis with a potential for therapeutic intervention.

Symposium 11-4

# Ontogeny and Function of Intratumoral High-Endothelial Venules

**Yichao Hua[1], Gerlanda Vella[1], Florian Rambow[1], Susan Schlenner[2], Junbin Qian[1], Diether Lambrechts[1], Jean-Christophe Marine[1], Gabriele Bergers[1,3*]**

[1] Center for Cancer Biology, VIB-KU Leuven, Belgium
[2] . Adoptive Immunology, Department of Microbiology, Immunology and Transplantation, UZ Leuven, Belgium
[3] Department of Neurological Surgery, Brain Tumor Research Center, Helen Diller Family, Comprehensive Cancer Center, UCSF, USA

*gabriele.bergers@kuleuven.vib.be*

High endothelial venules (HEVs) are endothelial cells (ECs) that are specialized to facilitate the trafficking of lymphoid cells into lymphoid organs. HEVs can also spontaneously occur in non-lymphoid organs where they enable the generation of tertiary lymphoid-like structures (TLS) due to their ability to recruit lymphocytes but the mechanistic underpinnings of HEV induction remain obscure. Spontaneous HEV/TLS formation in human cancers as well as therapeutically induced HEVs in mouse models of cancer correlate with tumor reduction and prolonged survival. Thus, intratumoral HEVs (TU-HEV) may help to eradicate malignant lesions by increasing T-cell influx and activity at the tumor site. In order to elicit the ontogeny, functional properties and regulation mechanisms of intratumoral HEVs, we used various approaches of single cell transcriptional profiling and fate tracing of therapeutically induced TU-HEVs in tumors undergoing combinations of antiangiogenic immunotherapies. We found that TU-HEVs share features with normal lymph node (LN) HEVs but also displayed prominent transcriptomic heterogeneity compared to LN-HEVs and tumor ECs. Gene ontology (GO) analysis further revealed that TU-HEVs up-regulate immune-related processes while they downregulate angiogenesis-related processes compared to tumor ECs. Trajectory analysis and in vivo fate mapping revealed that specific subsets of endothelial cells transition into TU-HEVs which revert upon cessation of treatment. We further identified a feed-forward loop in which TU-HEVs generate an accumulation of lymphocytes while lymphocytes are also critical in maintaining TU-HEVs. Thus, immunotherapies can create hubs of active immune centers by activating a vascular inflammatory program.

# KAI1 (CD82) in Pericytes Suppresses Angiogenesis by Inducing Leukemia Inhibitory Factor (Lif) as Well as by Direct Binding and Quenching VEGF/PDGF

**Yoo-Wook Kwon**[1*]

[1] Biomedical Research Institute, Seoul National University Hospital, Korea

*ywkwon@snu.ac.kr*

Little is known about endogenous inhibitors of angiogenic growth factors. We identified a novel endogenous VEGF angiogenesis inhibitor expressed by vascular cells and clarified its underlying mechanism and clinical significance.

To identify whether KAI1/CD82 would be an endogenous inhibitor of angiogenesis, we used Kai1 knock-out mice. We observed significantly enhanced angiogenesis in Kai1 KO mice. In Kai1-emGFP knock-in mice, KAI1 was mainly expressed in pericytes (PCs) rather than in endothelial cells (ECs).

We used these animal to study the mechanism how KAI1/CD82 did anti-angiogenic activity in pericytes. localized at the membrane surface after palmitoylation by zDHHC4 enzyme, and induced leukemia inhibitory factor (LIF) through the Src/p53 pathway. LIF released from PCs in turn suppressed Sox17 and other angiogenic factors in ECs as well as VEGF in PCs itself, leading to inhibition of angiogenesis. Interestingly, KAI1 had another mechanism to inhibit angiogenesis: it directly bound to VEGF and PDGF (but not fibroblast growth factor) and inhibited activation of their receptors. In the two different in vivo cancer models, KAI1 supplementation significantly inhibited tumor angiogenesis and growth.

KAI1 from PCs is a novel molecular regulator that counterbalances the effect of VEGF and angiogenic factors.

Symposium 11-6 Short Talk

# Vascular Niche Signals via Ceruloplasmin-Iron Ion Metabolism Promote Glioma Resistance to Anticancer Drugs

**Fumitaka Muramatsu[1], Hiroyasu Kidoya[2], Yumiko Hayashi[2], Yohei Tsukada[2], Nobuyuki Takakura[1,2*]**

[1] Signal transduction, IFRec, Osaka University, Japan
[2] Signal transduction, Research Institute for Microbial Diseases, Osaka University, Japan

*ntakaku@biken.osaka-u.ac.jp*

Drug-resistant cancer stem cells are distributed near blood vessels in general, known as vascular niche, but the details are not well understood. Since DNA alkylating agent, Temozolomide, is the only effective anticancer drug for glioma, it is important to suppress the emergence of resistant cells. In this study, we aimed to clarify novel microenvironment signals supporting drug-resistant cells through in vivo imaging analysis of the interaction between glioma cells and blood vessels.

We made GL261 glioma orthotopic transplantation model in vascular imaging mice and analyzed the distribution of temozolomide resistant glioma cells by using two-photon in vivo imaging technique. Thereafter, we performed RNA-Seq analysis to search for the signal molecules involved in temozolomide resistance induction. Under the co-culture system of glioma and endothelial cells, the target genes were examined by siRNA knockdown test using the expression of O-6-methylguanine-DNA methyltransferase (Mgmt) in glioma as an index.

After temozolomide treatment, the distribution and division points of glioma cells became closer to the blood vessels, increasing from 40% to 70% of the total. We confirmed increased Mgmt gene expression by qRT-PCR in such drug resistant glioma. Suppressing the expression of Ceruloplasmin in endothelial cells or additional iron ion chelators increased glioma cells sensitivity to temozolomide in vitro co-culture system. Osteopontin from GL261 cells increased the expression of ceruloplasmin in endothelial cells via PI3K signal pathway. Finally, it was confirmed that the extension of the overall survival of orthotopic transplant mouse models with the combination of iron ion chelators and temozolomide.

In vivo imaging analysis revealed that the vascular niche assisted in acquiring resistance to chemotherapy. Glioma exposed to temozolomide increased osteopontin production and induced ceruloplasmin expression in endothelial cells. Ceruloplasmin was found to be essential for Mgmt expression in gliomas by regulating iron ion metabolism in the tumor microenvironment.

# GPCRs in Fluid Sheer Stress Mechanotransduction

**Martin A Schwartz**[1*]

[1] Internal Medicine, Yale University / Yale School of Medicine / Yale Cardiovascular Research Center (YCVRC), USA

*martin.schwartz@yale.edu*

Fluid shear stress, the frictional force from flowing blood, is a critical determinant of morphogenesis and physiology in the vascular system. It is also a critical determinant of atherosclerosis, which arises selectively in regions of low and disturbed flow. We previously identified a complex consisting of PECAM-1, VE-cadherin and VEGFR2 that resides at cell-cell junctions and transduces forces from fluid flow into biochemical signals. We have also found that the matrix beneath the endothelial cells is a major modulator of the signals from flow, determining whether flow activates inflammatory vs. anti-inflammatory pathways and subsequent vascular remodeling or development of atherosclerotic plaque. I will present our latest work on elucidation of mechanisms by which endothelial cells sense fluid shear stress and their role in vascular remodeling and atherosclerosis.

Symposium 12-2

# Regulation of Endothelial Phenotype by Different Flow Patterns

**Stefan Offermanns**[1,2*]

[1] Pharmacology, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
[2] Center for Molecular Medicine, Goethe University Frankfurt, Germany

*stefan.offermanns@mpi-bn.mpg.de*

Vascular endothelial cells can acquire different phenotypes. One major determinant of the endothelial phenotype is the pattern of flow the endothelial cell is exposed to. In most areas of the arterial system, blood flows at high laminar flow, which promotes an anti-atherogenic endothelial phenotype. In contrast, in areas of disturbed or low flow, endothelial cells show signs of inflammation, and these areas of the arterial system are prone to atherosclerosis development. In the past, we have identified upstream signaling pathways activated by different flow patterns, which include the mechanosensitive cation channel Piezo1, the purinergic P2Y2 and the adrenomedullin receptor as well as their Gq/G11- and Gs-mediated downstream signaling pathways. While laminar flow induces through these pathways phosphorylation and activation of eNOS, disturbed flow rather inhibits eNOS activation and induces integrin-dependent activation of NF-κB. I will summarize our most recent findings and present data on new mechanisms mediating flow-induced eNOS activation and flow-dependent regulation of endothelial-to-mesenchymal transition.

Symposium 12-3

# Disturbed Flow Reprograms Endothelial Cells by Altering Transcriptomic and Epigenomic Landscapes in Mouse Carotid Artery in Vivo as Revealed by Single-Cell RNAseq and ATACseq Studies

**Hanjoong Jo**[1*]

[1] Biomedical Engineering, Emory University and Georgia Institute of Technology, USA

*hjo@emory.edu*

Atherosclerosis occurs preferentially in arterial regions of disturbed blood flow (d-flow) while the regions exposed to stable blood flow (s-flow) remain protected. To investigate the effect of d-flow on transcriptome expression and chromatin accessibility and organization in endothelium at a single-cell (sc) resolution, we used the partial carotid ligation model and performed a sc-RNA sequencing and sc-Assay for Transposase-Accessible Chromatin using Sequencing (ATAC)-sequencing. Two days or two weeks after the partial carotid ligation surgery of C57BL6 mice to induce d-flow in the left carotid (LCA) and s-flow in the right carotid (RCA), mouse carotids were dissected out and the lumens were digested with collagenase to obtain the luminal single cells and nuclei using the 10X system, and sc-RNAseq and sc-ATACseq were performed. Analyses of the sc-RNAseq and sc-ATACseq results showed that the luminal single cell populations were comprised mostly of endothelial cells (ECs) but also of smooth muscle cells, fibroblasts, and leukocytes. While the RCAs at 2 days and 2 weeks contained three "normal or healthy" EC populations, LCAs at 2 days contained increased number of immune cells, smooth muscle cells, fibroblasts, which further increased in LCAs at 2 week time points. This demonstrated that D-flow in LCAs induced a dramatic transition of EC clusters from "healthy" types to "activated or unhealthy" types in a time-dependent manner compared to the RCAs. Further, in depth analyses of these endothelial sub-clusters for transcript expression and chromatin accessibility changes illuminate their important role in flow-sensitive function in the pathophysiology of atherosclerosis.

Symposium 12-4

# Regulation of Angiogenic Sprouting by the Extracellular Matrix

**Britta Trappmann**[1*]

[1] Bioactive Materials Laboratory, Max Planck Institute for Molecular Biomedicine, Germany

*britta.trappmann@mpi-muenster.mpg.de*

A major challenge in the field of tissue engineering is the generation of new materials that can support angiogenesis, wherein endothelial cells from existing vasculature invade the surrounding matrix to form new blood vessels. This is largely due to a gap in our understanding of how extracellular matrix (ECM) properties affect angiogenic sprouting and ultimately, blood vessel formation. Due to the complex nature of native ECMs, it is difficult to identify the role of individual matrix properties. Here, we have developed synthetic hydrogels with independently tunable properties, which are integrated into a microfluidic platform that mimics the process of angiogenesis in vitro. In this talk, I will present our efforts to understand how matrix adhesiveness, as well as mechanical and degradative properties jointly regulate angiogenic sprouting, and importantly, vascular lumen formation.

# PAR-1 Is a Novel Mechano-Sensor Transducing Laminar Flow-Mediated Endothelial Signaling

**Chang-Hoon Woo[1*], Suji Kim[1], Jae Hyang Lim[2]**

[1] Pharmacology, Yeungnam University College of Medicine, Korea
[2] Microbiology, Ewha Womans University College of Medicine, Korea

*changhoon_woo@yu.ac.kr*

Recent studies have indicated that protease-activated receptor-1 (PAR-1) is involved in cytoprotective and anti-inflammatory responses in endothelial cells (ECs). However, the role of PAR-1 in laminar flow-mediated atheroprotective responses remains unknown. Herein, we investigated whether PAR-1 regulates laminar flow-mediated mechanotransduction in ECs.

To determine the cellular localization of PAR-1 in reponse to laminar flow, confocal and flow cytometry analysis were performed. In addition, the role of PAR-1 in laminar flow-induced mechanotransduction was addressed by PAR-1 knockdown with siRNA against PAR-1. At last, the physiological role of PAR-1 was addressed by genetic PAR-1 deficiency in a mouse model system by measuring vasomotor modulation and blood pressure.

Confocal analysis showed that PAR-1 was internalized into early endosomes in response to laminar flow. In addition, flow cytometry analysis showed that cell surface expression of PAR-1 was reduced by laminar flow, suggesting that PAR-1 was activated in response to laminar flow. Depletion of PAR-1 using human PAR-1 siRNA inhibited unidirectional laminar flow-mediated actin stress fiber formation and cellular alignment as well as atheroprotective gene expressions in HUVECs. Moreover, PAR-1 knockdown inhibited laminar flow-stimulated eNOS phosphorylation, and inhibited the phosphorylations of Src, AMPK, ERK5 and HDAC5. Furthermore, PAR-1 depletion inhibited laminar flow-mediated anti-inflammatory responses as demonstrated by reduced TNFα-induced VCAM-1 expression and by monocyte adhesion to HUVECs, and prevented laminar flow-mediated anti-apoptotic response. An investigation of the role of PAR-1 in vasomotor modulation using mouse aortic rings revealed that acetylcholine-induced vasorelaxation was diminished in PAR-1 deficient mice compared to littermate controls.

Taken together, these findings suggest that PAR-1 be viewed as a novel pharmacologic target for the treatment of vascular diseases, including atherosclerosis.

Symposium 12-6 Short Talk

# Impaired SMAD 1/5 Mechanotransduction and Inflammation Converge on Connexin37(Cx37) Enabling Arteriovenous Malformations

**Hanna Peacock[1], Ashkan Tabibian[1], Nathan Criem[1], Vincenza Caolo[2], Lauriane Hamard[3], Astrid Deryckere[4], Jacques-Antoine Haefliger[3], Brenda Kwak[5], An Zwijsen[1], Elizabeth Jones[1*]**

[1] Cardiovascular Sciences, KU Leuven, Belgium
[2] Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK
3 Department of Medicine, University of Lausanne, Switzerland
4 Department of Developmental Neurobiology, KU Leuven, Belgium
[5] Department of Pathology and Immunology, University of Geneva, Switzerland

*liz.jones@kuleuven.be*

Impaired ALK1/Endoglin/BMP9 signaling predisposes to arteriovenous malformations (AVMs). Activation of SMAD1/5 signaling can be enhanced by shear stress. In the genetic disease Hereditary Hemorrhagic Telangiectasia, which is characterized by AVMs, the affected receptors are those involved in the activation of mechanosensitive SMAD1/5 signaling. To elucidate how genetic and mechanical signals interact in AVM formation, we sought to identify targets differentially regulated by BMP9 and shear stress.

We use a combination of endothelial cells in culture exposed to shear stress and/or ligand to analyse gene expression regulation. We use embryo culture with exogeneous factors as an in vivo remodelling model.

We identify Connexin37 (Cx37) as a differentially regulated target of ligand-induced and mechanotransduced SMAD1/5 signaling. We show that stimulation of endothelial cells with BMP9 upregulated Cx37 whereas shear stress inhibited this expression. This signaling was SMAD1/5-dependent and in the absence of SMAD1/5, there was an inversion of the expression pattern. Ablated SMAD1/5 signaling alone caused AVM-like vascular malformations directly connecting the dorsal aorta to the inlet of the heart. In yolk sacs of mouse embryos with an endothelial-specific compound heterozygosity for SMAD1/5, addition of TNF〈, which downregulates Cx37, induced development of these direct connections bypassing the yolk sac capillary bed. In wildtype embryos undergoing vascular remodeling, Cx37 was globally expressed by endothelial cells but was absent in regions of enlarging vessels. TNF〈 and endothelial-specific compound heterozygosity for SMAD1/5 caused ectopic regions lacking Cx37 expression, which correlated to areas of vascular malformations. Mechanistically, loss of Cx37 impairs correct directional migration under flow conditions.

Our data demonstrate that Cx37 expression is differentially regulated by shear stress and SMAD1/5 signalling and that reduced Cx37 expression is permissive for capillary enlargement into shunts.

# The RNA Helicase Ddx21 Controls Developmental Lymphangiogenesis by Balancing Endothelial Cell Ribosome Biogenesis and p53-p22 Signalling

**Benjamin M. Hogan**[1*]

[1] PeterMac Cancer Centre, The University of Melbourne, Australia

*Ben.Hogan@petermac.org*

The correct assignment of cell fate within fields of multipotent progenitors is essential for accurate tissue diversification. The first lymphatic vessels arise from pre-existing veins after venous endothelial cells transdifferentiate into lymphatic endothelial cells. This event ultimately generates an entire, complex, second vascular network in the developing embryo and is thought to reinitiate in pathogenesis in settings that include inflammation and cancer metastasis. The transcription factor Prox1 is considered the master regulator of this key transdifferentiation event, yet remarkably the mechanisms by which Prox1 controls lymphatic fate are still unknown . We have used large scale genetic screens, live-imaging of development and single cell transcriptomics in zebrafish embryos to build an unexpected picture of the venous-lymphatic fate transition and the role of Prox1.

# Organotypic Specialization of Lymphatic Vessels

**Esther Hoppe[1], Nils Kirschnick[1], Friedemann Kiefer[1*]**

[1] Intravital Molecular Imaging, University of Münster / European Institute for Molecular Imaging, Germany

*fkiefer@uni-muenster.de*

Functional specialization of organs is reflected in the specialization of their vessel beds. Lymphatic vessels fulfil essential roles in the uptake and transport of interstitial fluid, leukocytes and dietary lipids. Initial lymphatics also referred to as lymphatic capillaries are formed by oak leave shaped endothelial cells and are equipped with specialized button-type junctions, which makes them optimally suited for uptake tasks. Lymphatic collecting vessels are comprised of spindle shape cells, which are connected by continuous zipper-type junctions and stabilized by mural cells. Valves that form in collectors in regular distances and ensure the uni-directionality of lymph flow complete their optimal adaptation for fluid transport.

We will report how in different organs, depending on their physiological function, these specialized lymphatic vessel types can either occur stratified in zones, fulfil their function side by side or even be intermingled. Data on the growth factors and their sources determining the functional specialization and spatial distribution of the specialized lymphatic vessels will be presented and molecular mechanisms active during their morphogenesis will be discussed.

# Endothelial Lipid Metabolism

**William Sessa**[1*]

[1] Vascular Biology and Therapeutics Program, Yale School of Medicine, USA

*william.sessa@yale.edu*

Endothelial cells lining all blood vessels are continuously bombarded with circulating lipids including lipoprotein derived triglycerides, cholesterol and free fatty acids. Little is known as to how endothelial cells process these diverse lipids under homeostatic conditions or the mechanism that promote lipid uptake and atherosclerosis. New insights will be presented examining the role of the LDL receptor and additional binding proteins, ALK1 and SCARB1, in LDL uptake, cholesterol distribution and LDL transcytosis under physiological and pathophysiological conditions.

[EVBO Award Lecture]

# Inside Out: PI3Ks Taking Control of Vessel Growth

**Mariona Graupera**[1*]

[1] Vascular Biology and Signalling Group, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, Spain

*mgraupera@idibell.cat*

The PI3K pathway is fundamental for endothelial cell function during sprouting angiogenesis, the most common type of blood vessel formation. Research in animal models has revealed key functions of PI3K family members and downstream effectors in angiogenesis. In addition, perturbations in PI3K signalling have been associated with aberrant vascular growth including tumour angiogenesis and vascular malformations. Together this highlights that endothelial cells are uniquely sensitive to fluctuations in PI3K signalling. We have now identified that PI3K also regulates pericyte biology during angiogenesis, albeit a different isoform, PI3K®, takes the control. I will discuss new results from our lab on the functions of PI3K hub in physiological and pathological blood vessel growth and the importance of being selective during this process.

# Axon Guidance Molecules Contribute to the Cerebrovascular Recovery Following Ischemic Damage

**Won-Jong Oh**[1*]

[1] Neurovascular Unit Research Group, Korea Brain Research Institute, Korea

*ohwj@kbri.re.kr*

Most major axon guidance molecules have been shown to play a role in guiding developing vascular network formation. Recent studies revealed that the guidance molecules are also involved in the vascular recovery in the damaged tissues beneficially or detrimentally. Given the distinct expression and function of diverse guidance cues, the underlying molecular mechanism of vascular recovery could be complicated and remains still unclear. In this study, we uncovered that the activation of Semaphorin 3E (Sema3E) and its receptor Plexin-D1 pair which is one of the traditional axon guidance cues are necessary for the functional cerebrovascular reconstruction after ischemic insult. We found that ischemic damage rapidly induces Sema3E expression in the penumbral neurons followed by a reappearance of Plexin-D1 in the ischemic vessels. Ablation of Sema3E or Plexin-D1 inhibited vascular recovery and worsened ischemic brain damage including neuronal loss and blood-brain barrier (BBB) disruption. Moreover, the lack of Plexin-D1 activated abnormal VEGF signaling, leading to vascular malformation and increased BBB permeability. These results demonstrate that Sema3E-Plexin-D1 signaling ameliorates ischemic damage and helps recovery in the adult brain as a beneficial mediator of vasculature formation.

Symposium 13-6 Short Talk

# aPKC Is a Key Regulator of Endothelial Proliferation by Modulating C-Myc via FoxO1 DNA Binding Ability

**Masanori Nakayama**[1*]

[1] Laboratory for Cell Polarity and Organogenesis, Max Planck Institute for Heart and Lung Research, Germany

*masanori.nakayama@mpi-bn.mpg.de*

Cell proliferation is tightly controlled during development and in tissue homeostasis, while unrestrained cell division is a hallmark of cancer. ECs expand rapidly in a tightly coordinated manner to form new vessels. Conversely, pathological EC proliferation occurs in multiple forms of vascular sarcoma, including angiosarcoma, a rare but malignant vascular neoplasm. However, molecular mechanisms controlling angiosarcoma cell remain elusive.

To identify the key signaling pathway controlling physiological and pathological endothelial cell proliferation, we analyzed aPKC KO mice and angiosarcoma patient derived samples.

We have found that aPKC directly phosphorylates FoxO1 at Ser218 within the DNA binding domain to suppress FoxO1 DNA binding ability . FoxO1 has DNA binding dependent and independent target genes. Although FoxO transcription factors are thought as typical tumor suppressors, they are often found to show strong nuclear localization in malignant cancer. In angiosarcoma, we have found that c-Myc is highly expressed even though FoxO transcription factors show strong nuclear localization. This is due to the high levels of aPKC, resulting in enhanced FoxO1 Ser218 phosphorylation. As a consequence, highly expressed c-Myc leads to unrestrained proliferation. Conversely, aPKC inhibition reduces c-Myc expression and proliferation of angiosarcoma cells. Moreover, aPKC-dependent FoxO1 phosphorylation correlates with patient prognosis.

Our findings may provide a potential therapeutic strategy for treatment of malignant cancers, like angiosarcoma.

# Endotheliopathy in COVID19: Yale Experience

**Hyung Joon Chun**[1*]

[1] Yale Cardiovascular Research Center, Internal Medicine, Yale University, USA

*hyung.chun@yale.edu*

An important feature of severe acute respiratory syndrome coronavirus 2 pathogenesis is COVID-19-associated coagulopathy, characterised by increased thrombotic and microvascular complications. Previous studies have suggested a role for endothelial cell injury in COVID-19-associated coagulopathy. To determine whether endotheliopathy is involved in COVID-19-associated coagulopathy pathogenesis, we assessed markers of endothelial cell and platelet activation in critically and non-critically ill patients admitted to the hospital with COVID-19. We show that endotheliopathy is widespread among hospitalised patients with COVID-19 and is more extensive in critically ill patients than in non-critically ill patients. We describe, for the first time, that concentrations of multiple endothelial factors, including soluble thrombomodulin, angiopoietin 2, and others, might predict mortality and other clinical outcomes in patients with COVID-19. Our findings identify a potential prognostic role for measurement of endothelial markers in patients with COVID-19 and suggest a need for future investigations of therapeutic strategies aimed at preserving endothelial function in COVID-19 and other related infectious processes.

Symposium 14-2

# Cell-to-Cell Communication Regulating Angiogenesis and Lymphangiogenesis

**Yoshiaki Kubota**[1*]

[1] Department of Anatomy, Keio University School of Medicine, Japan

*ykubo33@a3.keio.jp*

The two major circulatory systems of blood and lymphatic vasculature are discretely distributed throughout the body. While the former provides tissues with oxygen and nutrients, the latter drains the interstitial fluid from the tissue spaces to return it to the bloodstream. In this developmental process, diverse interactions between endothelial cells and other cell types contribute to the establishment of such tissue-specific vascular patternings. Our research is mainly focusing on vascular and lymphatic development in retina, bone, and skin. In this presentation I would like to discuss our latest findings regarding the cellular and molecular basis of cell-to-cell communication regulating angiogenesis and lymphangiogenesis.

# Single Cell Transcriptomic Characterization of Smooth Muscle Cell Transitions

**Thomas Quertermous**[1*]

[1] Medicine, Stanford University, USA

*tomq1@stanford.edu*

In the setting of vascular disease, smooth muscle cells (SMC) can de-differentiate, proliferate and migrate in a process known as phenotypic transition. However, the phenotype of modulated SMC in vivo during atherosclerosis and the influence of this process on coronary artery disease (CAD) risk are not well established. Using single cell RNA sequencing (scRNAseq), we comprehensively characterized the transcriptomic phenotype of transition SMC in vivo in both mouse and human arteries. We found that these cells transform into unique fibroblast-like cells that we term "fibromyocytes" (FMC) and not into a macrophage phenotype. SMC-specific knockout of Tcf21, a causal CAD gene, markedly inhibited SMC phenotypic modulation in mice, leading to fewer FMC within the lesion and the protective fibrous cap. In addition, we have shown with scRNAseq that these FMC can undergo a further transition, adopting a phenotype that is similar to chondrogenic cells that mediate endochondral bone formation, creating cells we have termed "chondromyocytes." This transition was highly enhanced in a mouse atheroscerlosis model with deletion of the aryl hydrocarbon receptor (Ahr) gene, which is involved in gene by environment interactions. Employing information from the mouse knockout models, and directionality afforded by the human association data, a tentative disease relationship of these two SMC transitions is that FMC are protective while CMC are detrimental toward CAD risk.

Symposium 14-4

# Endothelial microRNAs and Pulmonary Arterial Hypertension

**Beata Wojciak-Stothard**[1*]

[1] NHLI, Imperial College London, UK

*b.wojciak-stothard@imperial.ac.uk*

Pulmonary arterial hypertension (PAH) is a severe disorder of lung vasculature that causes right heart failure. Endothelial damage followed by proliferation of vascular endothelial and smooth muscle cells underlie the disease pathology and hypoxia and inflammation are known contributory factors. Homoeostatic effects of flow-activated transcription factor Krüppel-like factor 2 (KLF2) are compromised in PAH. Recently, microRNAs have emerged as important regulators of endothelial function by fine-tuning gene expression. We show that KLF2-induced exosomal microRNAs can act together to attenuate pulmonary vascular remodelling and restore vascular homoeostasis in PAH. An understanding of the role of flow-dependent microRNAs in endothelial activation and dysfunction may provide novel therapeutic opportunities for controlling pulmonary hypertension and other diseases associated with endothelial damage, inflammation, proliferation and propensity for vascular cell enlargement.

# The Renin-Angiotensin-Aldosterone System (RAAS) Is One of the Effectors by Which Vascular Endothelial Growth Factor (VEGF)/ Anti-VEGF Controls the Endothelial Cell Barrier

**Andrius Kazlauskas[1,2*], Yueru Li[1], Zhonghao Yan[1], Komal Chaudhry[2]**

[1] Ophthalmology/Physiology, University of Illinois at Chicago, USA
[2] Department of Physiology and Biophysics, University of Illinois at Chicago, USA
[3] School of Medicine, Southern Illinois University, USA

*ak20@uic.edu*

Leakage of retinal blood vessels, which is an essential element of numerous blinding conditions including diabetic retinopathy (DR), is driven by chronic elevation of vascular endothelial growth factor (VEGF). VEGF quickly (within minutes) relaxes the endothelial cell barrier by triggering signaling events that post-translationally modify preexisting components of intercellular junctions.

VEGF also changes expression of genes, some of which are known to regulate endothelial cell barrier function. The purpose of this project was to identify effectors by which VEGF and anti-VEGF control the endothelial cell barrier in cells that were chronically exposed to VEGF (hours instead of minutes).

Using in vitro models of diabetes-induced endothelial dysfunction, we discovered that the duration of exposure to VEGF influenced both barrier relaxation and anti-VEGF-mediated closure. Furthermore, the vast majority of VEGF-induced changes in gene expression were not reversed by anti-VEGF. Those VEGF-regulated genes that were also sensitive to anti-VEGF constitute VEGF effectors that are targets of anti-VEGF. By pursuing such candidates, we learned that VEGF used multiple, non-redundant effectors to relax the barrier in cells that were exposed to VEGF for a prolonged period.

Furthermore, one of these effectors was ACE (angiotensin converting enzyme), which is a member of the renin-angiotensin aldosterone system (RAAS). Pharmacologically antagonizing either ACE, or the receptor for angiotensin II attenuated VEGF-mediated relaxation of the barrier. Furthermore, activating the RAAS reduced the efficacy of anti-VEGF. These discoveries provide a plausible mechanistic explanation for the long-standing appreciation that RAAS inhibitors are beneficial for patients with DR and suggest that antagonizing the RAAS improves patients' responsiveness to anti-VEGF.

Symposium 14-6 Short Talk

# Fursultiamine Alleviates Choroidal Neovascularization by Suppressing Inflammation and Metabolic Reprogramming

**Dong Ho Park[1,2*], Ji Yeon Do[2], Juhee Kim[2], Mi-Jin Kim[2], Ryoji Yanai[3], In-kyu Lee[2,4], Sungmi Park[2]**

[1] Department of Ophthalmology, Kyungpook National University, Korea
[2] Leading-edge Research Center for Drug Discovery and Development for Diabetes and Metabolic Disease, Kyungpook National University Hospital, Korea
[3] Department of Ophthalmology, Yamaguchi University Graduate School of Medicine, Japan
[4] Department of Internal Medicine, Kyungpook National University, Korea

*sarasate2222@gmail.com*

To assess the anti-angiogenic effects of fursultiamine in choroidal neovascularization (CNV) by its modulation of inflammation and metabolic reprogramming in the retinal pigment epithelium (RPE)

The anti-angiogenic effects of fursultiamine were assessed by measuring vascular leakage and CNV lesion size in the murine laser-induced CNV model. Inflammatory responses were evaluated by qPCR, Western blot, and ELISA in both CNV mouse tissues and ARPE-19 cells subjected to LPS treatment or hypoxia. Mitochondrial respiration was assessed by measuring oxygen consumption in ARPE-19 cells treated with LPS ± fursultiamine, and lactate production was measured in ARPE-19 cells subjected to hypoxia ± fursultiamine.

In laser-induced CNV, fursultiamine significantly decreased vascular leakage and lesion size, together with choroidal and retinal inflammatory cytokines, including Interleukin (Il)-1β, Il-6, Il-8, and Tumor necrosis factor-alpha (Tnf-α). Furthermore, fursultiamine decreased proinflammatory cytokine secretion and Nuclear factor kappa-B (NF-κB) phosphorylation in LPS-treated ARPE-19 cells. Interestingly, fursultiamine significantly enhanced mitochondrial respiration in the LPS-treated ARPE-19 cells. Additionally, fursultiamine attenuated hypoxia-induced aberrations, including lactate production, inhibitory phosphorylation of pyruvate dehydrogenase, increased VEGF production, and HIF-1α activation.

Our findings show that fursultiamine is a viable putative therapeutic for neovascular AMD by modulating the inflammatory response and metabolic reprogramming by enhancing mitochondrial respiration in the RPE.

# Interactions Between Endothelial and Intimal Myeloid Cells in Homeostasis and the Initiation of Atherosclerosis

**Myron I. Cybulsky**[1,2*]

[1] Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada
[2] Toronto General Hospital Research Institute, University Health Network, Canada

*myron.cybulsky@utoronto.ca*

Myeloid cells reside in the artery intima at regions with disturbed blood flow that are predisposed to atherosclerosis, and throughout the entire adventitia. During homeostasis, the abundance of intimal myeloid cells is tightly regulated and these cells protect the intima from infection by intracellular pathogens. In the setting of hypercholesterolemia, intimal myeloid cells accumulate lipid to become the initial foam cells of nascent lesions, and over time the number of intimal myeloid cells gradually increases as a result of local proliferation and monocyte recruitment. We investigated whether colony stimulating factor 1 (CSF1) regulates the abundance of arterial myeloid cells. In osteopetrotic mice, which are deficient in CSF1, and in wild type C57BL/6 mice infused with a function blocking antibody to CSF1 via an osmotic pump, the abundance of both intimal and adventitial myeloid cells was reduced dramatically. To investigate which cells in the artery wall produce CSF1 that is required for maintenance of aortic myeloid cells, Csf1-floxed mice were bred to Ubc-CreER or Cad5-CreER mice to generate mice with inducible global or endothelial–specific deficiency of CSF1, respectively. Inducible global deficiency of CSF1 reduced both intimal and adventitial myeloid cells. In contrast, inducible endothelial deficiency of CSF1 reduced only intimal, but not adventitial, myeloid cells. Collectively these data indicate that distinct cellular sources of CSF1 regulate myeloid cell populations in the artery wall and that endothelial cells are critical for maintaining intimal the intimal myeloid cells in regions predisposed to atherosclerosis.

Symposium 15-2

# Systems Approach to Target Discovery for Vascular Disease: A Focus on Macrophage Activation

**Masanori Aikawa**[1*]

[1] Vascular Biology, Harvard Medical School, USA

*maikawa@bwh.harvard.edu*

Pro-inflammatory activation of macrophages contributes to the pathogenesis of atherosclerosis; however, its underlying mechanisms are incompletely understood. Despite the availability of potent drugs for modifiable risks, some of which can also suppress macrophage activation, complications of atherosclerosis remain global health threats. To challenge residual risk, my group explores novel mechanisms for macrophage activation as an important first stride towards the development of new therapies. While many potential targets have been proposed by others and us, the science community recognizes that complex mechanisms of human diseases involve intertwined crosstalk among many molecules and pathways, and understanding individual and collective contributions of components to macrophage activation is critical. Seemingly promising candidates that are established by a conventional reductionist approach may thus ultimately fail. We indeed face low success rates of the clinical development of new drugs after lengthy preclinical efforts for identifying and validating new targets. To facilitate the discovery process and improve success rates, we use a systems approach, involving multi-omics, our original bioinformatics programs, computational prediction by network analysis, and artificial intelligence. My keynote address will showcase novel targets for macrophage activation we have identified through such a strategy.

# Dietary Protein and Amino Acid-Mediated mTOR Signaling in Atherosclerosis

**Babak Razani**[1*]

[1] Medicine, Cardiology, Washington University School of Medicine, USA

*brazani@wustl.edu*

High protein diets which are commonly utilized for weight loss have been reported to raise cardiovascular risk. However, the mechanisms underlying this risk are unknown. First using mouse models, we show that high protein diets increase atherosclerotic lesion area with a particular rise in plaque complexity. In both in vitro and in vivo experiments, we demonstrate that mice ingesting diets high in protein develop an acute rise in circulating amino acids, which in turn distribute to the atherosclerotic plaque leading to activation of mTORC1 signaling. A prominent consequence of this mTOR response is suppression of autophagy/mitophagy, accumulation of dysfunctional mitochondria, and resultant macrophage apoptosis, reactive oxygen species (ROS), and inflammasome activation

In order translate these findings, we recapitulated several of these observations in cultured human monocyte derived macrophages (HMDMs). Akin to murine macrophages, amino acids significantly elevate mTORC1 activation in HMDMs, acutely suppress mitophagy, and trigger sequelae of mitochondrial dysfunction including increases in ROS, inflammatory signaling, and apoptosis. In ongoing work, we have now also demonstrated rapid stimulation of mTORC1 signaling in circulating monocytes derived from subjects ingesting a high protein meal.

Our data demonstrate that the relationship between dietary protein and amino acid-mediated mTORC1 activation is robust in both mouse models and humans. Furthermore, we provide the first mechanistic details of the deleterious effects of high protein diets on macrophages and atherosclerotic progression. Incorporation of these concepts in future human clinical trials will be an important step in mitigating the effects of dietary protein on cardiovascular disease.

Symposium 15-4

# Atheroprotective Roles of Myeloid Cells in the Pathogenesis of Atherosclerosis

**Goo Taeg Oh**[1*]

[1] Department of Life Sciences, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

Atherosclerosis is a chronic inflammatory disease that intense immunological pathways play an essential role. During the progression of atherosclerosis, large numbers of inflammatory and immune cells accumulate in intima. The accumulated immune cells, including T cells, macrophages, and dendritic cells (DCs), cross-talk each other, and affect the development of atherosclerosis. Importantly, we found DCs that were poorly phagocytic but were immune stimulatory in the steady state mouse aorta. By crossing Flt3-/- to Ldlr-/- mice, deficiency of classical CD103+ aortic DCs exacerbated atherosclerosis and fewer Foxp3+ Treg cells. These data indicate that functional DCs are dominant in normal aortic intima, and CD103+ classical DCs are associated with atherosclerosis protection. Also, we identified functional mouse and human pDCs in the aortic intima and showed that selective, inducible pDC depletion in mice exacerbates atherosclerosis. The function of CD137, a member of the tumor necrosis factor receptor superfamily, in mediating atherosclerosis plaque stability remains unknown. We found that activation of CD137 signaling decreases the stability of plaques via its combined effects on T cells, vascular smooth muscle cells, and macrophages. Recently, we show in vivo evidence that Ninjurin-1 (Nerve injury-induced protein, Ninj1) is directly cleaved by MMP9 and concomitantly its soluble form (sNinj1), which exhibits anti-atherosclerotic effects with MMP9 in mouse and human atherosclerosis.

# LncRNA-Mediated Control of Vascular Senescence and Atherosclerosis

**Mark Feinberg**[1*]

[1] Cardiology, Brigham and Women's Hospital / Harvard Medical School, USA

*mfeinberg@bwh.harvard.edu*

Long noncoding RNAs (lncRNAs) are emerging regulators of biological processes in the vessel wall; however, their role in atherosclerosis remains poorly defined.

We used RNA sequencing to profile lncRNAs derived specifically from the aortic intima of Ldlr–/– mice on a high-cholesterol diet during lesion progression and regression phases

We found that the evolutionarily conserved lncRNA small nucleolar host gene-12 (SNHG12) is highly expressed in the vascular endothelium and decreases during lesion progression. SNHG12 knockdown accelerated atherosclerotic lesion formation by 2.4-fold in Ldlr–/– mice by increased DNA damage and senescence in the vascular endothelium, independent of effects on lipid profile or vessel wall inflammation. Conversely, intravenous delivery of SNHG12 protected the tunica intima from DNA damage and atherosclerosis. LncRNA pull-down in combination with liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis showed that SNHG12 interacted with DNA-dependent protein kinase (DNA-PK), an important regulator of the DNA damage response. The absence of SNHG12 reduced the DNA-PK interaction with its binding partners Ku70 and Ku80, abrogating DNA damage repair. Moreover, the anti-DNA damage agent nicotinamide riboside (NR), a clinical-grade small-molecule activator of NAD+, fully rescued the increases in lesional DNA damage, senescence, and atherosclerosis mediated by SNHG12 knockdown. SNHG12 expression was also reduced in pig and human atherosclerotic specimens and correlated inversely with DNA damage and senescent markers.

These findings reveal a role for this lncRNA in regulating DNA damage repair in the vessel wall and may have implications for chronic vascular disease states and aging.

Symposium 15-6 Short Talk

# Exploring Human Atherosclerosis With Single-Cell Resolution

**Ljubica Matic[1*], Robert Wirka[2], Sampath Narayanan[1], Anton Gisterå[3], Thomas Quertermous[2], Ulf Hedin[1]**

[1] Dept Molecular Medicine and Surgery, Karolinska Institute, Sweden
[2] Division of Cardiovascular Medicine, Stanford University, USA
[3] Department of Medicine Solna, Karolinska Institute, Sweden

*Ljubica.Matic@ki.se*

Omics profiling of biomaterial from atherosclerotic patients has previously identified molecular pathways enriched in plaque instability. Emerging single-cell RNA sequencing (scRNAseq) studies revealed cell populations co-existing in atherosclerotic plaques. Here, we associated patient clinical characteristics with cellular fractions using the Biobank of Karolinska Endarterectomies (BiKE).

Deconvolution analyses were applied on BiKE plaques and peripheral blood monocytes (PBMCs) microarray data (n=127 patients) using the scRNAseq information generated from n=5 plaques (15 cell populations identified), or a public immune signature expression file (22 cell subtypes), respectively. Cell fractions were enumerated by the Cibersort software and stratified based on patient clinical characteristics and outcome.

Plaques from symptomatic patients were enriched with type 1 macrophages and pericytes, while asymptomatic contained more typical and modulated smooth muscle cells (SMCs), especially in female patients. Transitory ischemic attack was associated with more type 2 macrophages and less SMCs compared to retinal stroke symptoms, while statin treatment and diabetes showed no effect on plaque cell fractions. Corresponding analyses in PBMCs revealed the enrichment of CD8 T-cells in symptomatic patients and lower resting NK cells compared to asymptomatic. Here, statins associated with more CD4 memory resting and CD4 naïve T cells, but less CD8 and resting NK cells. Survival analysis showed that modulated SMCs, type 1 macrophages and T cells in plaques, and higher circulatory monocytes associate with future adverse cerebro- and cardio-vascular events. In addition, plaque SMCs, pericytes, type 2 macrophages and NK cells associated with plasma proinsulin levels, while endothelial and B cells with IL6 levels. Circulatory B cells associated with plasma fibrinogen, Hb, and s-creatinine.

Our approach combining microarray, scRNAseq and clinical data, provided insight into the associations between cell types and patient characteristics. These results lay ground for further investigations to elucidate the interplay of various cells in atherosclerosis, starting from human disease perspective.

# Carbohydrate Intakes, Dietary Patterns, and Risk of Cardiovascular Diseases in Asian Populations

**Rob Martinus van Dam**[1*]

[1] Saw Swee Hock School of Public Health, National University of Singapore, Singapore

*rob.van.dam@nus.edu.sg*

In past decades, dietary recommendations in many countries across the world were focused on the restriction of total fat intake for prevention of chronic diseases including type 2 diabetes (T2D) and cardiovascular diseases (CVD). However, scientific evidence from trials and cohort studies clearly shows that the quality of dietary fats is more important than the total amount of fat. Similarly, the quality of carbohydrates may affect the development of T2D and CVD. Whole grain consumption has been linked to a lower risk, whereas refined grains and a high dietary glycemic index or glycemic load have been suggested to increase risk of these diseases. In many Asian populations, refined grains such as white rice and noodles are the main staple foods raising the question whether the high consumption of these foods contributes to T2D and CVD risk in Asia. Several prospective cohort studies on white rice consumption and risk of T2D and CVD have been conducted in Asian populations, but findings have been inconclusive. In particular, high white rice consumption was associated with a higher T2D risk in some cohort studies, whereas no substantial association was observed in other studies. A key issue is that consumption of major staple foods cannot be considered in isolation, because groups with high versus low intake of these foods will have markedly different dietary patterns. Specifically, substitution foods will need to be considered in the analysis of staple foods and health outcomes. Careful consideration of evidence from epidemiological studies of dietary intakes and relevant biomarkers in relation to disease outcomes, trials of effects on intermediary biomarkers, and Mendelian randomization studies will be needed to inform dietary recommendations on optimal carbohydrate intakes in Asian countries.

Symposium 16-2

# Introduction of KSoLA Dietary Guideline and KDRI for Carbohydrate/Sugar Intakes

**YoonJu Song**[1*]

[1] Food Science & Nutrition, The Catholic University of Korea, Korea

*yjsong@catholic.ac.kr*

Recent dietary guidelines for cardiovascular disease tend to emphasize the overall dietary quality. It is based on the accumulating evidence that quality of fat or carbohydrate is more important than quantity of fat or carbohydrate. In the KSoLA Dietary Guideline, dietary fat and carbohydrate are recommended by specific type as well as optimal level of total amount. With total fat intake limited to within 30% of daily energy intake, saturated fatty acids (SFA) and n-6 polyunsaturated fatty acid (PUFA) should be less than 7% and 10% of energy intake, respectively while avoiding trans fatty acids. Regarding carbohydrate, total carbohydrates consume less than 65% of energy intake, along with adequate intake of 10~20% of dietary sugar. In addition, dietary fiber is recommended to consume more than 25g per day, emphasizing whole grains as a staple. In the 2015 Dietary Reference Intakes for Koreans (KDRIs), dietary carbohydrate is recommended 55~65% of energy and dietary sugar is 10~20% of energy in all ages. Regarding dietary sugar, added sugar is defined as a sugar in which monosaccharides and disaccharides are added to foods during food processing or cooking. Added sugar intake should be less than 10% of energy intake and major food sources are sucrose, honey, syrups, fruit juice concentrates. In this talk, evidence of dietary guideline and KDRI for carbohydrate and sugar intake will be reviewed and carbohydrate and sugar intakes for the Korean population will be evaluated.

# Rice and Cardiovascular Health: Focus on Amylose Content

**Koutatsu Maruyama**[1*]

[1] Department of Bioscience, Ehime University, Japan

*maruyama.kotatsu.rt@ehime-u.ac.jp*

Rice is a major food source of carbohydrate intake for Asian populations, particularly. Although some observational studies suggested that higher rice intake may cause type 2 diabetes which is a major risk factor of cardiovascular diseases, amylose which is one of the components of starch of rice may suppress the increment of postprandial glucose concentration due to reduce to digestion than the other starch components. Amylose is classified in "resistant starch" which has a potential health benefit, i.e. decreased glycemic response, and increased GLP-1 and insulin secretion. Some clinical trials showed that high resistant starch including amylose rice has a lower glycemic index, and increased insulin secretion or sensitivity. Although few studies examined the associations of amylose intake or amylose contents of rice with cardiovascular health, high amylose rice might have a lower risk of cardiovascular diseases due to lower risk of glucose abnormality compared with low amylose rice. Since it may be difficult to change the diet with high rice intake based on culture and climate, the replacement of high amylose rice may work to help our health. This presentation reviews the role of amylose and shows some findings by clinical and observational studies to think about the association between rice intake and cardiovascular health.

Symposium 16-4

# Dietary Guideline of Acceptable Macronutrient Distribution Range and Recommended Food Sources for Carbohydrate

**SuJin Song**[1*]

[1] Department of Food and Nutrition, Hannam University, Korea

*sjsong@hnu.kr*

Dietary carbohydrates account for a considerable part of daily energy intake and are provided from various food sources. It is well studied that a diet balanced in carbohydrate quantity and quality is important for cardiovascular health. For the optimal amount of carbohydrate intake, dietary guidelines suggest an Acceptable Macronutrient Distribution Range (AMDR) as a recommended percentage of energy from carbohydrate. For example, in the 2015 Dietary Reference Intakes for Koreans, the AMDR for carbohydrate is 55–65% of energy for all age groups. Based on epidemiological evidence, the carbohydrate intake exceeding the AMDR is reportedly associated with an increased risk of cardiovascular diseases. Moreover, the carbohydrate quality has received research attention, as it is known to be associated with nutrient intake, diet quality, as well as health outcomes. In previous studies, dietary glycemic index and load, dietary fiber intake, and food sources have been frequently used to assess the effects of carbohydrate quality on cardiovascular health. Epidemiologic research on food sources of carbohydrate have reported that the consumption of whole grains, fruits, or legumes is inversely associated with cardiovascular diseases risks as these foods contain low glycemic index but high fiber and other nutrients. By contrast, intakes of refined grains and sugar-sweetened beverages are associated with an increased risk for cardiovascular diseases. Balanced carbohydrate diets in terms of adequate amounts and healthy sources can be helpful to improve cardiovascular health. Specific dietary guidance considering both carbohydrate quantity and quality should be suggested based on the well-designed prospective and clinical studies. Further studies are necessary to examine the mechanism underlying the associations between the amount and sources of dietary carbohydrates and cardiovascular diseases.

# Regulating Glucose Delivery to the Body and Physiological Importance

**Byung-Hoo Lee**[1*]

[1] Food Science and Biotechnology, Gachon University, Korea

*blee@gachon.ac.kr*

For decades, quality of carbohydrate-based foods has been associated with the in vivo measured glycemic index or the in vitro digestion rate-based categories of rapidly digestible, slowly digestible, and resistant carbohydrates. Glycemic index has been related to health-based endpoints mostly through correlative or observational studies with mechanisms proposed, but not well established. Here, we bring forth the concept of locational delivery of glucose from dietary carbohydrates, especially starches, to the distal small intestine to elicit an "ileal brake" effect, which slow gastric emptying and, in turn, extend nutrient (i.e., energy) delivery to the body and may decrease appetite and promote weight management. Slowly digestible carbohydrates are currently a popular topic of research, though where they are digested and the released-glucose is delivered in the small intestine is not known. A proposal is to further study and establish this mechanism of appetite and food intake regulation so that carbohydrate-based ingredients and foods can be developed that promote the ileal brake mechanism.

Symposium 16-6 Short Talk

# Balanced Carbohydrate Diet: Focus on Intakes and Food Sources of Protein and Fat

**Kyungho Ha**[1*]

[1] Department of Food Science and Nutrition, Jeju National University, Korea

*kyungho.ha715@gmail.com*

Diets with extreme macronutrient intake, including high-carbohydrate or high-fat diets, are related to increasing cardiovascular disease risk. In the Asian diet, carbohydrate plays an important role and the average intake of carbohydrate is approximately 60% of total energy among adults from Korea and Japan.

Previous epidemiologic studies reported that avoiding excessive carbohydrate intake may reduce the risk of cardiovascular diseases such as metabolic syndrome and type 2 diabetes, especially in older adult populations. To lower carbohydrate intake, both protein and fat intakes can be increased without restriction or protein intake can be increased while allowing fat intake to reach an appropriate level.

According to several previous studies conducted in Western populations, low-carbohydrate diets showed different associations with cardiovascular diseases by food sources of protein and fat (e.g., animal or plant). However, few studies have explored association between a low-carbohydrate or moderate-carbohydrate diet and cardiovascular risk factors considering intakes and food sources of protein and fat in Asian populations.

In this presentation, recent studies which examined associations between a low-carbohydrate and moderate-carbohydrate diet with cardiovascular risk factors in Korean adults will be introduced.

# Ezetimibe

**Anselm Kai Gitt**[1*]

[1] Cardiology, Herzzentrum Ludwigshafen, Department of Cardiology, Germany

*GittA@klilu.de*

In 1964 data from the Framingham study described a correlation between the levels of plasma lipids and the development of coronary heart disease. Twenty years later, the first study on statins (4S trial) reported a significant benefit of lipid-lowering on cardiovascular complications in patients treated with simvastatin for secondary prevention. Additional randomized studies investigating other statins followed, and in 2004 the PROVE-IT trial demonstrated, that a more aggressive lowering of LDL-cholesterol by high doses of potent statins even further reduced adverse CV-events.

Ezetimibe inactivates the cholesterol transporter NPC1L1 (Niemann-Pick C1 Like 1 Protein) and provides a further reduction of LDL-cholesterol on top of statins. The outcome trial to test the impact of this new compound was the IMPROVE-IT trial published 2015 in the NEJM reporting a further benefit in CV outcome for the combination therapy of ezetimibe with simvastatin. Additional sub-analyses of this trial showing the benefit in patients on different risk levels will be demonstrated. In the PRECISE -IVUS Study the combination therapy of ezetimibe and atorvastatin demonstrated a significant better reduction in plaque volume as compared to statin therapy alone measured by intravascular ultrasound.

Based on the evidence for ezetimibe, current guidelines (e.g. ESC guidelines) recommend the use of the combination therapy in a step-wise approach. Data from large registries show, that combination therapy is not yet widely used and that there is still large space for improvement in daily clinical practice to bring down the event rates for the high-risk population.

Symposium 17-2

# PCSK9 Inhibitors in Familial Hypercholesterolemia A Revolution in Treatment

**Frederick J. Raal**[1*]

[1] Division of Endocrinology, University of the Witwatersrand, South Africa

*Frederick.Raal@wits.ac.za*

At one time atherosclerosis was thought to be an irreversible degenerative disease that was an inevitable consequence of ageing. Research in the past decade has shown that atherosclerosis is neither a degenerative disease nor inevitable. The long-term clinical benefits of reducing LDL-cholesterol, and its impact on decreasing coronary artery disease (CAD) morbidity and mortality, have been conclusively demonstrated. Major statin trials involving over 70 000 subjects have yielded reductions of about 30% in the rate of CAD death or non fatal myocardial infarction in both primary (WOSCOPS, AFCAPS-TexCAPS, HPS) as well as secondary (4S, CARE, LIPID) prevention, and in patients with mild to moderate as well as severe LDL-cholesterol elevation. However despite the use of high intensity statin therapy often in combination with other available lipid-lowering drugs such as ezetimibe, residual cardiovascular risk remains. In addition many patients with heterozygous familial hypercholesterolaemia (FH) and nearly all those with homozygous FH are unable to achieve acceptable LDL-cholesterol levels. The discovery of PCSK9 and its role in LDL metabolism has allowed for the rapid development of PCSK9 inhibitors. Monoclonal antibodies (mAbs) against PCSK9 and more recently the siRNA inclisiran, have been shown to be remarkable effective in reducing LDL-cholesterol levels by an additional 50-60% in non-FH and heterozygous FH patients and by 30% in those homozygous FH patients with residual LDL receptor activity. For the first time PCSK9 mAbs have allowed a substantial proportion of FH patients to achieve LDL-cholesterol levels below 70 mg/dL (1.8 mmol/L). More aggressive lipid modifying treatment and lower LDL-cholesterol targets which can be achieved with PCSK9-inhibitor therapy will very likely reduce the cardiovascular event rate and prolong life even further.

# Incretin-Based Therapeutics: DPP-4 Inhibitor and GLP-1RA

**Mansoor Husain**[1*]

[1] Ted Rogers Centre for Heart Research, University Health Network, Canada

*mansoor.husain@uhn.ca*

This presentation will highlight the increased risk of cardiovascular disease and major adverse cardiovascular events [MACE] in patients with type 2 diabetes, and discuss meta-analyses that show how previous generations of anti-diabetic therapies had few if any benefits on MACE, and may have contributed to increased risks of heart failure [HF]. The presentation will summarize data from more recent cardiovascular outcomes trials [CVOT] with incretin drugs, namely DPP 4 inhibitors [DPP4i] and GLP 1 receptor agonists [GLP-1RA], and briefly contrast these with data from the CVOT of SGLT-2 inhibitors. More focus will be paid to more recent studies of GLP 1 RAs dulaglutide and semaglutide, including oral semaglutide. Combined analyses of CVOT of both subcutaneous and oral semaglutide will be reviewed. After summarizing our current understanding of the putative cardiovascular mechanisms of action of the incretin drugs, the presentation will conclude with the clinical implications of the data to date and how they have influenced the latest treatment guidelines from major international societies.

# SGLT-2 Inhibitors

**Matthias Blüher**[1*]

[1] Medicine, University Hospital Leipzig, Germany

*Matthias.Blueher@medizin.uni-leipzig.d*

Recent cardiovascular outcomes trials (CVOT) have shown that specific medications from two new classes of glucose-lowering medications, sodium-glucose cotransporter-2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists (GLP-1 RA) can reduce cardiovascular outcome parameters significantly. Here, I focus on recent data and developments in SGLT2i treatments including their impact on cardiovascular events, effects on body weight, blood pressure, renal function, heart failure and relevant side effects. SGLT2i may be considered as a "multimodal diabetes treatment in a pill" because of their pleitropic beneficial effects on cardiometabolic risk factors. For patients with type 2 diabetes (T2D), with or without established cardiovascular disease, SGLT2i have demonstrated impressive reductions in hospitalization for heart failure and protective effects on renal function. Particularly for T2D patients with established cardiovascular disease, some SGLT2i demonstrated an additional benefit of reduced major adverse cardiac events, on top of reductions in hospitalizations for heart failure, renoprotection, and in some instances, mortality. The major question remains how SGLT2i exert their beneficial cardiometabolic effects. Studies on the mechanisms of action of SGLT2 inhibitors have focused on pathways linked to glucose metabolism and toxicity, liver fat, hemodynamic/volume, vascular and renal actions, and cardiac effects, including those on myocardial energetics. Results of many ongoing trials may help us understanding how SGLT2 inhibitors improve cardiovascular and renal outcomes and may also identify unexpected mechanisms suggesting novel therapeutic applications.

# Past, Present, and Future of NOACs in Patients with Atrial Fibrillation

**So-Ryoung Lee**[1*]

[1] Cardiology, Seoul National University, Korea

*minerva1368@gmail.com*

In a recent decade, direct oral anticoagulants (DOACs) have been introduced in the clinical practice and now, DOACs are widely prescribed in patients with non-valvular atrial fibrillation (AF). In this lecture, we overview the data from pivotal randomized clinical trials of DOACs and how DOACs perform in the real-world clinical setting, especially in Korean population. We also discuss the future direction of research in field of stroke prevention in patients with AF.

Symposium 17-6 Short Talk

# Comparison of the Effects of High-dose Statin alone versus Intermediate-does Statin combined with Ezetimibe on Major Adverse Cardiovascular Events in Patients with Acute Myocardial Infarction: A Nationwide Cohort Study

**Kihyun Kim**[1*]

[1] Cardiology, Gangneung Asan Hospital, Korea

*twogama2@gmail.com*

To compare the effects of high-intensity statin monotherapy with those of moderate-intensity statin-ezetimibe combination therapy on major adverse cardiovascular events in patients with acute myocardial infarction (AMI)

Using the Korean National Health Insurance Service database, we searched 82941 new patients with AMI undergoing percutaneous coronary intervention (PCI) from January 2013 to December 2018. Among them, we identified 9908 patients who treated with atorvastatin 40mg (A40, n=4041), atorvastatin 20mg + ezetimibe 10mg (A20/E10, n=233), rosuvastatin 20mg (R20, n=5251) or rosuvastatin 10mg + ezetimibe 10mg (R20/E10, n=383) and analyzed the risk of PCI with MI, PCI without MI, ischemic stroke, and all-cause death. The inverse probability of treatment weighting (IPTW) was used to balance covariates across each groups.

We compared a IPTW cohort of new patients with AMI who treated with A40 (reference group), A20/E10, R20, or R10/E10 after index PCI. There was no significant difference in the composite end point of PCI with MI, PCI without MI, ischemic stroke, and all-cause death (A20/E10: adjusted HR (aHR), 1.25; 95% confidence interval (CI), 0.82-1.89; R20: aHR 0.96, 95% CI, 0.84-1.08; R10/E10: aHR 0.92, 95% CI, 0.63-1.36). However, high-intensity statin monotherapy decreased the risk for PCI with MI (A20/E10: aHR, 1.73; 95% CI, 0.86-3.49; R20: aHR 0.97, 95% CI, 0.76-1.24; R10/E10: aHR 1.33, 95% CI, 0.70-2.52) and mortality (A20/E10: aHR, 1.25; 95% CI, 0.47-3.28; R20: aHR 0.87, 95% CI, 0.67-1.14; R10/E10: aHR 2.03, 95% CI, 1.07-3.85) compared with moderate-intensity statin-ezetimibe combination therapy.

In this real-world Asian population with AMI, there was no significant difference in the composite end point of PCI with MI, PCI without MI, ischemic stroke, and all-cause death between groups. However, high-intensity statin monotherapy might be associated with reduced risk of PCI with MI and all-cause death compared with moderate-intensity statin-ezetimibe combination therapy.

# Pericyte-Specific Vascular Expression of SARS-CoV-2 Receptor ACE2 – Implications for Microvascular Inflammation and Hypercoagulopathy in COVID-19

**Christer Betsholtz**[1*]

[1] Immunology, Genetics and Pathology, Uppsala University, Sweden

*christer.betsholtz@igp.uu.se*

We have found that within the vasculature, angiotensin converting enzyme 2 (ACE2) is specifically expressed by microvascular mural cells, in particular pericytes. ACE2 has recently been identified as the cellular receptor for SARS-coronavirus-2, the cause of the current COVID-19 pandemic. In my talk, I will show data on the organotypic and heterogeneous expression of ACE2 by pericytes. I will also show evidence that endothelial cells do not express ACE2. Thus, on order for the SARS-CoV-2 virus to reach pericytes, it would not only have to disseminate into the blood from primary infection sites in the airways and/or garstointestinal tract, but it would also have to passage across the endothelial layer, which in the healthy state is unlikely penetrable by particles the size of a corona virus. Thus, we hypothesise that virus may reach and infect pericytes only when the endothelial barrier is dysfunctional and leaky, such as in hypertension and inflammation. Using pericyte-deficient mice, we have further studied that consequences of pericyte loss for the endothelial cells and found that this leads to pro-coagulant and pro-inflammatory endothelial responses, which may be of relevance for the coagulopathy and systemic inflammation observed in severely ill COVID-19 patients.

Symposium 18-2

# Targeting the Endothelial Cell Barrier to Prevent Tumor Cell Metastasis

**Luisa Iruela-Arispe**[1*]

[1] Cell & Developmental Biology, Feinberg School of Medicine, Northwestern University, USA

*arispe@northwestern.edu*

The contribution of the endothelial barrier to tumor cell extravasation has been fully supported by gain- and loss-of-function studies highlighting the critical roles of junctional proteins and their regulators (VE-cadherin, tight junctions, Tie1, Tie2, and Notch1) in metastasis. While these studies have offered important proof-of-principle, the complex and pleotropic roles of those proteins in endothelial biology have prevented their exploration to inhibit metastasis. Using chemical libraries of FDA-approved compounds, we performed a high-content screen to identify molecules that may hinder tumor cell extravasation across an endothelial monolayer. Hits were further validated and characterized using a series of in vitro assays, zebrafish tumor cell extravasation studies and metastatic models in mouse. Niclosamide, a compound used as an anti-helminthic drug showed a remarkable ability to enhance the endothelial barrier and suppress tumor cell extravasation without affecting immune cell trafficking. Single-cell sequencing was used to gain mechanistic insights of drug effects on tumor and endothelial cell signaling, and to clarify the interaction network between both cell types. Mechanistically, niclosamide affected homotypic and heterotypic signaling critical to intercellular junctions, cell-matrix interactions, and cytoskeletal regulation. Proteomics on mouse plasma identified biomarkers associated with niclosamide function and revealed additional systemic effects consistent with barrier stabilization. Our findings designate niclosamide as an effective drug to restrict tumor cell extravasation through modulation of multiple signaling pathways and tumor-endothelial cell interactions.

# GPCR Regulation of Gene Expression During Vascular Development and Organotypic Specialization

**Timothy Hla[1*], Keisuke Yanagida[1], Eric Engelbrecht[1]**

[1] Vascular Biology Program, Boston Children's Hospital, Department of Surgery, Harvard Medical School, USA

*Timothy.Hla@childrens.harvard.edu*

Sphingosine 1-phosphate (S1P), a lysophospholipid produced from the metabolism of sphingomyelin, was originally proposed as an intracellular second messenger produced in response to growth factors and cytokines. Even though several proteins were proposed to be "intracellular receptors" for S1P, for example, TRAF2, HDAC1, hTERT, prohibitin-2 and atypical PKC, whether these purported targets truly mediate various physiological functions of S1P is presently unclear. Our laboratory discovered the first S1P receptor as an orphan G protein-coupled receptor (GPCR) from vascular endothelial cells. We now know that five widely-expressed GPCRs (S1P1-5) mediate most of the actions of this lysophospholipid mediator. Vertebrates secrete S1P into the extracellular environment via specific transporters (Spns2 and Mfsd2b). Extracellular S1P is chaperoned by circulating proteins such as HDL-bound ApoM and signals via cell-surface receptors. Functions of S1P include its essential role in vascular development, immune cell trafficking and neuronal development. Indeed, the two S1P receptor targeted drugs are now approved as oral medications in the treatment of multiple sclerosis. Novel S1P receptor-based therapeutics are being developed to control additional autoimmune diseases such as ulcerative colitis and psoriasis as well as in acute neurological conditions such as stroke and subarachnoid hemorrhage.

In the vascular system, S1P works together with VEGF to regulate early vascular development. S1P receptors 1,2 and 3, which have distinct as well as overlapping signaling pathways, cooperate to regulate vascular development. We recently characterized the retinal vascular development in S1P receptor compound knockout mice. Transcriptome and chromatin profiling showed that tip cell genes are induced concomitant with repression of organ-specific endothelial cell differentiation. We will present recent data on how S1P signaling sculpts endothelial chromatin to coordinate transcriptional gradients and organ-specific endothelial cell specialization.

Symposium 18-4

# Transcription Networks and Endothelial Heterogeneity

**Anna M. Randi**[1*]

[1] National Heart and Lung; Vascular Science, Imperial College London, UK

*a.randi@imperial.ac.uk*

Endothelial cells (EC) lining blood vessels show striking phenotypic heterogeneity in their structure and function, according to their location and exposure to distinct microenvironments. Deciphering the molecular basis for endothelial heterogeneity is a key step in the development of vascular bed-specific therapies. Transcription factors (TF) belonging to a handful of families, including ETS, GATA and KLF, play key roles in regulating endothelial lineage and function. Some of these factors are expressed almost uniquely in EC whilst others show a wider expression pattern. Recent expression profiling studies have shown that some TF appear to be present in all EC (pan-endothelial), whilst others have an organotypic pattern of expression, i.e. are only found in EC from selected tissues such as liver, lung or brain. Moreover, high/low shear stress and local growth factors can regulate expression and/or activation of TF. Control of endothelial gene expression is likely to result from a complex network of pan-endothelial, organotypic and stimulus-dependent factors. We are investigating this question using as model the pan-endothelial ETS factor ERG, an essential regulator of vascular development and endothelial homeostasis. Although ERG is found in most EC in the body, its deletion in a mouse models does not cause equally severe phenotypes in all organs or all vascular beds. This suggests that ERG may regulate its targets in an organotypic fashion, likely due to its ability to interact with different transcriptional partners. We focus on a set of ERG gene targets involved in a crucial function of the vascular endothelium, namely the control of haemostasis, and the regulation of pro- and anti-thrombotic genes, and investigate the transcriptional networks likely to confer organotypic specificity.

# Vascular Precision Biology and Mapping the Human Body at Single Cell Resolution

**Zorina Galis**[1*]

[1] National Heart Lung and Blood, National heart Lung and Blood Institute, USA

*zorina.galis@nih.gov*

New analysis, imaging, and computational tools are increasing our ability to characterize multiple biological activities of single cells, including in their natural context. Several efforts, including the NIH Human Biomolecular Atlas Program (HuBMAP) seek to comprehensively map the human body at cellular resolution
A key challenge for single cell mapping is the ability to precisely localize the position of individual cells within tissues. Defining a Common Coordinate Framework (CCF) for the human body calls for several desirable characteristics: 1) works across several scales (cell <> tissue <> body); 2) is applicable to all (most) body tissues; 3) can account for donor differences, e.g., size, shape, sex; 4) is useful/acceptable across specialty domains.
Capitalizing on newly obtained information, specifically endothelial single cell analyses and machine learning, we will present practical and theoretical considerations of how the vasculature could fulfil the main requirements and provide an anatomically relevant multiscale, "built-in" CCF for the human body.
Ultimately, the precision vascular mapping will enable generation and testing of new scientific hypothesis related to the integration of vasculature within various local functional units and across the body, lifespan, and the health-disease continuum.

Symposium 18-6 Short Talk

# Real-Time Intravital Characterization of Non-Classical Monocytes Mediating Resistance to Anti-Angiogenic Treatment in Cancers

**Keehoon Jung**[1*]

[1] Department of Anatomy and Cell Biology / Biomedical Sciences, Seoul National University College of Medicine, Korea

*keehoon.jung@snu.ac.kr*

Colorectal cancer (CRC) is the leading cause of cancer-related deaths worldwide. However, current anti-VEGF therapies for CRC provide limited survival benefit as tumors rapidly develop resistance to these agents.

We have developed a miniaturized confocal endomicroscopy technique for spontaneous CRC models in mice. We recently also established a novel abdominal imaging window. These unique systems enable us to monitor the CRC and its immune microenvironment longitudinally with a video-rate intravital multi-photon microscope.

Using these in vivo imaging methods and CRC models, we have uncovered an immunosuppressive role for non-classical Ly6Clow monocytes that mediates resistance to anti-VEGFR2 treatment. We found that the chemokine CX3CL1 was upregulated in both human and murine tumors following the VEGF signaling blockade, resulting in recruitment of CX3CR1+ Ly6Clow monocytes into the tumor. We also found that treatment with VEGF-A reduced expression of CX3CL1 in endothelial cells. Intravital microscopy revealed that CX3CR1 is critical for Ly6Clow monocyte transmigration across the endothelium in tumors. Moreover, Ly6Clow monocytes recruit Ly6G+ neutrophils via CXCL5 and produce IL-10, which inhibits adaptive immunity. Preventing Ly6Clow monocyte or Ly6G+ neutrophil infiltration into tumors enhanced inhibition of tumor growth with anti-VEGFR2 therapy. Furthermore, we developed a gene therapy using a nanoparticle formulated with a siRNA against CX3CL1, which reduced Ly6Clow monocyte recruitment and improved outcome of anti-VEGFR2 therapy in mouse CRCs.

Taken together, we identified immunosuppressive non-classical Ly6Clow monocytes as key players in tumor resistance to anti-angiogenic therapy in CRCs. We also revealed molecular mechanisms underlying anti-angiogenic treatment resistance, suggesting potential immunomodulatory strategies to enhance the long-term clinical outcome of anti-VEGF therapies, proven by state-of-the-art in vivo imaging modalities.

# New Insight of Vascular Calcification; Metabolic Reprogramming

**In-kyu Lee**[1*]

[1] Dept. of Int. Med, Kyungpook National University Hospital, Korea

*leei@knu.ac.kr*

Vascular calcification (VC), which is classified with intimal and medial calcification, depending on the site(s) involved within the vessel, is closely related to cardiovascular disease.

Medial calcification is commonly associated with chronic kidney disease and diabetes. Previous extensive research into VC uncovered that mechanism of VC is not merely a consequence of a high-phosphorous and –calcium milieu, but also occurs via delicate and well-organized biologic processes, including an imbalance between osteochondrogenic signaling and anticalcific events. In addition to traditionally established osteogenic signaling, dysfunctional calcium homeostasis is prerequisite in the development of VC.

Recent accumulating evidence showed microorganelle dysfunctions, including hyper-fragmented mitochondria, mitochondrial oxidative stress, defective autophagy or mitophagy, and endoplasmic reticulum (ER) stress, significantly associated with increased VC. In this presentation, I want to provide a detailed updated molecular mechanism of VC. This encompasses a vascular smooth muscle phenotypic of osteogenic differentiation, and multiple signaling pathways of VC induction, including the roles of inflammation and cellular microorganelle changes.

Symposium 19-2

# CAC Score to Guide CV Risk Assessment When Decision to "Statin" is Uncertain

**Erin D. Michos**[1*]

[1] Medicine (Cardiology), Johns Hopkins University School of Medicine, USA

*edonnell@jhmi.edu*

The most important way to prevent cardiovascular disease (CVD) is to follow a healthy lifestyle throughout one's lifetime. However, when considering drug therapy, estimation of risk helps facilitate matching the intensity of pharmacotherapy to one's absolute risk to maximize anticipated benefits and minimize harms of over-treatment. In 2019, the American College of Cardiology (ACC) and American Heart Association (AHA) published a new Guideline on the Prevention of CVD. In this Guideline, for adults aged 40-75 without known CVD, the Guideline recommends starting with a 10-year risk assessment using the Pooled Cohort Equation (PCE) to guide-risk based decisions. Individuals are then classified as low (<5%), borderline (≥5%-<7.5%), intermediate (≥7.5-19.9%), or high (>20%) 10-year risk. Statin therapy is recommended for high-risk patients, as well as intermediate-risk patients especially if risk enhancers are present, and may also be considered for borderline-risk individuals who have risk enhancers. However, the Guideline acknowledges that the PCE is imperfect and can both over – and under-estimate risk in certain populations. The Guideline recognizes that the possible benefits of lipid pharmacotherapy may remain uncertain even after consideration of risk-enhancing factors. Thus, among these intermediate-risk or selected borderline-risk individuals, it may be reasonable to measure atherosclerosis burden with a coronary artery calcium (CAC) score by non-contrast computed tomography to further refine risk estimation either upwards or downwards. CAC, a marker of total atherosclerotic burden, has emerged to be a superior marker to re-classify risk compared to traditional risk factors. It serve as a decision aide tool (not a screening tool) to guide shared decision-making. The objectives of this talk are to 1) Review recent updates on CAC for predicting CVD and its incorporation in the 2019 ACC/AHA Guideline and 2) Discuss which type of patients may benefit from CAC score to guide the clinician-patient risk discussion about treatment decisions.

# Intracranial Arterial Calcification

**Kwang-Yeol Park**[1*]

[1] Department of Neurology, Chung-Ang University, Korea

*kwangyeol.park@gmail.com*

Although calcification in arterial wall has been considered a passive phenomenon of aging and an inert end-point of atherosclerosis, it is an active process resembling bone formation. It has been reported to be distributed in various vascular beds including carotid, coronary, aorta and iliac artery whose extent increases with age. Arterial wall calcification is associated with hardening of arterial wall (arterial stiffness), pulsatility, mortality and cardiocerebrovascular diseases.

Among various vascular bed involved in wall calcification, intracranial arterial calcification (IAC) is a common neuroimaging finding in the elderly. The prevalence was reported to be about 40 – 90% in patients with acute ischemic stroke. It was most frequently and severely found in distal intracranial internal carotid artery followed by vertebral artery. Associated conditions of IAC are age, hypertension, diabetes, chronic kidney disease, dyslipidemia, vitamin D deficiency and so on. IAC is associated with increased arterial stiffness and larger pulsatile insult to the brain. Also, the presence of IAC is associated with neuroimaging correlates of cerebral small vessel disease such as white matter hyperintensities, lacunes, and deep cerebral microbleeds. One possible explanation is that vitamin D deficiency, which is prevalent in patients with stroke, brings about vascular calcification which makes arterial wall stiff, exaggerate hypertensive insult, and causes cerebral small vessel disease. The lack of vitamin K or the use of vitamin K antagonist is also associated with calcification through the inhibition of vitamin K dependent protein, matrix Gla protein which inhibits vascular calcification.

In summary, IAC is common in patients with stroke and is associated cerebral small vessel disease. Some possible links between IAC and small vessel disease are vitamin D deficiency and vitamin K antagonist. Future studies will be needed to confirm this hypothesis.

Symposium 19-4

# Pathophysiologic Aspect of Calcification in CKD

**Makoto Kuro-o**[1*]

[1] Anti-Aging Medicine, Jichi Medical University, Japan

*mkuroo@jichi.ac.jp*

During the evolution of skeletons, terrestrial vertebrates acquired the bone made of calcium-phosphate. By keeping the extracellular fluid in a supersaturated condition regarding calcium and phosphate ions, they create the bone when and where they want simply by providing a cue for precipitation. To secure this strategy, they acquired a new endocrine system to strictly control the extracellular phosphate concentration. In response to phosphate intake, fibroblast growth factor-23 (FGF23) is secreted from the bone and acts on the kidney through binding to its receptor Klotho to increase urinary phosphate excretion, thereby maintaining phosphate homeostasis. The FGF23-Klotho endocrine system, when disrupted, results in hyperphosphatemia and ectopic calcium-phosphate precipitation in mice and humans. In addition to disturbed phosphate homeostasis, mice lacking Klotho suffer from premature aging. They exhibit multiple organ atrophy, vascular calcification, sarcopenia, cardiac hypertrophy, frailty, and a shortened lifespan associated with chronic non-infectious inflammation, which are a phenocopy of patients with end-stage renal disease (ESRD). Besides calcium-phosphate deposition in extraosseous tissues, ESRD patients bear colloidal nanoparticles containing calcium-phosphate in the blood, which are termed calciprotein particles (CPPs). CPPs have the ability to induce cell damage and inflammation, and thus may be responsible for the accelerated aging. To test this hypothesis, we developed a CPP adsorption column and inserted it in hemodialysis circuit to remove CPPs from the blood and succeeded in improving survival in hemodialysis miniature pigs. These findings not only show that the CPP adsorption column is promising as a new therapeutic device for improving prognosis of hemodialysis patients, but also provide unequivocal evidence that CPPs are a causative agent for the aging-like phenotypes. Terrestrial vertebrates with the bone made of calcium-phosphate may be destined to age due to ectopic calcium-phosphate.

# Clinical Implication of Vascular Calcification in Patients With Diabetes

**Eun-Jung Rhee**[1*]

[1] Endocrinology and Metabolism, Sungkyunkwan University, Kangbuk Samsung Hospital, Korea

*hongsiri@hanmail.net*

Vascular calcification is considered as a great marker for vascular aging and atherosclerosis. For example, coronary artery calcium score (CACS)is a non-invasive and valuable marker for atherosclerotic burden in our body and shows good correlation with future cardiovascular events. Diabetes is a vascular disease that causes damage to macro- and microvasculature. Diabetes and chronic kidney disease are the main two disease conditions that cause medial calcification, differing from hypercholesterolemia or hypertension that cause intimal calcification. Recent guidelines recommend CACS as the tool for risk stratification in intermediate risk groups. However, in patients with diabetes, CACS is not firmly recommended as the first surrogate marker for risk stratification. In this talk, I would like to talk about current knowledge on vascular calcification in patients with diabetes and the role of CACS in prediction of cardiovascular events in patients with diabetes.

Symposium 19-6 Short Talk

# Zingerone Attenuates Pi-Induced Vascular Calcification via AMPK-Mediated TIMP4 Expression

**Won-Gu Jang**[1*]

[1] Dept. of Biotechnology, College of Engineering, and Research institute of Anti-Aging, Daegu University, Korea

*jangwg@daegu.ac.kr*

Vascular calcification requires vascular smooth muscle cells (VSMCs) differentiation into osteoblast-like cells. This phenomenon can be enhanced by inflammation and oxidative stress. Zingerone is one of the active ingredients present in ginger plant that has anti-inflammatory and anti-oxidant effects. However, vascular calcification by zingerone has not yet been elucidated. This study investigated the effect of zingerone on vascular calcification and its molecular mechanism.

The VSMCs were isolated from rats, and cells induced calcification with inorganic phosphate (Pi). To confirm the expression levels of osteogenic marker genes such as Dlx5, CBFA1 and ALP, we performed RT-PCR or Real-time PCR analysis and Western blot analysis was performed to determine protein expression levels. Calcium deposition was confirmed by alizarine red staining (ARS).

Zingerone induced α-SMA and SM22α (VSMC marker) gene expressions whereas decreased CBFA1 in a dose-dependent manner. Additionally, zingerone decreased Pi-induced the expression of Dlx5 and CBFA1 gene and protein levels. In addition, AMPK phosphorylation and TIMP4 expression were increased by zingerone. Inactivation of AMPK using comp C attenuated zingerone effects. The zingerone reduced Pi-induced ARS levels were recovered by comp C. The CA-AMPK treatment dramatically increased TIMP4 expression and also, zingerone-induced TIMP4 expression was suppressed by comp C.

Taken together, zingerone inhibits Pi-induced vascular calcification via regulating AMPK-TIMP4 signaling cascade in vascular smooth muscle cells.

# Cardiovascular Risk Heterogeneity in FH: How to Identify the Severe Forms?

**Raul D. Santos**[1,2*]

[1] Lipid Clinic Heart Institute (InCor), University of Sao Paulo, Brazil
[2] Academic Research Organizationi, Hospital Israelita Albert Einstein, Brazil

*rauldsf@gmail.com*

FH affects roughly 1/310 individuals and is associated with a 10-20-fold greater risk of atherosclerotic cardiovascular disease (ASCVD) than the one of normolipidemic subjects. This risk however is heterogeneous and not driven solely by gradients in LDL-C among individuals with FH. Indeed, genetic defects influence on ASCVD risk however not only the monogenic defects may influence this risk in FH. In addition to high LDL-C, factors like presence of previous ASCVD events and subclinical coronary atherosclerosis like coronary artery calcification (CAC) indicate higher risk FH individuals. Furthermore, presence of factors like smoking, low HDL-C, elevated lipoprotein(a) [Lp(a)] concentrations, family history of early ASCVD and late onset of lipid lowering treatment also point to higher risk individuals. Risk equations like SAFEHEART-RE may help identify higher risk FH individuals since it encompasses classical risk factors for atherosclerosis in addition to Lp(a) levels. One possibility to improve ASCVD risk discrimination might be the use of vascular age derived from CAC scores, rather than biological age, in addition to classical risk factors. This may help to better discriminate higher from lower risk individuals. Considering the cost ccontained health environment, higher risk FH individuals would be the adequate candidates for further LDL-C lowering with PCSK9 inhibitors.

Ref-
1-Prog Cardiovasc Dis. 2019 ;62(5):414-422
2-Eur Heart J Cardiovasc Imaging. 2020;21(3):251-257
3-Circulation. 2017;135(22):2133-2144

Symposium 20-2

# Pharmacotherapy of FH: Present and Future

**Sang-Hyun Kim**[1*]

[1] Cardiology, College of Medicine, Seoul National University, Korea

*shkimmd@snu.ac.kr*

The most important management strategy for people with familial hypercholesterolemia is to prevent cardiovascular disease and decrease cardiac death through the comprehensive management of risk factors, especially LDL cholesterol. The current treatment strategy for FH is to decrease LDL cholesterol with statin. New drugs including lomitapide, mipomersen and PCSK9 monoclonal antibodies are valuable for the treatment of FH. And injection therapies of anti-sense oligomer which prevents the synthesis of PCSK9 or lipoprotein (a) are currently under clinical trials and has shown favorable efficacy to reduce target molecules. Further research is required to ascertain whether anti-sense oligomer therapies for lowering PCSK9 or lipoprotein (a) levels can reduce cardiovascular disease in patients with FH.

# Discordance on FH Care: Lessons From the "Ten Countries Study"

**Brian Tomlinson**[1*]

[1] Faculty of Medicine, Macau University of Science & Technology, Macau

*btomlinson@must.edu.mo*

There have been several international guidelines and models of care for the management of familial hypercholesterolemia (FH) but there remains a continued discordance on various aspects of the care for FH patients in different countries. The "Ten Countries Study" was initiated in the Asia-Pacific region to investigate the various aspects of diagnostic, epidemiological and service approaches and the physician practices and patient experiences of FH in the region with the UK serving as the international benchmark. Centers from South Africa and Brazil subsequently joined the study. A series of questionnaires were completed by key opinion leaders from selected specialist centers. The estimated percentage of patients diagnosed with FH was low at <3% in all the countries, compared with approximately 15% in the UK. The under-detection of FH was thought to be associated with government expenditure on health care. The most commonly used methods for detecting FH patients were opportunistic and systematic screening and the Dutch Lipid Clinic Network criteria were commonly used. Genetic testing was infrequent and noninvasive imaging for risk assessment was underutilized. Patients with FH were often not adequately treated with an estimated <30% of patients achieving guideline recommended LDL cholesterol targets on conventional therapies. Other treatment gaps included suboptimal availability and use of lipoprotein apheresis and proprotein convertase subtilisin-kexin type 9 inhibitors. There was a deficit of FH registries, training programs and publications in less economically developed countries. Important gaps across the continuum of care for FH were identified, particularly in the less economically developed countries. It was concluded that wider implementation of primary and pediatric care, telehealth services, patient support groups, education and training programs, research activities, and health technology assessments are needed to improve the care of patients with FH in these countries.

Symposium 20-4

# Genetic Characteristics and Response to LLT in FH

**Sang-Hak Lee**[1*]

[1] College of Medicine, Yonsei University, Korea

*Shl1106@yuhs.ac*

Pharmacological treatment for FH has made considerable progress in the past two decades. Currently, pharmacological agents including statins, ezetimibe, and PCSK9 inhibitors are being used to treat FH in clinical practice. Clinical trials or registry studies conducted till date have reported that individual responses to LLT employing statins and/or PCSK9 inhibitors vary substantially. Individual difference in response to LLT is a crucial clinical issue as it can affect future cardiovascular outcomes. However, the reason underlying the variation in response to LLT among individuals is not yet completely understood.

Several clinical and genetic factors have been reported to influence the response in general population. Thus, in FH, it is probable that genetic variations affect an individual's response to LLT. Differences in cardiovascular risk due to genetic characteristics in FH may be partly explained if the presence or type of mutations could influence the response. Some studies have reported that the response to statins and PCSK9 inhibitors could differ depending on mutation types, such as defective mutation. However, contradictory studies have warranted the need for further studies to clarify the link between genetic characteristics and responses to statins and PCSK9 inhibitors.

Among the patients enrolled in the Korean FH registry, patients who had undergone appropriate LLT escalation and were followed-up for >6 months were analyzed for pathogenic mutations. Lipid-lowering regimens included statin, ezetimibe, or evolocumab. The achieved percentage of expected low-density lipoprotein-cholesterol (LDL-C) reduction (a primary variable for adjusted response) and achievement rates of LDL-C <70 mg/dL were assessed. The correlations between the treatment response and the characteristics of mutations were evaluated.

# Cardiovascular Risk and Effect of Lipid-Lowering Therapy in Koreans With Phenotype of Familial Hypercholesterolemia

**Chan Joo Lee[1], Sanghyun Park[2], Kyungdo Han[2], Sang-Hak Lee[1*]**

[1] Cardiology, Yonsei University College of Medicine, Korea
[2] Biostatistics, The Catholic University of Korea, Korea

*shl1104@yuhs.ac*

Familial hypercholesterolemia (FH) is a critical global health issue. However, a substantial portion of patients with FH are not diagnosed and treated properly, at least partly from insufficient data of specific area of the world. The aim of this study was to investigate the prevalence, cardiovascular risk, and treatment benefit of patients with FH using large-scale nationwide data in Korea.

From the Korean National Health Insurance Service, a nationwide population-based cohort of 2,644,004 adults aged >20 years, with lipid profile measurements, with or without atherosclerotic cardiovascular disease, were followed-up until the date of death or cardiovascular events (myocardial infarction, ischemic stroke, and coronary revascularization). The phenotype of FH was defined using the cutoff values of low-density lipoprotein-cholesterol >190, >225, or >260 mg/dL from prior domestic or international references. The intensity of lipid-lowering therapy (LLT) was classified to four grades (high, higher moderate, lower moderate, and low). In statin-users, baseline LDL-C levels were estimated by multiplying the value by 1.43.

Among 2,407,135 (91.0%) of statin-naïve individuals, 1.38%, 0.20%, and 0.04% showed to have LDL-C >190, >225, or >260 mg/dL, respectively. In this population, the adjusted risk ratio of coronary events compared to individuals with LDL-C <190 mg/dL were 1.56, 1.98, and 2.61, respectively, in patients with each LDL-C levels. Those of death were 1.50, 1.82, and 3.26 in patients with each LDL-C levels. Determinants of clinical outcomes including statin intensity in patients with FH phenotype will be presented in the congress.

The prevalence of Korean patients with FH was similar to international data, when the cutoff value of LDL-C was 225 mg/dL. Adjusted cardiovascular risk in this population was remarkably higher and was influenced by several clinical factors.

Symposium 20-6 Short Talk

# Characteristics of Korean Patients With Familial Hypercholesterolemia: KFH Registry Data 2020

**Hyoeun Kim[1], Chan Joo Lee[2], Sang-Hyun Kim[3], Jang Young Kim[4], Sung Hee Choi[5], Hyun-Jae Kang[3], Kyong Soo Park[5], Byung Ryul Cho[6], Byung Jin Kim[7], Ki Chul Sung[7], In-Kyung Jeong[8], Jin-Ok Jeong[9], Jang-Whan Bae[10], Ji Hyun Lee[11], Sang-Hak Lee[2*]**

[1] Health Promotion, Yonsei University College of Medicine, Korea
[2] Cardiology, Yonsei University College of Medicine, Korea
[3] Cardiology, Seoul National University College of Medicine, Korea
[4] Cardiology, Yonsei University Wonju College of Medicine, Korea
[5] Endocrinology, Seoul National University College of Medicine, Korea
[6] Cardiology, Kangwon University College of Medicine, Korea
[7] Cardiology, Sungkyungkwan University School of Medicine, Korea
[8] Endocrinology, Kyung Hee University School of Medicine, Korea
[9] Cardiology, Chungnam National University School of Medicine, Korea
[10] Cardiology, Chungbuk National University College of Medicine, Korea
[11] Clinical Pharmacology and Therapeutics, Kyung Hee University School of Medicine, Korea

*Shl1106@yuhs.ac*

Familial hypercholesterolemia (FH) is considerable clinical burden. This genetic disease cause phenotypes in common regardless of the ethnicities of affected individuals. However, characterization of patients in specific population is still important to properly diagnose and manage them. The aim of this study was to register and analyze clinical and genetic features of Korean patients with FH.

A total of 279 patients suspicious of heterozygous FH were enrolled in 16 university hospitals in Korea from 2009 to 2020. Inclusion criteria were any of three following conditions: 1) fulfillment of Simon Broome criteria, 2) low-density lipoprotein-cholesterol (LDL-C) levels >190 mg/dL plus family history of hypercholesterolemia or coronary artery disease (CAD), and 3) LDL-C levels >225 mg/dL. Sequencing of LDLR, APOB, and PCSK9 was performed using whole exome-sequencing or targeted exome sequencing. Determinants of pathogenic mutations were identified by multivariate analyses.

.The mean age of the patients was 51.2 years and males were 49.8%. Premature CAD were present in 20.1%, whereas 19.0% of the study population had tendon xanthoma. The mean LDL-C levels were 232 mg/dL. Pathogenic mutations in any of three genes were found in 102 patients (36.6%). Among them, 91 (89.2%), 5 (4.9%), and 6 (5.9%) revealed mutations in LDLR, APOB, and PCSK9 genes, respectively. Compared to mutation-negative patients, mutation carriers were younger and had more premature CAD and xanthoma. Their LDL-C levels were higher, whereas their triglyceride and high-density lipoprotein-cholesterol levels were lower. In multivariate analyses, age (hazard ratio [HR]: 0.97, p=0.029), xanthoma (HR: 3.06, p=0.019) and LDL-C levels (HR: 1.014, p=0.001) were identified as determinants of pathogenic mutations.

Korean patients with FH showed clinical and genetic features largely similar to those of other countries with minor differences. This largest-to date Korean FH data will be helpful to develop more tailored clinical guidelines for this population.

# Functional Diversity and Modulation of Macrophages in Organ Crosstalk and Cardiovascular Disease

**Ichiro Manabe**[1*]

[1] Disease Biology and Molecular Medicine, Chiba University Graduate School of Medicine, Japan

*manabe-tky@umin.ac.jp*

Macrophages are versatile cells that play central roles in physiological and pathological processes in the cardiovascular system. We previously showed that cardiac resident Ly6C$^{lo}$ macrophages are essential for the proper adaptive response to left ventricular pressure overload. Importantly, Ly6$^{lo}$ macrophage activation is regulated by the heart-brain-kidney network, suggesting that macrophages are the key component of the organ crosstalk. We have found that macrophages crucially contribute to cardiac homeostasis by multiple mechanisms, including the maintenance of electrical conduction. However, cardiac macrophages may also promote tissue remodeling and heart failure. Interestingly, cardiac and metabolic stress and aging modulate macrophage functions in the cardiovascular system, which may impair cardiovascular homeostasis. Moreover, we found that the organ system crosstalk involving the immune and nervous systems underlie the changes in macrophage functions and diversities in the cardiovascular system.

Symposium 21-2

# Single Cell Perspective of Cardiac Macrophages in Health and Disease

**Slava Epelman**[1*]

[1] Division of Cardiology, University Health Network, Canada

*slava.epelman@uhn.ca*

Cardiovascular disease is the leading cause of mortality worldwide. Cardiac injury, either ischemic or non-ischemic in origin, results in adverse remodeling and dysfunction which manifests clinically as heart failure. A key modulator of cardiac disease is the innate immune system. Macrophages (MFs) are the most abundant immune cells residing in the heart, orchestrating both inflammatory and reparative cascades. However, discriminating origins and functional responses of MFs remains challenging. Using single cell transcriptomics and genetic lineage tracing, we have shown that cardiac MFs are transcriptionally heterogeneous populations tracing back to divergent origins: Embryonic-derived resident MFs are established prenatally and persist into adulthood without monocyte input through self-renewal, whereas recruited MFs are continuously replenished by adult bone marrow monocytes. We use genetic fate mapping to precisely track self-renewing resident cardiac MFs to establish their functional role in the setting of ischemic injury or hypertensive challenge. Single-cell RNA sequencing of fate-mapped resident cardiac MFs revealed ten transcriptionally diverse cell states at the steady state. Hypertensive stress elicited numerical expansion and complex transcriptional reorganization with a preferential expansion of cell states enriched in reparative pathways and the upregulation of key reparative programs. Inducible depletion of resident MFs led to cardiac dysfunction and pathological fibrosis during chronic hypertension. Conversely, ischemic injury reduced resident cardiac MF abundance within infarcted tissue, while recruited monocyte derived MFs infiltrated the myocardium and adopted numerous cell fates, including those indistinguishable from resident MFs at the single cell level. However, inducible ablation of resident MFs led to impaired cardiac function and promoted adverse cardiac remodeling following myocardial infarction. Taken together, these data illustrate the dynamic reorganization and response of resident cardiac MFs upon cardiac injury and their cardioprotective functions.

# Phagocyte Regulation of Cardiac and Vascular Inflammation

**Edward Thorp**[1*]

[1] Pathology, Northwestern University, USA

*ebthorp@northwestern.edu*

Phagocyte macrophages accumulate in the ischemic and failing heart and contribute to cardioprotection or cardiac damage. The therapeutic potential of targeting cardiac macrophages hinges on the understanding of the molecular regulators of macrophage function and cross talk with the myocardium and cardiac vasculature. Cell mechanisms of macrophage function that are relevant to cardiac inflammation and crosstalk with cardiomyocytes and cardiac vessels will be discussed.

Symposium 21-4

# Single Cell Landscapes of Hyperlipidemic Cardiovascular Diseases

**Jae-Hoon Choi**[1*]

[1] Department of Life Science, College of Natural Sciences, Hanyang University, Korea

*jchoi75@hanyang.ac.kr*

Hyperlipidemia is a pivotal risk factor of various cardiovascular diseases including atherosclerosis and aortic valve sclerosis and stenosis. The infiltration of immune cells and local lipid accumulation are prominent features of atherosclerosis and aortic valve sclerosis. To understand the pathophysiology of hyperlipidemic cardiovascular diseases, we performed single cell RNA seq analyses on murine atherosclerosis and valvular sclerosis. First, scRNA-seq analysis of CD45+ leukocytes from murine atherosclerotic aorta revealed that there are macrophage subpopulations having distinct differentially expressed genes involved in various functional pathways. To specifically characterize the intimal foamy macrophages of plaque, we developed a lipid staining-based flow cytometric method for analyzing the lipid-laden foamy cells of atherosclerotic aortas. We employed the fluorescent lipid probe, BODIPY493/503, and detected side-scattered light (SSC) as an indication of cellular granularity. BODIPYhiSSChi foamy macrophages were residing in intima and expressed CD11c, and the foamy macrophage accumulation determined by flow cytometry was positively correlated with the severity of atherosclerosis. Bulk RNA-seq analysis showed that the foamy macrophages expressed few inflammatory genes but many lipid-processing genes compare to the non-foamy macrophages. Intimal non-foamy macrophages were the major population expressing IL-1β and many other inflammatory transcripts in atherosclerotic aorta. RNA-seq of intimal macrophages from atherosclerotic aorta reveals that lipid-loaded plaque macrophages are not likely the plaque macrophages that drive lesional inflammation. Second, we performed scRNA seq analysis on sclerotic aortic valves and found cellular heterogeneity of total valvular cells including immune and non-immune cells. I will also discuss about our new findings related pathogenesis of valve sclerosis.

# CGRP and Its Receptor, RAMP1 Regulate Lymphangiogenesis in the Pathological Conditions

**Masataka Majima**[1*]

[1] Department of Molecular Pharmacology, Graduate School of Medical Sciences, Kitasato University, Japan

*mmajima@med.kitasato-u.ac.jp*

Calcitonin gene-related peptide (CGRP) regulates inflammation via signaling through receptor activity-modifying protein (RAMP) 1. Here, we investigated the role of RAMP1 signaling in growth of lymphatic vessels during inflammation. Further we clarified the roles of RAMP1 in the development of endometriosis and the lipid absorption from intestines.

Lymphangiogenesis in the diaphragm of RAMP1-deficient (-/-) mice or their wild-type (WT) counterparts was induced by repeated intraperitoneal injection of lipopolysaccharide (LPS). In an endometriosis model, RAMP1-dependent lymphagiogenesis was tested. Further, lipid absorption was tested in RAMP1-/- mice.

Compared with WT mice, LPS-induced lymphangiogenesis in RAMP1-/- mice was suppressed. This was accompanied by the reduced expression of vascular endothelial growth factor (VEGF)-C and VEGF-D. The number of CD4+ cells in diaphragm tissue from WT mice was greater than RAMP1-/- mice. Removing CD4+ cells attenuated lymphangiogenesis and expression of VEGF-C and VEGF-D. CD4+ cells isolated from RAMP1-/- mice exhibited reduced expression of VEGF-C and VEGF-D. The number of CD11b+ cells from RAMP1-/- mice was higher than WT mice and was associated with the upregulated expression of genes related to pro-inflammatory macrophage phenotype and downregulation of reparative macrophage phenotype-related expression. When fluorescein isothiocyanate (FITC)-dextran was injected into the peritoneal cavity, the amount of residual FITC-dextran in WT mice was lower than that in RAMP1-/- mice. Endometriosis development was dependent on lymphngiogenesis. The development of endometriosis was hampered in in RAMP1-/- mice. RAMP1 signaling also regulates lipid absorption from intestines. RAMP1-/- mice showed obesity under high fat diets.

The present results suggest that RAMP1 signaling in immune cells plays a critical role in inflammation-related lymphangiogenesis, and further CGRP/RAMP1 regulated endometriosis development, and lipid absorption from intestines. CGRP/RAMP1 exhibits numerous pathophysiological roles in lymphangiogenesis-related conditions.

Symposium 21-6 Short Talk

# Reduction in Lung Vascular Leakage in Acute Respiratory Distress Syndrome (ARDS) by Tie2 Activation or VE-PTP Inhibition

**Peter Baluk[1*], Minah Kim[2], Gavin Thurston[3], Kevin G. Peters[4], Donald M. McDonald[1]**

[1] Cardiovascular Research Institute, Anatomy Department, University of California, San Francisco, USA
[2] Department of Pathology and Cell Biology, Columbia University, New York, USA
[3] Regeneron Pharmaceuticals, Tarrytown, USA
[4] Aerpio Therapeutics, Cincinnati, USA

*peter.baluk@ucsf.edu*

Acute respiratory distress syndrome (ARDS) is a life-threatening condition associated with COVID-19, influenza viral pneumonia, and sepsis. Impaired endothelial barrier function in ARDS leads to vascular leakage, edema, and reduced gas exchange in the lung. Angiopoietin-1 (Ang1) and its receptor Tie2 play key roles in regulating vascular permeability by modulating endothelial cell junction stability. Vascular endothelial protein-tyrosine phosphatase (VE-PTP) suppresses Tie2 signaling by reducing Tie2 phosphorylation, which can make blood vessels prone to leakage. Tie2 activation by Ang1 mimetics or VE-PTP inhibitors provide potential strategies for reversing endothelial barrier dysfunction. This study examined effects of viral infection on Tie2 signaling and plasma leakage in lungs of mice and determined whether lung vascular leakage in ARDS after endotoxin (lipopolysaccharide, LPS) can be reduced by Tie2 activation or VE-PTP inhibition.

Lung vascular leakage was assessed in mice at 7 days after H1N1 influenza virus A infection or 6 hours after LPS (5 mg/kg intratracheally). Tie2 activity was monitored by phospho-Tie2 Y992 immunoreactivity. Plasma leakage in the lung was assessed by extravasation of Evans blue dye or by fibrinogen immunoreactivity. Tie2 was activated by BowAng1 (25 mg/kg ip, Regeneron) or by inhibiting VE-PTP (AKB-9785, 30 mg/kg subcutaneously twice in 6 hours, Aerpio) before LPS.

Extravasated fibrinogen was sparse in the lung at baseline. At 7 days after influenza infection, widespread regions of extravasated fibrinogen coincided with areas of low phospho-Tie2, and Evans blue leakage was 1.7-fold baseline. Evans blue leakage at 6 hours after LPS was 6-fold baseline but was reduced 37% by BowAng1 and 35% by AKB-9785 ($P < 0.05$, ANOVA).

We conclude that lung vascular leakage in ARDS occurs at sites of low phospho-Tie2 in endothelial cells and can be reduced by activation of Tie2 phosphorylation with an Ang1 mimetic or VE-PTP inhibitor.

# Immunometabolic Fabric in Atherosclerosis

**Laurent Yvan-Charvet**[1*]

[1] Molecular Medicine, INSERM, France

*Laurent.YVAN-CHARVET@univ-cotedazur.fr*

Macrophages are strategically positioned throughout tissues to act as sentinels and maintain tissue homeostasis. Hypercholesterolaemia contributes to chronic inflammatory responses during atherosclerosis progression in part by promoting cholesterol accumulation in macrophages and other immune cells. However, macrophages handle changes in plaque microenvironment and face a substantial amount of other lipid or nutrients after ingestion of modified lipoproteins or apoptotic cells increasing their cellular contents and metabolic load during disease progression. Thus, the concept of metabolic shift in response to environmental stresses, initially attributed to cancer cells, has been extended to macrophages. This presentation explores whether and how metabolic load could shape the metabolic rewiring of macrophages in atherosclerosis to modulate immune- effector and tissue-reparative functions.

Symposium 22-2

# Endothelial Cell Regulation of Macrophage Plasticity

**Asrar Malik**[1*]

[1] Pharmacology & Regenerative Medicine, University of Illinois College of Medicine in Chicago, USA

*abmalik@uic.edu*

Macrophages demonstrate remarkable plasticity that is essential for host-defense and tissue repair. The tissue niche imprints macrophage identity, phenotype, and function. The role of vascular endothelial signals in tailoring the phenotype and function of tissue macrophages remains unknown. The lung is a highly vascularized organ and replete with a large population of resident macrophages. We found that in response to inflammatory injury, lung endothelial cells release the Wnt signaling modulator Rspondin3 which activates β-catenin signaling in lung interstitial macrophages and increases mitochondrial respiration by glutaminolysis. The generated tricarboxylic acid cycle intermediate α-ketoglutarate, in turn, serves as the cofactor for the epigenetic regulator TET2 to catalyze DNA hydroxymethylation. Notably, endothelial-specific deletion of Rspondin3 prevented the formation of anti-inflammatory interstitial macrophages in endotoxemic mice and induced unchecked severe inflammatory injury. Thus, the angiocrine-metabolic-epigenetic signaling axis specified by the endothelium is essential for reprogramming interstitial macrophages and dampening inflammatory injury.

# Longitudinal Intravital Cellular-Level Imaging of Vascular System

**Pilhan Kim**[1*]

[1] Graduate School of Medical Science and Engineering, KAIST, Korea

*pilhan.kim@kaist.ac.kr*

IVIM Technology's All-in-One IntraVital Microscopy system (IVM-C/CM/MS) enables dynamic 3D cellular-level imaging of various biological processes in living animals in vivo. It enables scientists to directly verify hypotheses derived from ex vivo or in vitro observations in natural physiological in vivo microenvironments. Using intravital microscopy, in vivo visualizations of gene expression, protein activity, cell trafficking, cell-cell / cell-microenvironment interactions and various physiological responses to stimuli have been achieved providing imperative novel insights, which have been impossible to obtain with conventional static 2D observation of ex vivo or in vitro samples. Additionally, it is possible to directly analyze the delivery and efficacy of new biopharmaceuticals such as antibodies, cell therapy, gene therapy, nucleic acids and exosome in an in vivo microenvironment.

IVIM Technology's IVM series are extensively optimized and carefully engineered to ensure superb performance in the intravital imaging of live animal models in vivo, providing multi-color sub-micron resolution ream-time fluorescence images. Key indispensable functionalities for intravital imaging are fully integrated into the All-in-ONE system with attentive design for smooth and easy operation. A versatile S/W, IVIM Engine and Studio, comes with unique features for intravital imaging including ultrafast image acquisition to capture fast real-time dynamics, high-precision tissue motion compensation enabled by optimized a registration algorithm, GPU-assisted parallel computing for rapid image processing. The world's first All-in-ONE intravital microscopy platform from IVIM Technology is a key solution that can explore complex dynamic behaviors of numerous cells inside a living body.

In this talk, intravital microscopic imaging of various organs including skin, liver, spleen, pancreas, kidney, small intestine, colon, retina, lung, heart, lymph node, and bone marrow will be briefly introduced. Subsequently, recent studies utilizing the real-time intravital imaging technique to investigate dynamic cellular-level pathophysiology of various human diseases will be introduced.

Keyword: Intravital microscopy, In vivo imaging, Fluorescence imaging, Confocal microscopy, Two-photon microscopy

Symposium 22-4 Short Talk

# Vascular Control of Metastatic Colonization

**Mahak Singhal[1,2], Nicolas Gengenbacher[1,2], Ashik Ahmed Abdul Pari[1,2], Miki Kamiyama[1,2], Ling Hai[3], Bianca Kuhn[4], Eva Besemfelder[1], Barbara Leuchs[6], Jeroen Krijgsveld[4], Matthias Schlesner[3], Junhao Hu[7], Stephen E. Moss[5], John Greenwood[5], Hellmut G. Augustin[1,2*]**

[1] Vascular Oncology and Metastasis, German Cancer Research Center, Germany
[2] Vascular Biology and Tumor Angiogenesis, European Center for Angioscience (ECAS), Germany
[3] Junior Group Bioinformatics and Omics Data Analytics, German Cancer Research Center, Germany
[4] Proteomics of Stem Cells and Cancer, German Cancer Research Center, Germany
[5] Department of Cell Biology, UCL, Institute of Ophthalmology, UK
[6] Vector Development & Production Unit, German Cancer Research Center, Germany
[7] Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China

—

*h.augustin@dkfz.de*

Metastasis is the primary cause of cancer-related mortality and the mechanistically least well understood step of the tumour progression cascade. Tumour cell interactions with cells of the vessel wall are decisive and rate-limiting for metastasis. The past decade has witnessed a fundamental change of paradigm from blood vessel wall-lining endothelial cells (EC) being conceived as merely supportive of angiogenesis to an active gatekeeper and modulator of the tumour microenvironment. The molecular nature of this crosstalk is beyond candidate gene approaches hitherto poorly understood. Employing surgical models of lung metastasis in temporal systems biology-based screens, we show here that primary tumours systemically reprogram the body's vascular endothelium to perturb homeostasis and to precondition the vascular niche for metastatic colonization. The vasculature with its enormous surface thereby served as amplifier of tumour-induced instructive signals. The combined endothelial transcriptomic and serum proteomic screen identified the TGFβ pathway signalling specifier LRG1 as an early instructor of metastatic colonization. Systemic upregulation of LRG1 promoted metastasis by increasing the number of prometastatic NG2+ perivascular cells. In turn, adjuvant LRG1 inhibition in primary tumour-resected mice delayed metastatic growth and increased overall survival. The study has thereby established the systems map of early primary tumour-induced vascular changes and identified LRG1 as a therapeutic target for metastasis.

- Comparative transcriptomic and proteomic screens identified LRG1 as one of the novel angiocrine instructors of metastasis
- Multi-organ vascular endothelium serves as an amplifier of primary tumor-derived instructive signals
- Administrating LRG1-neutralizing antibody improved overall survival in clinically-relevant therapeutic regimens

# The Transcription Factor SRF Regulates Pericyte Migration During Retinal Angiogenesis

**Michael Orlich[1,3*], Rodrigo Diéguez-Hurtado[2], Ralf H. Adams[2], Alfred Nordheim[1]**

[1] Molecular Biology, Interfaculty Institute for Cell Biology , Tuebingen University, Germany
[2] Department of Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Germany
[3] Max-Planck research school, IMPRS "From molecules to organisms", Tuebingen, Germany

*michael.orlich@gmx.de*

Serum Response Factor (SRF) is a ubiquitously expressed transcription factor, that regulates the transcription of about 1000 genes. Endothelial cell specific deletion of SRF has been shown to cause the formation of hemorrhages in the neonatal murine brain and microaneurysms in the retina, but its physiological role in mural cells (MCs) remains unknown. MCs wrap around blood vessels and play important roles in angiogenesis, vessel stabilization and homeostasis. They are essential to maintain the integrity of the blood brain barrier and play important roles in numerous diseases. To investigate the role of SRF in MCs, we established Srf-flex-1::Pdgfbr-CreERT2 mice and studied the consequences of MC-specific SRF deletion in the postnatal mouse retina.

Retinal angiogenesis, Microscopy, RNAseq

We found that MCs lacking Srf adopt an abnormal morphology, lose the expression of smooth muscle actin and fail to properly co-migrate with angiogenic vascular sprouts. Blood vessels at the sprouting front remain deprived from MCs, become dilated and appear to lose their barrier properties, as red blood cells extravasate in the surrounding tissue. Most interestingly, however, from postnatal day 12 (P12), these mice also develop arterio-venous shunts which become increasingly severe with age. Interestingly, those malformations resemble arterio-venous malformations described in animal models of Hereditary Hemorrhagic Telangiectasia (HHT), but develop substantially later. MCs that lack SRF accumulate around the malformed vessels, while overall MC coverage in the adjacent unaffected vasculature becomes reduced. In vitro experiments with primary isolated MCs suggest that a lack of Srf leads to cytoskeletal defects and a failure of MCs to migrate.

Taken together, our data suggest that, in the absence of Srf, MCs acquire a disease-promoting competence which is not phenocopied by MC ablation. Our ongoing studies now aim to investigate the involvement of MCs in the development of vascular malformations such as in HHT.

Symposium 23-1

# Macrophage Exosomes: New Mediators to Control Hematopoiesis, Monocyte Activation & Atherosclerosis

**Robert L. Raffai[1,2*], Laura Bouchareychas[1,2], Phat Duong[2], Sergio Covarrubias[3], Eric Alsop[4], Allen Chung[2], Michael Gomes[2], David Wong[2], Bessie Meechoovet[4], Allyson Capili[3], Ryo Yamamoto[5,6], Michael McManus[7], Susan Carpenter[3], Kendall Van Keuren-Jensen[4]**

[1] Department of Surgery, UCSF, USA
[2] Northern California Institute, for Research and Education & VA Medical center, USA
[3] Research and Education & VA Medical center, University of California Santa Cruz, USA
[4] Neurogenomics, The Translational Genomics Research Institute (TGen), Phoenix, USA
[5] Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, USA
[6] Department of Genetics, Stanford University School of Medicine, USA
[7] Department of Microbiology and Immunology, UCSF Diabetes Center, University of California, USA

*Robert.Raffai@ucsf.edu*

We tested whether exosomes produced by M2-like anti-inflammatory macrophages can control atherosclerosis in hyperlipidemic mice.

Exosomes were isolated from the cell culture medium of naïve bone marrow derived macrophages (BMDM-exo) and BMDM exposed to interleukin 4 (IL-4) to polarize them into an M2 phenotype (BMDM-IL-4-exo).

BMDM-IL-4-exo reduced Tnf and Il1b mRNA expression in cultured macrophage, while increasing their M2 differentiation including by fostering mitochondrial oxidative metabolism. Treatment of Apoe–/– mice fed a western diet with BMDM-IL-4-exo for 4 weeks resulted in their uptake and microRNA cargo delivery in numerous tissues including the bone marrow and aorta. This suppressed hematopoiesis in the bone marrow and reduced neutrophils and Ly6Chi monocytes in the circulation. This also increased the expression of M2-markers in peritoneal macrophages, improved inflammatory gene expression in Ly6Chi monocytes and suppressed lesion inflammation by reducing the necrotic core area and the number of macrophages in aortic root lesions. Unbiased RNA sequencing revealed an enrichment of microRNA-99a/146b/378a in BMDM-IL-4-exo. Gene editing to selectively knock down each one of these microRNA in IL-4 polarized macrophages resulted in exosomes that were less effective in reducing NF-κB signaling and TNFκexpression in recipient macrophages.

Our findings reveal that M2-like macrophage exosomes are potent anti-inflammatory mediators that can control atherosclerosis through the delivery of select microRNA to recipient cells within the hematopoietic system and vessel wall.

# Bacterial Exosomes as Next-Generation Cancer Immunotherapy

**Yong Song Gho**[1*]

[1] Department of Life Sciences, POSTECH, Korea

*ysgho@postech.ac.kr*

Communication between cells and environment is an essential process in living organisms. The secretion of extracellular vesicles is a universal cellular process occurring from simple organisms to complex multicellular organisms, including humans. Throughout evolution, both prokaryotic and eukaryotic cells have adapted to manipulate extracellular vesicles for intercellular communication via outer membrane vesicles in the case of Gram-negative bacteria and ectosomes (also known as microvesicles) or exosomes in eukaryotic cells. Recent progress in this area has revealed that extracellular vesicles play multiple roles in intercellular and interspecies communication, suggesting that extracellular vesicles are NanoCosmos, i.e., extracellular organelles that play diverse roles in intercellular communication (http://evpedia.info). This presentation will briefly introduce the state-of-art exosome research and our recent progress in novel mammalian and bacterial exosome-mimetic technology for targeted drug delivery, theranostics, and epigenetic reprogramming as well as for adjuvant-free, non-toxic vaccine delivery system against bacterial infection. Moreover, basic research and clinical application on bacterial exosome-based cancer immunotherapy will be introduced.

Symposium 23-3

# Circulating Extracellular Vesicles a Potential Prognostic Marker of Atherosclerosis-Related Lymphatic Dysfunction

**Catherine Martel**[1*]

[1] Medicine, Université de Montréal, Canada
[2] Research Center, Montreal Heart Institute, Canada

*catherine.martel@icm-mhi.org*

Cardiovascular events can occur in asymptomatic subjects devoid of traditional risk factors, emphasizing the need for the identification of new biomarkers for the prevention of cardiovascular disease (CVD). Lymphatic vessels are present in the adventitial layer of atherosclerotic arteries and their proper function has been shown to be essential to mobilize cholesterol out of the vessel wall before it reaches the blood circulation. Further, lymphatic dysfunction can occur before the atherosclerosis plaque onset, the main underlying cause of CVD, and sustaining the contractile capacity of collecting lymphatic vessels early on in the disease process delays plaque buildup and limits its progression. Thus, understanding the mechanisms responsible for this prompt defect in lymphatic function may contribute to the prevention and treatment of atherosclerosis. Extracellular vesicles (EVs) are released by cells under physiological and pathological and are thought to contribute to vascular endothelial dysfunction, one of the first step of atherosclerosis. EVs are present in mouse lymph, and their levels increase during atherosclerosis. Whereas current studies focus on role EVs in the onset and progression of atherosclerosis, the interaction between EVs and the lymphatic system during atherosclerosis is understudied. Regardless of how EVs would get in lymph, we believe they could disturb lymphatic function and therefore modify lymph flow. This could result in the accumulation of EVs within the lymphatic vessels, which would enhance the effect of EVs on lymphatic endothelial cells and create a feedback loop. Altogether, these sequential events would potentially contribute to the instigation of the lymphatic transport impairment that precedes the onset of atherosclerosis, and suggest that EVs could be at the interplay between lymphatic function and atherosclerosis.

# Engineered Exosomes for Intracellular Biologics: Principles and Application of EXPLOR Technology

**Chulhee Choi**[1*]

[1] ILIAS Biologics Incorporated, Korea

*ccatkaist@gmail.com*

Our group has recently developed an opto-genetically engineered exosome system, named 'exosomes for protein loading via optically reversible protein–protein interaction" (EXPLOR) that can deliver soluble proteins into the cytosol of target cells via controlled, reversible protein–protein interactions (PPI). By integrating a reversible PPI module controlled by specific wavelength of light with the endogenous process of exosome biogenesis, cargo proteins of our interest can be loaded into newly generated exosomes. Protein-loaded exosomes were shown to significantly increase intracellular levels of cargo proteins and their function in recipient cells in both a time- and dose-dependent manner. In this presentation, I will introduce the basic principles of EXPLOR technology and follow-up studies for therapeutic applications.

Symposium 23-5 Short Talk

# Tip Cell Invasive Behaviour Is Controlled by Myosin IIA-Dependent Inhibition of Arp2/3 Activity

**Claudio Franco**[1*]

[1] Vascular Morphogenesis Lab, Instituto de Medicina Molecular, Portugal

*cfranco@medicina.ulisboa.pt*

Sprouting angiogenesis is fundamental for development and disease, including cancer, diabetic retinopathy and cardiovascular diseases. Sprouting angiogenesis depends on the invasive properties of endothelial tip cells. However, the current concept assumes that sprouting angiogenesis is based on a universal endothelial tip cell invasive profile.

To identify how endothelial tip cells invade into tissues, we used reporter mouse models to visualise actin dynamics (LifeAct-GFP), membrane dynamics (R26-mTmG) and myosin-IIA (MIIA-GFP), in combination with genetic mouse models which allow selective inactivation of MII-isoforms and the actin-related protein 2/3 (Arp2/3) complex in endothelial cells. In addition, zebrafish embryos, optogenetics, and cell culture systems were used to investigate the molecular mechanisms regulating tip cell invasion behaviour.

We disclose that endothelial tip cells use long lamellipodia projections (LLPs) as a general mechanism to invade avascular tissues. LLPs and filopodia protrusions are balanced by myosin-IIA (MIIA) and actin-related protein 2/3 (Arp2/3) activity. Endothelial cell-autonomous ablation of MIIA promotes excessive LLPs formation in detriment of filopodia. Conversely, endothelial cell-autonomous ablation of Arp2/3 prevents LLPs development and leads to excessive filopodia formation.

Our discoveries identified how endothelial tip cells invade into tissues and demonstrate the existence of filopodia-sensitive and filopodia-insensitive sprouting angiogenesis in vivo. This work may pave the way towards new strategies to block invasive tip cells during sprouting angiogenesis.

# Customized Vasculatures – Lessons From Organoids and Embryos

**Ondine Cleaver[1*], Anne Ryan[1], Edward Daniel[1], Mitzy Cowdin1,Max Hiltabidle1, Berfin Azizoglu1, Christopher Chaney[1], Thomas Carroll[1]**

[1] Department of Molecular Biology, UT Southwestern Medical Center, USA

*Ondine.Cleaver@UTSouthwestern.edu*

Chronic kidney disease (CKD) and end stage renal disease (ESRD) are increasingly frequent and devastating conditions that have driven a surge in the need for kidney transplants. This stark shortage of organs has fueled the interest in generating viable replacement tissues ex vivo for transplantation. One promising approach has been self-organizing organoids, which mimic developmental processes and yield multicellular, organ-specific tissues. A recognized roadblock with this approach, however, is that many organoid cell types fail to acquire full maturity and function. We propose this is part due to their failure to properly vascularize. We comprehensively assess the vasculature of kidneys during development, and we examine the vasculature in two distinct kidney organoid models. Using a variety of methods, we show that organoids develop a full range of kidney cell types, as previously shown. However, while angioblasts and endothelial cells (ECs) arise during organoid differentiation, and the vasculature initially takes shape, it rapidly regresses over time in culture likely due to the absence of hemodynamic flow. Engraftment of embryonic kidneys or kidney organoids under the kidney capsule results in maintenance of vascularization. Interestingly, implanted organoid vasculature consists primarily of invading host vessels. Together this work demonstrates that kidney organoids can be used as a model system to define the complexities of vascular-nephron interactions, although rapid vascularization and blood flow are required for maintenance of the vasculature.

Symposium 24-2

# Identifying Novel Modifier Genes Related to Naturally-Occurring Heterogeneity of Lymphatics Within a Diverse Genetic Population

**Wenjing Xu**[1], **Kathleen Caron**[1*]

[1] Cell Biology and Physiology, Univ. of North Carolina at Chapel Hill, USA

*kathleen_caron@med.unc.edu*

Inter-individual and sex-based differences in lymphatic vessel density, anatomy and function have been described in humans. Moreover, several clinical studies illustrate that the naturally-occurring heterogeneity of lymphatics among individuals impacts the development and severity of lymphatic-related diseases.

To better characterize baseline levels of lymphatic variation and reveal potential modifier genes related to lymphatic heterogeneity, we exploited the powerful genetic resource of the Collaborative Cross (CC). Compared to commonly employed laboratory animal strains with limited genetic variation, the CC is a genetically diverse recombinant inbred panel derived from 5 common and 3 wild inbred strains, ultimately segregating ~42 million genetic variants, which is double that observed in human populations.

By performing quantitative analysis, we have characterized the wide distribution in dermal capillary lymphatic diameter, cardiac lymphatic density and distribution, and tail lymph flow rates for both male and female mice from 16 independent CC strains. More interestingly, we also discovered an effect of biological sex on the structure and function of lymphatics, which we further identify as an inherent factor associated with cardiac function and imparting protection against the development of myocardial edema. Genome-wide sequencing and genetic mapping permitted the identification of discrete chromosomal regions and loci that govern the heterogeneity of lymphatics at an individual level.

Our study is the first to demonstrate both the inter-individual differences and sex-dependent effects in mouse lymphatics within a diverse genetic population. Findings from this study provide foundational knowledge for revealing modifier loci that can influence lymphatic heterogeneity and function in humans and potentially reveal novel therapeutic targets for the fine-tuned control of lymphatic growth, development and function.

# Hypoxia-Inducible Transcription Factors Regulate Brain Capillary Development

**Wiebke Herzog[1,2*], Friedemann Kiefer[2], Claudia Quiñonez-Silvero[1,2]**

[1] Angiogenesis lab / Developmental Biology, Max Planck Institute for Molecular Biomedicine/University of Erlangen-Nuernberg, Germany
[2] University of Münster, Münster, Germany

*wiebke.herzog@uni-muenster.de*

Hypoxia inducible factors (Hif) transcriptionally can regulate many physiological processes, including vascular expansion and endothelial cell migration through the regulation of proangiogenic growth factors.
To better understand the role of Hif signaling during angiogenesis we developed a novel transgenic reporter line in zebrafish that allows us to detect Hif pathway activation in vivo at cellular resolution. The reporter consists of five repeats of the hypoxia-response elements (HRE) that upon binding of Hif induce transcription of UnaG, an oxygen-independent fluorophore.
Our reporter shows Hif activity during development under physiological conditions. We observe endothelial Hif activation in brain capillaries, starting when the sprouts migrate to invade the brain parenchyma. We do not observe activation of the pathway during sprouting and migration in other vascular beds, suggesting that endothelial Hif signaling specifically regulates brain angiogenesis. Global as well as endothelial specific overactivation of the Hif pathway results in dilated brain vessels, but does not affect vessel caliber in other vascular beds, in line with a brain specific function. Individual dilated cells in mosaic endothelial specific gain of function experiments suggest that the physiological response to Hif activation is cell autonomous. We can further dissect the mechanism of dilation to be linked to endothelial cell shape changes and vascular remodeling as well as to increased Kdrl/Vegf receptor 2 signaling. We are currently dissecting the signaling pathways that link Hif activation to the dilation and aim to resolve the function of Hif activation specifically in developmental brain angiogenesis.

Symposium 24-4 Short Talk

# Negative Regulation of VEGFC Transcription by Sox7 in Blood Vessels Is a Key Determinant to Lymphatic Morphogenesis

**Mat Francois**[1*]

[1] The Centenary Institute, The University of Sydney, Australia

*m.francois@centenary.org.au*

The functional assembly of the lymphatic vasculature in the embryo relies on its interplay with other tissues and the integration of growth signalling pathways. A key signalling axis of lymphatic vessels morphogenesis is vascular endothelial growth factor C (VEGFC) which instructs network patterning. Little is known about the cellular source of VEGFC during embryogenesis. The objective of this work is to identify a cellular source of VEGFC during embryogenesis

We take advantage of classic mouse gain and loss of function to assess the role of the SOX7 transcription factor in vivo in blood endothelial in the control of VEGFC transcription.

This work combines genetics, genomics and biophysics approaches to elucidate the molecular function of Sox7

Here, we show that genetic disruption of the blood-endothelial specific transcription factor Sox7 leads to an aberrant formation of dermal lymphatic vessels. Analysis of VEGFC mRNA levels after Sox7 gene depletion both in vivo and in vitro reveals that Sox7 negatively regulates Vegfc transcription in blood endothelial cells (BECs). In vivo ChIP-seq for SOX7 identifies a distal element 252Kb upstream of VEGFC which harbours repressive marks, suggesting a direct transcriptional repression. A further indicator of Sox7 inhibitory function is highlighted by a direct protein-protein interaction with HEY1, a key repressor of the Notch1 pathway. Akin to Notch1 gene disruption, endothelial-specific loss of Sox7 function deeply perturbs the organisation of LEC progenitors in the cardinal vein.

This study reveals that the heterotypic relationship between blood and lymphatic vessels is at least in part mediated by VEGFC, and that Sox7 is a molecular effector required to fine tune the transcription levels of this growth factor to govern lymphatic vessel morphogenesis.

# Transcriptional Regulation and Physiological Significance of ALK1 Signal Target Genes in Embryonic Vascular Endothelial Cells

**Osamu Nakagawa[1*], Yusuke Watanabe[1], Akihiro Urasaki[1], Toru Tanaka[1], Daiki Seya[1], Norika Liu[1], Shoko Tamura[1], Dai Ihara[1,2], Yukihiro Harada[1,2], Teruhisa Kawamura[2]**

[1] Molecular Physiology, National Cerebral and Cardiovascular Center Research Institute, Japan
[2] Biomedical Sciences, Ritsumeikan University College of Life Sciences, Japan

*osamu.nakagawa@ncvc.go.jp*

Bone morphogenetic protein 9 (BMP9)/BMP10-ALK1 receptor signaling plays essential roles in embryonic vascular development mainly through transcriptional regulation of downstream target genes.
Novel ALK1 signal target genes were identified by analyzing gene expression profiles in cultured human endothelial cells treated with BMP9, followed by the characterization of their transcriptional regulation and physiological significance in mouse embryos.
We demonstrated that SGK1 and TMEM100 were novel downstream targets of ALK1 signaling. Expression of SGK1, serum/glucocorticoid regulated kinase 1, in endothelial cells was rapidly up-regulated by the BMP9 treatment through SMAD-mediated transcriptional activation. Sgk1 expression enriched in endothelial cells of mouse embryos was significantly reduced in Alk1/Acvrl1 null embryos. TMEM100, transmembrane protein no. 100, encodes a small transmembrane protein of uncharacterized functions. Mouse embryos with the endothelial specific Tmem100 deletion died due to impaired vascular morphogenesis. TMEM100 expression was markedly but gradually increased by ALK1 signal activation in cultured human endothelial cells, which required de novo production of unspecified signaling protein(s) in addition to or downstream of SMAD-dependent transcription. Additionally, the expression of HEY1 and HEY2 was synergistically up-regulated by the Notch and ALK1 signals. Combined loss of Hey1 and Hey2 in endothelial cells, but not in smooth muscle cells, caused defective vascular formation and early lethality of mouse embryos. Impairment of pharyngeal arch artery development at late embryonic stages and resulting aortic anomalies observed in Hey1 null mice were also reproduced by the endothelial specific Hey1 deletion, but not that in smooth muscle cells. We identified novel endothelial enhancers for Sgk1, Tmem100 and Hey1, each of which was controlled by distinct transcription factors and/or upstream signaling pathways.
Characterization of novel target genes helps understand how ALK1 signaling is involved in normal vascular development and etiologies of human diseases.

Symposium 25-1

# Regulation of Cardiac Robustness by Reactive Sulfide Species

**Motohiro Nishida**[1,2*]

[1] Graduate School of Pharmaceutical Sciences, Kyushu University, Japan
[2] Exploratory Research Center on Life and Living Systems (NIPS), National Institutes of Natural Sciences, Japan

*nishida@nips.ac.jp*

Mitochondria are dynamic organelles that continuously undergo fission and fusion, which are necessary for maintaining bioenergetic homeostasis and robustness in heart. Mitochondrial fission and fusion cycle is precisely regulated by three GTP-binding proteins, dynamin-related protein 1 (Drp1), mitofusins (Mfn1 and mfn2) and optic atrophy 1 (Opa1), and these three G proteins have redox-sensitive cysteine (Cys) residues. Especially, mitochondria predominantly show tubular form in adult cardiomyocytes and are reported to be fragmented by exposure to electrophilic chemical substances. We revealed that depolysulfidation of Cys624 on Drp1, caused by endogenous or exogenous electrophiles, increased basal Drp1 GTPase activity as well as cardiac vulnerability to hemodynamic load in mouse hearts. Reactive sulfide species such as Cys persulfides that are produced through mitochondria-localized Cys tRNA synthetase (CARS2) preferentially contribute to electrophile metabolism. Protein persulfide detection assay revealed that endogenous Drp1 protein possesses several Cys persulfides in a CARS2) dependent manner, and exposure to environmental electrophiles such as methylmercury (MeHg) reduced Drp1 persulfide levels. Supplementation of sulfur to Cys-624 by exogenous treatment with NaHS completely abolished MeHg-induced sulfur deprivation of Drp1 protein as well as exacerbation of myocardial injury induced by mechanical stress. These results strongly suggest that formation of Drp1 Cys624 polysulfidation negatively regulates electrophile-mediated mitochondrial hyperfission and cardiac stress resistance against environmental stresses.

# Role of Mitochondrial Fusion During Cardiac Developments and Heart Energy Metabolism

**Arnaud Mourier[1*], Eduardo Silva Ramos[2], Nils-Göran Larsson[2]**

[1] BioDynaMit, Institut de Biochimie et de Génétique Cellulaires (UMR5095-CNRS), France
[2] Mitochondrial Biology, Max-Planck Institute For Biology of Ageing, Germany

*arnaud.mourier@ibgc.cnrs.fr*

Mitochondria are double membrane organelles, which hold a central role in cell metabolism as they produce the bulk part of the energy currency ATP through the oxidative phosphorylation (OXPHOS) system. Beside their role in energy transduction, mitochondria are also involved in some intermediary metabolism pathways, Calcium homeostasis, cell behaviour, and apoptosis. For yet unclear reasons, mitochondria are highly dynamics and constantly fuse and divide.

Our goal was to decipher the interplay between mitochondrial dynamics and energy metabolism in cardiac development and functions.

To this end we generated heart conditional KO for Mitofusin 1 and 2 to abolish mitochondrial fusion capacity.

The complete loss of mitochondrial fusion causes lethal cardiomyopathy and premature death at 5 weeks of age. The cardiac myopathy is associated with aberrant mitochondrial morphology and energy metabolism crisis. Interestingly, mitochondrial dysfunctions are caused by severe loss of mitochondrial genome.

Our recent investigation unraveled that mitochondrial fusion ensuring mitochondrial content-mixing is essential to ensure proper and efficient mitochondrial DNA replication. Impaired, mitochondrial fusion will prevent the mtDNA replication burst occurring during cardiac development.

Symposium 25-3

# Circulating Amylin Dysregulation Triggers Microvascular Dysfunction

**Florin Despa**[1*]

[1] Pharmacology, Neurology, Neuroscience, University of Kentucky, USA

*f.despa@uky.edu*

Amylin, a pancreatic hormone, crosses blood-brain barrier (BBB) and regulates satiety. Because amylin is overexpressed in individuals with Alzheimer's disease (AD) and accumulates in the brain, this may modify the course of AD. We created transgenic rats to uncover consequences of high blood amylin on AD pathology, and used human brain tissue and cerebrospinal fluid to assess translational potential of the results. Rats that co-express human amylin in pancreas and APPSwe/PS1ΔE9 in brain have accelerated behavior deficits. This was associated with brain amylin-Aβ cross-seeding, BBB injury, and hypoxic, mitochondrial and white matter changes. Intravenous injection of human amylin in APP/PS1 rats triggered brain hypoxia signaling. Suppression of amylin expression protected against AD effects. Analyses of human brain tissue showed that amylin modulates cerebral small-vessel-type pathology, hypoxia signaling and amyloid composition in both familial and sporadic forms of AD. These results suggest that amylin may be a therapeutic target for AD.

# Mitochondria and Cardiac Differentiation

**Sung Woo Cho**[1*]

[1] Department of Cardiology, Inje University College of Medicine, Ilsan Paik Hospital, Korea

*drswcho@hanmail.net*

Concomitant increase of myofibrils and mitochondria is a key process of cardiomyocyte differentiation from pluripotent stem cells (PSCs). Specifically, development of mitochondrial oxidative metabolic capacity in cardiomyocytes is essential to providing the energy necessary to sustain the beating function. Therefore, the connection between transcriptional and metabolic regulation is important for cardiomyocyte specification and differentiation, and a nucleus-mitochondria interaction is required to translate signals that regulate cardiomyogenesis. Although previous studies reported that mitochondrial function and oxidative metabolism have some correlation with the differentiation of cardiomyocytes, the mechanism by which mitochondrial oxidative metabolism is regulated and the link between cardiomyogenesis and mitochondrial function are still poorly understood. Additionally, reactive oxygen species modulate the differentiation of cardiomyocytes from embryonic stem cells (ESCs), but the exact role of redox signaling in PSC-derived cardiomyocyte differentiation remains controversial.

In the present study, we demonstrate that Cyclosporin A (CsA) promotes differentiation of functional cardiomyocytes from mouse ESC-derived Flk1+ mesodermal precursor cells (MPCs) by inhibition of mitochondrial permeability transition pore (mPTP). This increase in differentiation appears to result from activation of mitochondrial oxidative metabolism. In addition, we found that antioxidant treatment augmented the cardiomyogenic effect of CsA. These data thus constitute novel evidence that activation of mitochondrial oxidative metabolism via inhibition of mPTP and subsequent changes in redox signaling pathways are contributing factors in PSC fate determination toward the cardiac lineage.

Next, we investigated the stage specific transcriptome profiles and characteristics associated with mitochondrial function and metabolism at cardiac lineage commitment during cardiomyocyte differentiation from mouse ESCs. We induced and sorted out Flk1+ MPCs, PDGFRα+ cardiac lineage-committed cells (CLCs), PDGFRα+ cells without cardiac induction, and αMHC+ cardiomyocytes from mouse ESCs and performed microarray analysis of each cells. Gene ontology analysis showed that gene expression profiles of αMHC+ cardiomyocytes revealed a robust upregulation of genes associated with mitochondrial function and metabolism and ion channel activity compared with Flk1+ MPCs and PDGFRα+ CLCs. We identified differently upregulated genes associated with mitochondrial function and metabolism during cardiac differentiation in PDGFRα+ CLCs and αMHC+ cardiomyocytes.

Symposium 25-5 Short Talk

# TIMP3 Is a Potent Regulator of Cancer-Associated Fibroblasts-Mediated Angiogenesis in Invasive Breast Cancer.

**Sajita Shah[1,2], Hyunsook Lee[1,2], Kyu-Tae Kang[1,2*]**

[1] College of Pharmacy, Duksung Women's University, Korea
[2] Duksung Innovative Drug Center, Duksung Women's University, Korea

*kyutaekang7@gmail.com*

Breast cancer has two distinct phenotypes: invasive and non-invasive based on the molecular profiles as well as metastatic potential. Tumor vasculature is critical for tumor invasion and metastasis. Cancer-associated fibroblasts (CAFs) in tumor stroma are known to facilitate tumor angiogenesis. It has also been reported that 40% of endothelial cells in tumor tissue were derived from endothelial colony-forming cells (ECFCs) originated in bone marrow. Previously, the role of CAFs in tumor angiogenesis has been largely studied. However, the different characters between CAFs isolated from invasive breast cancer and those isolated from non-invasive breast cancer have not been studied intensively. Thus, we first compared angiogenic property of two types of CAFs, which were isolated from non-invasive luminal B subtype breast cancer tissue (NBFs) and isolated from invasive triple negative breast cancer tissue (IBFs).

Three-dimensional spheroid assay and molecular biology techniques were performed to measure angiogenic potential and underlying mechanisms.

Sprout number and length formed from ECFCs-spheroids were higher by treatment with conditioned medium of IBFs (IBFs-CM) when compared with NBFs-CM treatment. Matrix metalloproteinase-1 (MMP1) and vascular endothelial growth factor-A (VEGF-A) secretion were upregulated in IBFs when compared with NBFs. Interestingly, tissue inhibitor of matrix metalloproteinase-3 (TIMP3) was downregulated in IBFs compared with NBFs. TIMP3 is the endogenous inhibitor of a disintegrin and metalloproteinase 17 (ADAM17), which is a shedding protease leading to the cleavage and release of soluble ligands and growth factors. Indeed, IBFs showed higher ADAM17 activity compared with NBFs, and treatment with recombinant human TIMP3 protein (rhTIMP3) inhibited ADAM17 activity as well as secretion of MMP1 and VEGF-A in IBFs. rhTIMP3 effectively diminished sprout number and length in ECFCs-spheroids treated with IBFs-CM.

Our findings suggest that CAFs isolated from invasive breast cancer potentiate tumor angiogenesis by ADAM17-mediated MMP1 and VEGF-A secretion, which can be inhibited by exogenous TIMP3 treatment.

# HDL and Glucose Metabolism

**Alicia J. Jenkins**[1*]

[1] NHMRC Clinical Trials Centre, University of Sydney, Australia

*alicia.jenkins@ctc.usyd.edu.au*

To overview the modulation of glucose metabolism by HDL and HDL-targeted therapy in diabetes, with an emphasis on human studies

The presenter and her colleagues have conducted relevant clinical research and a literature review. HDL structure and function and the effects of diabetes are described, followed by the effects of HDL on glucose metabolism and of HDL-targeting interventions. Future research directions and tools are suggested. Results of our FIELD (fenofibrate) trial studies in adults with Type 2 diabetes are described.

There are complex bidirectional links between HDL and glucose metabolism. Glucose modifies HDL levels, composition and functions, including reverse cholesterol transport and its anti-inflammatory and anti-oxidant effects . HDL affects glucose metabolism via insulin dependent and independent effects, including beta cell survival and insulin secretion and peripheral insulin sensitivity and altered intracellular lipids.

There are divergent results re links between HDL and Type 2 diabetes risk. In existent Type 2 diabetes managed by lifesytle alone, our FIELD trial substudy (n=2608 with median of 5-yrs follow-up) showed that low HDL-C /ApoA1 and low HDL-C was associated with substantially slower progression to needing glucose control tablets or insulin.

Most major lipid drug classes impact HDL, (HDL-C change range 0 - 100%), yet there are differences between effects on glucose measures and type 2 diabetes incidence. Some drugs (statins and PCSK9i) increase risk of Type 2 diabetes. Effects of HDL on glucose and on CVD are not always aligned and may reflect different mechanisms.

HDL has multiple effects on glucose metabolism, often by effects on beta cell mass, insulin secretion and insulin sensitivity. In established Type 2 diabetes fenofibrate can retard progression to need for glucose drugs by 2-years. Lipid drugs have divergent effects on HDL and glucose and CVD.

# International Lipid Expert Panel (ILEP) 2020 Guidelines on the Definition and Management With Statin Intolerance

**Maciej Banach**[1*]

[1] Department of Cardiology, Polish Mother's Memorial Hospital Research Institute (PMMHRI) in Lodz, Poland

*maciej.banach@icloud.com*

Statin intolerance is a clinical syndrome whereby adverse effects associated with statin therapy (most commonly muscle symptoms) result in the discontinuation of therapy. Statin discontinuation is associated with significant increased risk of adverse cardiac outcomes. Most of the patients (even 95%) who initially experience adverse effects are able to tolerate statin therapy to some extent. Careful stepwise diagnosis and management of individuals who experience adverse effects is essential to enable optimal reduction of cardiovascular risk.
In this Position Paper of the International Lipid Expert Panel (ILEP), we present a step by step approach to the diagnosis and practical management of statin intolerance resulting from muscle symptoms, and other adverse effects with demonstrated statin causality.
Methods: Relevant clinical evidence (including new trials, meta-analyses and cohort studies) and international clinical guidelines were discussed and assimilated by ILEP members. Consensus was used to formulate recommendations for the diagnosis and management of statin intolerance.
Consensus resulted in the adoption of three parts to the recommendation. 1) definition and diagnosis of Statin Intolerance; 2) advice for management of all patients with statin intolerance, including the place of the new and perspective lipid-lowering drugs; 3) specific advice for patients who have partial (rather than complete) statin intolerance. Patients with partial statin intolerance are likely to make up the vast majority (even 95%) of statin-intolerant individuals. Each part of the recommendation consists of a four-step process and has an associated acronym to aid memory (see attached recommendations). We adopted the Banach and Mikhailidis four-step approach to diagnosis and we developed novel recommendations for management.
We present very practical recommendations, which will enable clinicians to distinguish between rare, severe adverse effects; true statin intolerance, and symptoms incorrectly attributed to statin therapy. In each case we summarize guidelines, clinical evidence and expert opinion pertaining to the optimal management of cardiovascular disease in these patients.

# HDL and Cognitive Decline in Older People With Diabetes Mellitus

**Timothy Kwok**[1*]

[1] Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Hong Kong

*tkwok@cuhk.edu.hk*

Older people with diabetes mellitus (DM) are at risk of cognitive decline and dementia. A post hoc analysis of a negative randomized trial of vitamin B12 supplementation in older people with DM and mild vitamin B12 deficiency found a significant association between baseline serum HDL-C and decline in executive function over two years. We further analyzed the archived serum at month 0, 9 and 27 for Apo A1, ABCA1 mediated cholesterol efflux capacity, and examined their associations with clinical dementia rating (CDR) scale, memory and executive function over 27 months. Serum HDL-C and Apo A1 were significantly correlated (R2=0.57), but their respective correlations with cholesterol efflux were weak ($R^2$= 0.202 and 0.211 respectively). Statin use and lower serum creatinine were associated with higher serum Apo A1 at baseline.

Out of 271 subjects at baseline, 234 of them (86.3 %) were followed up at month 27. Of these, 39 subjects (16.7 %) had cognitive decline as defined by an increase in CDR global score. ABCA1 mediated cholesterol efflux capacity declined significantly with time, but was not associated with cognitive decline. The comparisons of decliners and non-decliners showed no significant differences in serum HDL-C and Apo A1 at baseline and month 27, except that decliners had significantly lower Apo A1 at month 27 (144.3 ± 29.5 versus 156.1 ± 26.2 g/L, P = 0.034). After adjustment for confounders, baseline serum HDL-C and ApoA1 were significantly associated with changes in executive function (beta = 0.444, P < 0.001; beta = 0.004, P = 0.001) and memory (beta = 0.384, P = 0.012; beta = 0.003, P = 0.052) at month 27. It was concluded that serum Apo A1 and HDL-C but not ABCA1 mediated cholesterol efflux capacity were associated with decline in executive function and memory in older people with DM.

Symposium 26-4

# High-Density Lipoprotein Cholesterol and the Risk of Myocardial Infarction, Stroke, and Cause-Specific Mortality

**Yeoree Yang[1,2*], Kyungdo Han[3], Mee Kyoung Kim[4], Kun-Ho Yoon[1], Seung-Hwan Lee[1]**

[1] Division of Endocrinology and Metabolism, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Korea
[2] Catholic Smart Health Care Center, The Catholic University of Korea, Korea
[3] Department of Statistics and Actuarial Science, Soongsil University, Korea
[4] Division of Endocrinology and Metabolism, Department of Internal Medicine, Yeouido St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Korea

*lambten@gmail.com*

The linear inverse association between high-density lipoprotein cholesterol (HLD-C) concentration and the risk of cardiovascular disease has been well-demonstrated by previous observational epidemiologic studies. However, randomized controlled pharmacological intervention studies failed to show the benefit of increasing HDL-C level, raising a question the HDL-C as a causal risk factor. Moreover, recent large cohort studies, which covered the whole general population and the entire range of HDL-C level, have suggested that an extremely high level of HDL-C is paradoxically associated with the increased risk of mortality. There was a U-shaped risk pattern between HDL-C level and all-cause death, making new insight into extremely high HDL-C as a CVD risk factor. However, in most studies, a significant U-shaped risk pattern was definite only in all-cause mortality. Only the Copenhagen cohort showed that this U-shaped association was also significant with cardiovascular mortality. In this presentation, I will summarize the recent population-based cohort studies that investigated the relationship between extremely high HDL-C level and cardiovascular outcomes, including our recent analysis in the general South Korean population. Also, I will briefly discuss the possible explanations for the inconsistent findings from previous studies.

# Altered Lipid Droplet Metabolism in the Endothelium Causes Vascular Dysfunction

**Boa Kim[1], Ayon Ibrahim[1], Soon Tang[2], Jian Li[1], Garret FitzGerald[2], Zoltan Arany[1*]**

[1] Cardiovascular Institute, University of Pennsylvania, USA
[2] Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, USA

*zarany@pennmedicine.upenn.edu*

Endothelial cells (ECs) transport nutrients from circulation into parenchyma in most tissues. Surprisingly, little is known about 1) how ECs process and transport lipid and 2) what happens when lipid droplet (LD) metabolism in ECs is altered to endothelial function. Metabolic syndromes are interconnected in a way that is not fully understood. We hypothesized that the fat intake-mediated hypertension is due to lipid mishandling in the endothelium and therefore EC-specific deletion of adipose triglyceride lipase (ATGL) causes hypertension.

The role of excessive lipid in endothelium was tested by oleic acid (OA) treatment in vitro or ex vivo, and by olive oil gavage followed by en face staining of aorta in vivo. To test the role of ATGL-mediated regulation of LD turnover in ECs, we used knockdown system in vitro and ATGL ECKO mice in vivo.

Excessive lipid treatment dynamically increases LD biogenesis in ECs. Canonical pathways of LD metabolism present in ECs, ATGL being the first step in the TG hydrolysis pathway. OA-mediated LD accumulation is elevated by deletion of ATGL. Strikingly, eNOS expression and systemic NO production were compromised in the ECKO mice. Accordingly, the KO mice became hypertensive upon salt challenge while WT mice remained normotensive. Mechanistically, forskolin treatment that enhances lipolysis and therefore reduces the LD size rescued eNOS expression in ATGL knockdown ECs. In addition, blocking the TG synthesis pathway by targeting either ACSL (initial step) or DGAT (terminal step) rescued eNOS expression in ATGL knockdown ECs.

ECs incorporate fatty acids into LD and ATGL is the main lipase.

Lack of ATGL reduces eNOS expression and NO availability.

ATGL ECKO mice are predisposed to hypertension upon salt challenge.

Dynamics of LD turnover impacts eNOS expression explaining the longstanding and yet still unexplained relationship between the metabolic syndrome and vascular dysfunction.

Symposium 27-1

# Integrative Functional Genomics for Regulatory Mechanisms of Coronary Artery Disease

**Thomas Quertermous**[1*]

[1] Medicine, Stanford University, USA

*tomq1@stanford.edu*

A significant portion of the attributable risk for coronary artery disease (CAD) is genetic, likely reflecting variation in the expression and function of genes expressed in the blood vessel wall that regulate disease risk related pathways. Advances in genome wide association studies have identified over 160 loci that harbor variation that modulates CAD risk, providing the opportunity to characterize vascular wall processes that may be targeted to ameliorate disease. Given that many of the GWAS candidate genes are primarily functional in smooth muscle cells, and much of the disease risk appears to be contributed by this cell type, we have undertaken extensive genomic studies in human coronary artery smooth muscle cells (HCASM) to map causal variants and causal genes. We have collected primary cultured HCASMC from over 60 individuals, isolated DNA and performed whole genome sequencing and variant calling. We have performed RNA sequencing, ATACseq mapping of open chromatin regions, and chromatin immunoprecipitation for candidate CAD associated genes that are transcription factors (TFs), under standard culture and stimulation conditions. Further, we have used a pooling approach with these cells to identify quantitative trait loci that regulate binding of the TCF21 transcription factor, modulate chromatin accessibility and chromosomal looping genome-wide. These combined studies have allowed the identification and study of the mechanisms of causality for a number of CAD associated TFs, including TCF21, SMAD3, and AHR. These studies show that CAD associated TFs contribute to a disease related transcriptional network that allows the regulation of smooth muscle cell fate and disease risk

# Genomic Approaches to Identify Novel Therapeutic Targets for Cardiovascular Disease

**Nathan Stitziel**[1,2,3*]

[1] Medicine, Washington University, USA
[2] Genetics, Washington University, USA
[3] McDonnell Genome Institute, Washington University, USA

*nstitziel@wustl.edu*

Human genetic studies have the potential to successfully identify genes and pathways relevant to both human biology and disease pathogenesis. There are still challenges, however, in mapping causal genes for complex forms of disease. For example, while studies of common genetic variation have successfully identified numerous loci associated with coronary artery disease (CAD), most of the associated variants lie in non-coding sequence, making it difficult to pinpoint causal genes. We will review the results of an exome-wide screen that found coding polymorphisms which significantly associated with risk for (or protection from) CAD, implicating *ANGPTL4* and *SVEP1* as novel genes for CAD. We will review the emerging evidence implicating lipoprotein lipase and its regulators in risk for atherosclerosis along with new evidence demonstrating that SVEP1 is a proatherogenic extracellular matrix protein and that its inhibition may be therapeutically beneficial for the treatment and prevention of CAD.

Symposium 27-3

# Using Proteomics and Metabolomics for a Precision Medicine Approach in Cardiovascular Disease

**Lars Lind**[1*]

[1] Dept of Medical Sciences, Uppsala University, Sweden

*lars.lind@medsci.uu.se*

Precision medicine has been defined as: "An approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person."

In prevention of cardiovascular disease (CVD) it is essential to quantify the degree of risk for the individual subject. So far, the combination of data from traditional risk factors, like lipids, blood pressure and smoking, have been combined into scores. The most commonly used is Framingham risk score, but the predictive power at the individual level is far from perfect.

One strategy to increase the predictive power is to add information on genetics to traditional risk factors. However, despite that >100 genetic loci have been linked to myocardial infarction, the predictive power is only marginally improved. Another strategy is to find new biomarkers being more powerful than the traditional ones. By the technical improvement in proteomics and metabolomics measurements in recent years, we have used these techniques during the last 6 years in several population-based longitudinal cohorts.

Using the anti-body based CVD-1 chip from OLINK, we have searched for novel biomarkers for the major CVD as well as their risk factors. A great number of associations have been disclosed and will be presented. We have also used mass spectroscopy (MS)-based metabolomics in a similar fashion.

To summarize: 1. Using proteomics and metabolomics new biomarkers that predict the common CVDs have been identified. 2. Proteomics has so far delivered more new biomarkes than metabolomics. 3. Some of these new biomarkers add predicitive power on top of traditional risk factors. 4.

The increase in C-statistics (predictive power) when adding these new biomakers is however small. In conclusion, Proteomics and metabolomics are useful techniques to find new biomarkers for CVD, but still the predictive power is poor to use these data in precision medicine.

# The Pilot Project for National Bio Big Data of Republic of Korea

**Hyun-Young Park**[1*]

[1] Center for Genome Science, Korea National Institute of Health, Korea

*mdhypark@gmail.com*

Advances in genomics science and the emerging of new omics, data collection and storage, computational analysis, and mobile health applications over the last decade are changing culture of medical research and practice. Recently the concept of precision medicine was emerged. According to the Precision Medicine Initiative, precision medicine is 'an approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person'. To realize the precision medicine, prospective cohort that have the ability to identify biomarkers and causative factors contributing to future diseases is needed.

The Korean government has planned to set up the 'national bio big data' to build a comprehensive scientific knowledge base by creating a network of scientists and embarking on a national study of one million Korean to expand our understanding of health and disease. Disease patients such as rare diseases, cancer, cardiovascular diseases, and other intractable diseases as well as general population are considered as study subjects. The Ministry of Science and ICT, the Ministry of Health and welfare, and the Ministry of Trade, Industry and Energy launched two-year pilot project this year. Five government agencies including KNIH are managing the project, and medical community, scholars, businesses, and patients' organizations also actively involved.

The goals of the pilot project are as follows; 1) to establish the governance structure and advisory committees, 2) to set up the participant engagement process, especially for rare disease patients, 3) to set up the data collection process from the participants and data management, 4) to establish the whole genome sequencing and analysis pipeline, 5) to establish the bio-banking process such as sample collection, processing, storage, and biochemical analysis and/or shipment to analytic laboratories, 6) to build up the data storage, and access process.

Symposium 27-5 Short Talk

# Novel Scoring System to Predict Coronary Artery Calcification

**Samel Park[1], Nam-jun Cho[1], Sungwan Chun[1], Eun-young Lee[1], Hyo-Wook Gil[1]***

[1] Department of Internal Medicine, Soonchunhyang University Cheonan Hospital, Korea

*hwgil@schmc.ac.kr*

Given the association between traditional risk factors of cardiovascular disease and coronary artery calcium (CAC), we built a model to predict CAC score > 0 and > 100 based on patients' demographic and laboratory data.

This retrospective study enrolled patients who performed cardiac computed tomography (CT) at Soonchunhyang University Cheonan Hospital (Cheonan, Korea) between January 2010 and July 2012. The patients who aged 18 – 69 and with estimated glomerular filtration rate (eGFR) > 60 ml/min per 1.73 $m^2$ were finally enrolled. A total of 1134 patients were evaluated. A model was built using a multivariable ordinal proportional regression model. We entered variables including age, eGFR, body mass index (BMI), sex, hypertension, diabetes, and total cholesterol level into a model. During the backward elimination process, however, the total cholesterol level was excluded from the final model. Then, we multiplied 3.76972 to make the highest level of the scoring system to 20 and rounded up the value to make integral. As a results, scoring system with age (< 40 year, reference; ≥ 40 and < 50 year, 3; ≥ 50 and < 60 year, 6; ≥ 60 and < 70 year, 10), eGFR (≥ 90 ml/min per 1.73 $m^2$, reference; ≥ 60 and < 90 ml/min per 1.73 $m^2$, 1), BMI (< 25 kg/$m^2$, reference; ≥ 25 kg/$m^2$, 1), Sex (female, reference; male, 3), hypertension (absence, reference; presence, 2), and diabetes (absence, reference; presence, 3) was built. The area-under-curve for CAC score > 0 and > 100 were 0.747 and 0.761, respectively.

Our study showed that CAC score > 0 and > 100 could be predicted only based on demographic and laboratory data. It could be helpful for clinicians to make decision to start or not the preventive medical therapy.

# An Update on Anti-Inflammatory Therapy for Atherosclerosis: CANTOS and Beyond

**Peter Libby**[1*]

[1] Cardiovascular Medicine, Brigham and Women's Hospital - Harvard Medical School, USA

*PLIBBY@BWH.HARVARD.EDU*

All phases of atherosclerosis involve inflammation – from the initiation, through the long phase of progression, and ultimately the thrombotic complications that cause myocardial infarctions and many ischemic strokes. Reports of experimental results in this field often end with a promissory note regarding translation. Yet, a gap yawns between the elegant scientific findings in genetically modified mice, and the reduction to practice. Statin therapy, an intervention that has anti-inflammatory actions independent of lipid lowering, limits cardiovascular complications. Even on maximum current therapy, however, including high dose statin, many with established atherosclerosis remain at risk for recurrent events. Thus we need new approaches to addressing this residual burden of risk. Inflammation furnishes a possible therapeutic target in this regard. Targeting the prominent inflammatory mediator interleukin-1 (IL-1) beta is the first direct anti-inflammatory therapy that showed improved cardiovascular outcomes. Cholesterol crystals found in plaques selectively activate the inflammasome, the molecular machinery that converts the inactive precursor of IL-1 beta to its functional pro-inflammatory form. CANTOS, a >10,000 clinical trial showed that treatment with a monoclonal antibody that neutralizes IL-1 beta can reduce cardiovascular events in patients, stable post acute coronary syndrome, on a full standard of care secondary prevention regimen including statin therapy, with persistent inflammation indicted by above median C-reactive protein. The COLCOT trial showed that low dose colchicine can limit recurrent events post myocardial infarction. The application of biological insights about inflammation in atherogenesis has already sharpened risk prediction, aided targeting of therapy, and has provided new targets for therapy now proven efficacious in large scale clinical trials.

# Non-Canonical Functions of Nuclear microRNAs in Endothelial Protection

**Christian Weber**[1*]

[1] Institute for Cardiovascular Prevention (IPEK), Institut für Prophylaxe und Epidemiologie der Kreislaufkrankheiten, Germany

*Christian.Weber@med.uni-muenchen.de*

MicroRNAs (miRNAs) are versatile regulators of gene expression with profound implications for human disease including atherosclerosis, but whether they can exert post-translational functions to control cell adaptation and whether such non-canonical features harbor pathophysiological relevance is unknown. Here we show that miR-126-5p sustains endothelial integrity in the context of high shear stress and autophagy. Bound to Ago2, miR-126-5p forms a complex with Mex3a, which occurs on the surface of autophagic vesicles and guides its transport into the nucleus. Mutational studies and biophysical measurements demonstrate that Mex3a binds to the central U- and G-rich regions of miR-126-5p with nanomolar affinity via its two KH domains. In the nucleus, miR-126-5p dissociates from Ago2 and binds to caspase-3 in an aptamer-like fashion with its seed sequence, preventing dimerization of the caspase and inhibiting its activity to limit apoptosis. The anti-apoptotic effect of miR-126-5p outside of the RNA-induced silencing complex is important for endothelial integrity under conditions of high shear stress promoting autophagy: ablation of Mex3a or ATG5 in vivo attenuates nuclear import of miR-126-5p, aggravates endothelial apoptosis, and exacerbates atherosclerosis. In human plaques, we found reduced nuclear miR-126-5p and active caspase-3 in areas of disturbed flow. The direct inhibition of caspase-3 by nuclear miR-126-5p reveals a non-canonical mechanism by which miRNAs can modulate protein function.

Symposium 28-3

# Mechanisms Meditating Platelet-Neutrophil Interactions in Thromboinflammationp: Role of Platelet PDI

**Jaehyung Cho**[1*]

[1] Department of Pharmacology, University of Illinois at Chicago, USA

*thromres@uic.edu*

Evidence is mounting that neutrophils adherent to activated endothelial cells support platelet adhesion and induce microthrombus formation, leading to microvascular occlusion under inflammatory conditions. Platelet-neutrophil association primarily results from the interaction of platelet P-selectin and glycoprotein Ibα (GPIbα) with neutrophil P-selectin glycoprotein ligand-1 (PSGL-1) and αMβ2 integrin, respectively. While the binding of P-selectin to PSGL-1 initiates cell-cell contact and drives transmigration of neutrophils, the interaction between GPIbα and αMβ2 integrin is required for the firm attachment of platelets to neutrophils. We have demonstrated that extracellular protein disulfide isomerase (PDI), a prototypic thiol isomerase, promotes the ligand-binding function of cell surface receptors, including integrins and GPIbα, and contributes to neutrophil-endothelial cell and neutrophil-platelet interactions and vascular occlusion in inflammation. GPIbα is a platelet-specific receptor that is believed to be constitutively active for ligand-binding. Recently, we found that extracellular PDI was critical for GPIbα-mediated platelet adhesive function. Studies using genetic and pharmacologic approaches revealed that the oxidoreductase activity of platelet surface-bound PDI was required for the ligand-binding function of GPIbα. Using Biacore and mass spectrometry, we demonstrated a direct interaction between PDI and GPIbα on the platelet surface and found PDI-mediated cleavage of two allosteric disulfide bonds in GPIbα. In vivo intravital microscopy suggested that PDI-regulated GPIbα function was essential for platelet-neutrophil interactions and vascular occlusion in vascular inflammation. Our studies demonstrate that platelet-released PDI modifies allosteric disulfide bonds in GPIbα and enhances the ligand-binding activity, leading to platelet-neutrophil interactions and vascular occlusion under inflammatory conditions.

Symposium 28-4 Short Talk

# Endothelial Phenotype Regulation by ECM

**Sanguk Yun**[1], **Martin Schwartz**[2*]

[1] Department of Biotechnology, Inje University, Korea
[2] Department of Internal Medicine, Yale University, USA

*martin.schwartz@yale.edu*

Fibronectin (FN) is deposited in subendothelial basement membranes in athero-prone regions of arteries with disturbed flow, where it promotes endothelial NFκB activation and expression of inflammatory mediators. We previously reported that integrin α5 cytoplasmic tail directly bound phosphodiesterase (PDE) 4D. Mice bearing mutation of the integrin α5 cytoplasmic tail or the integrin binding site in PDE4D had markedly reduced atherosclerosis. Further analysis showed binding of PDE4D to α5 led to dephosphorylation of PDE4D by the phosphatase complex PP2A, which increased PDE and NFκB activity. Further, PDE4D directly bound the B55α subunit of PP2A to localize PP2A to sites of adhesion. These experiments unexpectedly showed that the PDE4D promoted the PP2A-B55α complex assembly and led us to hypothesize that focal adhesion-localized PP2A may have other substrates.

We therefore performed phosphoproteomic analysis to compare phosphoprotein profiles between (1) ECs on FN vs. matrigel (MG, basement membrane matrix) or (2) ECs expressing WT PDE4D vs. a mutant PDE4D with deletion of the PP2A-binding domain.

Among 205 hits showing >1.5-fold changes in phosphorylation from FN vs. MG analysis, 63 phosphorylation events were also changed in PDE4D WT vs. mutant analysis. Functional annotation for the hits with differential phosphorylation from FN vs MG analysis revealed that 'cell-cell adhesion' and 'mRNA splicing' are the top two GO terms. These terms were also ranked top two from PDE4D-wild type vs mutant analysis.

This suggests that PDE4D-depenent PP2A activation could be a major mechanism for FN roles in inflammatory signaling and also mediate novel function of FN on endothelial junctional molecules and alternative splicing.

# Endothelial Sharpin Regulates Vascular Leakage in Inflammation

**Anne Pink[1], Elina Kiss[1], Laura Hakanpää[1], Emilia Peuhu[2], Johanna Ivaska[2], Pipsa Saharinen[1,3,4]***

[1] RPU, Translational Cancer Medicine program, University of Helsinki, Finland
[2] Turku Centre for Biotechnology and Department of Biochemistry, University of Turku and Abo Akademi University, Finland
[3] Wihuri Research Institute, Biomedicum, Finland
[4] Department of Biochemistry and Developmental Biology, Faculty of Medicine, University of Helsinki, Finland

*pipsa.saharinen@helsinki.fi*

Dynamic control of vascular permeability is essential for normal inflammatory response, allowing the transient passage of fluid and macromolecules from the circulation into the tissues. The endothelial barrier function is regulated via endothelial cell-cell junctions and actin cytoskeleton, which couples the endothelial cells to underlying basement membrane via integrin-mediated adhesions.

We have previously demonstrated that inflammatory agents promote endothelial permeability via β1-integrin mediated endothelial cell contractile forces. Inflammation induced the formation of active β1-integrin-containing fibrillar adhesions, which promoted inflammation-induced loss of VE-cadherin and the formation of actin stress. Here, we study, whether a cytoplasmic adapter protein Sharpin, a β1-integrin inhibitor, and a component of LUBAC, could play a role in endothelial barrier function and vascular leakage. We used conditionally targeted Sharpinflox/flox mice to delete Sharpin in the endothelial cells in adult mice (SharpiniECKO).

Vascular leakage was assessed following VEGF, TNF-α and LPS administration, and vascular markers using whole-mount staining. Genetic deletion of Sharpin increased agonist-induced, but not basal, vascular leakage in SharpiniECKO mice, by decreasing VE-cadherin in endothelial cell-cell junctions.

However, Sharpin deletion did not affect LPS-induced endothelial inflammation. In line with these results, SHARPIN-silenced endothelial cells displayed increased activation of β1-integrin, and decreased cell surface expression of VE-cadherin. In summary, our results indicate that Sharpin regulates endothelial β1-integrin, and loss of Sharpin in adult mice predisposes the vasculature to inflammation-induced leakage.

Symposium 29-1

# Defective D-Lactate Metabolism Induce Methylglyoxal Accumulation and Cause Cardiomyopathy

**Chan Bae Park**[1*]

[1] Physiology, Ajou University School of Medicine, Korea

*pcbkaist@gmail.com*

Methylglyoxal is a highly reactive a-oxoaldehyde that is formed in cells primarily from the triose phosphate intermediates of glycolysis, dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. In diabetic heart, hyperglycemia triggers enhanced production of methylglyoxal, one consequence of which is the rapid modification of proteins and other substrates to generate what are called advanced glycation end products, AGE. One mechanism known to detoxify MG is the glyoxalase system, composed of two enzymes, glyoxalase 1 and glyoxalase 2, which act sequentially to convert MG into D-lactate. We discovered that protein X is responsible for D-lactate metabolism. Here, we describe that deficiency of protein X gene increased the level of D-lactate and induced accumulation methylglyoxal. Mouse strains deficient in protein X gene develop cardiomyopathy at the age of 40 weeks.

# Rebalancing Metabolism to Improve Cardiac Function in Diabetes: "Walking the Tightrope" Between Glucose and Fatty Acid Metabolism

**Lisa Heather**[1*]

[1] Dept Physiology, Anatomy and Genetics, University of Oxford, UK

*lisa.heather@dpag.ox.ac.uk*

Metabolic dysfunction in the type 2 diabetic heart is associated with diabetic cardiomyopathy and diastolic dysfunction. The incidence of ischemia is increased by type 2 diabetes, yet the heart is less resilient to this challenge and less able to adapt following reperfusion. Over reliance on fatty acids and decreased utilisation of glucose make the diabetic heart metabolically abnormal, which has consequences of cardiac function and post-ischaemic recovery. In light of this, targeting metabolism may provide a fruitful avenue to improve the heart in diabetes. Here we discuss strategies to rebalance metabolism in diabetes. We have taken a variety of novel pharmacological approaches to reduce fatty acid metabolism and promote glucose utilisation in diabetes. These include directly targeting key transporters and proteins within the metabolic pathway, as well as targeting transcription factors central to metabolic regulation.

Symposium 29-3

# Endothelial Cells Control Muscle Regeneration Through Angiocrine Lactate

**Katrien De Bock**[1*]

[1] Health Sciences and Technology, ETH Zurich, Switzerland

*katrien-debock@ethz.ch*

Endothelial cell (EC) derived signals contribute to organ regeneration, but angiocrine metabolic communication is not described. We found that EC-specific loss of the glycolytic regulator pfkfb3 reduced ischemic hindlimb revascularization and impaired muscle regeneration. This was caused by the reduced ability of macrophages to adopt a proangiogenic and proregenerative M2-like phenotype. Mechanistically, loss of pfkfb3 reduced lactate secretion by ECs and lowered lactate levels in the ischemic muscle. Addition of lactate to pfkfb3-deficient ECs restored M2-like polarization in an MCT1-dependent fashion. Lactate shuttling by ECs enabled macrophages to promote proliferation and fusion of muscle progenitors. Moreover, VEGF-production by lactate-polarized macrophages was increased resulting in a positive feedback loop that further stimulates angiogenesis. Finally, increasing lactate levels during ischemia rescued macrophage polarization and improved muscle reperfusion and regeneration, whereas macrophage-specific mct1 deletion prevented M2-like polarization. In summary, ECs exploit glycolysis for angiocrine lactate shuttling to steer muscle regeneration from ischemia.

# Loss of Dscr-1 Accelerates Hypercholesterolemia Leading to Corneal Neovascularization and Opacity

**Masashi Muramatsu[1], Suguru Nakagawa[2,3], Tsuyoshi Osawa[4], Akiyoshi Uemura[5], Hiroyasu Kidoya[6], Nobuyuki Takakura[6], Sandra Ryeom[7], Takashi Minami[1*]**

[1] Div. Molecular and Vascular Biology, IRDA, Kumamoto University, Japan
[2] Dept. Ophthalmology, Graduate School of Medicine, The University of Tokyo, Japan
[3] Div. Genome Science, RCAST, The University of Tokyo, Japan
[4] Integrative Nutriomics and Oncology, RCAST, The University of Tokyo, Japan
[5] Dept. Retinal Vascular Biology, Nagoya City University, Japan
[6] Dept. Signal transduction, RIMD, Osaka Univeristy, Japan
[7] Dept. Cancer Biology, Perelman School of Medicine, University of Pennsylvania, USA

*t-minami@kumamoto-u.ac.jp*

The calcineurin-nuclear factor for activated T cells (NFAT)-Down syndrome critical region (DSCR)-1 pathway plays a crucial role as the downstream effector of VEGF-or thrombin-mediated angiogenesis, inflammation and anti-oxidative stress. However, a role for DSCR-1 in lipid metabolism and ocular manifestations is not well understood.

We generated double-knockout mouse, lacks both apolipoprotein (*Apo*) *E* and *Dscr-1*, by crossing each gene-deficient mouse to investigate physiological function of DSCR-1 under the hyperlipidemic condition. In addition to *ApoE*-deficient model, we also utilized other hypercholesterolemic model such as overexpression of proprotein convertase subtilisin/kexin type (PCSK) 9 which binds to the low-density lipoprotein (LDL) receptor and accelerates its degradation. To assess molecular mechanism of pathological neovascularization regulated by DSCR-1, we performed suture model, genome-wide screening, neutralizing experiment and clinical specimens test.

We found that *Dscr-1*-null condition accelerates severe hypercholesterolemia in all models. Loss of both *ApoE* and *Dscr-1* showed higher ceruloplasmin and lower scavenger receptor in aorta compared with *ApoE*-deficient, that induce a dramatic increase in serum cholesterol but controversially reduced atherosclerotic plaque. Thus, loss of both *Dscr-1* and *ApoE* was sufficient to increase peripheral LDL causes spontaneous lipomas, corneal neovascularization and severe corneal opacity. In suture model with wild-type, *Dscr-1*-deficient and *Dscr-1*-transgenic mice, corneal angiogenesis was significantly increased in Dscr-1-null condition whereas decreased in overexpressed condition. Genome-wide screening revealed that *Dscr-1* deficiency triggers stromal derived factor (SDF)-1-CXCR4 axis upregulation into the cornea and VEGF secretion from macrophages by oxidative LDL stimulation. Therefore, neutralizing SDF-1 efficiently suppressed corneal neovascularization induced by *Dscr-1* deficiency. Moreover, we observed DSCR-1 expression indicating NFAT signal activation in endothelium of the ophthalmological diseases.

Our study provides new molecular insights that DSCR-1 regulates pathological corneal angiogenesis in hypercholesterolemia-inducing corneal opacity.

Symposium 29-5 Short Talk

# Angiopoietin-2–Integrin α5β1 Signaling Enhances Vascular Fatty Acid Transport and Prevents Ectopic Lipid–Induced Insulin Resistance

**Hosung Bae[1], Gou Young Koh[1*]**

[1] Center for Vascular Research, IBS, Korea

*gykoh@kaist.ac.kr*

The distribution of body fat towards subcutaneous adipose tissue (SAT) is an important factor for metabolic health since impaired uptake of circulating lipids by SAT can induce ectopic fat accumulation in major glucose-consuming organs leading to insulin resistance. Numerous approaches have focused on ameliorating ectopic fat accumulation, which have reported some controversial results presumably due to systematic approach, which targets the whole circulatory system, thus could not promote effective fat distribution into local tissue specifically.

By thorough analyses with various tissue-specific KO mouse models and mechanistic studies in primary cultured cells, we show that adipocyte-produced Angpt2 regulates endothelial FA transport via CD36 and FATP3 through integrin α5β1 signaling. We utilized the Ribo-Tag method to isolate endothelial cells and confirmed that integrin α5β1 expression was restricted in SAT endothelium compared to other metabolic organs through RNA-sequencing. We provide clinical relevance thorough comparative analyses of non-diabetic obese (NDO) and diabetic obese (DO) individuals by uncovering angiopoietin-2 (Angpt2) as the only secretory molecule that is specifically enriched in SAT of NDO individuals.

Adipocyte-specific deletion of Angpt2 markedly reduces fatty acid uptake and storage in SAT, leading to ectopic lipid accumulation in glucose-consuming organs including skeletal muscle and liver and to systemic insulin resistance. Mechanistically, Angpt2 activates integrin α5β1 signaling at the endothelium and triggers fatty acid transport via CD36 and FATP3 into SAT. Genetic or pharmacological inhibition of the endothelial integrin α5β1 recapitulates adipocyte-specific Angpt2 knockout phenotypes.

Our findings demonstrate critical roles for Angpt2–integrin α5β1 signaling at SAT endothelium in regulating whole-body fat distribution for metabolic health and highlight adipocyte–endothelial crosstalk as a potential target for prevention of ectopic lipid deposition–induced lipotoxicity and insulin resistance.

# Contribution of Arterial Stiffness to Cardiovascular Disease

**Teemu Niiranen**[1,2*]

[1] Department of Health, Finnish Institute for Health and Welfare, Finland
[2] Department of Medicine, Turku University Hospital, Finland

*teemu.niiranen@thl.fi*

Increased arterial stiffness, most commonly measured using aortic pulse wave velocity, has been shown to be a risk factor for cardiovascular disease events, such as stroke and myocardial infarction. In this presentation, we will review the current evidence on the association between arterial stiffness and cardiovascular disease. We will also assess the extent to which arterial stiffness may improve cardiovascular disease risk prediction beyond conventional risk factors.

Symposium 30-2

# Left Ventricle and Arterial System Interaction in Heart Failure with Preserved Ejection Fraction

**Chi Young Shim**[1*]

[1] Departement of Internal Medicine/Division of Cardiology, Severance Cardiovascular Hospital, Seoul, Korea

*CYSPRS@yuhs.ac*

Heart failure with preserved ejection fraction (HFpEF) has recently been recognized as the single greatest unmet need in cardiovascular medicine. Among the heterogeneous pathophysiologic mechanisms, arterial stiffness and its influence on the left ventricular function are key concepts for understanding the pathogenesis of HFpEF. Diverse noninvasive imaging methods have been proposed for assessing arterial stiffness, ventricular stiffness and ventricular arterial coupling. In this lecture, we will review the pathophysiologic and clinical importance of ventricular arterial interaction and share a few research works.

# Effects of Systemic Arterial Pulsatile Function on Cerebral Small Vessel Disease

**Naoki Saji**[1*]

[1] Center for Comprehensive Care and Research on Memory Disorders, National Center for Geriatrics and Gerontology, Japan

*sajink@nifty.com*

Cerebral small vessel disease (SVD), including silent lacunar infarcts, white matter hyperintensities, and microbleeds, poses a risk for cerebrovascular disease, cognitive impairment, and geriatric syndrome via effects on arterial stiffness. However, the vascular, physiological, and metabolic roles of arterial stiffness in cerebral small vessel diseases remain unclear.

Arterial stiffness is independently associated with all components of cerebral small vessel disease including silent lacunar infarcts, white matter hyperintensities, and microbleeds, although there are some methodological differences among the various surrogate markers. Evidence of arterial stiffness indicates microvessel arteriosclerosis presenting with vascular endothelial dysfunction. Further, vascular narrowing due to atherosclerosis and vascular stiffness due to lipohyalinosis can accelerate the pulse waves. This hemodynamic stress, pulsatile pressure, or blood pressure variability can cause a 'Tsunami effect' towards the cerebral parenchyma and lead to cerebral small vessel disease. Previous studies have shown that silent lacunar infarcts and white matter hyperintensities are strongly associated with arterial stiffness. However, the association between microbleeds and arterial stiffness remains controversial, as there are two vessel mechanisms related to microbleeds: cerebral amyloid angiopathy and hypertensive small vessel disease.

Cerebral SVD with associated arterial stiffness is a risk factor for silent cerebral lesions, stroke, and cognitive impairment. Improvement of living environment, management of risk factors, and innovation and development of novel drugs that improve arterial stiffness, may suppress the progression of cerebral SVD, and may reduce the risk of stroke and dementia.

Symposium 30-4

# The Role of Aortic Stiffness in Orthostatic Hypotension

**Hack-Lyoung Kim**[1*]

[1] Division of Cardiology, Internal Medicine, Seoul National University, Korea

*khl2876@gmail.com*

Orthostatic hypotension (OH) is an excessive drop in blood pressure (BP) during the upright position. OH is clinically important, because it is common in the elderly and associated with an increased risk of falls, syncope, and cerebrovascular events. It has been reported that OH is also associated with hypertension, coronary heart disease and stroke in middle-aged subjects. Impaired compensatory baroreflex response has been considered a main factor for the development of OH. However, underlying pathophysiology has not yet been fully understood. Arterial stiffening is one of the characteristics of arterial aging and arteriosclerosis. Decreased reserve function and blunted baroreflex sensitivity in a stiffened artery have shown the possibility of its important contribution to orthostatic BP response. Indeed, both arterial stiffness and OH prevalence increase with age. Irrespective of their ages, patients with OH also have increased arterial stiffness. Recent study has shown that there was a significant and independent correlation between invasively measured aortic pulse pressure (APP) and OH. In this study, 200 patients (age 64.3 ± 10.9 years, 62.5% males) who underwent invasive coronary angiography was prospectively recruited. OH was defined as systolic blood pressure drop ≥20 mmHg or diastolic blood pressure drop ≥10 mmHg within 3 minutes of the standing position compared to the supine position. Hemodynamic parameters were measured at the ascending aorta using a pig-tail catheter immediately before ICA. APP was calculated as a difference between the aortic peak systolic pressure and the end-diastolic pressure. The study results demonstrated that diabetes and higher APP were associated with OH even after controlling for potential confounders. These results support the hypothesis that aortic stiffness plays an important role in the hemodynamic response to orthostatic challenge and results in the development of OH. Interfering the activation of baroreceptor response and reduced vasoconstricting potential by arterial stiffening may be suggested mechanisms underlying he association between high APP and OH. Further prospective or interventional studies with large sample size are needed to confirm these results.

# The Role of Arterial Stiffness in Chronic Kidney Disease

**Hyo Jin Kim**[1*]

[1] Nephrology, Internal medicine, Pusan National University Hospital, Korea

*kimhj923@gmail.com*

Arterial stiffness increases in chronic kidney disease (CKD) patients which involves many factors, including lots of uremia-related factors. Increased arterial stiffness is a marker of vasculopathy in patients with CKD, suggesting a significant cardiovascular damage. In this lecture, we overview the relationship between arterial stiffness and CKD, as well as clinical implications of increased arterial stiffness and the potential therapeutic options to reduce arterial stiffness in patients with CKD.

Symposium 30-6 Short Talk

# Overexpression of the Amyloid-β Precursor Protein Exerts a Direct Corticosterone-Dependent Effect On In Vivo Arterial Stiffness Without Affecting Ex Vivo Arterial Wall Biomechanics

**Jhana O. Hendrickx[1*], Sofie De Moudt[1], Debby Van Dam[2,3], Guido R. Y. De Meyer[1], Paul Fransen[1]**

[1] Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Lab of Physiopharmacology, University of Antwerp, Antwerp, Belgium
[2] Department of Biomedical Sciences, Laboratory of Neurochemistry and Behaviour, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium
[3] Department of Neurology and Alzheimer Research Center, University of Groningen and University Medical Center Groningen, Groningen, Netherlands

*jhana.hendrickx@uantwerpen.be*

The Amyloid-β precursor protein (APP) is a highly evolutionary conserved and ubiquitously expressed type I transmembrane protein in many different tissues. Overexpression of the protein, carrying specific genetic mutations, are linked to the familial Alzheimer's Disease (AD) pathology. Given the suggested interplay between arterial stiffness (AS) and AD, we aimed to investigate AS by APP overexpression (APP23+/-) in C57BL/6 mice and an endothelial dysfunction mouse model of AS (eNOS-/-).

C57BL/6 (male; n= 10), APP23+/- (male; n= 9), eNOS-/- (male; n=14) and eNOS-/-/APP23+/- (male; n= 11) were studied at the age of 6 months. AS was determined in vivo by aortic pulse wave velocity (aPWV, VEVO2100) and ex vivo by aortic Peterson Moduli (Ep) through the in-house developed Rodent Oscillatory Tension set-up for Arterial Compliance (ROTSAC). Corticosterone levels were analysed on blood serum via ELISA. Data are represented as mean ± SEM.

Increased aPWV were observed upon APP overexpression (C57BL/6 = 2.9 ± 0.1; APP23+/- = 4.1 ± 0.3; eNOS-/- = 4.6 ± 0.3; eNOS-/-/APP23+/- = 5.0 ± 0.9 m/s). Similarly, corticosterone levels were elevated by APP overexpression (C57BL/6 = 2.5 ± 1.4; APP23+/- = 10.1 ± 3.0; eNOS-/- = 13.1 ± 4.4; eNOS-/-/APP23+/- = 16.5 ± 2.4 µg/mL), which led to a positive Pearson correlation with aPWV values (p=0.0048, r=0.6335). Aortic Ep values at different extrapolated physiological pressures showed no effect of the APP overexpression in C57BL/6 or eNOS-/- mice at baseline (KREBS), α1-adrenergic receptor-dependent contracted (phenylephrine (PE)) and fully contracted (PE and Nω-Nitro-L-arginine methyl ester hydrochloride) conditions.

These observations suggest that the APP protein might exert an indirect corticosterone-dependent effect on in vivo arterial stiffness without affecting ex vivo arterial wall biomechanics.

# The Gut Microbiota in Atherosclerosis and Atherothrombosis

**Christoph Reinhardt**[1*]

[1] University Medical Center Mainz, Johannes Gutenberg-University Mainz, Germany

*christoph.reinhardt@unimedizin-mainz.de*

Atherosclerosis and its most severe complication atherothrombosis is largely influenced by cholesterol metabolism and the vascular immune response. Both, cholesterol metabolism as well as inflammatory tone are influenced by the gut microbiota. Therefore, it is important to causally resolve how gut microbial communities impact atherosclerotic lesion development and arterial thrombosis. Our data on the germ-free (GF) low-density lipoprotein receptor (Ldlr)-deficient mouse model showed that the presence of a gut microbiota lowers plasma lipoprotein levels dependent on diet. Conventionally raised (CONV-R) Ldlr-deficient mice had increased signs of vascular inflammation relative to their GF counterparts, such as elevated myeloid cell counts in whole blood and increased numbers of adhering leukocytes to the carotid artery plaques. However, in late atherosclerosis, at 16 weeks of high-fat diet (HFD), analysing the lesions in the carotid artery, the aortic root, and the aortic arch, we did not find differences in atherosclerotic lesion size. Interestingly, the absence of a gut microbiota reduced ultrasound-triggered plaque rupture-induced thrombus growth in the common carotid artery of late atherosclerotic Ldlr-deficient mice. In line, thrombus growth was also reduced the injured carotid artery of GF C57BL/6J mice on a standard lab diet, indicating that colonisation with a gut microbiota promotes arterial thrombosis. Comparing anticoagulated whole blood form HFD-fed GF Ldlr-deficient mice with HFD-fed CONV-R Ldlr-deficient controls, standardized flow chamber experiments revealed a diminished adhesion-induced platelet activation on type I and type III collagen matrices, as indicated by reduced phosphatidylserine exposure. Collectively, our results demonstrate that the gut microbiota does not influence atherosclerotic lesion size at 16 weeks of HFD in a hyperlipidemia mouse model of late atherosclerosis, but the gut microbiota promotes arterial thrombus growth at various diets and in different carotid artery thrombosis models.

Symposium 31-2

# Dietary Sucrose Induces Metabolic Inflammation and Atherosclerotic Cardiovascular Diseases More Than Dietary Fat in Mice: Role of the Gut Microbiota

**André Marette[1*], Lais Perazza[1]**

[1] Medicine, Laval University, Canada

*andre.marette@criucpq.ulaval.ca*

Poor dietary habits contribute to the obesity pandemic and related cardiovascular diseases but the respective impact of high saturated fat versus added sugar consumption remains debated. Herein, we aimed to disentangle the individual role of dietary fat versus sugar in cardiometabolic disease progression. Methods: We fed pro-atherogenic LDLr–/– ApoB100/100 mice either a low-fat/high-sucrose (LFHS) or a high-fat/ low-sucrose (HFLS) diet for 24 weeks. Weekly body weight gain was registered. 16S rRNA gene-based gut mi- crobial analysis was performed to investigate gut microbial modulations. Intraperitoneal insulin (ipITT) and oral glucose tolerance test (oGTT) were conducted to assess glucose homeostasis and insulin sensitivity. Cytokines were assessed in fasted plasma, epididymal white adipose tissue and liver lysates. Heart function was evaluated by echocardiography. Aortic atheroma lesions were quantified according to the en face technique. Results: HFLS feeding increased obesity, insulin resistance and dyslipidemia compared to LFHS feeding. Conversely, high sucrose consumption decreased gut microbial diversity while augmenting inflammation and the adaptative immune defense against metabolic endotoxemia and reduced macrophage cholesterol efflux capacity. This led to more severe cardiovascular complications as revealed by remarkably high level of ather- osclerotic lesions and the early development of cardiac dysfunction in LFHS vs HFLS fed mice. Conclusion: we uncoupled obesity-associated insulin resistance from cardiovascular diseases and provided novel evidence that dietary sucrose, not fat, is the main driver of metabolic inflammation accelerating severe atherosclerosis in hyperlipidemic mice.

# Probiotics and Targeted Therapeutics in Atherosclerosis

**Sae Hun Kim**[1*]

[1] Food Bioscience and Technology, Korea University, Korea

*saehkim@korea.ac.kr*

Cholesterol is a lipid found in animal products such as meat and eggs. Cholesterol accumulation by high consumption of these foods leads to diseases such as hypercholesteremia, atherosclerosis, liver damage, and obesity. A former study on the immunity of Maasai male to coronary heart disease revealed that high milk intake was inversely related to cholesteremia. The study was the beginning of numerous studies examining the functions of milk and milk products. For instance, a 90-day consumption of buffalo milk products fermented with probiotics attenuated serum TC, LDL-C, VLDL-C elevations, lowered atherogenic risk, and prevented hepatic lipid accumulation in a hypercholesteremia rat model. Also, another study proved that the co-consumption of probiotics enriched milk and oats had hypocholesterolemia lowering actions by reducing triglyceride, cholesterol, LDL, and VLDL levels in an albino rat model. Among various mechanisms proposed, the most prevalent mode for probiotic milk products to lower cholesterol is the secretion of bile salt hydrolase (BSH). BSH is an enzyme secreted by some Lactobacillus strains that hydrolyzes conjugated bile salts to deconjugated bile salts. Deconjugated bile salts are excreted through the host's feces, and due to excretion more cholesterol is used to synthesize new bile acids. The production of new bile acids therefore leads to the reduction of total cholesterol levels in the host. Further studies are still on-going for a more thorough understanding on the mechanism of cholesterol lowering effects of probiotic milk products.This study aimed to determine the cardiovascular health benefits of Maillard Reaction Proteins (MRPs) fermented by Lactobacillus strains. MRPs are made by the chemical reaction between amino groups and reducing sugars, and are known to have antioxidant, antihypertensive, and antimicrobial activities. Sprague-Dawley rats were given a high cholesterol diet for 6 weeks, and MRPs (1500mg/kg/day) fermented by Lactobacillus strains were co-administered during this period. As a result, the co-treatment of high fat diet and Lactobacillus fermented MRPs showed that Lactobacillus fermented MRPs were able to attenuate serum cholesterol levels and thrombotic activity, improve liver enzyme activities, and regulate mRNA expressions of cholesterol metabolism related genes. Moreover, lipid accumulation in the liver and aorta was reduced by Lactobacillus fermented MRP treatment.Altogether, these results proved that fermented products by probiotic strains are potential candidates for cardiovascular disease (CVD) treatments. However, further studies are needed to determine the connections between milk products and the host's microbiota, and the definite mechanism related.

Symposium 31-4

# Single Cell Genomics of Beige Adipogenesis in Germ Free Mouse

**Je Kyung Seong[1*], Jong Kyoung Kim[2], Yun Hee Lee[3], Daehee Hwang[4]**

[1] Laboratory of Developmental Biology and Genomics and Research Institute of Veterinary Science, College of Veterinary Medicine, Interdisciplinary Program for Bioinformatics and Korea Mouse Phenotyping Center, Seoul National University, Seoul, Republic of Kore
[2] Department of New Biology, DGIST, Daegu, Republic of Korea
[3] College of Pharmacy Seoul National University, Seoul, Republic of Korea
[4] Department of Biological Science, Seoul National University, Seoul, Republic of Korea

*snumouse@snu.ac.kr*

Beige adipocytes are thermogenic adipocytes that appear in white adipose tissues and have a potential to counteract obesity and metabolic diseases. Although multiple molecular players in beige adipogenesis have been identified, understanding of transcriptional dynamics in beige adipogenesis is limited due to heterogeneity in cellular status of differentiating adipocyte progenitors.

Also gut microbiota is one of key component controlling energy balance and glucose homeostasis. It can be mediated by beige adipogenesis and activated brown adipocytes. Here, we conducted transcriptomic profiling of PDGFRA+ single cells from inguinal adipose tissue of control mice and mice treated with a beta3 adrenergic receptor agonist, CL316243. This single cell-level analysis defined subpopulations that undergo proliferation and adipogenic differentiation under germ free conditions. The cell populations influencing beige adipogenesis in iWAT of germ free mouse has been newly identified. These finding provided the new insight for cellular dynamics of beige adipogenesis.

Keywords : scRNA seq, beige adipogenesis, germ free mouse, iWAT

# Current Clinical Trials With Gut Microbiota for Prevention of Atherosclerosis

**Sang Hoon Kim**[1*]

[1] Internal Medicine/Cardiology, CHA University School of Medicine, Korea

*kimsang978@naver.com*

For even one clinical trial, many previous basic laboratory studies and animal studies should be performed previously. Therefore, searching currently conducted clinical trials helps understanding and follow current trend and future perspectives of associated study categories. In this secession I want to review clinical trials about gut microbiota and atherosclerosis. There were many clinical trials for gut microbiota were conducted. Among them 12 studies were about gut microbiota and atherosclerosis. Two studies about TMAO modulation by gut microbiota modification were completed and can be searched in Pubmed and showed favorable results. Also, many studies about gut microbiota and metabolic disease were conducted, they were metformin related, probiotics for changing metabolic factors like insulin sensitivity, some fecal microbiome transplantation for diabetes and so on. Gut microbiota for vascular diseases are ongoing, some studies for control hypertension by gut microbiota change with probiotics are conducted and TMAO related myocardial infarction study was published. Gut microbiota modification for treating Coronary artery disease and stroke are also proceeding now. Many studies about gut microbiota, atherosclerosis, metabolic disease, vascular disease are waiting results. In phase 1 and preclinical studies some of them showed favorable results, especially TMAO modulation related aspect, we hope we can use some of them as a effective therapeutic agents for cardiovascular disease patients in the future.

Symposium 31-6 Short Talk

# Effects of Oral Porphyromanas Gingivalis Infection on Intestinal Microbiota and Atherosclerosis

**Inyeong Kim[1], Young Mi Park[1*], Su-jung Han[2], Kyung Ryul Seo[2], Jongwon Ha[2]**

[1] College of Medicine, Ewha Womans University, Korea
[2] College of Medicine, Yonsei University, Korea

*parkym@gmail.com*

P.gingivalis is a gram-negative anaerobic bacterium. It is an important pathogen associated with chronic periodontitis. Recent researches have shown that the increase in pro-inflammatory cytokine in periodontitis causes intestinal dysbiosis and there have been increasing evidences for the association between intestinal dysbiosis and atherosclerosis. There is positive correlation between periodontitis and atherosclerosis. However, the mechanism by which P.gingivalis promotes atherosclerosis and the mechanism of how the oral P.gingivalis infection affects intestinal microbiota have not been clearly defined.

Investigated the effects of P.gingivalis infection by inoculating P.gingivalis (10^9 CFU, daily administration for 3 weeks) into oral cavities of ApoE-/- mice after 8 weeks of western diet and maintaining 4 weeks of western diet thereafter. We collected feces from the western diet-fed ApoE-/- mice with or without oral P.gingivalis infection and analyzed the intestinal microbiota using 16S rRNA gene-based microbiome taxonomic profiling program in the Ezbiocloud.

Serum analyses for lipids showed that total cholesterol and HDL were lower in ApoE-/- mice with oral P.gingivalis infection and the oil-red-O staining of the en face aortae of the mice revealed that ApoE-/- mice with P.gingivalis infection had larger atherosclerotic lesions. The alpha diversities in the microbiome were analyzed by using CL community™ version 3.43 . The results showed that oral P.gingivalis infection attenuates the western diet-induced increase of Firmicutes, however, the relative abundance of Bacteroidetes was not affected by P.gingivalis infection. P.gingivalis infection augmented the western diet-induced increase in Deferrebacteres. Microbiome alpha diversities were decreased just after the 3 week administration of P.gingivalis (at week 11) and recovered to be similar to ApoE-/- mice without infection after 4 weeks.

P.gingivalis oral inoculation significantly changed intestinal microbiota and exacerbated atherosclerosis. Future studies would be warranted to unveil the mechanisms by which P.gingivalis infection alters the microbiota and of how the P.gingivalis infection promotes atherosclerosis.

# Comparison of Recent Guideline Statement

**Alberico Catapano**[1*]

[1] Parmacologial and Biomolecular Sciences and Mulrimedica IRCCS, University of Milano, Italy

*alberico.catapano@unimi.it*

In response to the need for expert synthesis and guidance on the use of newer data on the management of lipid disorders for the prevention of clinical ASCVD, expert panels were convened in the United States and Europe, resulting in the publication of the 2018 American Heart Association/American College of Cardiology/Multi-Society (AHA/ACC/MS) Guideline on the Management of Blood Cholesterol1 and the 2019 European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) Guidelines for the Management of Dyslipidemias: Lipid Modification to Reduce Cardiovascular Risk2. Both documents employ rankings of classes of recommendations and an assessment of supporting evidence, and advise preventive treatments in accordance with the estimated risk of the patient. These guidelines are based on data from Mendelian randomization, other genetic, epidemiological and clinical studies that show, in agreement with a wealth of data derived from basic research, that excessive circulating concentrations of apolipoprotein B (apoB) lipoproteins are a key driver of the atherosclerotic process3 and that reduction of low-density-lipoprotein (LDL) cholesterol (-C) using interventions that decrease LDL-C by increasing LDL receptor expression reduce ASCVD risk, with the greatest benefit observed in those with a history of ASCVD, higher baseline LDL-C, diabetes and other established risk factors. While there are many similarities between the two documents, there are also differences in interpretation of the evidence, resulting in different recommendations for lowering of LDL-C. In my presentation I will highlight the similarities and differences between these documents and the divergent perspectives that result in these differences

# Recent ESC-EAS Guideline

**Kausik Ray**[1*]

[1] Department of Primary Care and Public Health, Imperial College London, UK

*koshray@gmail.com*

The 2019 ESC/EAS guidelines made several critical updates. Firstly the goals for patients with very high, high, moderate risk were lowered to LDL-C of < 55mg/dl, <70mg/dl, <100mg/dl respectively. This should be achieved with earlier use of ezetimibe and/or PCSK9 inhibitors after maximising statin therapy. This must be achieved in high and very high risk patients in addition to at least a 50% reduction in LDL-C lowering. For LDL-C > 190mg/dl no risk scoring is recommended but merely moving to pharmacotherapy. There is also a recommendation for enhancing factors which can reclassify people in high risk groups. This includes imaging such as CIMT and CAC and Lp(a).

# JAS Guidelines for the Prevention of Cardiovascular Disease

**Hidenori Arai**[1*]

[1] National Center for Geriatrics and Gerontology, Japan

*harai@ncgg.go.jp*

Japan is entering a super aging society, which leads to a rapid increase in deaths due to cardiovascular and cerebrovascular diseases. Atherosclerosis, which is the basis of these disorders, is increasingly important in the future; therefore, its prevention and the establishment of therapeutic strategies are an urgent issue. Started as hyperlipidemia clinical practice guidelines in 1997, the Japan Atherosclerosis Society (JAS) has revised the guidelines for the diagnosis and prevention for atherosclerotic cardiovascular disease (ASCVD) every five years. In the latest 2017 version, a systematic review was performed on the following four issues; 1) dyslipidemia in risk factor assessment, 2) absolute risk of coronary artery disease (CAD) and lipid management target, 3) diet therapy in improving lifestyle habits and 4) drug therapy, and the response to clinical question is described with the evidence and recommendation levels. For the risk assessment of CAD, we adopted that of 10-year incidence of CAD based on the Suita score in the guidelines. The strict management of LDL-cholesterol (LDL-C) levels in high-risk ASCVD patients in secondary prevention is strongly emphasized and high-risk cases such as familial hypercholesterolemia and acute coronary syndrome are classified requiring management LDL-C levels to 70 mg/dl or less. After the guidelines 2017 were published, a couple of randomized controlled trials have been reported. One of them is REAL-CAD, which showed 4 mg of pitavastatin reduced cardiovascular events by 19 % compared to 1 mg of pitavastatin in patients with CAD, which is consistent with the TNT study. Another one is EWTOPIA-75 study, which we showed ezetimibe reduced cardiovascular events by 34 % compared to the control group in primary prevention patients 75 years old or over. We plan to revise the guidelines in 2022 and hope that the onset and recurrence of ASCVD will be prevented in Japan by managing the atherosclerotic risks based on the JAS guidelines.

Symposium 32-4

# Korean Guidelines for the Management of Dyslipidemia

**In-Kyung Jeong**[1*]

[1] Endocrinology and Metabolism, Kyung Hee University, Korea

*jik1016@naver.com*

Cardiovascular disease (CVD) is one of the leading causes of death in Korea. Among the major modifiable CVD risk factors including hypertension, dyslipidemia, diabetes, and smoking, the prevalence of dyslipidemia and diabetes is on the rise. Also, dyslipidemia is a primary and major risk factor of CVD in Korea. Therefore the management of dyslipidemia is very important to prevent CVD. Since the Korean Society of Lipid and Atherosclerosis (KSoLA) published the first edition of the Guidelines for the management of dyslipidemia in 1996, recently fourth edition was published in end of 2018. In this treatment guideline, we maintained the differentiating target LDL-C and non-HDL-C goals based on the level of CVD risk factors, as previously used in Korea. There are some different points from previous version. In very high risk group, it is recommended to administer statin immediately after acute MI regardless of the baseline LDL-C concentration. In high risk group, for diabetes patients with target organ damage or major CVD risk factors, the target could be lowered depending on the case. In diet therapy, limitation of daily cholesterol intake to 300 mg is recommended for patients with hypercholesterolemia. In pharmacological therapy, if statin therapy is not sufficient to achieve the target LDL-C, combination therapy with ezetimibe or PCSK9 inhibitor is recommended. Recently, we evaluated the appropriateness of our guideline by use of Korean National Health Insurance Service (NHIS) database. This lecture will summarize the recent Korean guideline for the management of dyslipidemia and the results from Korean NHIS data.

# Statin Therapy in HIGH Risk Patients With NORMal Coronary Arteries: HIGH-NORM Study

**Kyeong-Hyeon Chun[1], Chan Joo Lee[1], Jaewon Oh[1], Sungha Park[1], Seok-Min Kang[1], Sang-Hak Lee[1*]**

[1] Division of Cardiology, Yonsei University College of Medicine, Korea

*Shl1106@yuhs.ac*

Coronary imaging has been widely adopted in cardiovascular medicine. In contemporary lipid guidelines, statin is recommended for patients with high cardiovascular risk. However, the value of statin in patients with normal coronary arteries, and many physicians have resistance to use statins in this population. The aim of this study was to evaluate the effect of statin on clinical outcomes in patients with high cardiovascular risk but normal coronary arteries.

This was retrospective, propensity score-matched study and data were acquired between 2005-2019 in a tertiary university hospital. Of patients who underwent coronary CT angiography, 24,820 patients with normal or near normal coronary arteries (<30% luminal narrowing) were selected. After exclusion of those with other vascular disease or with low-moderate cardiovascular risk, 3,389 patients were chosen. Among them, patients who newly received statins (n=313) and 1:2 matched controls (n=626) were finally analyzed. Primary outcome variables include major adverse cardiovascular and cerebrovascular events (MACCE: cardiovascular death, nonfatal myocardial infarction, percutaneous coronary intervention, or ischemic stroke) and all-cause death.

Mean age of study population was 60.4 years and males were 58.4%. Baseline mean total cholesterol level was 193 mg/dL. At a median follow-up of 5.8 years, rates of MACCE were not different between statin users and controls (7.4 vs. 6.0 events/1000 patient-years; hazard ratio: 1.12; p=0.74). However, statin users had lower rates of all-cause death (0.7 vs 3.9 events/1000 patient-years; hazard ratio: 0.19; p=0.025). The incidences of each component of events did not significantly differ between the groups. In multivariate analysis, age, smoking status, and diabetes mellitus were identified as determinants for higher MACCE.

Statin therapy was not associated with lower risk of cardiovascular events, but with lower all-cause death in individuals with high cardiovascular risk and normal coronary arteries. In this specific population, decision of statin use is needed to be more refined

Symposium 32-6 Short Talk

# Assessing Validity for Extreme Risk Group of Cardiovascular Disease: A Nationwide Population-Based Study

**Kyung-Soo Kim[1], Kyungdo Han[2], Cheol-Young Park[3*]**

[1] Department of Internal Medicine, CHA Bundang Medical Center, CHA University School of Medicine, Korea
[2] Department of Statistics and Actuarial Science, Soongsil University, Korea
[3] Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Korea

*cydoctor@chol.com*

Extreme risk group was proposed in the latest guidelines but there were few studies for assessing validity of its definition. The aim of this study is to validate the criteria of extreme risk group of cardiovascular disease (CVD).

The study subjects were Koreans who conducted health exams supported by the Korean National Health Insurance Corporation in 2009 (baseline). After excluding individuals who were aged < 90 years, LDL-C ≥ 400 mg/dL, and had missing information, we followed 35,464 individuals with established atherosclerotic CVD (ASCVD). We investigated an incident CVD (myocardial infarction [MI] or ischemic stroke) to validate the criteria of extreme risk group defined as the presence of diabetes mellitus (DM), chronic kidney disease (CKD), and history of premature ASCVD (< 55 male, < 65 female).

The fully adjusted hazard ratio (HR) of patients with DM, CKD, and history of premature ASCVD for MI in extreme risk group were 1.62 (95% CI 1.45-1.81), 1.56 (1.36-1.78), and 1.30 (1.13-1.48), respectively. The HR of patients with DM, CKD, and history of premature ASCVD for ischemic stroke were 1.39 (1.29-1.50), 1.12 (1.02-1.22), and 2.08 (1.87-2.31), respectively. Compared to patients without DM, CKD, and history of premature ASCVD, patients with all three components were 3.90 times (3.45-4.40) more likely to have an incident CVD after fully adjustment.

DM, CKD, and history of premature ASCVD were associated with elevated risk for an incident CVD in individuals with established ASCVD. Consequently, the extreme risk category could be considered reasonable.

# Nutritional Therapy with Polyunsaturated Fatty Acids: Results from Clinical Trials

**Philip C. Calder**[1*]

[1] Facuty of Medicine, University of Southampton, UK

*P.C.Calder@soton.ac.uk*

There are two families of polyunsaturated fatty acids (PUFAs), the omega-6 (n-6) and the omega-3 (n-3). Members of both families can come from either plant or animal/fish sources. The plant n-6 PUFA linoleic acid lowers total and LDL-cholesterol concentrations, especially when replacing saturated fatty acids. This effect is due to regulation of hepatic LDL-receptor levels, in part via effects of SREBPs and in part by decreased PCSK9 levels. Thus, linoleic acid is expected to lower cardiovascular risk. The fish n-3 PUFAs beneficially modify as number of cardiovascular risk factors including as blood triglycerides and HDL-cholesterol, blood pressure, heart rate and heart rate variability, platelet aggregation, endothelial function, and inflammation. Thus, fish n-3 PUFAs are expected to lower cardiovascular risk. Long-term prospective cohort studies consistently demonstrate an association between higher intakes of fish, fatty fish and fish n-3 fatty acids (EPA + DHA) or higher levels of EPA and DHA in the body and lower risk of developing cardiovascular disease (CVD), especially coronary heart disease (CHD) and myocardial infarction (MI), and cardiovascular mortality in the general population. However, evidence for primary prevention of CVD through randomised controlled trials (RCTs) is relatively weak. In high-risk patients, especially in the secondary prevention setting (e.g., post-MI), a number of large RCTs support the use of EPA + DHA (or EPA alone) to reduce cardiovascular outcomes including mortality, as confirmed through a recent meta-analysis.

Symposium 33-2

# Telomere, Cardiovascular Aging, and Lifestyle Modification

**Inkyung Baik**[1*]

[1] Department of Foods and Nutrition, Kookmin University, Korea

*ibaik@kookmin.ac.kr*

It was reported that leukocyte telomere length (LTL), an indicator of biological aging, is associated with the risk of cardiovascular disease (CVD) and its risk factors including carotid plaque (CP). However, data regarding the effects of CVD and CP on LTL changes are limited. This study aimed to evaluate LTL changes during a 6-year period in middle-aged or older adults with CP or diagnosed CVD and analyze the association of CP and CVD with LTL changes. In addition, whether lifestyle factors modify this association was examined. The study participants were 1781 Korean adults aged 49-78 years, who underwent health examination including carotid ultrasound assessment and a questionnaire-based interview. Assays of relative LTL were conducted during the same period of health examination (baseline) and repeated after a mean follow-up time of 6 years. Mean values of 6-year LTL changes (differences between follow-up and baseline values) were 0.04 in participants who were free of both CP and CVD, -0.02 in those with CP who did not report CVD diagnosis, and -0.07 in those who reported CVD diagnosis. Participants with CVD were more likely to have LTL attrition compared with those who were free of both CP and CVD as well as with those with CP. Furthermore, CVD was significantly associated with LTL attrition even after the adjustment of potential risk factors (p-value=0.011). In particular, such an association was stronger among smokers (p-value=0.024), alcohol drinkers (p-value=0.022), and participants who did not consume green tea (p-value=0.009) compared with a proper comparable group for each lifestyle factor. These findings may suggest the role of CVD in short telomere, which may reflect vascular aging or cell senescence in other tissues, and can be supportive data to promote lifestyle modification for patients with CVD.

# The Role of Taste Receptors in CVD Risk

**Emma Feeney**[1*]

[1] Institute of Food and Health, University College Dublin, Ireland

*emma.feeney@ucd.ie*

The study of diet and health is moving away from a single nutrient and single outcome focus to a more holistic view, studying foods and dietary patterns. While the link between saturated fat (SFA) and heart health was established using the traditional reductionist approach, recent evidence shows that this link is more nuanced, and food-source dependent. Some food sources that are rich in SFA do not have the same impact as others. As research seeks to better understand dietary intake as a whole, the role of taste in food choice is of particular interest. Genetic differences in taste receptors can result in very different taste perception, and subsequently, taste preferences. On the other hand, dietary intake can also impact the regulation of gene expression in response to food consumption. This talk summarises some of the recent work being done at UCD in this area, highlighting the importance of considering individual differences in taste receptors both in dietary choice and in response to intake, within the umbrella of personalised nutrition.

Symposium 33-4

# Diverse Biological Functions of Ectopic Taste Receptors

**Sung-Joon Lee**[1*]

[1] Department of Biotechnology, School of Life Sciences & Biotechnology for BK21 PLUS, Department of Food Bioscience & Technology, College of Life Sciences & Biotechnology, Korea University

*junelee@korea.ac.kr*

Taste receptors are mainly expressed in the gustatory sensory cells and are stimulated by tastants, and oral gustatory systems mediate chemical signals from tastants via nerve systems to the brain. Taste receptors are also expressed in extra-oral tissues, and this expression has often been described as ectopic. However, various papers have reported widespread expression of taste receptors in many tissues, suggesting that extra-oral expression of taste receptor is normal. Extra-oral taste receptors have tissue-specific biological functions. Among taster receptors, sweet, umami, bitter and potentially fat taste receptors are classified as G protein-coupled receptors, which are major drug targets in the pharmaceutical industry, thus ectopic taste receptors may be attractive potential novel therapeutic targets for a broad range of clinical and industrial application. For example, bitter tastants (e.g. quinine and denatonium benzoate) act on TAS2Rs expressed on adipocytes and on enteroendocrine cells in the gut to reduce appetite and body weight, with increased plasma levels of enteroendocrine hormones such as GLP-1 in mice. Fat taste receptor such as free fatty acid receptor 4 (FFAT4) has been implicated in the ability to taste fats, has shown potential therapeutic application in the treatment of metabolic diseases. These findings suggest that ectopic taster receptors have tissue-specific functions including energy metabolism and hormone secretion and may be important as novel therapeutic targets.

# Role of Taste Receptor in Health Behaviours Outcome: Focusing on Epidemiological Evidence

**Jeong-Hwa Choi**[1*]

[1] Department of Food Science and Nutrition, Keimyung Univeristy, Korea

*jhchoi@kmu.ac.kr*

Human dietary behaviour is the comprehensive consequence of multiple factors including gender, socio-economic and psychological status, and education. Recent findings suggest the regulatory mechanism of taste perception, and add more information to understand the human dietary behaviour and disease aetiology. Major taste properties human could perceive, bitterness, sweet and umami taste are mediated by a family of G protein-coupled receptors, taste receptors type 1 and 2. Those chemosensing proteins in lingua tissue are the key mediators in the individuals' sensitivity and preference for taste, and, therefore, could modify the level for food, alcohol and tobacco consumption. The expression of taste receptors in extra-oral tissues including from brain, airways, thyroid and to the digestive tract have also been observed, and their physiological roles are revealed recently. A growing body of evidence elucidates that those extra-oral taste receptors are the critical in the identification of intra-/extra- stimuli, and hence lead to the differential subsequent metabolism by activating hormonal and neuronal cascades systems. The epidemiological findings also supported the idea: taste receptor are the associated with the susceptibility for cancer and other diseases with genetic variations as a modifying factor. Taken all together, taste and its genetics are the interesting key to understand human diet, health and disease. However studies are still in its infancy. More experimental and epidemiological evidence are required to expand the area, and will provide the potential application for a pharmaceutical and nutritional target as well as in food industry.

Symposium 33-6 Short Talk

# Real-World Evidence of Cardiovascular Effects of Calcium Supplementation: A Nationwide Cohort Study

**Kyoung Jin Kim[1,2], Nam Hoon Kim[2], Min Sun Kim[3], Juneyoung Lee[3], Sin Gon Kim[2*]**

[1] Division of Endocrinology and Metabolism, Severance Hospital, Korea
[2] Division of Endocrinology and Metabolism, Korea University College of Medicine, Korea
[3] Department of Biostatistics, Korea University College of Medicine, Korea

*k50367@korea.ac.kr*

To investigate the impact of calcium supplementation on the risk of major cardiocerebrovascular outcomes in patients with osteoporosis especially with low daily calcium intake in a real clinical setting.

In this Korean nationwide cohort study, we screened patients with diagnosed osteoporosis between 2004 and 2013. Among them, 16,231 participants who had used calcium supplementations for at least three months (Ca-treated group) were matched on propensity score in a 1:1 ratio with participants who had never received daily calcium supplementation (untreated group). Composite cardiocerebrovascular events including ischemic heart disease (IHD), ischemic stroke (IS), and cardiocerebrovascular death were assessed by Cox proportional hazards models.

During a median follow-up of 34.2 months (interquartile range, 13.7-50.5), 1229 and 1113 composite cardiocerebrovascular events occurred in the Ca-treated group and untreated group respectively. Calcium supplementation over three months was associated with increased risk of composite cardiocerebrovascular events (hazard ratio [HR], 1.11; 95% CI, 1.02-1.20).There were also significantly differences in terms of IHD (HR, 1.16; 95% CI, 1.00-1.34) and IS (HR, 1.12; 95% CI, 1.02-1.24) in the Ca-treated group compared to that in the untreated group. However, the risk of death from cardiovascular causes was significantly lower in the Ca-treated group than in the untreated group (HR, 0.61; 95% CI, 0.53-0.71).

This real-world database analysis showed that supplementary calcium intake was associated with increased ischemic cardiocerebrovascular risks including IHD and IS, while decreased CVD death among osteoporotic patients who have insufficient daily calcium intake.

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# IVBM Joint Session

September 9 (Wed) ~ October 10 (Sat), 2020 | Virtual Meeting (On demand)
September 9 (Wed) ~ 12 (Sat), 2020 | IVBM 2020 LIVE for Korean

KSoLA
The Korean Society of Lipid and Atherosclerosis

KVBM
Korean Society for Vascular Biology and Medicine

AAVBM-1

# Effect of Stroke Beyond the Brain

**Connie Wong**[1*]

[1] Medicine, Monash University, Australia

*connie.wong@monash.edu*

Stroke is a leading contributor of death and disability around the world. Despite its recognised debilitating neurological deficits, a devastating clinical complication of surviving stroke patients that needs more attention is infection. Up to half of the patients develop infections after stroke, and a high proportion of them will die as a direct consequence. Major clinical trials that examined preventive antibiotic therapy in stroke patients have demonstrated this method of prevention is not effective as it does not reduce incidence of post-stroke pneumonia or improve patient outcomes. Therefore, there is an urgent need for more effective therapeutic options that target the underlying mechanisms of post-stroke infections. In this talk, I will present our research on stroke-induced pathways outside the brain that result in the development of post-stroke infections.

AAVBM-2

# Vascular Specification and Homeostasis in Development

**Yulong He**[1*]

[1] Cyrus Tang Hematology Center, Soochow University, China

*heyulong@suda.edu.cn*

Mechanisms underlying vein specification and homeostasis remain imcompletely elucidated. In this presentation, I will mainly discuss the key pathways involved in vein development and maintenance, including: 1) Using genetically modified mouse models targeting Tek, we have shown that TIE2 plays an essential role in vein specification and the parallel alignment of veins with arteries. Biochemical analysis further reveals that TIE2 is required for the identity of venous endothelial cells via AKT-mediated stabilization of COUP-TFII, a key regulator in venogenesis. 2) Loss of endothelial cell-derived follistatin-like protein 1 (FSTL1) leads to an increase of pulmonary vascular resistance, resulting in the heart regurgitation especially with tricuspid valves. There is excessive alpha-smooth muscle actin (αSMA) associated with atrial endocardia, heart valves and veins after the endothelial FSTL1 deletion. The SMAD3 phosphorylation is significantly enhanced and the treatment with a TGFβ pathway inhibitor reduces the abnormal association of αSMA with the atria and venous vessels in Fstl1ECKO mutant mice. The findings imply that endothelial FSTL1 is critical for the homeostasis of vascular walls and its insufficiency may favor cardiovascular fibrosis leading to heart failure.

# Lymphatic Vasculature and Cholesterol: A Love Affair?

**Veronique Angeli**[1*]

[1] Microbiology and immunology department, Immunology Programme, Yong Yoo Lin School of Medicine, National University of Singapore, Singapore

*micva@nus.edu.sg*

In the last decade, research has provided evidence for a bidirectional crosstalk between lymphatic vessels and cholesterol. Lymphatic vessels are essential for proper cholesterol clearance from tissues while excess cholesterol in tissues may lead to morphological and functional alterations in the lymphatic vasculature. Although adventitial lymphangiogenesis has been associated with atherosclerosis in humans and mice, the role of lymphatic drainage in this disease is not well understood and the functionality of the new vessels has not been assessed. Therefore, we developed new tools to assess the functionality of aortic lymphatic vessels in experimental atherosclerosis, and further define the contribution of lymphatic drainage in atherogenesis. Our results suggest that despite the growth of lymphatic vessels in atherosclerotic artery, aortic lymphatic drainage is severely compromised and contribute to atherosclerotic plaque progression. Therefore, promoting lymphatic drainage seems a promising additional therapeutic strategy for atherosclerosis.

AAVBM-4

# Tumor Vasculature as a Target for Anticancer Radiotherapy

**G-One Ahn**[1*]

[1] College of Veterinary Medicine, Seoul National University, Korea

*goneahn@snu.ac.kr*

Although less realized, radiation therapy is a powerful antiangiogenic therapy for cancer. We have previously demonstrated that clinically relevant radiation doses effectively inhibit tumor vasculature and that re-vascularization involves with bone marrow-derived monocyte/macrophages. These myeloid cells (monocytes and macrophages) not only secrete proteases such as matrix metalloproteinase-9 but also vascular endothelial growth factor. Furthermore, we have recently reported that these cells contribute to hypoxia and glycolysis thereby complicating anticancer treatments. In this talk, I will highlight some new angles where we can approach to inhibit tumor vasculature in combination with anticancer radiotherapy.

# Regulatory T Cell (Treg) Control of Cardiovascular Regeneration

**Kathy Lui**[1*]

[1] Chemical Pathology, The Chinese University of Hong Kong, Hong Kong

*kathyolui@cuhk.edu.hk*

To study the functional role of CD4+FOXP3+ Treg in the regeneration of blood vessel and heart muscle after injuries.

We employed a gain-of-function model by adoptive transfer of hCD2 expressing Treg derived from the Foxp3hCD2 knockin mice where expression of hCD2 is driven under the Treg specific transcription factor Foxp3; and a loss-of-function model via Foxp3 driven ablation of Treg after diphtheria toxin treatment in the Foxp3-DTR mice. We also utilised single cell RNA-sequencing (scRNA-Seq) to study the molecular signatures of individual T cells during cardiovascular regeneration. Finally, we developed a patient specific humanized mouse model to study the therapeutic potential of humanized antibodies in the expansion of human Treg for promoting vascular regeneration in vivo.

We found that CD4+ Th subsets and CD8+ cytotoxic T cells are detrimental in cardiovascular regeneration after ischemic injury in vivo. Interestingly, the developmental trajectory analyzed by scRNA-Seq uncovers that these cells are plastic and can be posed to differentiate towards the angiogenic or cytotoxic phenotype in the regenerating or non-regenerating blood vessels, respectively. Moreover, we found that Treg facilitate the regeneration of heart muscle and blood vessels by potentiating their proliferation in a paracrine manner. Furthermore, scRNA-Seq analyses also showed that targeting PD-1 signaling can expand Treg in vivo. Finally, we showed that human Treg can be expanded in vivo for facilitating vascular function by humanized antibodies.

Our studies might give clinically relevant insights into development of immune regulatory medicine for potential treatment of cardiovascular diseases.

References:

Leung OM... Lui KO, Cell Reports, 2018

Leung CS... Lui KO, Genome Medicine, 2018

Li J... Lui KO, Theranostics, 2019

Liang C... Lui KO, Theranostics, 2020

Li J... Lui KO, Biomaterials, in revision

AAVBM-6 Short Talk

# VEGF-Mediated Unique Epigenetic Modifications Initiated Accurate Angiogenesis via Bivalent Marked Gene-Set Activation in Endothelium

**Takashi Minami**[1*]

[1] IRDA, Div. Molecular and Vascular Biology., Kumamoto University, Japan

*t-minami@kumamoto-u.ac.jp*

Endothelial cells (EC)s display phenotypic heterogeneity in our body, which is mainly regulated by epigenetic regulation, at least, by site-specific and time-dependent differences in gene transcription. We have previously reported EC specific transcriptional regulation genome-widely via ChIP-seqs (EMBO. J. 2011, Mol. Cell.Biol. 2011, Cell Rep. 2013, Nuc.Acids.Res. 2017, Plos.Genet. 2018). VEGF, best-known angiogenic and EC maturation factor, activates calcium-NFAT signaling. Considering the genetic factors for EC activation, NFAT is believed to be a key mediator for acute turning on the VEGF-angiogenic switch via changing the chromatin structure.

Thus, we examined global mapping of genome-wide NFAT binding events, as well as major histone-code profiling in VEGF-stimulated ECs with ChIP-seqs, and categorized them by combination with whole mRNA-seqs treated with VEGF in detailed time points.

Remarkably, essential transcription factors for vascular sprouting and proliferation, almost exclusively obtained embryonic stem-like bivalent epigenetic marks after the VEGF stimulus within 1 hour. Our newly found NFAT associated epigenome modifier, PTIP, directed these critical angiogenic gene transcriptions by the H3K4me3-accelerater over the H3K27me3-brake. Moreover, EC specified polycomb complex variant (PRCI.3) specifically bound and allow the H3K27me3-brake marks enriched bivalent angiogenic genes to active transcription only the VEGF-stimulated ~15 minutes just before the conventional PRCI association. EC-specific knockout mice of these epigenome modifier birthed normally on the embryo, but abrogated aberrant neo-vessel formations and xenografted tumor growth via turning off the acute angiogenic transcription factors.

Collectively, uncovering such a time dependent dynamic epigenome landscape in VEGF treated endothelium would lead emerging theory for understanding the exact EC activation status as well as epigenetically modified therapeutic ways against the various vasculopatic diseases.

# Involvement of Vital NETosis in the Progression of High Fat Diet-Induced Atherogenic Vascular Inflammation

**Masayuki Yoshida**[1*]

[1] Dept Life Science and Bioethics, Tokyo Medical and Dental University, Japan

*masa.vasc@tmd.ac.jp*

Exceed fat intake has been shown to trigger the development of atherosclerosis. However, direct molecular link between high fat diet (HFD) and vascular inflammation is not completely understood. Previously, we reported a dominant role of complement C5a and subsequent neutrophil activation in HFD–induced vascular inflammation in wild type mice (Osaka et al. Sci Rep 2016). To further extend our knowledge from vascular inflammation toward atherosclerosis development, we utilized LDL receptor deficient LDLR-/- mice fed HFD to observe atherosclerosis development. As previously reported, LDLR-/- mice (age of 7weeks, male) fed HFD (20% fat, 1.25%cholesterol) for 12weeks developed significant atherosclerotic lesion formation judged from Oil red O staining. Interestingly, neutrophil depletion by specific antibody significantly reduces lesion formation under HFD suggesting a role for neutrophil in atherosclerosis development in LDLR-/- mice. Peripheral MPO and NE assay supported neutrophil activation as early as 4 weeks of HFD in LDLR-/- mice. Moreover, citrullinated histone, a marker of neutrophil extracellular traps (NETs), was detected in the aorta of LDLR-/- mice prior to lesion development. Ex vivo analysis confirmed that sera from HFD fed LDL-/- mice significantly induced histone citrullination in BM-derived neutrophils. These line of evidence strongly supported that neutrophil activation plays a key role in HFD-induced atherosclerosis. Further study will be necessary to understand the molecular consequence behind this phenomenon.

JK Joint Session 1-2

# Role of Mitochondrial UQCRB in Tumor Angiogenesis

**Ho Jeong Kwon**[1*]

[1] Department of Biotechnology, Yonsei University, Korea

*kwonhj@yonsei.ac.kr*

The Complex III of mitochondrial respiratory chain has been highlighted as a crucial regulator in hypoxia-induced angiogenesis through mitochondria-derived reactive oxygen species (ROS) involved oxygen sensing. Recently, we identified the ubiquinol-cytochrome c reductase binding protein (UQCRB), a subunit of the mitochondrial Complex III, is responsible for anti-angiogenic activity of terpestacin, a natural anti-angiogenic small molecule. Notably, terpestacin binding to UQCRB inhibited hypoxia-induced ROS generation, subsequently HIF-1 activation and angiogenesis in vivo, without inhibiting mitochondrial respiration. Accordingly, small molecules targeting UQCRB can suppress tumor angiogenesis without acting as a respiratory poison. Functional knock down of UQCRB by siUQCRB as well as UQCRB morpholino in zebrafish dose-dependently inhibited angiogenesis both in vitro and in vivo. Furthermore, vascular endothelial growth factor (VEGF) levels decreased with UQCRB inhibitor treatment and UQCRB morpholino injection in zebrafish model. Moreover, co-treatment with terpestacin and bevacizumab, a VEGF signaling inhibitor, resulted in additive inhibition of angiogenesis both in vitro and in vivo. These results demonstrate that UQCRB could be a new target for treating angiogenesis disregulated diseases including cancer and retinopathy. Based on these new structural and biological information, new small molecules that specifically regulate the function of UQCRB were developed by using the smart chemical library designed by pharmacophore-based virtual screening. Collectively, these studies provide new insights into the role of UQCRB in angiogenesis and small molecules targeting UQCRB provide new mitochondrial ROS inhibitors with novel modes of action for treating angiogenesis disregulated diseases.

# Targeting Senescent Cells for the Treatment of Lifestyle-Related Disease

**Tohru Minamino**[1*]

[1] Department of Cardiovascular Biology and Medicine, Juntendo University Graduate School of Medicine, Japan

*tminamino@med.niigata-u.ac.jp*

Epidemiological studies have shown that age is the dominant risk factor for lifestyle-related diseases. The incidence and the prevalence of diabetes, heart failure, coronary heart disease and hypertension increase with advancing age. However, the molecular mechanisms underlying the increased risk of such diseases that is conferred by aging remain unclear. Cellular senescence is originally described as the finite replicative lifespan of human somatic cells in culture. Cellular senescence is accompanied by a specific set of phenotypic changes in morphology and gene expression including negative regulators of the cell cycle such as p53. Primary cultured cells from patients with premature aging syndromes are known to have a shorter lifespan than cells from age-matched healthy persons. It is also reported that the number of senescent cells increases in various tissues with advancing age. Interestingly, such accumulation of senescent cells in aged animals is attenuated by caloric restriction that regulates the lifespan regulatory system and delays age-associate phenotypes. I therefore hypothesize that cellular senescence in vivo contributes to the pathogenesis of age-associated disease and have shown a critical role of cellular senescence in age-related pathologies. However, a direct inhibition of cellular aging signaling would lead to the increased incidence of cancer, so we need to develop anti-senescent therapy without cancer development. Here I will show our recent data on a novel strategy of anti-senescent therapy for lifestyle-related disease by targeting cellular senescence (Seno-antigens, Seno-metabolites, SASP), which would not promote tumorigenesis.

JK Joint Session 1-4

# Heat Shock Protein 70 as a Potential Therapeutic Target for Diabetic Erectile Dysfunction

**Kalyan Ghatak[1], Guo Nan Yin[1], Ji-Kan Ryu[1*], Jun-Kyu Suh[1]**

[1] Urology and Research Center for Sexual Medicine, Inha University School of Medicine, Korea

*rjk0929@inha.ac.kr*

Penile neurovascular dysfunction is a major cause of erectile dysfunction (ED) in patients with diabetes, which is responsible for poor response rate to oral phosphodiesterase-5 inhibitors. Heat shock protein 70 (Hsp70) is one of the molecular chaperones and play an indispensable role for the regulation of cell proliferation, survival, and angiogenesis. However, the role of Hsp70 in ED has not been studied yet. Here, we determined whether and how Hsp70 restores erectile function in diabetic mice. Intracavernous administration of Hsp70 restored erectile function in the diabetic mice, which reached up to 90% of control values. Hsp70 protein induced significant restoration of cavernous contents of endothelial cells, pericytes, and neuronal cells in the diabetic mice in vivo; promoted tube formation in primary cultured mouse penile endothelial cells under high-glucose condition in vitro; and accelerated neurite sprouting from major pelvic ganglion under high-glucose condition ex vivo, by regulating the expression of neurotrophic factors (BDNF, NGF and NT-3). Transcriptome analysis of penile endothelial cells revealed that cystathionine gamma-lyase (Cth) is a major molecular target for HSP70. Inhibition of Cth abolished the HSP70-mediated penile angiogenesis and neural regeneration in the diabetic mice. Hsp70 significantly improved the erectile function through recovery of damaged penile blood vessels and nerves in diabetic condition. The dual angiogenic and neurotrophic effects of Hsp70, especially local therapy in the form of therapeutic protein, will open a new avenue to treat diabetic ED.

# Identification of the Master Regulators of Aortic Valve Calcification

**Tomohisa Sakaue[1,2*], Mika Hamaguchi[3], Jun Aono[3], Koh-ichi Nakashiro[4], Fumiaki Shikata[5], Yusuke Oshima[6], Mie Kurata[7], Junya Masumoto[7], Osamu Yamaguchi[3], Shigeki Higashiyama[2], Hironori Izutani[1]**

[1] Department of Cardiovascular and Thoracic Surgery, Ehime University Graduate School of Medicine, Ehime University, Japan
[2] Department of Cell Growth and Tumor Regulation, Proteo-Science Center, Ehime University, Japan
[3] Department of Cardiology, Pulmonology, Hypertension, and Nephrology, Ehime University Graduate Schoo, Ehime University, Japan
[4] Department of Oral and Maxillofacial Surgery, Ehime University, Graduate School of Medicine, Ehime University, Japan
[5] Department of cardiovascular surgery, Nagano children's hospital, Japan
[6] Department of Gastroenterological and Pediatric Surgery, Oita University, Japan
[7] Department of Pathology, Division of Analytical Pathology, Ehime University, Japan

*sakaue@m.ehime-u.ac.jp*

AS (aortic valve stenosis) is the most common in valvular heart diseases. Accordingly, aortic valve replacements are frequently required due to calcification and fibrosis mainly related to ages and morphological features like bicuspid valves. However, medical therapies have not been established for progression of aortic stenosis. In this present work, we aim to identify the master regulator of calcification and clarify the molecular mechanisms of AS using comprehensive gene expression analysis.

Calcified aortic valves were surgically excised from AS patients (n=5) who required aortic valve replacement. The valve interstitial cells adhering to the dish bottom were cultured with DMEM in humidified 10% $CO_2$/air under 2% oxygen concentrations for 3 weeks. Total RNAs were extracted from calcified and non-calcified VICs and microarray analysis were performed. Up-regulation of gene expression were confirmed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), Western blotting (WB) and immunohistochemical (IHC) analysis. Functional analyses of identified proteins were performed by in vitro calcification assays.

We found 87 genes showing greater than a twofold change in calcified tissues. Among these genes, 68 were downregulated and 19 were upregulated. Prostaglandin G/H synthase 1 (PTGS1) messenger RNA and protein levels were upregulated in VICs from calcified tissues. The PTGS1 messenger RNA and protein levels in VICs were also strongly increased by stimulation with osteoblast differentiation medium. Although Alizarin Red staining-positive nodules were observed in control siRNA-transfected cells, these phenotypes were completely abrogated by transfection with PTGS1 siRNAs.

The VIC-specific PTGS1 played a crucial role in calcification by promoting osteoblast differentiation in aortic valve tissues.

JK Joint Session 1-6 Short Talk

# YAP and TAZ Regulate Lymphatic Valve Development

**Boksik Cha[1,2*], Yen-Chun Ho[1], Xin Geng[2], Riaj Mahamud[2], R. Sathish Srinivasan[2], Lijuan Chen[2]**

[1] New Drug Development Center, Daegu-Gyeongbuk Medical Innovation Foundation, Korea
[2] 2Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, USA

*boksik-cha@dgmif.re.kr*

The lymphatic vasculature regulates fluid homeostasis by returning lymph to the blood circulation. Lymph travels through lymphatic vessels containing lymphatic valves (LVs). LVs function as gates that prevent the backflow of lymph. Lymph finally return to blood circulation via two pairs of lymphovenous valves (LVVs) located bilaterally at the intersection of jugular and subclavian veins.

Defects in LVs and LVVs cause lymphedema, a disease in which tissues swell due to fluid accumulation. However, the mechanisms that regulate LV and LVV formation and maintenance remain incompletely understood. Here, we have identified that the transcriptional co-activators YAP and TAZ are necessary for the maintenance of valvular endothelial cells. In vivo the nuclear localization and transcriptional activity of YAP/TAZ gradually increase in LVs and LVVs during valve development. Tissue-specific deletion of Yap and Taz in mouse embryos did not affect LV and LVV initiation. However, the loss of Yap and Taz resulted in the degeneration of valves. Furthermore, VEGF-C/VEGFR3 signaling pathway is necessary for valve morphogenesis and VEGF-C can enhance YAP and TAZ activity. Our results have identified VEGF-C/YAP/TAZ as a previous unknown molecular pathway that regulates valve morphogenesis within the mammalian lymphatic vasculature.

# Fibroblast Growth Factor 12, a New Regulator of Vascular Remodeling in Pulmonary Arterial Hypertension

**Wonhee Suh**[1*]

[1] College of Pharmacy, Chung-Ang University, Korea

*wsuh@cau.ac.kr*

Loss of bone morphogenic protein (BMP) signaling induces the phenotype switch of pulmonary arterial smooth muscle cells (PASMCs), which is the pathological basis of pulmonary vascular remodeling in pulmonary arterial hypertension (PAH). Here, we identified fibroblast growth factor 12 (FGF12) as a novel regulator of the BMP-induced phenotype change in PASMC and elucidated its role in pulmonary vascular remodeling during PAH development.

The expression levels of FGF12 were analyzed in lung tissues of two different murine models of PAH and patients with PAH. A role of FGF12 during PAH-mediated vascular remodeling process was investigated in gain of function and loss of function experiments using tissue-specific conditional transgenic mice, adenoviral transfection, and siRNA-mediated knockdown.

Using murine models of PAH and tissue specimens of patients with PAH, we observed that FGF12 expression was significantly reduced in PASMCs. In human PASMCs, FGF12 expression was increased by canonical BMP signaling. FGF12 knockdown blocked the anti-proliferative and pro-differentiation effect of BMP on human PASMCs, suggesting that FGF12 was required for the BMP-mediated acquisition of quiescent and differentiated phenotype of PASMCs. Mechanistically, FGF12 regulated this phenotype change by inducing myocyte enhancer factor 2a (MEF2a) phosphorylation via p38MAPK, thereby modulating the MEF2a target gene expression involved in cell proliferation and differentiation. Furthermore, we observed that transgenic mice with SMC-specific FGF12 overexpression were protected from chronic hypoxia-induced PAH development, pulmonary vascular remodeling, and right ventricular hypertrophy. Consistent with in vitro data using human PASMC, FGF12 transgenic mice showed high levels of MEF2a phosphorylation and a substantial change in MEF2a target gene expression compared to that in wild type controls.

Overall, our findings suggested FGF12 as a potential molecular target for the development of therapeutics directed toward pulmonary vascular remodeling in PAH.

JK Joint Session 2-2

# New Insights Into Drug-Resistance: Abnormality in Tumor Endothelial Cells

**Kyoko Hida**[1*]

[1] Graduated School of Dental Medicine, Hokkaido University, Japan

*khida@den.hokudai.ac.jp*

Drug resistance is a major problem in anticancer therapy. It has been considered that resistance is caused in cancer cells, however, recent studies have revealed that tumor endothelial cells are also resistant to several drugs. We have reported that tumor endothelial cells (TECs) have abnormal chromosome and centrosome (Hida et al., Cancer Res 2004) Furthermore, TECs showed resistance to paclitaxel via an elevated level of ABCB1(p-glycoprotein) (Akiyama et al, Am J Pathol 2012), which is one of drug transporter. Furthermore, ABCB1 expression is induced in tumor blood vessels by inflammatory change during chemotherapy (Kikuchi et al., Cancer Res 2020). This may be another mechanism of acquired resistance. TECs also plays a role in tumor immunity. TECs secret damage-associated molecular patterns (DAMPs) such as IL-6, IL-1beta, and biglycan.
We have shown that IL-6 and biglycan alter tumor microenvironment to suppress tumor immunity or reduce drug efficacy. Resistant TEC can sustainably support cancer cells during chemotherapy. Thus, targeting these abnormal TEC may provide a promising strategy.

# Roles of TGF-β Family Signals During Formation and Maintenance of Blood and Lymphatic Vascular Systems

**Tetsuro Watabe**[1*]

[1] Department of Biochemistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Japan

*t-watabe@umin.ac.jp*

Blood and lymphatic vasculatures play important roles not only in the maintenance of fluid homeostasis. Blood and lymphatic vessels undergo anatomical and physiological changes during inflammation and aging. While lymphatic endothelial cells (LECs) undergo mesenchymal transition in response to transforming growth factor-β (TGF-β), the molecular mechanisms underlying endothelial-to-mesenchymal transition (EndMT) of LECs remain largely unknown. We examined the effect of TGF-β2 on EndMT using human skin-derived lymphatic endothelial cells (HDLECs). TGF-β2-treated HDLECs showed increased expression of SM22α, a mesenchymal cell marker accompanied by increased cell motility and vascular permeability, suggesting HDLECs to undergo EndMT. Furthermore, TGF-β2 induced the production of Activin A while decreasing the expression of its inhibitory molecule Follistatin, and thus enhancing EndMT. The tumor microenvironment (TME) consists of various components including cancer cells, tumor vessels, cancer-associated fibroblasts (CAFs). These components interact with each other via various cytokines, which often induce tumor progression. Thus, a greater understanding of TME networks is crucial for the development of novel cancer therapies. Many cancer types express high levels of TGF-β, which induces EndMT of blood vascular endothelial cells (BECs), leading to formation of CAFs. Although we previously reported that CAFs derived from EndMT promoted tumor formation, the molecular mechanisms underlying these interactions remain to be elucidated. Human umbilical aortic endothelial cells (HUAECs) underwent EndMT in response to TGF-β. In addition, treatment of ECs with TGF-β exhibited sustained activation of Smad2/3 signals, which was presumably induced by elevated expression of TGF-β2 and Activin A, suggesting that TGF-β induces EndMT by augmenting TGF-β family signals. Furthermore, oral cancer cells underwent epithelial-to-mesenchymal transition (EMT) in response to humoral factors produced by TGF-β-cultured ECs. This EndMT-driven EMT was blocked by inhibiting the action of TGF-βs. Collectively, our findings suggest that TGF-β induces EndMT of both BECs and LECs, which contributes to progression of various diseases.

JK Joint Session 2-4

# Epigenetic Regulation of Vascular Calcification

**Hyun Kook**[1*]

[1] National Research Laboratory for Heart and Muscle Diseases, Vascular Remodeling Research Center, Department of Pharmacology, Chonnam National University, Korea

*kookhyun@chonnam.ac.kr*

Calcium deposition to vascular smooth muscle matrix, vascular calcification (VC), makes the vessels rigid, which results in the increase in the morbidity and mortality of the patients with cardiovascular diseases or renal diseases. Previously, we suggested that histone deacetylase (HDAC) 1 prevents VC, whereas its E3 ligase, mouse double minute 2 homolog (MDM2) exaggerates it by inducing the polyubiquitination of HDAC1. The aim of the current work is to find the transcriptional regulation mechanism of MDM2 in response to vascular calcification stresses. By promoter mapping analysis, we found the Pi-responsive element exist in -1.0~-1.5 kb upstream of MDM2 promoter. Further analysis showed that -1331~-1327 region, an Msh homeobox (Msx) binding element is critical for the activation of MDM2. Both Msx1 and Msx2 can bind to the Pi-responsive element in a sequence-specific manner and can induce the activation of MDM2 promoter as well as the increase in MDM2 protein/mRNA levels. By in vivo animal models, we further demonstrated that both Msx1 and Msx2 enhance the calcification of aorta in mice. As an alternative key epigenetic regulator, non-coding RNAs are under extensive investigation in association with VC. By microRNA array, we found that miR-2A and miR-3B were dysregulated. miR-2A targets activating transcription factor 3 to inhibit VC, whereas miR-3B affects HDAC5 to induce VC. By RNA sequencing, we found some candidate circular RNAs that are expected to affect the VC. We investigated that circ4S, a circular RNA, have anti-calcification roles by working as miRNA sponges. Taken together, the epigenetic regulation of both Msx/MDM2/HDAC1 axis and non-coding RNA participate in the development of VC, which will be novel therapeutic targets of the diseases.

# Analysis of the Angiopoietin-1 Expressing Macrophage in Mouse Wound Healing Model

**Seiji Yamamoto[1*], Erika Azuma[1,2], Masashi Muramatsu[3], Takeru Hamashima[1], Noriko Okuno[1], Masao Hayashi[1], Naruho Okita[1], Naotaka Yamauchi[1], Masabumi Shibuya[4], Shumpei Niida[5], Masakiyo Sasahara[1]**

[1] Departmento of Pathology, University of Toyama, Japan
[2] Department of Technology Development, Astellas Pharma Tech Co., Ltd., Japan
[3] Institute of Resource Development and Analysis, Kumamoto University, Japan
[4] Institute of Physiology and Medicine, Jobu University, Japan
[5] Bio Bank, National Center for Geriatrics and Gerontology, Japan

*seiyama@med.u-toyama.ac.jp*

In wound healing process, angiogenesis plays important roles in wounded area for supplying oxygen and nutrients, and recruitment of the inflammatory related cells, such as macrophages. Macrophages are generally classified two groups, M1 and M2, by their function and expressing markers; however, the contribution of such macrophages to angiogenesis on wounded area is still unclear.

Here, we clearly demonstrated that the CD206$^{+}$ M2 macrophage promotes neovascularization in wounded area of skin utilizing virtually macrophage deficient mice, Csf1$^{op/op}$.

In Csf1$^{op/op}$ mice, severely suppressed sprout formation and vascular density, and aberrant blood vessel formation could be observed in wounded area. We found that CD206$^{+}$ macrophages secreted angiopoietin-1. In vitro, by supplementing angiopoietin-1 to HUVECs, WAS and ITGAV mRNAs were upregulated, which have been considered important roles in filopodia formation and binding to ECM.

These findings suggest that CD206$^{+}$ macrophages modulate endothelial cells to proangiogenic status by secreting Angiopoietin-1, and mediate appropriate angiogenesis in wound healing site. We conclude that bone-marrow derived M2 macrophage recruited in wounded area plays crucial roles in angiogenesis.

JK Joint Session 2-6 Short Talk

# Role of AIM2 Inflammasome in Post-Stroke Cognitive Impairment

**Hwa Kyoung Shin**[1,2*]

[1] Korean Medical Science, Pusan National University, Korea
[2] Korean Medical Science Research Center for Healthy-Aging, Pusan National University, Korea

*julie@pusan.ac.kr*

Although over one-third of stroke patients may develop post-stroke cognitive impairment (PSCI), the mechanisms underlying PSCI remain unclear. We explored here, the involvement of post-stroke inflammasomes in long-term PSCI development.

We induced ischemic brain injury using a 45 min-middle cerebral artery occlusion (MCAO)/reperfusion. Immunohistological assessment was performed at 1, 3, or 7 days and cognitive function test at 28 days post-stroke. Evaluation of inflammasome sensor gene expression in aged mouse brains showed dominant expression of absent in melanoma 2 (Aim2) in 6-, 12-, and 18-month-old mouse brains. AIM2 mRNA and protein increased until 7 days post-stroke. PSCI decreased anxiety in elevated plus maze tests and impaired spatial learning and memory functions in Morris water maze tests 28 days post-stroke. AIM2 and other inflammasome subunit immunoreactivities, including those for caspase-1, interleukin (IL)-1β, and IL-18, were higher in the hippocampus and cortex of the PSCI than in those of the sham group 7 days post-stroke. AIM2 immunoreactivity of the PSCI group was primarily co-localized with Iba-1 (microglial marker) and CD31 (endothelial cell marker) immunoreactivities but not NeuN (neuronal marker) and GFAP (astrocyte marker) immunoreactivities, suggesting that microglia or endothelial cell-induced AIM2 production mediated PSCI pathogenesis. Additionally, inflammasome-induced pyroptosis might contribute to acute and chronic neuronal death after stroke. AIM2 knockout (KO) and Ac-YVAD-CMK-induced caspase-1 inhibition in mice significantly improved cognitive function and reversed brain volume in the hippocampus relative to those in stroke mice.

AIM2 inflammasome-mediated inflammation and pyroptosis likely aggravated PSCI; therefore, targeting and controlling AIM2 inflammasome could potentially treat PSCI.

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# ICoLA Joint Session

September 9 (Wed) ~ October 10 (Sat), 2020 | Virtual Meeting (On demand)
September 9 (Wed) ~ 12 (Sat), 2020 | IVBM 2020 LIVE for Korean

KSoLA
The Korean Society of Lipid and Atherosclerosis

KVBM
Korean Society for Vascular Biology and Medicine

# Clinical Aspects of Genetic Architecture of the Circulating Very Long-Chain Ceramides (24:0 and 22:0)

**Linda R. Peterson**[1*]

[1] Medicine, Washington University, USA

*lpeterso@wustl.edu*

Ceramides are waxy lipids that have been implicated in the pathogenesis of cardiovascular disease due to obesity and type 2 diabetes, as well as other 'lipotoxic' disorders. In animal models of excessive fat uptake and lipotoxicity, ceramide enhances apoptosis and organ dysfunction. However, recent studies by our group and others have shown that the length of the fatty acyl chain in the ceramide molecule affects its effects.

Total ceramide concentrations are linked with increased insulin resistance and cardiac dysfunction. However, recent large studies demonstrate that plasma concentrations of specific very long-chain fatty ceramides (e.g., C24:0 and C22:0) are associated with reduced incidence of coronary heart disease and/or all-cause mortality. We hypothesized that specific genetic loci are associated with plasma C22:0 and C24:0 concentrations.

Our group investigated the genetic underpinnings of these very long-chain plasma ceramides, C24:0 and C22:0 in the FHS. Multivariable heritability studies showed that both very-long chain ceramides were heritable (C22:0, 0.42, $p<0.001$; C24:0, 0.25, $p<0.001$). Genome-wide association studies showed 19 single nucleotide polymorphisms (SNPs) were significantly associated with C22:0 concentrations; all on chromosome 20; with the closest gene to these variants was SPTLC3. The lead SNP (rs4814175) was significantly associated with 3% lower plasma C22:0 concentrations ($p=2.83E-11$). Nine SNPS were significantly associated with C24:0 ceramide; All 9 were on chromosome 20, close to SPTLC3. All 9 also significantly related to plasma C22:0. The lead SNP (rs168622) significantly associated with 10% lower plasma C24:0 ceramide concentrations ($p=9.94E-09$).

SNPs near SPTLC3, the gene encoding 'serine palmitoyltransferase long chain base subunit 3' (which is a part of the enzyme that catalyzes the rate-limiting step of de novo sphingolipid synthesis) are associated with plasma C22:0 and C24:0 ceramide concentrations. These results are biologically plausible and suggest that SPLTC3 may be a potential therapeutic target for C24:0 and C22:0 ceramide modulation.

# Novel Sphingolipid Mediators of Cardiovascular Pathology

**L. Ashley Cowart**[1*]

[1] Biochemistry and Molecular Biology, Virginia Commonwealth University, USA

*Lauren.Cowart@vcuhealth.org*

Serine palmitoyltransferase, the first committed step in sphingolipid biosynthesis, occurs as a multi-subunit enzyme. The canonical serine palmitoyltransferase is comprised of subunits Sptlc1 and Sptlc2 and utilizes serine and palmitoyl-CoA as substrates to generate an 18-carbon sphingoid base. This base serves as a scaffold upon which all downstream sphingolipids are built, including ceramides. Ceramides are generated by the action of a family of 6 Ceramide Synthase enzymes (CerS). Our previously published work has demonstrated that in a mouse model of lipotoxic cardiomyopathy, CerS5 and CerS2 likely contribute to aberrant autophagy and mitochondrial damage, respectively. These data demonstrate that in the context of cardiac pathophysiology, distinct sphingolipid species have specific roles. Recently, an additional subunit of serine palmitoyltrasferase, Sptlc3, was recently discovered. Inclusion of Sptlc3 in the serine palmitoyltransferase complex broadens the Acyl-CoA selectivity of the enzyme, enabling utilization of myristoyl-CoA and thereby producing a 16-carbon sphingoid base. While Sptlc1 and 2 are essential for viability, Sptlc3 has low constitutive expression in nearly all tissues and organs examined. We previously demonstrated, however, that Sptlc3 and cardiomyocyte production of d16-base sphingolipids are induced in a mouse obesity model. New unpublished results indicate that cardiac ischemia strongly induces Sptlc3 in humans, as does left anterior descending artery ligation in mouse hearts. We found that in cardiomyocytes, hypoxia, nutrient deprivation, or fatty acid treatment induced Sptlc3 and d16-based sphingolipids. Hypoxia induced Sptlc3 via Hif-1 〈. Importantly, d16 sphingoid bases showed alternative routing through the sphingolipid metabolic pathway. In contrast to d18-base sphingolipids, which drove mitochondrial dysfunction and maladaptive autophagy, overexpression of Sptlc3 in cardiomyocytes promoted apoptosis. Molecular mechanisms involve d16-base sphinoglipid interactions with proteins that regulate cell fate. We propose Sptlc3 is an inducible subunit of the serine palmitoyltransferase complex that determines cell fate in response to physiological, disease-relevant cell stress conditions.

# Oxidative Stress Related With Ca2+ Overload in Phosphate-Induced Vascular Calcification

**Kyu-Sang Park**[1*]

[1] Physiology, Yonsei University Wonju College of Medicine, Korea

*qsang@yonsei.ac.kr*

Hyperphosphatemia is a risk factor for vascular calcification that is associated with cardiovascular morbidity and mortality. Recent evidence showed that oxidative stress by high inorganic phosphate (Pi) mediates calcific changes in vascular smooth muscle cells (VSMCs). However, intracellular signalings responsible for Pi-induced oxidative stress remain unclear. Here, we investigated molecular mechanisms of Pi-induced oxidative stress related with intracellular $Ca^{2+}$ ($[Ca^{2+}]i$) disturbance, which is critical for calcification of VSMCs.

VSMCs isolated from rat thoracic aorta or A7r5 cells were incubated with high Pi-containing medium. Extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin were activated by high Pi that was required for vascular calcification. High Pi upregulated expressions of type III sodium-phosphate cotransporters, PiT-1 and -2, and stimulated their trafficking to the plasma membrane. Interestingly, high Pi increased $[Ca^{2+}]i$ exclusively dependent on extracellular Na+ and $Ca^{2+}$ as well as PiT-1/2 abundance. Furthermore, high Pi induced plasma membrane depolarization mediated by PiT-1/2. Pre-treatment with verapamil, as a voltage-gated $Ca^{2+}$ channel (VGCC) blocker, inhibited Pi-induced $[Ca^{2+}]i$ elevation, oxidative stress, ERK activation and osteogenic differentiation. These protective effects were reiterated by extracellular $Ca^{2+}$ free condition, intracellular $Ca^{2+}$ chelation or suppression of oxidative stress. Mitochondrial superoxide scavenger also effectively abrogated ERK activation and osteogenic differentiation of VSMCs by high Pi.

We concluded that high Pi activates depolarization-triggered $Ca^{2+}$ influx via VGCC, and subsequent $[Ca^{2+}]i$ increase elicits oxidative stress and osteogenic differentiation. PiT-1/2 mediates Pi-induced $[Ca^{2+}]i$ overload and oxidative stress, but in turn, PiT-1/2 is upregulated by consequences of these alterations.

KSoLA-SHVM 4

# Metabolic Impairment of Akt1 Signaling and Consequences in Angiogenesis

**Sunsik Bae**[1*]

[1] Pharmacology, Pusan National University, Korea

*sunsik@pusan.ac.kr*

Intercellular communication of vascular smooth muscle cells (VSMCs) with endothelial cells (ECs) plays an essential role in EC function. Therefore, Akt-mediated signaling cascade in VSMCs might regulate the expression of paracrine factors. The present study was designed to explore isoform specificity of Akt, identification of key paracrine factors, and signaling cascade of Akt in VSMCs. Retinal angiogenesis was delayed in VSMC-specific Akt1 deficient mice but not in Akt2 deficient mice. Vascular endothelial growth factor (VEGF)-stimulated corneal angiogenesis and melanoma cell-induced tumor angiogenesis were significantly retarded in mice lacking Akt1 from VSMCs. Proliferation of ECs, recruitment of pericytes, and coverage of VSMCs to endothelium were defected in VSMC-specific Akt1 deficient mice. Expression of angiopoietin 1 (Ang1) was reduced in aortic tissues isolated from VSMC-specific Akt1 deficient mice whereas expression of Ang2 was enhanced. Silencing of Akt1 in VSMCs led to the downregulation of Ang1 and upregulation of Ang2 whereas overexpression of Akt1 led to the opposite results. Activation of Notch3 in VSMCs was significantly reduced in retinas from VSMC-specific Akt1 deficient mice. Silencing of Akt1 suppressed the activation of Notch3. Silencing of Notch3 downregulated Ang1 whereas overexpression of Notch3 intracellular domain (NICD3) enhanced the expression of Ang1. Nuclear localization and transcriptional activity of yes-associated protein (YAP) were affected by the expression level of Akt1. Silencing of YAP downregulated Ang2 expression whereas overexpression of YAP showed opposite results. Ang1 antibody and Ang2 suppressed endothelial sprouting of wild type aortic tissues whereas Ang2 antibody and Ang1 facilitated endothelial sprouting of aortic tissues from mice lacking Akt1 in VSMCs. Finally, severe hemorrhage was observed in VSMC-specific Akt1 deficient mice and further facilitated under streptozotocin (STZ)-induced diabetic condition. These results suggest that Akt1-Notch3/YAP-Ang1/2 signaling cascade in VSMCs might play an essential role in paracrine regulation of endothelial function.

# Lipidomics and Mitochondrial Dysfunction in Cardiometabolic Disease

**Gary Sweeney**[1*]

[1] Department of Biology, York University, Canada

*gsweeney@yorku.ca*

Our lab has studied the mechanisms responsible for adiponectin's cardioprotective and anti-diabetic actions. We have shown that adiponectin elicits these effects via binding to two cell membrane receptors (AdipoR1 & AdipoR2) which propagate signaling via binding to the adapter protein APPL1 and activating AMPK. Transgenic APPL1 overexpressing mice are resistant to high fat diet (HFD) induced cardiomyopathy and lipidomic analysis showed reduced accumulation of specific ceramide and DAG species in response to HFD. More recently we have shown that adiponectin potently stimulates autophagy. Increased myocardial autophagy has been established as an important stress-induced cardioprotective response. We generated cardiomyocyte-specific autophagy deficient mice via deletion of Atg-7 on a wt or adiponectin knockout Ad-KO background. We observed that adiponectin protected against pressure overload induced cardiac dysfunction in Ad-KO mice but not those lacking Atg7. Interestingly, we found that cardiomyocyte autophagy deficient (AKO) mice had increased body weight and fat mass without altered food intake. Glucose and insulin tolerance tests indicated reduced insulin sensitivity in AKO mice. In work which will be presented here, we conclude that cardiac autophagy deficiency alters myocardial-adipose crosstalk via decreased atrial natriuretic peptide levels with adverse metabolic consequences.

KSoLA-APSAVD - 2

# The Role of Lp(a) in Premature Vascular Disease

**Richard O'Brien[1*], Daniel Raffout[1]**

[1] Medicine, Austin Health, University of Melbourne, Australia

*robrien@unimelb.edu.au*

Lipoprotein(a) {Lp(a)} is a lipoprotein consisting of a protein, apoprotein (a), covalently linked to the apo B moiety of LDL. Lp(a) has some structural homology with plasminogen, giving it a potential role in thrombosis and atherosclerosis. Recent studies have suggested a continuous relationship between Lp(a) and CV risk, independent of other risk factors. Because the circulating level of Lp(a) is predominantly determined by the apo(a) gene, it has been possible to confirm these findings in Mendelian randomization studies.

Approximately 20% of the population have Lp(a) levels above 50 mg/dL, a level defined as significantly elevated in some guidelines. Elevated Lp(a) is more prevalent in younger patients with ischemic heart disease. We have previously shown that 37% of acute coronary syndrome patients had an Lp(a) over 50 mg/dL, with 19% having a level >100 mg/dL (approx. 95th percentile). The relationship between Lp(a) and CVD was examined in 12,236 participants in the Heart Protection Study. Two single-nucleotide polymorphisms, strongly related to lipoprotein(a) levels, were significantly more prevalent in subjects who developed ischemic heart disease (IHD) and peripheral vascular disease (PVD) but not in subjects with stroke. However, a recent retrospective study from China suggested a significant association between elevated Lp(a) and ischemic stroke in men.

We have recently studied younger patients (age<70) presenting to Austin Health (Melbourne, Australia) with stroke, and found no increased prevalence of high Lp(a) levels. In 80 consecutive stroke patients, the prevalence of Lp(a) over 50mg/dL was 15%. Although this is a small study, there was no trend to significance in any sub-group, suggesting the Australian situation mirrors the findings from the Heart Protection Study. Lp(a) is becoming increasingly recognized as an important CV risk factor, with strong associations with IHD and PVD. It's role in stroke requires further studies, particularly examining potential ethnic variation.

References

Burgess S et al. JAMA Cardiol. Published online June 20, 2018

Clarke R et al. N Engl J Med 2009; 361:2518–2528.

Danesh J et al. Circulation 2000; 102:1082–1085

Fu H et al. Ann Transl Med 2020;8(5):212

Hopewell JC et al. Circulation Cardiovasc genetics. 2011;4:68–73

Kamstrup PR, et al. JAMA 2009; 301:2331–2339

Nordestgaard BG et al. European Heart Journal 2010; 31: 2844–2853

OO HP et al. Heart, Lung and Circulation (e-pub) 2020

Viney NJ et al. Lancet 2016; 388: 2239–53

# Cardiovascular Fate of Metabolically Healthy Obese Population in Asia

**Yu Mi Kang**[1*]

[1] Internal Medicine, Yale New Haven Health; Bridgeport Hospital, USA

*dryumikang@gmail.com*

Metabolically healthy obesity (MHO) is a subgroup displaying favorable metabolic profiles despite the excess body weight. A complex interconnection among genetic, environmental, and behavioral factors is thought to be the underlying mechanism of MHO phenotype. Proposed features of the preserved metabolic health in the MHO state include a healthier lifestyle, greater incretin response to meals, less abdominal fat distribution, less visceral and ectopic fat accumulation, lower levels of inflammation, and greater insulin sensitivity. When the concept of MHO was initially suggested, hopeful data existed potentially indicating a similar risk of cardiovascular morbidity and mortality compared to individuals with normal body weight. However, the long-term prognosis of MHO state is still in debate, as more recent data indicates its association with poorer cardiometabolic outcomes. The controversy has been attributed to multiple factors, such as ethnicity, age, inconsistent definitions of the MHO state, and the dynamic nature of MHO. This session will review up-to-date perspectives on MHO – definition, epidemiology, natural course, suggested mechanisms, and clinical implications in the context of cardiovascular prognosis – among Asian populations.

KSoLA-APSAVD - 4

# Association of Arachidonic Acid-Derived Lipid Mediators with Subsequent Onset of Acute Myocardial Infarction in Patients with Coronary Artery Disease

**Chin-Chou Huang**[1*]

[1] Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital, Taiwan

*cchuang4@vghtpe.gov.tw*

Polyunsaturated fatty acids (PUFAs) have been suggested for cardiovascular health. Oxylipins, a type of bioactive lipid mediators, are derived from the catalysis of PUFA substrates via lipoxygenases, cyclooxygenases, or cytochrome P450s. This study was conducted to investigate the prognostic impacts of oxylipins on clinical outcomes in coronary artery disease (CAD). The current study is associated with the "Development of New Biosignatures for Atherosclerosis Cardiovascular Diseases" study, which is a multicenter study which enrolled a series of patients with stable CAD in 9 medical centers in Taiwan. A total of 2,239 patients with stable CAD were prospectively enrolled and followed up regularly. Among them, twenty-five consecutive patients with new onset of acute myocardial infarction (AMI) within 2-year follow-up were studied. Another 50 gender- and age-matched patients without clinical cardiovascular events for more than 2 years were studied for control. Oxylipins were analyzed by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (UP-LC-ESI- MS/MS). Baseline levels of specific arachidonic acid metabolites were significantly higher in patients with subsequent AMI than in the controls. In Kaplan-Meier analysis, the incidence of future AMI was more frequently seen in patients with higher baseline levels of 8-hydroxyeicosatetraenoic acid (HETE), 9-HETE, 11-HETE, 12-HETE, 15-HETE, 19-HETE, 20-HETE, 5,6-epoxyeicosatrienoic acid (EET), 8,9-EET, 11,12-EET, or 14-15-EET when compared to their counterparts (all the $P < 0.01$). Further, serum levels of these specific HETEs, except for 11,12-EET, were positively correlated to the levels of some inflammatory and cardiac biomarker such as tumor necrosis factor-α and N-terminal pro B-type natriuretic peptide. In conclusion, CAD patients with future acute myocardial infarction had higher baseline levels of arachidonic acid metabolites when comparing to those without future cardiovascular events. The results imply the potential roles of arachidonic acid derived lipid mediators as clinical predictors for cardiovascular events in patients with CAD.

# Role of Renin-Angiotensin System Inhibitor in Hypertensive Patients with Cardiometabolic Disorder

**Sang Min Park**[1*]

[1] Department of Internal Medicine, Eulji University Medical Center, Korea

*samipark@hanmail.net*

The data of the life expectancy at birth around 2030 predict both the Korean women and man will have longest life span in the world. There are mounting evidence that hypertension (HTN) is one of major cardiovascular (CV) risk factors and most notorious precipitating factor of CVD during whole CV continuum. Antihypertensive medication had been begun to develop in 1950s. After first JNC report at 1977 remarkable advancement of antihypertensive drugs had been introduced. Major guideline recommended blockade of renin-angiotensin system in hypertensive patients with cardiometabolic (CM) derangement. Most hypertensive patients with CM disorder are obese and diabetic. The prevalence of resistant HTN is 12-14% in treated hypertensive population. The CM patients are often prone to be have resistant HTN. Especially, angiotensin receptor type1 blocker (ARB) is a very popular to Korean society because it is very effective and safe despite the current COVD19 situation. In this review, we will address the promising aspect of ARB in hypertensive patients with CM disorder, which will be focused on corrective function of ARB on CM derangement.

# Lipid Abnormalities Among Young Myocardial Infarctions

**Sazzli Kasim**[1*]

[1] Consultant Cardiologist, University Technology Mara, Malaysia

*sazzlikasim@gmail.com*

Myocardial infarction in the young can be devastating. The burden is between 5-10% in most OECD countries in those below the age of 45years old. We observe a higher incidence of myocardial infarctions in the young from a heterogeneous Asian population in South East Asia.

Half of these patients are known smokers, a direct risk factor for the onset of plaque rupture. We investigate the role of cholesterol efflux capacity and small dense LDL cholesterol in young patients with myocardial infarction.

# Diabetic Dyslipidemia and Coronary Heart Disease

**Shinji Koba**[1*]

[1] Department of Medicine, Division of Cardiology, and Department of Perioperative Medicine, Division of General Medicine, Showa University, Japan

*skoba@med.showa-u.ac.jp*

Diabetes is a significant risk factor for atherosclerotic cardiovascular disease (ASCVD). LDL cholesterol (LDL-C) is the best predictor of ASCVD in even diabetic patients, and aggressive LDL-C lowering dramatically reduced the incidence of CVD in diabetes populations. On the other hand, diabetic dyslipidemia is often characterized by hypertriglyceridemia. According to the observational cohort studies of Japanese diabetic patients showed that triglycerides are a leading predictor of coronary heart disease (CHD), comparable to LDL-C. Hypertriglyceridemia, especially elevated large VLDL triglyceride, is metabolically associated with the formation of small dense (sd) LDL particles. In metabolic syndrome, abdominal fat volume measured by CT scan was positively associated with sdLDL-C but inversely associated with large buoyant LDL-C. Therefore, we can call sdLDL as "Metabolic LDL". SdLDL particles are thought to be more atherogenic than large-buoyant LDL particles as a result of their better penetration of the arterial wall, lower binding affinity for the LDL receptor, longer plasma half-life, and increased susceptibility to oxidation. Previous cohort studies have reported that sdLDL-C is a better marker for CHD risk than LDL-C. It has been reported that intestinal expression of Niemann-Pick C1-Like 1 (NPC1L1) and microsomal triglyceride transfer protein (MTP) increase, and that of ATP binding cassette (ABC) proteins G5/G8 reduce, that is resulting in high prevalence of hypercholesterolemia as well as hyperchylomicronemia, in patients with diabetes. Adding ezetimibe onto usual-dose statin treatment may be the first-line therapy for diabetic patients in Japan. On the other hand, recent randomized controlled trials of Japanese diabetic patients showed that intensive lipid-lowering therapy did not reduce CHD events but prevented ischemic cerebrovascular events. In this lecture, I will present a significance of sdLDL and large HDL particles in diabetic dyslipidemia and for the risk of CHD.

KSoLA- JAS-2

# Lipid Management in Diabetes Mellitus for Secondary Prevention

**Katsumi Miyauchi**[1*]

[1] Cardiovascular Medicine, Juntendo University, Japan

*ktmmy@juntendo.ac.jp*

My view point is the clinical significance of lipid management in coronary artery disease patients with diabetes from the following standpoints: 1) The high risk for cardiovascular morbidity and mortality in diabetic patients; 2) How to Reduce the burden of cardiovascular disease in diabetes; (3) discuss the relationship between LDL-C and plaque regression or CV events. We review the clinical trials all over the world and our data. In this discussion, we conclude that diabetes is an important risk factor as a residual risk. In diabetic patients, advanced atherosclerosis is more common and plaque burden and instability is greater. Tight glucose control shows no beneficial outcomes. Reducing the burden of cardiovascular disease in diabetes should begin with treatment of LDL-C. Statins are the preferred treatment, and high-intensity statin therapy may be necessary to meet the current goal of less than 70 mg/dl. In diabetic patients, further reduction of LDL-C was associated with a significantly greater reduction in cardiovascular events.

# Impact of Gender on Cardiovascular Disease in Diabetes

**Hyun Min Kim**[1*]

[1] Department of Endocrinology, Chung-Ang University, Korea

*alsdl81@gmail.com*

The incidence of cardiovascular disease (CVD) is usually lower in women than in men of similar age. However, this "female advantage" seems to diminish or disappear in people with diabetes. In approaching the cardiovascular (CV) risk by gender, both common risk factors and gender-specific risk factors should be considered. There is no clear explanation yet as to why women with diabetes lose the benefits observed in men and women without diabetes. The differences in the traditional CV risk factors, whether diabetes is present or male or female, will be a partial explanation. A few studies suggest that women with diabetes are more likely to accumulate a higher cardiometabolic risk burden during the transition from normoglycemia to diabetes. Therefore, women diagnosed with type 2 diabetes already have more significant endothelial dysfunction, low-grade inflammation, and hypercoagulability states than men with diabetes. There are also views that gender-specific differences exist in the use of new anti-diabetes drugs, statins, and antiplatelet agents, which affect the risk and prognosis of CVD. It would be essential to clarify the biological mechanisms that cause gender-related differences in CV risk. More efforts are needed to reduce the possible disparity in medical access and the use of evidence-based pharmacological therapy for the prevention and treatment of CVD.

KSoLA- JAS-4

# Approach to Patients With Diabetes and Hypertriglyceridemia Whose LDL Cholesterol Is at Goal

**Sung Hee Choi**[1*]

[1] Endocrinology and Metabolism, Internal Medicine, Seoul National University & Seoul National University Bundang Hospital, Korea

*shchoimd@gmail.com*

In patients with diabetes, hypertriglyceridemia is a important feature of dyslipidemia due to underlying insulin resistance. Insulin resistance induced lipolysis in the peripheral fat tissues and cased the overflow phenomenon of free fatty acid into the liver. The hepatic VLDL production and CETP activation is prominent feature of diabetes.

In addition, the concept of residual risk for the major cardiovascular events or mortality, we have to think about the role of ApoB-rich lipoprotein particles not only for the level of LDL-cholesterol. In this lecture, review of the results of clinical trials to lower TG in patients with diabetes and share the share the idea of clinical implications of lowering TG in diabetes.

# Spillover Infections of Coronavirus From One Health Perspective

**Daesub Song**[1*]

[1] College of Pharmacy, Korea University, Korea

*songdaesop@gmail.com*

Zoonosis from wildlife represents the most significant threat to global health by spillover of zoonotic disease to human population. Passing from members of one species into human population, alien pathogen thrives and spreads among it and finally result in emergence. Currently unknown pathogens to cause human disease could lead a serious international epidemic, as did SARS-CoV, MERS-CoV, influenza and COVID19. The outbreaks have emphasized the critical need for health monitoring of human and animal, and identification of new, potentially zoonotic pathogens in wildlife population as a one-health measure for emerging infectious disease.

Here, we review the evolution of emerging viral diseases and the progress of developing vaccines with a focus on possibility of success and their challenges. Currently, no single effective vaccine is available against SARS-CoV, MERS-CoV and COVID19 yet. However, advances in recombinant gene technology have sped up the establishment of a variety of vaccine platforms, and numerous vaccines are under development, accordingly. To advance the time of vaccine development, strong collaboration with variety of research field are required, and this limits further damage due the Disease X.

Special Session-2

# COVID-19 and Cardiovascular Disease

**Kyung Woo Park**[1*]

[1] Cardiovascular Center, Seoul National University Hospital, Korea

*kwparkmd@snu.ac.kr*

COVID-19, a clinical disease caused by a strain of coronavirus, is a global pandemic that has infected over 26 million people worldwide, has caused mortality in almost 900,000 patients, and has affected the daily lives of billions of people around the world. There have been several reports regarding the bi-directional association between COVID-19 and cardiovascular disease. Patients with various cardiovascular conditions such as hypertension, diabetes, heart failure, and coronary artery disease are at increased risk of poor prognosis, death, and experiencing a severe form of the disease including severe acute respiratory failure. Further, the virus that causes COVID-19, the SARS coronavirus 2 (SARS-CoV-2) target endothelial cells leading to increased permeability, potentiation of inflammation, and cytokine storm. The virus mechanism of entry may be through binding of the viral spike protein to the angiotensin-converting enzyme 2 on the surface of the host cell. The fact that many patients with cardiovascular disease take drugs that may affect this enzyme may complicate treatment even further. In addition, COVID-19 may result in not only direct myocardial injury but also the activation of the coagulation cascade which may lead to thrombosis and subsequent bleeding when the activation of one's internal countermeasures result in the vicious cycle of disseminated intravascular coagulation. We will need to understand the interplay between the virus, the clinical syndrome that it causes, and the cardiovascular system to better manage the patients infected with the SARS-CoV-2.

# COVID-19 and DM

**Jun Sung Moon**[1*]

[1] Division of Endocrinology and Metabolism, Department of Internal Medicine, Yeungnam University, Korea

*mjs7912@yu.ac.kr*

Since the unprecedented outbreak of coronavirus disease-19 (COVID-19), the global pandemic continues, including Korea, and it is reported that several comorbidities are related to the prognosis of COVID-19 patients. Among the non-communicable disease, Diabetes Mellitus has been associated with more severe outcomes and higher mortality in COVID-19 patients compare to morbidity and mortality in patients without diabetes. Several mechanisms may play a role in this greater morbidity and mortality, especially uncontrolled hyperglycemia, an impaired immune system, pre-existing proinflammatory states, multiple comorbidities, and dysregulated angiotensin-converting enzyme 2 signaling. Thus, the diabetes medical community emergently needs to know about COVID-19 and its effects on patients with diabetes, as they must take precautions to carefully manage these patients during the COVID-19 pandemic. Several academic authorities such as the Korean Diabetes Association provides some guidance and practical recommendations for the management of diabetes during the pandemic. In this lecture, I will share insight into the association between diabetes, obesity and COVID-19, proper management of diabetes in patients with COVID-19 and evidence-based suggestion for managing the COVID-19 outbreak.

Special Session-4

# Treatment Strategy for COVID-19: Vaccine and Antiviral Drug

**Pyoeng Gyun Choe**[1*]

[1] Department of Internal Medicine, Seoul National University Hospital, Korea

*pgchoe@gmail.com*

In December 2019, a new strain of betacoronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), that cause coronavirus disease 2019 (COVID-19), emerged in Wuhan, China. Subsequently, the virus quickly spread worldwide, and the World Health Organization declared COVID-19 as a global pandemic on March 11, 2020. As of August 4, 2020, there were more than 18.29 million confirmed cases worldwide, with total deaths exceeding 694,233.

As of August 4, more than 165 vaccines against SARS-CoV-2 are being developed, and 27 vaccines are under clinical trials. ChaDOx1 is a vaccine that is based on a chimpanzee adenovirus. Their Phase I/II trial reported that the vaccine was safe, causing no severe side effects. It raised antibodies against the coronavirus as well as other immune defenses. The vaccine is now in Phase II/III trial in England, as well as Phase III trials in Brazil and South Africa.

Presently, there are no drugs or other therapeutics approved for the treatment of COVDI-19, although remdesivir, an investigational antiviral drug, is available through an FDA emergency use authorization. In a randomized, double-blind trial in 1,063 adults hospitalized with COVDI-19, a 10-day course of intravenous remdesivir was superior to placebo in shortening the time to recovery, from a median of 15 days among placebo recipients to 11 days among those receiving remdesivir. A trend toward lower mortality among patients who received remdesivir (7.1%) than among those who received a placebo (11.9%) was also observed, but the difference did not reach statistical significance. Definitive clinical trial data are needed to identify safe and effective treatments for COVID-19.

KSoLA-CSATVB-1

# The Effect of Altered Sphingolipid Acyl Chain Length on ER Stress and Fatty Acid Synthesis

**Joo-Won Park**[1*]

[1] Department of Biochemistry, Ewha Womans University, Korea

*joowon.park@ewha.ac.kr*

The endoplasmic reticulum (ER) is not only important for protein synthesis and folding but is also crucial for lipid synthesis and metabolism. We investigated the contribution of ER stress to nonalcoholic fatty liver disease (NAFLD), and demonstrated an important role of ceramide synthases (CerS) in ER stress and NAFLD progression. Ceramide acyl chain length is determined by a family of six CerS in mammals. More specifically, CerS2 generates C22-C24 ceramides, and CerS5 or CerS6 produces C16 ceramide. To gain insight into the role of CerS in NAFLD, we used a high-fat diet (HFD)-induced NAFLD mouse model. Decreased levels of CerS2 and increased levels of CerS6 were observed in steatotic livers during HFD. In vitro experiments with Hep3B cells indicated the protective role of CerS2 and detrimental role of CerS6 in the ER stress response induced by palmitate treatment. Particularly, CerS6 overexpression increased sterol regulatory element binding protein-1 (SREBP-1) cleavage with decreased levels of Insig-1, leading to increased lipogenesis. Blocking ER stress abrogated the detrimental effects of CerS6 in palmitate-induced SREBP-1 cleavage. In accordance with the protective role of CerS2 in the palmitate-induced ER stress response, CerS2 knockdown enhanced ER stress and SREBP-1 cleavage, and CerS2 heterozygote livers exhibited a stronger ER stress response and higher triglyceride levels following HFD. Finally, treatment with a low-dose of bortezomib increased hepatic CerS2 expression and protected the development of NAFLD following HFD. These results indicate that CerS and its derivatives impact hepatic ER stress and lipogenesis differently and might be therapeutic targets for NAFLD.

KSoLA-CSATVB-2

# Energy Sensing Regulates Immunometabolism in Atherosclerosis

**Morgan Fullerton**[1*]

[1] Biochemistry, Microbiology and Immunology, University of Ottawa, Canada

*morgan.fullerton@uottawa.ca*

Cardiometabolic diseases remain a global health burden and show no signs of slowing in prevalence. Immunometabolism encompasses the complex and ubiquitous entanglement of cellular metabolism and immunity. Atherosclerosis, which precedes and predicts cardiovascular disease, is both a metabolic and inflammatory condition and while there are numerous cell types involved, macrophages (innate immune cells) can play a driving role.

Cellular metabolism is coupled to energy requirements and is tightly regulated. The ancient energy sensor AMP-activated protein kinase (AMPK) acts as a cellular gauge and signals to conserve energy in multiple ways. In macrophages, we and others have shown that AMPK directly regulates mitochondrial biogenesis, inflammation and lipid metabolism. The importance of AMPK signaling in atherosclerosis is context and cell-type specific; however, the role of macrophage AMPK signaling in the progression of atherosclerosis is still unclear. Here, I will highlight the part that AMPK plays in regulating macrophage lipid and lysosomal homeostasis, the effects of both deleting and activating macrophage AMPK on the progression of atherosclerosis and finally how energy sensing directs atherosclerosis via the interaction of AMPK and the mevalonate pathway.

# Roles of ELOVL5 in Lipid Metabolism

**Young Ah Moon**[1*]

[1] Molecular Medicine, Inha University, Korea

*yamoon15@inha.ac.kr*

Elongation of very long chain fatty acids (ELOVL) 5 is one of the seven mammalian fatty acid condensing enzymes involved in microsomal fatty acid elongation and a critical component to maintain cellular very-long chain polyunsaturated fatty acid (PUFA) levels. Studies using liver microsomal protein from wild-type and knockout mice demonstrated that the elongation of gamma-linolenic (C18:3, n-6) to dihomo-gamma-linolenic (C20:3, n-6) and stearidonic (C18:4, n-3) to omega 3-arachidonic acid (C20:4, n-3) required ELOVL5 activity. Tissues of Elovl5-/- mice accumulated the C18 substrates of ELOVL5 and the levels of the downstream products, arachidonic acid (C20:4, n-6) and docosahexaenoic acid (DHA, C22:6, n-3), were decreased. A consequence of decreased cellular arachidonic acid and DHA concentrations was the activation of sterol regulatory element-binding protein (SREBP)-1c and induction of its target genes involved in fatty acid and triglyceride synthesis, which culminated in the development of hepatic steatosis in Elovl5-/- mice. High rates of liver triglyceride synthesis led accumulation of both triglycerides and cholesterol in liver and would result in dyslipidemia. Contribution of the ELOVL5 activity in atherosclerosis need to be studied.

KSoLA-CSATVB-4

# Numeric and Pathologic Significance of Smooth Muscle Cell Foam Cells in Atherosclerosis

**Gordon Francis**[1*]

[1] Medicine/Endocrinology and Metabolism, UBC, Canada

*gordon.francis@hli.ubc.ca*

Cholesterol-overloaded foam cell formation in atherosclerosis has previously been thought to primarily involve monocyte-derived macrophages. Early studies in human lesions suggested, however, that cholesterol accumulation in early atherosclerosis was primarily in intimal smooth muscle cells (SMCs). Low levels of the main cholesterol exporter, ABCA1, in intimal SMCs also suggested these cells have a defect in cholesterol efflux, contributing to their tendency to form foam cells. We have performed studies using gentle tissue digestion to release atheroma cells followed by lipid and CD45, a leukocyte-specific marker not expressed by SMCs, staining and flow cytometry analysis. Our recent studies suggest ~65% of foam cells in human coronary and aortic atheromas are CD45negative, have very low levels of ABCA1 expression compared to macrophage foam cells, and contribute at least 50% of the excess cholesterol accumulated in plaque. Similarly, in non-lineage tracing or SMC-lineage tracing apoE-deficient mice fed a Western diet for 6 weeks, ~70% of foam cells isolated in the same way are SMC-derived. Further studies indicate that SMC foam cells primarily hold their excess cholesterol in the lysosomal compartment, and have a defect in lysosomal processing of endocytosed lipoprotein cholesteryl esters. These combined findings suggest SMCs rather than macrophages contribute the majority of foam cells in human and mouse atherosclerosis, have specific defects in their ability to process and efflux lipoprotein-derived cholesterol, and represent a novel target to reduce the formation and induce the regression of atherosclerosis in ischemic vascular disease.

KSoLA-CSATVB-5

# Clonal Hematopoiesis as a Novel Risk Factor for Cardio-Metabolic Disorders

**Changhee Jung**[1*]

[1] Department of Internal Medicine, University of Ulsan, Korea

*chjung0204@gmail.com*

The accumulation of somatic mutations in hematopoietic stem/progenitor cells (HSPCs) is known to be an inevitable consequence of the process of aging. Some of these random mutations confer a competitive advantage to the mutant cells, leading to clonal expansion. This phenomenon is called as age-related clonal hematopoiesis (CH). The genes that were most commonly mutated in CH were DNMT3A, TET2 and ASXL1. A number of studies have associated with CH with an increase in all-cause mortality. Although the presence of CH was associated with the increased risk of hematologic cancer, this only affected 0.5% to 1% of mutation carriers each year and did not explain the marked increase in all-cause mortality. Instead, the increased all-cause mortality was attributable to increased risk of cardiovascular disease (CVD). Based on these epidemiological data, several groups tried to elucidate the possible molecular mechanism underlying the presence of CH and CVD such as atherosclerosis, myocardial infarction and heart failure. In addition to the causal link between CH and CVD, a modest and significant association between CH and type 2 diabetes was observed. In this talk, I'd like to introduce the possible causal link between the loss of function in DNMT3A, the most frequently mutated gene in CH, and metabolic dysfunction including adipose tissue inflammation in mouse.

Publication Committee Session -1

# Persistent Organic Pollutants (POPs), Lipids and Atherosclerosis

**Monica Lind**[1*]

[1] Occupational and Environmental Medicine, Uppsala University, Sweden

*monica.lind@medsci.uu.se*

POP denotes chemicals with known or suspected adverse health effects in animals or humans and with chemical properties that make them accumulate in the environment, including animals or humans. Lipid-soluble POPs, like dioxins, polychlorinated biphenyls (PCBs) and organochlorine pesticides are transported by lipoproteins and accumulate in adipose tissue. High levels of these compounds in the circulation have been associated with elevated cholesterol and triglycerides in cross-sectional studies and with an increase in mainly LDL cholesterol in a longitudinal study. Also, non-lipid-soluble POPs, such as perfluoroalkyl substances (PFAS) compounds have been associated with increased total cholesterol levels. Carotid artery atherosclerosis has been related to elevated levels of mainly highly chlorinated PCBs and to highly fluorinated PFASs, but in this case only in women. Both cross-sectional and prospective studies have shown dioxins, PCBs, as well as PFASs to be linked to cardiovascular disease and mortality. In conclusion, as highlighted in this review, several lines of evidence support the view that POPs of different chemical classes could be linked to lipid abnormalities, carotid atherosclerosis and overt cardiovascular disease like myocardial infarction and stroke.

# Association Between Serum Bilirubin and the Progression of Carotid Atherosclerosis in Type 2 Diabetes

**Inkuk Lee[1,2*], Hyeok-Hee Lee[1,2], Yongin Cho[2,3], Young Ju Choi[4], Byung Wook Huh[4], Byung-Wan Lee[1,2,5], Eun Seok Kang[1,2,5], Seok Won Park[1,5], Bong-Soo Cha[1,2,5], Eun Jig Lee[1,2,5], Yong-ho Lee[1,2,5], Kap Bum Huh[4]**

[1] Department of Internal Medicine, Yonsei University, Korea
[2] Graduate school, Yonsei University College of Medicine, Korea
[3] Department of Endocrinology and Metabolism, Inha University School of Medicine, Korea
[4] Huh's Diabetes Center and the 21st Century Diabetes and Vascular Research Institute, Huh's Diabetes Center and the 21st Century Diabetes and Vascular Research Institute, Korea
[5] Institute of Endocrine Research, Yonsei University College of Medicine, Korea

*inkuklee@gmail.com*

This study investigated whether serum bilirubin levels can predict the progression of carotid atherosclerosis in individuals with type 2 diabetes mellitus (T2DM).

This observational study included 1,381 subjects with T2DM in whom serial measurements of carotid intima-media thickness (CIMT) were made at 1- to 2-year intervals for 6–8 years. The progression of carotid atherosclerosis was defined as newly detected plaque lesions on repeat ultrasonography. After dividing total serum bilirubin levels into tertiles, the association between total serum bilirubin at baseline and plaque progression status was analyzed.

Among 1,381 T2DM patients, 599 (43.4%) were categorized as having plaque progression in their carotid arteries. Those with plaque progression were significantly older; showed a higher prevalence of hypertension, abdominal obesity, and chronic kidney disease; and had a longer duration of T2DM, higher levels of total cholesterol (TC), triglycerides, and insulin resistance, and lower total bilirubin concentrations than those with no plaque progression. When total serum bilirubin levels were divided into tertiles, the highest tertile group was younger than the lowest tertile group, with higher levels of TC and high-density lipoprotein cholesterol. Multiple logistic regression analysis demonstrated that higher serum bilirubin levels were associated with a significantly lower risk of CIMT progression (odds ratio, 0.584; 95% confidence interval, 0.392–0.870; p=0.008). Age (p<0.001), body mass index (p=0.023), and TC (p=0.019) were also associated with the progression of carotid atherosclerosis in T2DM patients. Total serum bilirubin is independently associated with progression of atherosclerosis in the carotid arteries in T2DM patients.

Publication Committee Session -3

# Associations of Dietary Intake with Cardiovascular Disease, Blood Pressure, and Lipid Profile in the Korean Population: a Systematic Review and Meta-Analysis

**Jeongseon Kim[1], Tung Hoang[1*], So Young Bu[2], Ji-Myung Kim[3], Jeong-Hwa Choi[4], Eunju Park[5], Seung-Min Lee[6], Eunmi Park[7], Ji Yeon Min[8], In Seok Lee[9], So Young Youn[10]**

[1] Cancer Biomedical Science, National Cancer Center Graduate School of Cancer Science and Policy, Korea
[2] Department of Food and Nutrition, Daegu University, Korea
[3] Food and Nutrition Major, Division of Food Science and Culinary Arts, Shinhan University, Korea
[4] Department of Food and Nutrition, Keimyung University, Korea
[5] Department of Food and Nutrition, Kyungnam University, Korea
[6] Department of Food and Nutrition, Yonsei University, Korea
[7] Department of Food and Nutrition, Hannam University, Korea
[8] Dietetics and Nutrition Services Team, Asan Medical Center, Korea
[9] Nutrition Support Team, Kyung Hee University Medical Center, Korea
[10] Clinical Nutrition Part, Samsung Medical Center, Korea

*75256@ncc.re.kr*

Previous studies have separately reported the contributions of dietary factors to the risk of cardiovascular disease (CVD) and its markers, including blood pressure (BP) and lipid profile. This study systematically reviewed the current evidence on this issue in the Korean population.

Sixty-two studies from PubMed and Embase were included in this meta-analysis. We performed a random-effects model to analyze pooled odds ratios (ORs) and hazard ratios (HRs) and their 95% confidence intervals (CIs) for the consumption of 14 food items, three macro- and eight micro-nutrients, two dietary patterns, and three dietary indices.

An analysis of pooled effect sizes from at least four individual study populations showed significant associations between coffee consumption and CVD (OR/HR, 0.71; 95% CI, 0.52–0.97) and elevated/high triglycerides (TG) (OR, 0.84; 95% CI, 0.78–0.90), sugar-sweetened beverage intake and elevated BP (OR/HR, 1.20; 95% CI, 1.09–1.33), and milk and dairy intake and elevated/high TG and low high-density lipoprotein cholesterol (HDL-C) (OR/HR, 0.82; 95% CI, 0.76–0.89 for both). Carbohydrate consumption and the low-carbohydrate-diet score were consistently related to an approximately 25% risk reduction for elevated TG and low HDL-C. A lower risk of elevated total cholesterol, but not low-density lipoprotein, was additionally observed for those with a higher low-carbohydrate-diet score. A healthy dietary pattern was only associated with a reduced risk of elevated TG in the Korea National Cancer Screenee Cohort (OR, 0.81; 95% CI, 0.67–0.98).

This study showed that milk and dairy and coffee had protective effects for CVD and its risk factors, such as BP and lipid profile, while sugar-sweetened beverages exerted harmful effects.

# Hepatic Expression of Serine Palmitoyltransferase Subunit SPTLC2 Reduces Lipid Droplets in the Liver by Activating VLDL Secretion

**Tae-Sik Park**[1*]

[1] Life Sciences, Gachon University, Korea

*pts9918@gmail.com*

Ceramide is a signaling molecule that contributes to insulin resistance and hepatosteatosis. In the present study, we activated de novo ceramide synthesis by inducing the hepatic expression of Sptlc2 to investigate the role of ceramide in glucose and lipid metabolism.

We first constructed an adenovirus containing Sptlc2 (AdSptlc2), which encodes a major catalytic subunit of serine palmitoyltransferase (SPT). We then infected hepatocytes and mice fed a regular diet with AdSptlc2 to activate de novo ceramide biosynthesis. The liver-specific effects of ceramide biosynthesis on glucose and lipid metabolism were investigated by measuring changes in insulin signaling, lipid droplet formation, and very low-density lipoprotein (VLDL) secretion.

In HepG2 hepatocytes, adenoviral Sptlc2 expression inhibited insulin signaling and increased ceramide levels via activation of c-Jun N-terminal kinase and serine phosphorylation of insulin receptor substrate 1. In contrast, in mice, AdSptlc2 infection decreased plasma glucose levels by downregulating gluconeogenic genes and increased plasma triglyceride levels by increasing VLDL secretion. In mice infected with AdSptlc2, glucose intolerance and insulin sensitivity improved, while pyruvate utilization via gluconeogenesis decreased.

Hepatic ceramide was found to modulate hepatosteatosis and the insulin response via increased VLDL secretion and inhibition of gluconeogenesis in vivo. Although inhibition of the insulin response was observed in vitro, the compensatory mechanism of relieving ceramide-induced stress and reducing ceramide levels resulted in improvements of glucose and lipid metabolic profiles in vivo. This discrepancy between in vitro and in vivo regulation mechanisms suggests that ceramide plays a role in non-alcoholic fatty liver disease and insulin resistance.

Publication Committee Session -5

# Preoperative Cessation of Both Dual Anti-Platelet Agents Is Safe in Patients Receiving Percutaneous Coronary Intervention After 1-Year

**Sang-Ho Jo**[1*]

[1] Internal Medicine, Hallym University Sacred Heart Hospital, Korea

*sophi5neo@gmail.com*

The aim of this study was to investigate the atherothrombotic and bleeding risk of discontinuing both components of dual antiplatelet therapy (DAPT) before surgery in patients with an intracoronary stent after 1 year.

We retrospectively enrolled 212 patients who received an evaluation of perioperative cardiac risk and underwent surgery from March 2017 to March 2019. We divided them into 2 groups: the discontinuation of both antiplatelet agents group (DCAP, no use of any antiplatelet agent) and the continuation of at least 1 antiplatelet agent group (CAP). The primary composite endpoint was the occurrence of major adverse cardiovascular events (MACE), including death, angina, postoperative coronary angiography, stroke, and readmission within 30 days postoperatively.

The second endpoint was bleeding requiring the transfusion of ≥2 packs of red blood cells (RBCs) A total of 136 patients were enrolled in the study, with 68 in the DCAP group and 68 in the CAP group. The occurrence of MACE did not significantly differ between the groups (25% vs. 17.6%, p=0.295). The incidence of bleeding that required a transfusion was higher in the CAP group (16.2% vs. 30.9%, p=0.044). The postoperative change in hemoglobin levels (–1.9 g/dL vs. –1.8 g/dL, p=0.742), and the number of transfused packs of RBCs (3.5 vs. 5.3, p=0.347) were not significantly different between the groups.

Preoperative discontinuation of DAPT did not increase the risk of MACE. However, continuation of at least 1 antiplatelet agent increased the incidence of bleeding requiring RBC transfusion. Further research with a large cohort is warranted.

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# Breakfast Symposium

September 9 (Wed) ~ October 10 (Sat), 2020 I Virtual Meeting (On demand)
September 9 (Wed) ~ 12 (Sat), 2020 I IVBM 2020 LIVE for Korean

KSoLA
The Korean Society of Lipid and Atherosclerosis

KVBM
Korean Society for Vascular Biology and Medicine

# Strategic Cardiovascular Risk Reduction in Diabetes– What You Need to Focus

**Jun Hwa Hong**[1*]

[1] Division of Endocrinology, Eulji University Hospital, Korea

*lammoth@naver.com*

The recent ADA 2020 Guideline recommends SGLT2 inhibitors or GLP-1 receptor agonists in patients with established atherosclerotic cardiovascular diseases and those with a need for weight loss promotion. Aligned with this change, recently the Korean Diabetes Association has also changed its recommendation level of SGLT2-inhibitors. A more recent addition to the class of SGLT2 inhibitors is Ertugliflozin. Ertugliflozin is a novel highly selective and potent SGLT2-inhibitor with proven HbA1C reduction data seen in the VERTIS Trials- VERTIS-Mono, VERTIS-MET, VERTIS-SITA, VERTIS-SITA2, VERTIS FACTORIAL, VERTIS ASIA. The efficacy of Ertugliflozin has also been compared in indirect comparison studies using methods of NMA (Network Meta-Analysis) and MBMA (Model-Based Meta-Analysis). Ertugliflozin also has an indication and have been studied in combination studies with Sitagliptin, the most commonly prescribed DPP4 inhibitor in Korea and also worldwide. In this talk, recent clinical data of Ertugliflozin will be reviewed.

# The Benefits of Early Initiation of SU and CV Safety of Gliclazide MR for Type 2 Diabetes With CV Risk

**So Hun Kim**[1*]

[1] Endocrinology and Metabolism, Inha University College of Medicine, Korea

*shoney@inha.ac.kr*

Sulfonylureas have a long history of use in the treatment of hyperglycemia in type 2 diabetes. However, their use has been associated with controversy, due to their effect on increased hypoglycemia, weight gain and possible cardiovascular risk. However, recent studies have shown the cardiovascular safety of sulfonylrueas and the potent glucose lowering effect of this drug class. Among the sulfonylureas, gliclazide is reported to be associated with lower incidence of severe hypoglycemia and to have beneficial impact on cardiovascular mortality among the sulfonylurea class drugs. The current role of sulfonylurea in the treatment of diabetes and the effects of gliclazide in patients with type 2 diabetes and cardiovascular risk will be reviewed.

# Why Is It Imperative to Consider Maximum Tolerable Statin as a First Treatment Option for ASCVD Patients?

**Hyun-Jae Kang**[1*]

[1] Internal Medicine, Seoul National University Hospital, Korea

*nowkang@snu.ac.kr*

With widening indications and uses of ezetimibe and PCSK-9 inhibitors in recent clinical practices, importance of evidence-based usage of lipid lowering drugs for cardiovascular disease prevention become more important in terms of risk benefit and wells as cost benefit. In this presentation, the role of statin in prevention of cardiovascular disease will be discussed. And the evidences from clinical studies with statins and evidence-based usage of statins also will be discussed.

# EMPA-REG OUTCOME: Does Glucose Lowering Have Anything to Do with the CV Benefits of this Glucose Lowering Drug?

**Silvio Inzucchi**[1*]

[1] Endocrinology, Yale University, USA

*silvio.inzucchi@yale.edu*

Empagliflozin was the first SGLT2 inhibitor to demonstrate a clear cardiovascular (CV) benefit in high risk patients with CV disease. The benefits included a 38% risk reduction in CV mortality, 35% in heart failure (HF) hospitalization, and 39% in CKD progression. Because HbA1c differences between the active therapy and placebo groups was small, it appeared unlikely that glucose lowering explained these impressive effects. Several theories have emerged as to why this medication improves CV outcomes, including plasma volume reduction with resultant left ventricular off-loading, alterations in energy utilization by the heart, and direct cardiac effects. Of these possibilities, there appears to be the largest body of evidence for the former - that the drug's diuretic actions are important in this regard. I will present the results of a mediation analysis that suggests this to be the case, and new data from a mechanistic study demonstrating a clear reduction in plasma volume from empagliflozin in HF patients. Notably, this effect occurs without the usual neurohumoral activation from traditional diuretics. More studies will be necessary before we fully understand how this and other SGLT2 inhibitors exert their CV benefits.

# The Power of One Pill, NesinaAct for T2DM Patients With Cardiovascular Disease Risk

**Changhee Jung**[1*]

[1] Department of Internal Medicine, University of Ulsan, Korea

*chjung0204@gmail.com*

The various combination therapy of oral anti-diabetic drugs for the management of type 2 diabetes mellitus (T2DM) are suggested. We consider the beneficial combination based on not only insulin resistance with protecting and/or restoring β-cell function but also insulin secretion. And the most important factor of treatment is to control glucose level.

For being synergistic partner in the treatment of T2DM, illustrated the potential benefits of combination therapy with pioglitazone and DPPIVi with respect to: (1) addressing pathophysiologic mechanisms underlying T2DM; (2) maintenance of sustained and effective glycemic control; (3) overall safety benefit; and (4) effect on CV risk and long-term outcomes.

Pathophysiologically, it is logical to combine insulin sensitizers with incretin agents/metformin, and these different mechanisms of action are covered with the combination of alogliptin, pioglitazone and metformin. By protecting β-cell function early on, it may be possible to prevent later deterioration and type 2 diabetes complications. Also, both of alogliptin and pioglitazone demonstrated CV safety and have abundant evidences (EXAMINE/ PRO-active) including studies of effect on atheroma volume regression and CIMT (SPEAD-A/ CHICAGO/PERISCOPE)

In this lecture, I attempt to address the synergistic benefits with DPPIVi and pioglitazone combination, that should be considered when providing optimized treatment and suggesting therapeutic strategies for better outcomes in T2DM patients.

Breakfast Symposium 6

# Lipid Management for ACS Patients: Resetting the Goals and Optimizing the Treatment

**Alberico Catapano**[1*]

[1] Parmacologial and Biomolecular Sciences and Mulrimedica IRCCS, University of Milano, Italy

*alberico.catapano@unimi.it*

Patients with ACS are at very high risk or recurrent CV events and often not at their therapeutic goal. Guidelines favor the use of high-intensity, or maximally-tolerated, statins as the first step in lipid-lowering pharmacotherapy (level IA ). The ESC/EAS Guidelines provide a recommendation for the use of ezetimibe in those not achieving their LDL-C goals despite maximally tolerated statins (class I, B) and recommends PCSK9 inhibitors for very-high risk patients not achieving the LDL-C goals despite maximally tolerated statins and ezetimibe (class I, A). An additional recommendation of the ESC/EAS Guideline is for patients on maximally-tolerated statins who experience a vascular event followed by a second vascular event within two years is that treatment to an LDL-C goal of <1 mmol/L (40 mg/dL) be considered (class IIb, C). Secondary treatment targets include also non-HDL-C and apoB, with goals being defined depending on the risk category: non-HDL-C <2.2 mmol/L (<85 mg/dL) and apoB <65 mg/dL for people at very high CV risk, and non-HDL-C <2.6 mmol/L (<100 mg/dL) and apoB <80 mg/dL for people at high CV risk, respectively. The need of an intensive approach in ACS patiens is clear

# Ezetimibe/Rosuvastatin Combination for CVD Prevention

**Chan Joo Lee**[1*]

[1] Cardiology, Yonsei University College of Medicine, Korea

*zanzu@yuhs.ac*

Dyslipidemia is one of the major cardiovascular risk factors. LDL-cholesterol lowering by statin has been proven to be beneficial in primary and secondary prevention for cardiovascular disease. Recent guidelines suggest target LDL-C levels according to cardiovascular risk and recommend statin therapy to reach LDL-C to the target level as treatment of choice. However, statin monotherapy cannot reduce LDL-C to the target level in some patients with very high cardiovascular risk. They had a residual risk for cardiovascular disease in spite of statin therapy. Adding a non-statin agent can be helpful to reduce LDL-C. demonstrated that when ezetimibe was used in combination with a statin, it further reduced LDL-C and further reduced the incidence of cardiovascular disease. In addition, ezetimibe can decrease the incidence of adverse effects caused by high-intensity statin. Therefore, statin and ezetimibe combination therapy is very useful in terms of efficacy and safety.

Breakfast Symposium 8

# Comparison of the Efficacy, Safe, and Cost-Effectiveness of Atorvastatin 10mg and 20mg in High-Risk Asian Patients with Hypercholesterolemia, The PEARL Study: A Randomized Controlled Multicenter Trial

**Ji Bak Kim[1*], Woo Hyuk Song[2], Jong Sung Park[3], Tae-Jin Youn[4], Yong Hyun Park[5], Shin-Jae Kim[6], Sung Gyun Ahn[7], Joon-Hyung Doh[8], Yun-Hyeong Cho[9], Jin Won Kim[10]**

[1] Division of Cardiology, Gimpo Woori Hospital, Korea
[2] Division of Cardiology, Department of Internal Medicine, Korea University Ansan Hospital, Korea
[3] Department of Cardiology, Dong-A University Hospital, Korea
[4] Division of Cardiology, Department of Internal Medicine, College of Medicine, Seoul National University and Cardiovascular C, Korea
[5] Cardiovascular Center, Division of Cardiology, Department of Internal Medicine, Pusan National University Yangsan Hospital, Korea
[6] Department of Cardiology, Ulsan University Hospital, University of Ulsan College of Medicine, Korea
[7] Division of Cardiology, Department of Internal Medicine, Wonju Severance Christian Hospital, Korea
[8] Department of Cardiology, Inje University Ilsan Paik Hospital, Korea
[9] Department of Internal Medicine, Myongji Hospital, Korea
[10] Cardiovascular Center, Korea University Guro Hospital, Korea

*dr3120@gmail.com*

Although accumulating evidence suggests a more extensive reduction of low-density lipoprotein cholesterol (LDL-C), it is unclear whether a higher statin dose is more effective and cost-effective in the Asian population. This study compared the efficacy, safety, and cost-effectiveness of atorvastatin 20 and 10 mg in high-risk Asian patients with hypercholesterolemia. A 12-week, open-label, parallel, multicenter, Phase IV randomized controlled trial was conducted at ten hospitals in the Republic of Korea between October 2017 and May 2019. High-risk patients with hypercholesterolemia, defined according to 2015 Korean guidelines for dyslipidemia management, were eligible to participate. We randomly assigned 250 patients at risk of atherosclerotic cardiovascular disease to receive 20 mg (n = 124) or 10 mg (n = 126) of atorvastatin. The primary endpoint was the difference in the mean percentage change in LDL-C levels from baseline after 12 weeks. Cost-effectiveness was measured as an exploratory endpoint. LDL-C levels were reduced more significantly by atorvastatin 20 mg than by 10 mg after 12 weeks (42.4% vs. 33.5%, $p < 0.0001$). Significantly more patients achieved target LDL-C levels (<100 mg/dL for high-risk patients, <70 mg/dL for very high-risk patients) with atorvastatin 20 mg than with 10 mg (40.3% vs. 25.6%, $p < 0.05$). Apolipoprotein B decreased significantly with atorvastatin 20 versus 10 mg (–36.2% vs. –29.9%, $p < 0.05$). Lipid ratios also showed greater improvement with atorvastatin 20 mg than with 10 mg (total cholesterol/high-density lipoprotein cholesterol ratio, –33.3% vs. –29.4%, $p < 0.05$; apolipoprotein B/apolipoprotein A1 ratio, –36.7% vs. –31.4%, $p < 0.05$). Atorvastatin 20 mg was more cost-effective than atorvastatin 10 mg in terms of both the average and incremental cost-effectiveness ratios. Safety and tolerability of atorvastatin 20 mg were comparable to those of atorvastatin 10 mg. In high-risk Asian patients with hypercholesterolemia, atorvastatin 20 mg was both efficacious in reducing LDL-C and cost-effective compared with atorvastatin 10 mg.

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# Luncheon Symposium

September 9 (Wed) ~ October 10 (Sat), 2020 I Virtual Meeting (On demand)
September 9 (Wed) ~ 12 (Sat), 2020 I IVBM 2020 LIVE for Korean

KSoLA
The Korean Society of Lipid and Atherosclerosis

KVBM
Korean Society for Vascular Biology and Medicine

# 3D Imaging and Analysis Platform for Angiogenesis

**Taewoo Kim**[1*]

[1] Protein and Cell Analysis, Bioscience division, Thermo Fisher Scientific, Korea

*taewoo.kim@thermofisher.com*

3D cell culture is emerging field because it can provide more natural cell phenotype and representative cell condition like in human body. Many researchers moving to 3D cell culture and developing new methods using new cell sources i.e 3D Organoid. Imaging and analysis is one of the best solution for 3D cell model because understanding phenotype of 3D cell can provide many important answers for particular biological effects and clues new drug development.

High-Content Screening (HCS) is integrated platform for phenotypic screening allow to do sophisticate analysis of cells. Here, we will introduce our HCS platform, CX7 LZR which has 7 laser, confocal and powerful analysis software, HCS studio. 7 laser and confocal will allow to do real 3D Spheroid or Organoid imaging and powerful software, HCS studio can detect and analysis of detail of morphological and subcellular changes in both 2D and 3D.

Addition to hardware, we will introduce powerful reagents to detect subcellular changes for angiogenic study up to 3D imaging

# IVIM Technology's IntraVital Microscopy (IVM): In Vivo Live Cell Imaging

**Pilhan Kim**[1*]

[1] Graduate School of Medical Science and Engineering, KAIST/IVIM Technology, Korea

*pilhan.kim@kaist.ac.kr*

IVIM Technology's All-in-One IntraVital Microscopy system (IVM-C/CM/MS) enables dynamic 3D cellular-level imaging of various biological processes in living animals in vivo. It enables scientists to directly verify hypotheses derived from ex vivo or in vitro observations in natural physiological in vivo microenvironments. Using intravital microscopy, in vivo visualizations of gene expression, protein activity, cell trafficking, cell-cell / cell-microenvironment interactions and various physiological responses to stimuli have been achieved providing imperative novel insights, which have been impossible to obtain with conventional static 2D observation of ex vivo or in vitro samples. Additionally, it is possible to directly analyze the delivery and efficacy of new biopharmaceuticals such as antibodies, cell therapy, gene therapy, nucleic acids and exosome in an in vivo microenvironment.

IVIM Technology's IVM series are extensively optimized and carefully engineered to ensure superb performance in the intravital imaging of live animal models in vivo, providing multi-color sub-micron resolution ream-time fluorescence images. Key indispensable functionalities for intravital imaging are fully integrated into the All-in-ONE system with attentive design for smooth and easy operation. A versatile S/W, IVIM Engine and Studio, comes with unique features for intravital imaging including ultrafast image acquisition to capture fast real-time dynamics, high-precision tissue motion compensation enabled by optimized a registration algorithm, GPU-assisted parallel computing for rapid image processing. The world's first All-in-ONE intravital microscopy platform from IVIM Technology is a key solution that can explore complex dynamic behaviors of numerous cells inside a living body.

In this talk, intravital microscopic imaging of various organs including skin, liver, spleen, pancreas, kidney, small intestine, colon, retina, lung, heart, lymph node, and bone marrow will be briefly introduced. Subsequently, recent studies utilizing the real-time intravital imaging technique to investigate dynamic cellular-level pathophysiology of various human diseases will be introduced.

Keyword: Intravital microscopy, In vivo imaging, Fluorescence imaging, Confocal microscopy, Two-photon microscopy

# Time in Range International Consensus and the Application in Clinical Practice in Korea

**Sang Soo Kim**[1*]

[1] Department of Internal Medicine, Pusan National University Hospital, Korea

*drsskim7@gmail.com*

The utility of A1c, reflects of average glucose over the recent 2-3 months, is further enhanced when applied as a complement to glycemic data measured by continuous glucose monitoring (CGM). Great advances in sensor technology have led to growing adoption of CGM over the past few years in real practice. International panel of expert clinicians and researchers defined core metrics for assessing CGM data in real world clinical practices. The consensus panel identified "time in ranges (TIR)" as a key factor among many metrics of glycemic control that provides more patient-centered management goals. In clinical trial, CGM improved glycemic control of both patients with type 1 and type 2 diabetes by expanding the TIR and reducing the time spent in hyperglycemia and hypoglycemia. Now, we need to understand and interpret the CGM data and metrics to provide better outcomes for diabetic patients. Furthermore, these new technologies might cover the potential use as a behavior modification tool for lifestyle changes in heathy and pre-diabetic individuals. In future, advanced technological solutions will make it easier to apply this CGM system in real clinical setting.

Luncheon Symposium 5

# Complications and Management in Diabetes Patients With Coronay Heart Disease

**Jae-hyuk Lee**[1*]

[1] Endocrine Internal Medicine, Myongji Hospital, Korea

*jaehyugy93@naver.com*

The recent guideline based on glucose lowering drugs.

An explanation of four main points :

1) Diabetes and complications
   - A large proportion of patients with T2D have CV disease such as ischemic heart disease, stroke, unstable angina and myocardial infarction.

2) Shifted the paradigm for T2DM patients management
   - CV effects of 21st century T2D agents.

3) Choice in second-line therapy in patients where ASCVD/HF/CKD predominates.
   - SGLT2 inhibitors effect on ASCVD/HF/CKD from ADA-EASD Consensus Report-2019

4) What is the role of SGLT-2 inhibitors in guideline?
   - SGLT2 inhibition with Empagliflozin has led to changes in clinical practice guidelines for the management of T2DM patients.

# Additional Benefits of Anagliptin Beyond Glycemic Control

**Jeong Hyun Park**[1*]

[1] Internal Medicine, Inje Univeristy, Korea

*pjhdoc@chollian.net*

DPP-IV inhibitors are used to treat type 2 diabetes mellitus. Because of their superior safety and reasonable efficacy for glycemic control, they are now regarded as one of the main oral anti-diabetic agents. DPP-IV inhibitors act by blocking the enzymatic activity of CD26 (DPP-IV) to catalyze GLP-1, thus increasing the blood concentration of active GLP-1. GLP-1 can augments the glucose level dependent insulin secretion, and suppresses paradoxically increased glucagon secretion in type 2 diabetes patients. However, DPP-IV inhibitors possess pleiotrophic effects, and are being involved in many kinds of biologic phenomenon. Recent researches showed that anagliptin, one of the DPP-IV inhibitors used to treat diabetes, directly inhibited apoptosis of HUVEC (human umbilical vein endothelial cell) exposed to severe hyperglycemia, through Sirt1 and NOX4 signaling, regardless of glycemic control. Our lab performed experiments that anagliptin prevent the increased oxidative stress induced apoptosis and SIAS (stress induced accelerated senescence) of HUVEC. Today, I will briefly review about the characteristics and the results of recent clinical trials on DPP-IV inhibitors, and talk about the possible beneficial effects of these agents, beyond glycemic control.

Luncheon Symposium 7

# The Importance of Earlier and Lower LDL-C Reduction With Evolocumab in ASCVD Patients

**Jeehoon Kang**[1*]

[1] Cardiology, Seoul National University Hospital, Korea

*medikang@gmail.com*

It has been clearly demonstrated that elevated low-density lipoprotein cholesterol (LDL-C) is a key risk factor for atherosclerotic cardiovascular disease and lipid-lowering drugsare beneficial for the primary and secondary prevention of cardiovascular disease. Because LDL-C is both causal and cumulative, the longer you maintain that difference of LDL cholesterol, the greater the benefit of that difference over time.

In fact, lipid lowering trials over the last 30 years have achieved progressively lower LDL-C levels from statins to PCSK9 inhibitors.

Based on these trials, recent dyslipidemia guidelines, particularly 2019 ESC/EAS guidelines, emphasized the importance of earlier and lower LDL-C reduction in ASCVD patients.

2019 ESC/EAS guidelines recommend aggressive LDL-C goals, ≥50% LDL-C reduction from baseline AND LDL-C <55 mg/dL, for ASCVD patients and even below 40mg/dL if patients have a recurrent ACS event within 2 years.

Evolocumab, a PCSK9 inhibitor, lowered LDL-C levels by approximately 60% from a median baseline value of 92 mg/dL to 30 mg/dL and significantly reduced the primary endpoint by 15%in just 2.2 years. More importantly, evolocumab showed a greater risk reduction in patients with recent MI (<12 months), 19% RRR and 3.2% ARR, in the primary endpoint.

In conclusion, the appearance of evolocumab has strongly supported LDL-C treatment concept – "the lower, the better and the earlier, the better" and will be expected to prove itslong-term clinical outcomes in future.

# How Can We Manage LDL-Cholesterol? – From the Past to the Future

**Anselm Kai Gitt**[1*]

[1] Cardiology, Herzzentrum Ludwigshafen, Germany

*GittA@klilu.de*

In the 1960s, first reports from observational studies showed a correlation between the level of plasma lipids and the development of coronary heart disease. The first study to address this correlation testing simvastatin versus placebo showed a significant reduction in cardiovascular risk in favor of LDL lowering with simvastatin. Since then, more than 25 large randomized controlled trials with different statins have confirmed these findings. For a long time it was discussed that this benefits partly might be due to specific action of statins and not only due to LDL lowering. The first trial of a non-statin (ezetimibe) confirmed in 2015 the importance of further LDL lowering on top of the treatment with statins. In the past years the development of PCSK9 inhibitors further advanced the treatment of hyperlipidemia in high-risk patients. This presentation will provide an overview of the past landmark studies and give a future perspective of new compounds in the fields such as bempedoic acid, icosapent ethyl and Inclisiran, all of which are expected to enter clinical routine availability in the near future.

# See the Unseen; SGLT2 Inhibitor's Potential Beyong Glucose Control

**Soo Lim**[1*]

[1] Seoul National University Bundang Hospital, Korea

*limsoo@snu.ac.kr*

SGLT2i CVOTs showed dramatic benefits of this class for the reduction of CVD, mortality, heart failure hospitalization, and diabetic kidney disease protection in type 2 diabetes patients, Guidelines recommend early SGLT2 inhibitor use in high CV risk patients with T2D for disease prevention.

DECLARE-TIMI 58 trial which used dapagliflozin as the active intervention, was very unique in enrolled patients characteristics compared with the other trials. Different from EMPA-REG and CANVAS trials, which included very high CV risk type 2 diabetes, majority of the enrolled diabetes patients in DECLARE-TIMI 58 trial did not have previous cardiovascular diseases, but had only risk factors.

# Dyslipidemia Management in High-Risk Patients: "Rosuvastatin+Ezetimibe" Combination Therapy

**Weon Kim**[1*]

[1] Cardiology of Internal Medicine, Kyung Hee University, Korea

*mylovekw@hanmail.net*

There is a strong relationship between increased CVD risks and increased LDL-C levels and high prevalence rate of dyslipidemia in diabetes. Several trials demonstrated that the reduction in cardiovascular events is associated with lower LDL-C levels. Effect of rosuvastatin on LDL-C reduction and CV prevention showed in multiple trials such as STELLAR, ASTEROID, COSMOS, JUPITER, HOPE-3, etc. IMPROVE-IT study demonstrated that combination therapy of ezetimibe/statin provided additional reduction of the incidence of cardiovascular events compared with statin monotherapy [HR 0.936 (95% CI, 0.89–0.99, p=0.016]. Based on the results of the IMPROVE-IT, FOURIER and ODYSSEY trials recent guidelines recommend aggressive LDL-C lowering and the addition of non-statin treatment such as ezetimibe or PCSK9i on maximally tolerated statin for the management of dyslipidemia. Benefits of CREZET, which have uual action of Rosuvastatin/Ezetimibe combination, significantly reduced LDL-C and improved lipid parameters more than statin monotherapy and has low CYP3A4-mediated metabolism and improved patient compliance with once-daily dosing regardless of time.

Luncheon Symposium 11

# The Benefits of Intensive Lipid Lowering Therapy

**Jong-Young Lee**[1*]

[1] Division of Cardiology, Department of Internal Medicine Kangbuk Samsung Hospital, Korea

*jyleeheart@naver.com*

Control of blood cholesterol level is one of the most effective strategies for atherosclerotic cardiovascular disease (ASCVD) prevention. In fact, many clinical trials have clearly demonstrated that low-density lipoprotein cholesterol (LDL-C) lowering, primarily with statins, reduces major ASCVD risk and mortality. However, available data indicate that a lot of patients fail to achieve LDL-C goals, and this is particularly frequent in patients at very high ASCVD risk. Furthermore, owing to side effects, a significant percentage of patients cannot tolerate high intensity statin therapy.

Ezetimibe is the first of a new class of cholesterol absorption inhibitors that impairs dietary and biliary cholesterol absorption at the brush border of the intestine without affecting the absorption of triglycerides or fat-soluble vitamins. Ezetimibe added to statin therapy results in an additional 15–20% reduction in LDL in primary hypercholesterolemia. Moreover, combination therapy of ezetimibe with the lowest statin dose was shown to be as effective as statin monotherapy at the highest dose and ezetimibe has been well tolerated.

IMPROVE-IT trial clarified the benefits of extremely tight lipid control with ezetimibe and statin combination therapy for individuals at very high or extreme risk Additionally, studies have recently shown that the concentration of apoB and apoB/A1 in the blood are risk factors for atherosclerosis and are more potent predictors than LDL-C in predicting the risk of cardiovascular disease. In a local study, the Rosuvastatin/Ezetimibe combination treatment showed a statistically significant decrease in the rate of change(%) of ApoB/ApoA1 compared to the Rosuvastatin monotherapy.

In patients with Hypercholesterolemia who require thorough lipid management to prevent cardiovascular events, the combination of Rosuvastatin and Ezetimibe is considered to effectively control lipid levels through the inhibition of the synthesis of lipid and the suppression of absorption.

# All Statins Are the Same in Safety and Outcome for Asian?

**Hyuk-Sang Kwon**[1*]

[1] Endocrinology and Metabolism, The Catholic University of Korea, Korea

*drkwon@catholic.ac.kr*

Elevated low-density lipoprotein cholesterol(LDL-C) is a major risk factor for cardiovascular events, and lowering LDL-C with statins has proved effective for primary and secondary prevention of Coronary Artery Disease(CAD). However, no clear evidence for more versus less statins has been established in an Asian population. In Japan, Three strong statins(atorvastatin, pitavastatin and rosuvastatin) have been used for hypercholesterolemic patients at high-risk of cardiovascular disease and recommended as the first-line therapy for ACS(acute coronary syndrome) patients. Among three statins, pitavastatin is characterized for its potent LDL-C lowering as well as TG-lowering and stable HDL-C elevating effects. Moreover, In TOHO-LIP trial published in Internal Journal of Cardiology 2020, Pitavastatin therapy compared with atorvastatin more prevents cardiovascular events in hypercholesterolemic patients with one or more risk factors for atherosclerotic diseases despite similar effects on LDLC levels. Moreover, clinical trials performed in Europe and Japan so far have not shown adverse effect of pitavastatin on glucose metabolism. In this presentation, the role of pitavastatin in management of diabetic dyslipidaemia patients with special emphasis on Asian population will be discussed.

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# E-Poster

September 9 (Wed) ~ October 10 (Sat), 2020 I Virtual Meeting (On demand)
September 9 (Wed) ~ 12 (Sat), 2020 I IVBM 2020 LIVE for Korean

KSoLA
The Korean Society of Lipid and Atherosclerosis

KVBM
Korean Society for Vascular Biology and Medicine

# E-POSTER

## 1. Angiogenesis

# E-POSTER

# E-POSTER

# E-POSTER

# E-POSTER



## 2. Atherosclerosis

# E-POSTER

# E-POSTER

# E-POSTER

# E-POSTER



## 3. Vascular Functions

# E-POSTER

# E-POSTER

# E-POSTER



## 4. Vascular Diseases

# E-POSTER

# E-POSTER

# E-POSTER

# E-POSTER

# E-POSTER



## 5. Vascular Metabolism

# E-POSTER

# E-POSTER



## 6. Lymphangiogenesis and Inflammation

# E-POSTER

## 7. Technical Advances of Vascular Biology

# E-POSTER

## 8. Others

# E-POSTER

# E-POSTER

# E-POSTER

# 1. Angiogenesis

PO-102

## A Novel Transmembrane Protein Tmem100: Regulatory Mechanisms of Endothelial-Specific Expression and Cellular Functions During Vascular Formation

**Norika (Mengchia) Liu[1], Yumi Kingasa Katayama[1], Yusuke Watanabe[1], Yuji Arai[1,2], Yoshie Isomoto[2], Atsushi Nakano[2], Mami Uemura-Kamata[3], Yoko Fukushima[4], Yoshiaki Kubota[5], Akiyoshi Uemura[6], Yoshiakira Kanai[3], Osamu Nakagawa[1*]**

[1] Molecular Physiology, National Cerebral and Cardiovascular Center Research Institute, Japan
[2] Animal Experiment and Medicine Management, National Cerebral and Cardiovascular Center Research Institute, Japan
[3] Agricultural and Life Sciences, The University of Tokyo, Japan
[4] Graduate School of Medicine, Osaka University, Japan
[5] School of Medicine, Keio University, Japan
[6] Graduate School of Medical Sciences, Nagoya City University, Japan

*osamu.nakagawa@ncvc.go.jp*

Tmem100, transmembrane protein no. 100, is specifically expressed in endothelial cells of large caliber arteries in mouse embryos, and its null mice show embryonic lethality due to impaired vascular development. The present study aimed to unveil regulatory mechanisms of Tmem100 expression and cellular functions during vascular formation.
The BAC transgenic mouse reporter analysis was performed to search for an endothelial enhancer of Tmem100. Influence of the Tmem100 deficiency at the cellular level was analyzed in the retinal vasculature of neonatal mice with the endothelial-specific inducible gene deletion (iECKO).
We identified a distal Tmem100 enhancer that was specifically active in endothelial cells of large caliber arteries in mouse embryos. CRISPR/Cas9-based deletion of the enhancer substantially decreased endothelial Tmem100 expression and caused partial lethality in mouse embryos. Although the TMEM100 expression is induced by ALK1 signaling in cultured human endothelial cells, the ALK1 receptor does not share the unique pattern of endothelial Tmem100 expression highly specific to large caliber arteries in mouse embryos. Consistently, the Tmem100 enhancer activity was not regulated by SMAD but rather controlled by some key endothelial transcription factors in mouse embryos. The equivalent region in the human genome, however, showed an identical enhancer activity in mouse embryos, suggesting a common regulatory mechanism for the endothelial specificity of Tmem100 expression in the mouse and human. Phenotype analyses of retinal angiogenesis in Tmem100 iECKO mice revealed that Tmem100 had important morphogenic roles in arterial endothelial cells, which might explain severe defects of vascular remodeling in Tmem100 null mouse embryos.
We demonstrated that the endothelial Tmem100 expression was controlled through a distant enhancer conserved in the mouse and human. The study also suggested that Tmem100 was indispensable for the maintenance of arterial endothelial cell morphology.

PO-104

# Evaluation of the Impact of Peroxidasin On in Vitro and In Vivo Vessel Formation

**Hong Seok Choi[1], Purevjargal Naidansuren[1,2], Seung-Woo Lee[1,2], Hyun-Kyung Kim[1,2], Kyung A Ham[1,2,3], Young Ae Joe[1,2,3*]**

[1] Cancer Research Institute, College of Medicine, The Catholic University of Korea, Korea
[2] Department of Medical Life sciences, College of Medicine, The Catholic University of Korea, Korea
[3] Department of Biomedicine & Health Sciences, The Catholic University of Korea, Korea

*youngjoe@catholic.ac.kr*

Peroxidasin (PXDN) crosslinks the C-terminal non-collagenous domains of collagen IV (Col IV) by forming covalent sulfilimine bond. PXDN is one of the genes upregulated during capillary morphogenesis in three-dimensional collagen matrices. Recently, we have reported that PXDN is required for endothelial cell survival and growth signaling by sulfilimine crosslink-dependent matrix assembly. However, the importance of PXDN in vessel formation is unclear. In this study, we addressed the role of PXDN in vessel formation in in vitro and in vivo models.

To determine the effect of PXDN depletion, small interfering RNAs (siRNAs) were transfected into human umbilical vein endothelial cells (HUVECs) or rat aortic vessels and analyzed in vitro and in vivo. To investigate the role of PXDN in in vivo vessel formation, the tissues originated from the wild type or Pxdn knockout (KO) mice were examined.

By western blot and RT-PCR analyses, PXDN expression was found to be specifically regulated by growth factors in HUVECs. Upon PXDN depletion in HUVECs by using siRNAs, cellular growth, migration, tube formation and 3D microfluidic angiogenesis sprouting were markedly decreased. PXDN knockdown also led to suppressed microvessel sprouting from rat aortic vessel in vitro. PXDN-deficient cells by siRNA silencing also displayed reduced vascular network formation in vivo in Matrigel plug assay. In the murine hindlimb ischemia model, expression of PXDN, fibronectin and Col IV was increased in the regenerated tissues. In the Pxdn KO mice, no difference in vessel formation was found between the homozygous mice and the heterozygous mice. However, angiogenic sprouting was markedly decreased in the homozygous mice compared with the heterozygous mice in in vitro aortic ring assay.

PXDN dependency in endothelial cell function and angiogenesis is different in in vitro and in vivo conditions, and in vitro angiogenesis is highly dependent on PXDN.

PO-105

# Association Between Erythrocyte Dynamics and Vessel Remodelling in Developmental Vascular Networks

**Miguel O. Bernabeu[1*]**

[1] Usher Institute, The University of Edinburgh, UK

*miguel.bernabeu@ed.ac.uk*

Sprouting angiogenesis is an essential vascularisation mechanism and consists of two phases: sprouting and remodelling. The remodelling phase is driven by rearrangements of endothelial cells (ECs) in response to flow-induced wall shear stress (WSS). However, the question of how the presence

of red blood cells (RBCs), and their profound impact on microvascular haemodynamics, affect vascular remodelling has not been addressed.

Confocal images of mouse retinas labelled for ICAM2 (indicating lumenised vessels) and Collagen IV (Col.IV) were acquired. Col.IV images were used to construct computational flow models and ICAM2 images used to assess the state of remodelling. We simulated blood flow as a suspension of deformable RBCs within selected regions of interest (ROIs) using the HemeLB flow solver. Furthermore, we carried out simultaneous live imaging of vessel remodelling and RBC dynamics in the zebrafish caudal vein plexus 48-72h post-fertilisation. We compared control morpholino oligomer (MO) fish with normal RBC perfusion and gata1 MO fish not carrying RBCs in their bloodstream.

Our simulations predicted blood flow rates and RBC velocities/fluxes that were in good agreement with in vivo data. A striking finding from these simulations was the highly heterogeneous distribution of RBC throughout the ROIs. Furthermore, we found a strong correlation between RBC depletion and vessel regression. We demonstrate plasma-skimming as the mechanism for RBC depletion. Live imaging in a developmental zebrafish model confirms this association.

Our study reports a new mechanism for enhancement of the WSS differences driving vascular remodelling during development, which arise due to the highly heterogeneous distribution of RBCs within the primitive plexus. Additionally, we speculate that vascular remodelling driven by the principle of removing RBC-poor vessels will lead to a network layout that avoids portions of the tissue being vascularised but poorly oxygenated. This can importantly contribute to the optimal patterning of vascular networks during development.

PO-106

# Inhibition of MEK1/2 Promotes Myocardial Arterialisation in Vivo

**Elisa Avolio[1*], Anita Thomas[1], Andrea Caporali[2], Rajesh Katare[3], Marco Meloni[2], Massimo Caputo[1], Paolo Madeddu[1]**

[1] Bristol Medical School, University of Bristol, UK
[2] University/BHF Centre for Cardiovascular Science, University of Edinburgh, UK
[3] Department of Physiology, University of Otago, New Zealand

*elisa.avolio@bristol.ac.uk*

Objective: To uncover new pro-arteriogenic approaches by exploiting cardiac pericytes (PCs), mural cells endowed with arteriogenic capacity.

Methods: Human perivascular CD31neg CD34pos PCs were identified in situ and extracted from myocardial samples of adult subjects. PC differentiation into vascular smooth muscle cells (VSMCs) was investigated. Molecular studies were undertaken to identify signalling responsible for pericytes VSMC potential and drugs able to foster this phenomenon. An in vivo study in mice was carried out to confirm the pro-arteriogenic properties of the identified compound.

Results: The withdrawal of EGF and bFGF from the PC culture media induces the differentiation of PCs into contractile VSMCs. Molecular investigations of pathways associated with EGF and bFGF showed that the MEK1/2-ERK1/2 signalling inhibits the expression of contractile VSMC genes in PCs. Screening of compounds able to interfere with this pathway revealed that PD0325901 - a potent MEK1/2 inhibitor (MEKi) - promotes the VSMC phenotype in PCs. Next, we validated the effect of PD0325901 on cardiac arteriogenesis in vivo. Adult C57BL6/J mice received the MEKi 10 mg/kg/day or vehicle (DMSO), orally for 14 days. At the

endpoint, echocardiographic evaluation of left ventricle (LV) function (n=6/group) showed no difference in comparison with respective baselines. Histological analyses of the hearts (n=5/group) showed an increase in small arterioles (diameter < 20μm) density in the LV of PD-mice compared with the DMSO group (16.4 vs 11.7 art/mm2). No change was observed for the capillary density. The PD treatment reduced the fraction of small arterioles covered with a CD34pos layer (53% vs 70% of total arterioles), suggesting a contribution of PCs to the arteriolar remodelling. Finally, the drug improved the LV myocardial perfusion in the PD- vs the DMSO-group (6.8 vs 5.3 ml/min/g of LV tissue, n=6/group).

Conclusion: We propose a new, fascinating approach to promote therapeutic myocardial arterialisation.

PO-107

# Transdifferentiation of Human Adult Fibroblasts Into Authentic Endothelial Cells by Defined Factors

**Youngchul Shin[1*], Jung-Kyu Han[1], Min-Hwan Sohn[2], Saet-Byeol Choi[1], Dasom Shin[1], Jong-Yeon Shin[3], Jeong-Sun Seo[3], Hyo-Soo Kim[1]**

[1] Center of Cell-& Bio-Therapy for Heart, Diabetes, and Cancer, Seoul National University Hospital, Korea
[2] Department of Biomedical Sciences, Seoul National University Graduate School, Korea
[3] Precision Medicine Center, Seoul National University Bundang Hospital, Korea

*inycshin@gmail.com*

Previously, we reported direct conversion of adult fibroblasts (FBs) into endothelial cells (ECs) using defined factors in mice. Here, we assessed whether this approach can be applied for transdifferentiation of human adult dermal fibroblasts (aHDFs) to authentic ECs.

Lentiviral vectors encoding endothelial transcription factors (TFs) were constructed. We examined whether 5 TFs (FOXO1, ER71, KLF2, TAL1, and LMO2) used for the generation of mouse induced ECs (iECs) could convert aHDFs into human iECs. Twenty-eight days after transduction, 32.1±5.1% cells expressed VE-cadherin. Factor screening revealed that only three factors (3F: ER71, KLF2, and TAL1) were necessary to induce VE-cadherin (+) cells.

Mature iECs double-positive for VE-cadherin/PECAM1 (DP cells) with a cobblestone appearance were obtained at the frequency of only 5.1±0.6%. Using whole transcriptome analysis, potential factors blocking conversion were screened. Among candidates, knockdown of TWIST1 enhanced efficiency of conversion. Rosiglitazone, an inhibitor of epithelial-mesenchymal transition (EMT), also improved this efficiency. We found 2nd stage conversion process where we incubated VE-cadherin (+) cells for 2 additional weeks after sorting leading to enhanced efficiency. The final 6 weeks' protocol resulted in conversion of 19.6±3.0% aHDFs into human iECs defined by DP cells that showed the authentic natures of mature ECs in various analyses.

Our iEC protocol showed the most efficient EC conversion rate, compared with the protocols suggested by other groups. Furthermore, unlike other studies in which EC-like cells were defined as only one single marker such as VE-cadherin, CD31 or VEGFR-2, our study suggests a new perspective that the use of double markers can purify mature iECs which exclude immature iECs that arise during the transdifferentiation process.

aHDFs can be converted into authentic ECs through transduction of three TFs (ER71, KLF2, and TAL1) with two EMT inhibitors (siTWIST1 and rosiglitazone) followed by 2nd stage conversion.

PO-108

# Endothelial Rho-GEF Trio Is Essential for Angiogenesis by Reorganizing the Actin Cytoskeleton via RhoG and Rac1

**Lanette Kempers[1], Jeffrey Kroon[2], Ilse Timmerman[3], Martijn Nolte[1], Jaap Van Buul[1,4*]**

[1] Molecular and cellular Hemostasis, Sanquin Research, Netherlands
[2] Vascular Medicine, Academic Medical Centrum University of Amsterdam | AMC, Netherlands
[3] Hematopoiesis, Sanquin Research, Netherlands
[4] Molecular Cytology, Leeuwenhoek Centre for Advanced Microscopy (LCAM), Swammerdam Institut, Netherlands

*j.vanbuul@sanquin.nl*

Upon vascular damage or occlusion, newly formed vessels sprout from pre-existing ones, a process known as angiogenesis. Endothelial cells lining the vessel wall are instrumental in angiogenesis. They initiate this process and remodel their actin cytoskeleton to facilitate proliferation and migration out of pre-existing vessels. However, the mechanisms that coordinate the remodeling of the endothelial actin cytoskeleton to promote angiogenesis remains poorly understood.

The Rho-GEF Trio is known to activate both Rac1 and RhoG, both modulators of the actin cytoskeleton. We show that the Rho-GEF Trio is crucial for angiogenic sprouting in vitro as well as in vivo.

Inhibition of Trio activity reduced sprout formation from the developing segmented vessel in a zebrafish model as well as in vitro sprouting assays. Moreover, a strong reduction in the length of developing sprouts was observed in a murine retinal explant assay. Inhibition of both RhoG and Rac1 showed the same sprouting dysfunction as Trio silencing, indicating indeed that the Trio signaling axis works through activation of the actin cytoskeleton regulators Rac1 and RhoG.

Our data reveal that silencing of Trio affects multiple steps of the angiogenic process which all require adjustments of the actin cytoskeleton, including endothelial cell adhesion, migration and permeability.

PO-109

# Epithelial Vegfa Specifies a Distinct Endothelial Population in the Mouse Lung

**Lisandra Vila Ellis[1], Jichao Chen[1*]**

[1] Pulmonary Medicine, MD Anderson Cancer Center, USA

*jchen16@mdanderson.org*

The lung microvasculature – long considered homogenous – must coordinate its growth with the development of the epithelial surface in order to conduct efficient gas exchange. We recently found that Vascular endothelial growth factor A (Vegfa) is expressed by alveolar type 1 (AT1) epithelial, and that the disruption of AT1 cell development leads to reduced lung angiogenesis. These findings led to our central hypothesis that AT1 cell-derived VEGFA is required to specify a subset of endothelial cells (ECs).

We generated an epithelial-specific Vegfa knockout mouse, and used state-of-the-art imaging to analyze the vascular and epithelial phenotypes. We also isolated lung ECs and performed single cell RNA-sequencing (scRNA-seq) to identify the presence of different subsets of endothelial cells, and conducted analysis of their gene expression as well as their trajectory to identify their ontogeny.

Single cell RNA-seq identified 15-20% lung ECs are transcriptionally distinct – marked by Carbonic anhydrase 4 (Car4), and arise from bulk ECs, as suggested by trajectory analysis. Car4 ECs, unlike bulk ECs, have extensive cellular projections and are separated from AT1 cells by a limited basement membrane without any intervening pericytes. Car4 ECs are specifically lost upon epithelial Vegfa deletion; without Car4 ECs, the alveolar space is aberrantly enlarged despite the normal appearance of myofibroblasts.

Lung vascular growth is a unique and complex process that is yet to be fully understood. Our findings support a signaling role of AT1 cells and shed light on alveologenesis. We believe that a better understanding of the interaction between the epithelium and the vasculature will offer new insights to diseases where this process is disrupted, such as bronchopulmonary dysplasia.

PO-110

# Establishment of a Cell Culture Model Derived From Human Corpus Cavernosum for In Vitro Erectile Function Study

**Guo Nan Yin[1], Choi Min Ji[1], Kwon Mi Hye[1], Ock Ji Yeon[1], Kalyan Ghatak[1], Anita Limanjaya[1], Ji-Kan Ryu[1], Jun-Kyu Suh[1]***

[1] National Research Center for Sexual Medicine and Department of Urology, Inha University Hospital, Inha University School of Medicine, Korea

*jksuh@inha.ac.kr*

To establish a simple and nonenzymatic technique to isolate endothelial cells (EC) and pericytes from human corpus cavernosum tissue and to establish an in vitro model of erectile dysfunction (ED) for the study of high-glucose-induced angiopathy.

For primary human cavernous EC culture, cavernous tissues were implanted into Matrigel in cell culture dishes. For primary human cavernous pericyte culture, cavernous tissues were settled by gravity into cell culture dishes. We performed immunocytochemistry to determine phenotype and morphologic changes from passage 1 to 5. To establish an in vitro model of diabetic ED, the primary cultured cells were exposed to a normal-glucose (5 mmoL) or a high-glucose (30 mmoL) conditions.

We successfully isolated high-purity EC and pericytes from human corpus cavernosum tissue. Primary cultured EC showed highly positive staining for vWF, and pericyte revealed positive staining for NG2 and PDGFR-β. Primary cultured EC and pericytes maintained their cellular characteristics up to passage 2 or 3. However, we observed significant changes in their typical phenotype from the passage 4 and morphological characteristics from the passage 3. Human cavernous EC or pericytes can formed well-organized capillary-like structures in normal-glucose condition, whereas severely impaired tube formation was detected in high-glucose condition.

This study provides a simple and nonenzymatic method for primary culture of human cavernous EC and pericytes. An in vitro model for diabetic ED will aid us to understand the pathophysiology of diabetic ED, and also be a valuable tool for determining the efficacy of candidate therapeutic targets.

PO-111

# Vasohibin-1 Inhibits Angiogenesis via the Increase of Detyrosinated α-Tubulin

**Miho Kobayashi[1,3], Ikumi Wakabayashi[1,2], Yasuhiro Suzuki[3,4], Yasufumi Sato[3,4], Tetsuro Watabe[1*]**

[1] Department of Biochemistry, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Japan
[2] Laboratory of Cardiovascular Medicine, Tokyo University of Pharmacy and Life Sciences, Japan
[3] Department of Vascular Biology, Institute of Development, Aging and Cancer (IDAC), Tohoku University, Japan
[4] New Industry Creation Hatchery Center (NICHe), Tohoku University, Japan

*t-watabe@umin.ac.jp*

Vasohibin-1 (VASH1) was identified as a negative-feedback regulator of angiogenesis. Pro-angiogenic factor, such as VEGF, stimulates VASH1 production in endothelial cells (ECs). On the other hand, it was recently reported that VASH1 has an enzymic activity for detyrosination on α-tubulin. Detyrosination of α-tubulin is one of the post-translational modification of microtubules. Detyrosinated α-tubulin (ΔY-tubulin) play an important role for several physiological function, but unknown in angiogenesis. The aim of this study is a revealing the role of ΔY-tubulin for anti-angiogenic effects by VASH1.

ΔY-tubulin is re-tyrosinated by tubulin tyrosine ligase (TTL) in cells, and ΔY/Y reaction on α-tubulin is reversible. Overexpression of VASH1 increased ΔY-tubulin, and co-expression of VASH1 and TTL reduced the level of ΔY-tubulin to nearly level of basal control in ECs. Thus, the intracellular level of ΔY/Y is able to manipulate by overexpression of VASH1 or/and TTL. Here we examined the function of ΔY-tubulin in VASH1-induced anti-angiogenic effects through the employing a combination of VASH1 and TTL.

VEGF-induced VEGFR2 signaling, ECs migration and angiogenesis were inhibited by the increase of ΔY-tubulin by overexpression of VASH1. However, the co-expression of VASH1 and TTL canceled out these anti-angiogenic effects by VASH1. VEGF-VEGFR2 downstream signaling activation requires endocytosis of VEGFR2. Increase of ΔY-tubulin suppressed VEGF-induced VEGFR2 endocytosis, but the combination of VASH1 and TTL showed no significant change compared with control. Furthermore, VASH1-induced ΔY-rich microtubules exhibited a characteristic alteration on the working of dynein, which is endocytic motor protein.

From our results, it was revealed that VASH1-induced ΔY-tubulin inhibits VEGF-signaling leading to ECs migration and angiogenesis through the inhibition of VEGFR2 endocytosis by the changing of dynein function on MTs. Therefore, it was suggested that VASH1 provokes anti-angiogenic effects by the hypofunction of microtubules as a rail for endocytosis.

PO-112

# Protrudin Regulates Angiogenesis

**Amita Arora[1], Annukka Kivelä[1], Juuso Taskinen[1], Annika Koponen[1], Camilla Raiborg[2], Harald Stenmark[2], Vesa Olkkonen[1*]**

[1] Minerva Foundation Institute for Medical Research, Minerva Foundation Institute for Medical Research, Finland
[2] Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Montebello, Oslo, Norway, Norway

*vesa.olkkonen@helsinki.fi*

Protrudin is a membrane contact site protein and is well reported in promoting neurite outgrowth. The aim of the present study is to understand the role of Protrudin in endothelial functions and angiogenesis per se.

Functional assays like in vitro tube formation assay, western blot and confocal microscopy were applied to understand the role of Protrudin regulates angiogenic signaling pathways in human umbilical vein derived endothelial cells (HUVECs) and human aortic endothelial cells (HAECs). Furthermore, transcriptome profiling using NextGen RNA sequencing analysis was done to investigate the genes and pathways affected in HUVECs upon Protrudin knockdown.

Functional assays demonstrate that Protrudin regulates angiogenic signaling pathways in HUVECs and HAECs. Knockdown of Protrudin inhibits angiogenic tube formation in HUVECs. Deletion of Kinesin-binding and endosomal-binding domains of Protrudin showed similar inhibition of angiogenic tube formation, suggesting that Protrudin-mediated peripheral translocation of endosomes is crucial for endothelial tube formation. Furthermore, NextGen RNA sequencing data suggests 'cell movement' and 'cell survival' as key pathways affected in HUVECs upon Protrudin knockdown. Experimental validation demonstrates that Protrudin affects cell migration by regulating the activity and distribution of the focal adhesion molecules paxillin and FAK (Focal adhesion kinase). We observed that Protrudin regulates expression of the transcription factor Erg, a master transcriptional regulator for endothelial cell function and homeostasis including angiogenesis and vascular stability. Downregulation of Erg further resulted in the suppression of downstream target genes including VE-Cadherin and e-NOS.

Overall, our data suggests Protrudin plays key role in endothelial cell biology. It seems to regulate multiple pathways of angiogenesis including cell migration and vascular stability. Our study unravels a novel role of Protrudin in regulating vascular homeostasis and opens up the possibility of therapeutic targeting of Protrudin to promote vascular health.

PO-113

# Endothelial Cell-Cell Contact Determines Tip Cell Induction During Sprouting Angiogenesis

**Jung Hyun Bae**[1,2], **Gou Young Koh**[1,2*]

[1] Biomedical Research Center, KAIST, Korea
[2] Center for Vascular Research, IBS, Korea

*koh57@kaist.ac.kr*

Sprouting angiogenesis involves a series of coordinated morphogenic events in the formation of tip, stalk and phalanx cells. The dynamics of endothelial cell sprouting is understood by the balance of Delta-like 4 (Dll4)/Notch signaling between endothelial cells (ECs) by a gradient of vascular endothelial growth factor (VEGF). Dll4/Notch feedback loop is sufficient to explain a random pattern of EC specification into tip and stalk cells, but the mechanism of how one particular EC is induced as tip cell is not fully addressed. Referring to Cao et al, we questioned whether different stability of endothelial cell-cell contact is decisive for tip cell induction during sprouting angiogenesis. Merlin (Moesin-ezrin-radixin-like protein, also known as schwannomin) is a tumor suppressor encoded by neurofibromatosis type-2 gene NF2 that coordinates contact-dependent inhibition of proliferation by sensing cell-cell contact and mediating receptor-dependent mitogenic signaling at plasma membrane in vitro. Therefore, we proposed that a simultaneous, contact-dependent interaction of Merlin to both signaling and adhesion receptors would define the mechanism of tip cell induction during sprouting angiogenesis.

In order to study sprouting angiogenesis, 100 μg of tamoxifen was injected into the stomach daily from postnatal day 2 (P2) to P4 by intraperitoneal injec-

tion. Retina and brain were harvested at P6 and P9, respectively.

Endothelial-restricted deletion of Nf2 in mice induced hyperplastic vascular front with accompanying filopodial burst and high ANGPT2 (Angiopoietin2) expression. Interestingly, endothelial Nf2/Merlin blocks tip cell formation through Hippo independent pathway, but through VEGFR2 signaling. NF2/Merlin physically interacts with VEGFR2 and regulates the quantity and the duration of VEGFR2 internalization in a contact dependent manner.

Our findings identify endothelial Nf2/Merlin as an inhibitor of tip cell induction, which is dependent on the cell-cell contact and define novel YAP/TAZ independent pathway that governs tip cell formation.

PO-114

# LRG1-Dependent Angiogenesis Induced by IL6-STAT3 Signalling Axis

**Athina Dritsoula[1], Laura Dowsett[1], Marie O'Connor[1], Stephen Moss[1], John Greenwood[1*]**

[1] Institute of Ophthalmology, University College London, London, UK

*j.greenwood@ucl.ac.uk*

Leucine rich α-2-glycoprotein 1 (LRG1) is a secreted glycoprotein predominantly produced by the liver under physiological conditions. In disease, LRG1 overexpression promotes pathogenic neovascularization and its inhibition through genetic modification or the use of neutralising antibodies leads to reduced dysfunctional angiogenesis. We aimed to examine the mechanisms underlying LRG1-dependent pathological angiogenesis and the impact of interleukin-6 (IL6), which has an established role in stimulating angiogenesis with impaired pericyte coverage.

Immunostaining of ex vivo cultures of wild type and Lrg1-/- metatarsal bones and aortic rings were used to study angiogenesis and pericyte coverage. Transcriptional activity using luciferase reporter vectors and chromatin immunoprecipitation assays were performed to study the transcriptional activity of the human LRG1 promoter.

IL6 treatment induced significantly LRG1 expression in the mouse brain endothelial cells as well as in the metatarsal and the aortic ring assays ($p<0.05$). IL6 promoted significantly angiogenesis and sprouting in both the metatarsal and aortic ring assays, respectively, and this effect was reduced in the Lrg1-/- metatarsal bones compared to wild type ($p<0.01$). IL6 treatment caused a significant drop in the alpha smooth muscle actin-positive (aSMA+) pericytes in the metatarsal assay ($p<0.001$), but not in the aortic ring assay highlighting tissue-dependent vascular bed differences. In addition, IL6 activated LRG1 transcription through the binding of the phosphorylated STAT3 on the conserved consensus binding site on the human promoter. Deletion of the STAT3 binding site abolished the transcriptional activation.

Our data confirmed that IL6 activates transcription of LRG1 gene through the phosphorylation and binding of STAT3 transcription factor on the gene promoter leading to LRG1-dependent dysfunctional angiogenesis. Blocking LRG1 and its vascular corrupting effects could be of therapeutic benefit and avoid potential IL6-STAT3 mediated housekeeping processes. Prevention of pathogenic neovascularization may have utility in conditions like neovascular age-related macular degeneration and cancer.

PO-115

# Endothelial Toll-Like Receptor 2 Promotes Angiogenesis and Pro-Angiogenic Immune Cell Recruitment via Endogenous Ligand Signaling

**Michael McCoy[1*], Rakhylia Murtazina[1], Daniel Nascimento[1], Tatiana Byzova[1]**

[1] Neuroscience, Cleveland Clinic Foundation, USA

*mccoym6@ccf.org*

To determine whether or not endothelium-derived TLR2 mediates angiogenesis.

We utilize an endothelial cell-specific knockout in order to determine what the effects of TLR2 signaling are at the transcriptional, protein, and organ level. We then employ the use of a wound healing and tumor model in order to assess what these relevant effects are in vivo.

We demonstrate with RNA-seq of whole mouse hearts in sterile conditions that TLR2 in the endothelium is continuously signaling and involved in a range of vascular homeostasis functions. In an aortic tissue culture model, we further establish that endogenous and exogenously sourced TLR2 ligands are capable of stimulating angiogenesis and analysis of endothelial cell secretion profiles reveals a reduction in multiple pro-angiogenic cytokines in knockout endothelial cells. In a knockout mouse model, we found evidence that wound healing is delayed alongside a reduction in monocytes and tumor growth is reduced alongside the recruitment of Ly-6G/C+, Tie2+, and CD11b+ pro-tumorigenic leukocytes. Both experimental models exhibited a reduction in neovessel growth and a reduction in the generation of the endogenous the TLR2 ligand carboxyethylpyrrole (CEP) via oxidative stress. Stimulation of TLR2 in endothelial cells leads to the induction of a cascade of endothelial cell adhesion molecules expression: P-selectin, E-selectin, ICAM-1, and VCAM-1. Disruption of the generation of CEP at early stages of tumor growth attenuated tumor neovascularization and leukocyte infiltration.

These data presented indicate that the endothelium is not a passive player in innate immunity but indeed functions as an extension of the immune system itself. These observations reveal that endothelial TLR2 in pro-inflammatory conditions is partially responsible for both wound healing and tumor angiogenesis. Our work implies that novel therapeutics that aim to reduce oxidative stress or infection may be useful in reducing pathological angiogenesis.

PO-116

# A Possible Biomechanical Mechanism Underlying Enhancement of Angiogenic Vessel Elongation by Pericytes

**Yasuyuki Hanada[1,2], Akiyoshi Uemura[3], Toyoaki Murohara[1], Koichi Nishiyama[2*]**

[1] Department of Cardiology, Nagoya University, Japan
[2] International Research Center for Medical Sciences, Kumamoto University, Japan
[3] Department of Retinal Vascular Biology, Nagoya City University, Japan

*nkanako@kumamoto-u.ac.jp*

Angiogenesis is a dynamic morphogenesis in which new vessels emerge from pre-existing ones. In the process, endothelial cells (ECs) move coordinately, changing their speed at every moment, to

form 3D vascular structure with lumen. Although perivascular pericytes that wrap EC branches is known to stabilize vessels, it is still unknown how pericytes influence the initial stage of angiogenesis.

To dissect the issue, first we analyzed additional effects of pericytes on angiogenesis using an originally established 3D reconstitution assay. We observed that pericytes co-culture enhanced EC branch elongation and made the branches thinner. These effects were also recapitulated in vivo by comparing the differences between with and without pericytes removal in murine retinal angiogenesis. To clarify the cellular mechanism, we performed time-lapse imaging of the reconstitution assay.

Then, we found that efficient forward EC migration was strengthened by coculturing pericytes. We further observed that forward EC migration halted irrespective of the presence or absence of pericytes when vascular lumen and diameter abruptly expanded, while the incidence became quite lower by coculturing pericytes. Recently, we discovered that circumferential tension externally applied to the vascular wall inhibited angiogenic forward EC migration without pericyte wrapping (in preparation). Also, theoretical calculation can estimate that vascular wall tension increases while vascular lumen and diameter abruptly expands. Based on these results, we hypothesized that pericytes might control vascular wall tension by preventing excess expansion of vascular lumen and diameter via some mechanisms, one of which may be stiffening of extravascular tissues. Therefore, we finally examined the possibility, and found that the component of extracellular matrix around vessels was changed by coculturing pericytes. Microrheological measurement further showed that this change stiffened extravascular tissue.

Collectively, these results suggest that pericytes facilitate angiogenic vessel elongation by keeping branches thin, which can control vascular wall tension increase.

PO-117

# Unc5b Is a Target of Notch Signaling and a Potential Effector of Notch-Mediated Vascular Barrier Enhancement

**Qanber Raza[1*], Bhairavi Swaminathan[1], Jing Du[1], Seock-Won Youn[1], Kevin Boye[2], Anne Eichmann[2], Jan Kitajewski[1]**

[1] Physiology and Biophysics, University of Illinois at Chicago, USA
[2] Yale Cardiovascular Research Center, Yale University, USA

*qraza@uic.edu*

Notch signaling acts as a central regulator of vascular development in vertebrates and is essential for suppression of excessive endothelial sprouting during angiogenesis. Nevertheless, Notch dependent mechanisms of angiogenesis and the downstream genetic effectors which control tip/stalk cell behaviour in sprouting endothelial cells (ECs) have not been fully characterized. Our objective is to identify downstream effectors of Notch signaling which function to supress endothelial migration and promote junctional integrity.

We conducted unbiased transcriptomic profiling of three independent Notch manipulations in endothelial cells: in vitro Notch activation via tethered ligand DLL4, in vitro rapid Notch activation with EGTA, and in vivo Notch inhibition combined with RiboTag-based EC transcriptomic analysis. All profiles were examined at the earliest stages of transcriptional changes to enrich for direct Notch targets. Notch dependent function of identified genes were tested using in vivo and in vitro developmental angiogenic assays.

Consensus targets regulated in all screens included established Notch target genes such as DLL4, HEY1

and EFNB2. Novel Notch targets were also identified, including UNC5B, a conserved guidance signalling receptor that has critical functions during angiogenesis but has not previously been recognized as a Notch target. We show that, like Notch signaling, Unc5b is strongly expressed in retinal arteries, and constitutive Notch activation results in ectopic upregulation of Unc5b across the retinal endothelium. We demonstrate that Unc5b colocalizes with VE-Cadherin at the cell-cell junctions and, similar to Notch signaling, is required for stabilization of endothelial cell-cell junctions and suppression of EC migration in vitro and in vivo.

Collectively, our data suggests that Notch signaling upregulates Unc5b to inhibit cell migration and promote vascular barrier integrity in ECs and hence Unc5b functions as a mediator of stalk cell behaviour downstream of Notch during developmental angiogenesis.

PO-118

# The Chicken or the Egg: MSC-Endothelium Dialogue in 2d Angiogenesis Model

**Irina Beloglazova[1*], Ekaterina Zubkova[1], Victoria Stepanova[3], Konstantin Dergilev[1], Yelena Parfyonova[1,2]**

[1] Institute of Experimental Cardiology, National Medical Research Center for Cardiology, Russian Federation
[2] Faculty of Fundamental Medicine, Lomonosov Moscow State University, Russian Federation
[3] Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, USA

*irene.beloglazova@gmail.com*

The aim of the work was to study communication between endothelial (EC) and mesenchymal stromal cells (MSC) in in vitro model of angiogenesis.

We used a 2D model of human umbilical vein endothelial cells (HUVEC) co-culture with human adipose stromal cells (ASC) without exogenous matrix addition. Using this model we estimated the self-assembled network formation (SNF) by HUVEC within 48h of culture in the presence or absence of inhibitors. Co-cultured cells were separated by FACS sorting, thereafter RT PCR was used to study mRNA expression changes in HUVEC and MSC in the course of co-culture.

We discovered that co-cultivation of HUVEC with ASCs leads to changes in mRNA expression of extracellular matrix (EM) proteins, Notch pathway components, and proteases in both types of cells. Inhibitory analysis revealed that avb3 integrin is the most significant player in SNF. Blocking antibodies to av subunit not only prevented SNF formation but also altered mRNA expression of VEGF receptors, Notch signaling and proteolytic system components in monoculture of EC. Gamma-secretase inhibitor decreased SNF formation by 46% and upregulated DLL1, VEGFR2, uPAR and downregulated DLL4, HES1 and HEY1 mRNA expression in monoculture of EC. As a surprise VEGFR2 inhibitor completely abolished SNF formation although didn't influence on the expression of components of Notch signaling or proteolytic system.

Thus we can conclude that formation of SNF is the coordinated work of both cell types. One of the key factors in the formation of SNF is the interaction of HUVEC with the EM through integrin av. Our results may have important implications in the optimization of cell-based strategies to promote angiogenesis during tissue reparation. The reported study was funded by RFBR according to the research projects № 19-015-00511 and 19-29-04164.

PO-119

# DIX Domain Containing 1(DIXDC1) Regulate VEGFR2 and Wnt/Beta-Catenin Signaling in Early Angiogenesis

**Yeaji Kim[1], Dong Young Kim[1], Haiying Zhang[1], Yeomyung Kim[1], Young-Guen Kwon[1*]**

[1] Department of Biochemistry, Yonsei University, Korea

*ygkwon@yonsei.ac.kr*

VEGF-A/VEGFR-2 and canonical Wnt/β-catenin signaling appears to play a very important role in cellular responses involved in angiogenesis. However, the interaction between the two signaling in early angiogenesis remains unclear. Here, we suggest that DIXDC1(DIX domain containing 1) regulate VEGFR2 and Dvl2 (dishevelled homolog-2) in post-translational manner that result in the expression level of both VEGF-A/VEGFR-2 and canonical Wnt/ â-catenin signaling.

In order to determine the function of DIXDC1 in vivo, globally knocked down DIXDC1 mice was analyzed in both embryonic and post-natal stages, and ex-vivo assay was carried out to examine the angiogenic ability of the mice with eliminated DIXDC1 expression. DIXDC1 expression was reduced in HUVECs to elucidate its role in early angiogenesis.

Deficiency of DIXDC1 in mice display delayed angiogenesis in both embryo and post-natal stages-marked reduction in intersomitic vessels, carotid artery and primary head veins in embryo and reduced radial length, vessel density and number of filopodia in post-natal retinae. Furthermore, elimination of DIXDC1 in mice effectively suppressed pathological angiogenesis in wound-healing and OIR (Oxygen Induced Retinopathy) models. Suppression of DIXDC1 in HUVEC (Human Umbilical Vein Endothelial Cell) significantly reduced proliferation, migration, tube-like structure formation and sprouting. The basal level of VEGFR2 and Dvl2 expression was regulated in post-translationally by DIXDC1 and its interaction with Dvl2 also resulted in binding of Dvl2 and VEGFR2.

These results demonstrate that DIXDC1 is a crucial regulator of the most important signals in angiogenesis by fine-tuning the expression of basal VEGFR2 and Dvl2 which might be applicable in pathological condition involving angiogenesis.

PO-120

# Induction of Angiogenic Genes by Plasmodium Berghei Through Hypoxia-Induced Manner

**Kyung-Yoon Jeon[1], Mi-Kyung Park[1], Yeonchul Hong[2], Mee Sun Ock[1], Hee-Jae Cha[1*]**

[1] Parasitology, genetics, Kosin University College of Medicine, Korea
[2] Parasitology and Tropical Medicine, Kyungpook National University School of Medicine, Korea

*hcha@kosin.ac.kr*

Malarial infection is expected to induce tissue hypoxia in host by destruction of red blood cells. Tissue hypoxia by malarial infection may increase the activity of HIF-1α through an intracellular oxygen sensing pathway. Activation of HIF-1α also may induce the VEGF to trigger angiogenesis. In order to investigate whether malarial infection actually generate the hypoxia-induced angiogenesis. we analyzed the hypoxic condition, expression of hypoxia related angiogenic factors, and number of blood

vessels in various tissues infected with Plasmodium berghei.

Infection in mice was initiated by intraperitoneal injection of 2 x 106 parasitized red blood cells. We checked parasitemia and survival conditions after infection. We analyzed hypoxic condition, number of blood vessels and expression of hypoxia related angiogenic factor including VEGF and HIF-1α by Western blot, immunofluorescence (IF) analysis, and immunohistochemistry (IHC) at various tissues from P. berghei infected mice.

Survival conditions showed that 10% mortality was recorded after over 10% parasitemia and about 50% mortality at 30% parasitemia, respectively, Expression of VEGF and HIF-1α was increased by infection degree-dependent manner in various malaria infected-tissues. Additionally, the number of blood vessel was significantly increased in each tissue of malaria infected groups compared to non-infected control groups.

These results suggest that the malarial infection of mice model potentially induces the hypoxic condition and activates hypoxia-induced angiogenesis by stimulation of HIF-1α and VEGF in various tissues.

PO-121

# Allin as Angiogenesis Inhibitor: Possible Mechanism of Action and Cost Effectiveness Perspective

**Eka Nurdina Inayatussholeha[1], Danan Budi Primadi[2*], Rifqi Akhdan Pradipta[3], Sekar Salma Putri[4], Mochamad Afifudin[2]**

[1] Undergraduate Program of Chemical Analysis, Universitas Islam Indonesia, Indonesia
[2] Undergraduate Program of Medical Education, Universitas Islam Indonesia, Indonesia
[3] Undergraduate Program of Chemical Engineering, Universitas Islam Indonesia, Indonesia
[4] Undergraduate Program of Statistic, Universitas Islam Indonesia, Indonesia

*18711093@students.uii.ac.id*

Angiogenesis is the generation of new capillary blood vessel and it has an essential role in cancer metastatic. The mechanism of angiogenesis in cancer regulate by Vascular Endothelial Growth Factor (VEGF) and hypoxia condition. The main anti-angiogenic based-therapeutical strategy has widely discussed due to resistance and adverts effect problems. The combination of chemotherapy and herb-drugs have a promising effect on the pathogenic pathway and cost-effectiveness. Allin is a bioactive compound of Allium sativum (Garlic) extract and has an antibacterial, immunomodulatory, and antioxidant properties. The aims of study is to review the underlying mechanism of Allin as angiogenic inhibitor and cost-effectiveness perspective.

Basic/translational and clinical studies were analyzed. A total 9 studies was include conducted from PubMed, Google Scholar, Science Direct, and Online Wiley with the keywords i.e.: Angiogenesis AND Allin AND Mechanism of Action OR Cost-Effec-

tiveness. Data were extracted and critical appraisal performed.

Allin induces caspase-3 upregulation which can lead to decrease VEGF-dependent survivin. VEGF has also indirectly regulated by Myosin Phosphatase Target Subunit 1 (MYPT1). Allin has a potential role in microRNA-30d downregulation as tumor angiogenic factor via MYPT1/c-JUN/VEGF pathway. IL-6 expression has negative feedback on Allin intervention which may turn to impairment of hypoxia-induce angiogenesis. The effect of various thiosulfinate-enriched Allium Sativum extracts on cell viability of the colon cancer cell lines Caco-2 and HT-29, after 24 h incubation showed that maximum concentration of the combination is 125 µg/ml. The weight cost of chemotherapy on metastatic cancer raised to € 2380.68. Allin using as herb-drugs compound on metastatic cancer combination therapy which characterized by angiogenesis-dependent mechanism might to be saving 43.3% of therapeutical cost-effectiveness.

Allin has a potential role as an angiogenesis inhibitor via VEGF and IL-6-dependent mechanism indirectly. The use of Allin as adjuvant herb-drugs on chemotherapy might reduce therapeutical expanses.

PO-122

# Analysis of Rab5C/Vegfr2 Signaling During Sprouting Angiogenesis

**Yuki Wakayama[1], Ivo van der Bijl[2], Charita Furumaya[2], Lanette Kempers[2], Iris De Cuyper[2], Aldo Jongejan[3], Marije Kat[2], Anne-Marieke van Stalborch[2], Antonius L. van Boxtel[4], Dirk Geerts[3], Dirk de Korte[4], Wiebke Herzog[1*], Coert Margadant[2]**

[1] Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Germany
[2] Molecular Cell Biology, Sanquin Research, Netherlands
[3] University of Amsterdam, Department of Clinical Epidemiology, Netherlands
[4] Cancer Biology and Genetics and Department of Medicine, Memorial Sloan Kettering Cancer Center, USA

*Wiebke.Herzog@mpi-muenster.mpg.de*

Sprouting angiogenesis is fundamental for developmental and pathophysiological processes and is promoted by Vascular Endothelial Growth Factor (VEGF) / VEGFR2 signaling. VEGFR2 signaling stimulates gene expression, cell migration, proliferation, and the specification of tip and stalk cells. Accumulating evidence suggests that endosomes are important platforms for VEGFR2 signaling. However, the endosomal machinery that regulates VEGFR2 endosomal retention versus degradation, or its impact on tip cell formation and sprouting angiogenesis, remains largely unknown.

To investigate VEGF/VEGFR2 signaling, we used HUVECs and zebrafish.

We identify the GTPase Rab5C, but not its related isoforms Rab5A or Rab5B, as a crucial regulator of VEGFR2 protein turnover. Depletion of Rab5C or the Rab5-GEF RIN2 disturbs VEGFR2 recycling and causes accelerated VEGFR2 degradation, thus abolishing VEGF signaling and the expression of VEGF target genes, including several proteins required for

tip cell identity. Consequently, disruption of Rab5C activation disturbs tip cell formation in vitro and in the intersegmental vessels in zebrafish, and impairs sprouting angiogenesis.

We identify a novel endosomal feedforward loop uniquely controlled by RIN2/Rab5C, that prevents VEGFR2 degradation and maintains an endosomal VEGF signaling window required for VEGF-induced gene expression, tip cell formation, and sprouting angiogenesis.

PO-123

# VEGFR2-Nf2/Merlin Axis Governs Tip Cell Induction Depending on the Stability of Cell-Cell Contact

**Jung Hyun Bae[1,2], Yoo Hyung Kim[3], Yoshiaki Kubota[4], Gou Young Koh[1,2*]**

[1] Biomedical Research Center, KAIST, Korea
[2] Center for Vascular Research, Institute for Basic Science (IBS), Korea
[3] Division of Endocrinology and Metabolism, Seoul National University Hospital, Korea
[4] Laboratory of Vascular Biology, Keio University, Japan

*gykoh@kaist.ac.kr*

Sprouting angiogenesis involves a series of coordinated morphogenic events in the formation of tip, stalk and phalanx cells. Upon tip cell induction, activated endothelial cells (ECs) by a gradient of vascular endothelial growth factor (VEGF) express Delta-like 4 (DLL4) rapidly or highly in a random pattern. However, the mechanism of how one particular endothelial cell(EC) is selected as tip cell is not fully understood. Considering the paper of unbalanced relative VE-cadherin concentration at angiogenic front, we hypothesized whether different stability of endothelial cell-cell contact is decisive for tip cell induction during sprouting angiogenesis. Interestingly, Nf2/Merlin coordinates contact-dependent inhibition of proliferation by sensing cell-cell contact and mediating receptor-dependent mitogenic signaling in vitro. Therefore, we investigated how endothelial cell-cell contact govern tip cell induction through Nf2/Merlin.

To specifically delete Nf2 in EC during sprouting angiogenesis, we generated $Nf2^{i\Delta EC}$ mice by crossing VE-cadherin-$CreER^{T2}$ mice and conditional $Nf2^{flox/flox}$mice, and treat tamoxifen at postnatal day 2.

EC-specifically Nf2 deleted mice exhibited hyperplastic vascular front with accompanying filopodial burst at the angiogenic front, indicating Nf2/Merlin regulates tip cell formation. Interestingly, Nf2/Merlin inhibits tip cell formation through YAP/TAZ-independent pathway, but through VEGF/VEGFR2 signaling. We also confirmed that Nf2/Merlin blocks tip cell induction under VEGF signaling at stable cell cell contact in vivo. Underlying mechanism of regulation of tip cell formation by Nf2/Merlin depending on cell-cell contact was that NF2/Merlin physically interacts with VE-cadherin and VEGFR2, coordinating the quantity of and duration of VEGFR2 internalization both in vitro and in vivo. Fianlly, we observed that Nf2/Merlin blocks VEGFR2 endocytosis during sprouting angiogenesis.

Endothelial Nf2/Merlin, regulated by cell-cell contact, inhibits tip cell induction by regulating the quantity and the duration of VEGFR2 internalization during sprouting angiogenesis.

PO-125

# Regulation of BACH1/NRF2 by Hypoxia and the Impact on Adventitial Pericyte Function

**Sadie Slater[1*], Valeria Alvino[1], Annabelle Fricker[1], Paolo Madeddu[1]**

[1] Translational Health Science, University of Bristol, UK

*sadie.slater@bristol.ac.uk*

To demonstrate hypoxia regulates BACH1, but not NRF2 expression, causing modulation of factors involved in angiogenesis and the antioxidant response.

Using adventitial pericytes (APC) exposed to hypoxia (72h, 2% $O_2$), expression of targets was determined using immunofluorescent staining (IF), Western blotting, qPCR and ELISA.

Hypoxia caused a significant increase in APC BACH1 protein expression (n=6, p<0.02). HO1, an antioxidant and well-characterised target of BACH1, was not detectable by Western blotting, however HO1 gene expression decreased in response to hypoxia (n=6, p<0.0003). IF staining showed little HO1 expression under normoxic or hypoxic conditions. Secretion of the newly identified BACH1 target ANGPT1 into conditioned media significantly decreased with hypoxia (n=6, p<0.01). Additionally, expression of NRF2 (a transcriptional activator which competes with BACH1 for the same binding sequences on target genes) was investigated. Hypoxia significantly decreased NRF2 (n=3, p<0.006) and increased KEAP1 (a regulator of NRF2) gene expression (n=4, p<0.008). IF showed KEAP1 had strong cytoplasmic expression, which was not changed by exposure to hypoxia. NRF2 had considerably less expression under both conditions along with no notable change in expression or localisation. Similarly, dual staining for KEAP1 and NRF2 confirmed KEAP1/NRF2 form a complex in the cytoplasm, which was unchanged by hypoxia. NRF2 was not detectable by Western blotting. Furthermore, there was no change in gene expression of SOD3, an antioxidant target of NRF2, in response to hypoxia.

Here we confirm hypoxia, via BACH1, modulates expression of the pro-angiogenic factor ANGPT1. Furthermore, we demonstrate decreased or no change in expression of antioxidant factors HO1 and SOD3. We believe this is due BACH1 repressing their transcription in conjunction with hypoxia decreasing NRF2 expression. These data demonstrate the importance of BACH1 in regulating angiogenic and antioxidant responses in APC, suggesting BACH1 could be a potential therapeutic target for reparative angiogenesis.

PO-126

# Regulating Optimal Angiogenesis and Leukocyte Adhesion of Human Endothelial Cells by Gradient Nanopore Substrate

**Soon Jun Hong[1*]**

[1] Cardiology Department, Korea University Anam Hospital, Korea

*psyche94@gmail.com*

Understanding signals in the microenvironment that regulate endothelial cell behavior are important in tissue engineering. Although a lot of studies have examined the cellular effects of nanotopography, few of study have addressed to investigate the functional regulation of human endothelial cells grown on nano-sized gradient pore substrate.

We examined the cellular response of human umbilical vein endothelial cells (HUVECs) by using a gradient nanopore substrate (GPS) with three hole type nanopore pattern (HP): which diameters were described in HP1, 120-200 nm; HP2, 200-280 nm; HP3, 280-360 nm.

The HP2 GPS increased the attachment and proliferation of HUVECs. Moreover, gene expression of focal adhesion markers in HUVECs were most significantly increased on HP2 GPS. In vitro tube formation assay showed the enhancement of tubular network formation of HUVECs after priming on GPS compared to Flat. Furthermore, leukocyte adhesion was also reduced in the HUVECs in a pore-diameter dependent manner.

Functional regulations of HUVECs were achieved by nanopore substrate with 200-280 nm-sized pores.

PO-127

# Target Identification and Validation of Voacangine Through a Systematic Combination of DARTS and MSI and Its New Derivative

**Sung Min Cho[1], Yonghyo Kim[2], György Marko-Varga[2], Ho Jeong Kwon[1*]**

[1] Chemical Genomics Global Research Lab., Department of Biotechnology, College of Life Science and Bi, Yonsei University, Seoul, Korea
[2] Clinical Protein Science & Imaging, Department of Biomedical Engineering, Lund University, Lund, Sweden

*kwonhj@yonsei.ac.kr*

Although natural products are important sources of drugs and drug leads, identification and validation of their target proteins have proven difficult.

Here, we report the development of a systematic strategy for target identification and validation employing drug affinity responsive target stability(DARTS) and mass spectrometry imaging(MSI) without modifying or labeling natural compounds.

Using label-free voacangine, an antiangiogenic alkaloid molecule as a model natural compound, DARTS analysis revealed vascular endothelial growth factor receptor 2 (VEGFR2) as a target protein. Voacangine inhibits VEGFR2 kinase activity and its downstream signaling by binding to the kinase domain of VEGFR2, as was revealed by docking simulation. Through cell culture assays, voacangine was found to inhibit the growth of glioblastoma cells expressing high levels of VEGFR2. Specific localization of voacangine to tumor compartments in a glioblastoma xenograft mouse was revealed by MSI analysis. The overlap of histological images with the MSI signals for voacangine was localized in the tumor regions expressed VEGFR2. In addition, synthesis of new derivatives based on ring structure of voacangine led to identify a lead compound inhibiting pVEGFR2 signaling. Currently, we focused on the inhibitory activities of a lead compound using histological images such as H&E and CD31 staining in vivo.

In summary, the strategy employing DARTS and MSI to identify and validate the targets of a natural compound as demonstrated for voacangine is expected to streamline the general approach of drug discovery and developing a lead compound using other biomolecules including natural products.

PO-128

# Circulating miR-4435 as a Potential Biomarker of Colorectal Cancer Associated with UQCRB

**Eunji Gwak[1], Jungmin Kim[1], Jiwon Hong[1], Ho Jeong Kwon[1*]**

[1] Chemical Genomics Global Research Lab, Department of Biotechnology, Yonsei University, Seoul, Korea

*kwonhj@yonsei.ac.kr*

Recently, we reported that ubiquinol-cytochrome c reductase (UQCRB), a subunit of the mitochondrial complex III, is highly expressed in tissues from Korean colorectal cancer patients. It is also known that UQCRB is involved in the generation of mitochonrial reactive oxygen species (mROS)-and hypoxia-inducible factor (HIF)-mediated angiogenesis and regulates vascular endothelial growth factor receptor 2 (VEGFR2) signaling-induced angiogenesis.

In this study, we further investigated miRNAs from mutant UQCRB-expressing cell lines to identify new miRNA biomarkers related with UQCRB mediated colorectal cancer, using microRNA sequencing.

After sequencing miRNAs in the mutant UQCRB-expressing cell lines, miR-4435 was selected as a potential biomarker candidate from the six up-regulated miRNAs. The expression level of miR-4435 was increased in exosomes isolated from cell culture medium, suggesting that miR-4435 is closely related to colon cancer. Additionally, exosomes extracted from the serum samples of colorectal cancer patients showed increased miR-4435 levels depending on the cancer progression stage. Furthermore, miR-4435 is related to an oncogenic function in UQCRB related disease, CRC, and that effects migration and invasion on mutant UQCRB-expressing cell lines and colorectal cancer cell.

In conclusion, our study demonstrated that miR-4435 as a potential circulating miRNA biomarker of colorectal cancer associated with UQCRB.

PO-129

# ALK2/ACVR1 Mediates BMP Signaling in Developmental and Pathological Angiogenesis

**Boryeong Pak[1], Sukwon Jin[1*]**

[1] School of life sciences, Gwangju institute of Science and Technology, Korea

*sukwonjin@gist.ac.kr*

We wish to uncover molecular underpinning of venous specific pro-angiogenic BMP signaling by identifying essential receptor in mammalian vessels.

Using vena cava and dorsal aorta explant assay, we demonstrated that BMP signaling selectively induces angiogenesis from the venous ECs but not from the arterial ECs in mammals. To identify the key receptor which enables venous specific pro-angiogenic effects of BMP signaling, we generated mice carrying an endothelial specific inducible deletion of each BMP type I receptor.

We found that endothelial specific deletion of Alk2 but not Alk3 selectively impairs venous angiogenesis. In particular, deletion of Alk2 substantially decreased BMP-induced proliferation of venous ECs. Mechanistically, ALK2 induces transcription of NR2F2/COUP-TFII via SMAD1/5/9 phosphorylation, and therefore promotes cell cycle progression and angiogenesis.

Our data demonstrate that BMP signaling functions as a venous specific pro-angiogenic cue in mammalian vessels in vivo. In addition, our results indicate that ALK2 modulates endothelial behaviors in response to BMP signaling therefore enables venous selective pro-angiogenic function of BMP signaling in mammals.

PO-130

# Regulation of Endothelial Cell Trafficking and Secretion of Soluble VEGFR1: Implications for Angiogenic Sprout Guidance

**Karina Kinghorn[1*], Allison Marvin[2], Ferdinand Le Noble[4], Victoria Bautch[2,3]**

[1] Cell Biology and Physiology, University of North Carolina at Chapel Hill, USA
[2] Biology, University of North Carolina at Chapel Hill, USA
[3] McAllister Heart Institute, University of North Carolina at Chapel Hill, USA
[4] Cell and Developmental Biology, Karlsruhe Institute of Technology, Germany

*k_kinghorn@unc.edu*

Although soluble vascular endothelial growth factor receptor 1 (sVEGFR1) is essential for proper angiogenesis, little is known about its trafficking and secretion from endothelial cells (EC). This work focuses on the underlying molecular mechanisms of sVEGFR1 trafficking and secretion from EC.

Endogenous sVEGFR1 trafficking and secretion in primary human umbilical vein endothelial cells was assessed via pharmacological inhibition (brefeldin-A, chlorpromazine, dynasore, cyclodextrin, chloroquine) and siRNA-mediated knockdown of trafficking proteins (ARF1 and RAB27a). Localization of sVEGFR1 in angiogenic vessels is assessed via an in vitro 3D angiogenic sprouting assay and in vivo in zebrafish.

sVEGFR1 secretion into media was inhibited after treatment with brefeldin-A, chlorpromazine, or dynasore. Chloroquine led to an increase in sVEGFR1 secretion, while cyclodextrin had no effect. siRNA-mediated knockdown of both ARF1 and RAB27a inhibited sVEGFR1 secretion. Polarity of 3D angiogenic sprouts was validated by marking the apical area (luminal) and basement membrane (ablumenal) of lumenized vessels with moesin and collagen IV, respectively. A mutant HA-tagged sVEGFR1 construct (lacking VEGF-A binding domain) was injected into single-cell zebrafish embryos, and mosaic expression within the vasculature was validated via immunofluorescence.

sVEGFR1 is secreted via vesicle packaging and trafficking from the Golgi, followed by cellular transport to the plasma membrane through interactions with RAB27a, clathrin, and dynamin. Lumenized vessels form and polarize in 3D sprouts, allowing further studies of polarized sVEGFR1 secretion. With successful mosaic expression of tagged mutant sVEGFR1 in zebrafish, localization and secretion can be assessed in vivo. This knowledge will impact our understanding of spatial regulation of angiogenesis, and may provide new avenues for treating vascular disorders linked to sVEGFR1 mis-regulation, such as preeclampsia.

PO-131

# Filopodia Speed Up Notch Lateral Inhibition Tip Cell Selection In Vivo and In Silico

**Bahti Zakirov**[1*]

[1] Cellular Adaptive Behaviour Lab, Francis Crick Institute, UK

*bahti.zakirov@crick.ac.uk*

How do cells make efficient collective decisions during tissue morphogenesis? Humans and other organisms utilize feedback between movement and sensing known as 'sensorimotor coordination' or 'active perception' to inform behaviour, but active perception has not before been investigated at a cellular level within organs.

Here we provide the first proof of concept in silico/in vivo study demonstrating that filopodia (actin-rich, dynamic, finger like cell-membrane protrusions) play an unexpected role in speeding up collective endothelial decisions during the time-constrained process of 'tip cell' selection during blood vessel formation(angiogenesis).

We first validate simulation predictions in vivo with live imaging of zebrafish intersegmental vessel growth. Further simulation studies then indicate the effect is due to the coupled positive feedback between movement and sensing on filopodia conferring a bistable switch-like property to Notch lateral inhibition, ensuring tip selection is a rapid and robust process. We then employ measures from computational neuroscience to assess whether filopodia function as a primitive ('basal') form of active perception and find evidence in support. By viewing cell behaviour in tissues through the 'basal cognitive lens' we acquire a fresh perspective on not only the well-studied tip cell selection process, revealing a hidden, yet vital, time-keeping role for filopodia, but on how to interpret and understand cell behaviour in general, opening up a myriad of new and exciting research directions.

PO-132

# STAT3 Precedes HIF1alpha Transcriptional Responses to Oxygen and Oxygen and Glucose Deprivation in Human Brain Pericytes

**Robert Carlsson**[1*]

[1] Translational Neurology, IKVL, Scientist, Sweden

*Robert.Carlsson@med.lu.se*

Brain pericytes are important to maintain vascular integrity of the neurovascular unit under both, physiological and ischemic conditions. Ischemic stroke is known to induce an inflammatory and hypoxic response due to the lack of oxygen and glucose in the brain tissue. How this early response to ischemia is molecularly regulated in pericytes is largely unknown and may be of importance for future therapeutic targets.

Here we evaluate the transcriptional responses in in vitro cultured human brain pericytes after oxygen and/or glucose deprivation.

Hypoxia has been widely known to stabilise the transcription factor hypoxia inducible factor 1-alpha (HIF1α) and mediate the induction of hypoxic transcriptional programs after ischemia. However, we find that the transcription factors Jun Proto-Oncogene (c-JUN), Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells (NFκB) and signal transducer and activator of transcription 3 (STAT3) bind genes regulated after 2hours (hs) of omitted glucose and oxygen before HIF1α. Potent HIF1α responses require 6hs of hypoxia to substantiate transcriptional regulation comparable to either c-JUN or STAT3. Phosphorylated STAT3 protein is at its highest after 5 min of oxygen and glu-

cose (OGD) deprivation, whereas maximum HIF1α stabilisation requires 120 min.

We show that STAT3 regulates angiogenic and metabolic pathways before HIF1α, suggesting that HIF1α is not the initiating trans-acting factor in the response of pericytes to ischemia.

PO-133

# The Role of RUNX3 in Hypoxia-Induced Cell Proliferation and Antiapoptosis in Early Tumorigenesis

**Sun Hee Lee[1,2,3], Do Young Hyeon[4], Soo-Hyun Yoon[1,2,3], Ji-Hak Jeong[2,3], Saeng-Myung Han[1,3,5], Ju-Won Jang[6], Minh Phuong Nguyen[1,3,5], Sojin An[7], Kyung-gi Hyun[7], Hee-Jung Jung[8], Ji-Joon Song[7], Suk-Chul Bae[6], Woo-Ho Kim[9], Daehee Hwang[4,8], You Mie Lee[1,2,3*]**

[1] BK21 Plus KNU Multi-Omics based Creative Drug Research Team, College of Pharmacy, Kyungpook National University, Korea
[2] Research Institute of Pharmaceutical Sciences, College of Pharmacy, Kyungpook National University, Korea
[3] National Basic Research Laboratory of Vascular Homeostasis Regulation, College of Pharmacy, Kyungpook National University, Korea
[4] School of Biological Sciences, Seoul National University, Korea
[5] School of Life Sciences and Biotechnology, Kyungpook National University, Korea
[6] Department of Biochemistry, School of Medicine, Institute of Tumor Research, Chungbuk National University, Korea
[7] Department of Biological Science, KI for the BioCentury, KAIST, Korea
[8] Center for Plant Aging Research, Institute for Basic Science, DGIST, Korea
[9] Department of Pathology, Seoul National University College of Medicine, Korea

*lym@knu.ac.kr*

Tumor suppressor Runt-related transcription factor 3 (RUNX3) plays an important role under hypoxia during early tumorigenesis. However, post-translational modifications (PTM) based mechanism for the inactivation of RUNX3 under hypoxia is largely unknown. Here, we investigate a mechanism that G9a, lysine-specific methyltransferase (KMT), modulates RUNX3 through PTM under hypoxia.

Quantitative real-time PCR (qRT-PCR), western blot, Immunofluorescence (IF), and immunohistochemical (IHC) assays were employed to detect RUNX3 and G9a in human gastric cancer cell lines and tissues. The biological functions of RUNX3 and G9a were demonstrated using in vitro and in vivo experiments. Histone methyltransferase assay, protein methyltransferase assay, luciferase reporter assay, chromatin immunoprecipitation (ChIP), TUNEL assay, cell proliferation assay, and colony formation assay were used to explore the mechanism of RUNX3 and G9a action. ChIP-seq data analysis, microarray analysis, enrichment analysis of GO biological processes, and reconstruction of network model were used to screen the function of RUNX3.

G9a protein level was significantly increased under hypoxia and G9a interacted with RUNX3 Runt domain, which led to increased methylation of RUNX3 at K129 and K171. This methylation inactivated transactivation activity of RUNX3 by reducing interactions with CBFβ and p300 cofactors, as well as reducing acetylation of RUNX3 by p300. Hypoxia-induced methylation of RUNX3 by G9a enhanced cancer cell proliferation by increasing cell cycle or cell division, while suppressed immune response and apoptosis. These ultimately promoted tumor growth during early tumorigenesis.

Inactivation of RUNX3 by G9-mediated methylation facilitates cell proliferation and anti-apoptosis under hypoxia, suggesting that RUNX3 can be a therapeutic or preventive target to control tumor growth during early tumorigenesis.

PO-134

# Developmental Endothelium Specific Molecule (DESM) Regulates Angiogenesis and Tumor Progression

**Yuri Miyamura[1], Masashi Muramatsu[1], Takashi Minami[1*]**

[1] Division of Molecular and Vascular Biology, Institute of Resource Development and Analysis (IRDA), Kumamoto University, Japan

*t-minami@kumamoto-u.ac.jp*

Vascular diseases are mainly occurred by disruption of endothelial cells (ECs) homeostasis. Thus, comprehensive analyses of the EC-derived genes and their regulatory systems are useful for understanding the mechanisms of pathological angiogenesis. We recently identified a novel EC-specifically expressed gene, named *Developmental Endothelium Specific Molecule* (*DESM*), from genome-wide expression arrays with cultured cell-sets in whole tissues. Predetermined EC unique regulatory element; GATA/ERG/FOXC2 binding region was existed at the proximal promoter of DESM, and the promoter activities were exclusively detected in the various cultured ECs. DESM was expressed with ~50kDa protein, but have not any characterized their biological function.

To investigate the role of Desm *in vivo*, Desm knock-out and *lac*Z knock-in mice, $Desm^{-/-}$, were generated. DESM expression levels and its physiological functions were evaluated by the X-gal staining and the phenotypic screening, respectively.

*LacZ* and CD31 merged staining indicated that strong and uniform Desm expression was detected specifically in EC layers in embryos. Such the unique expression starts at the embryonic day (E) 8.5. Sharp contrast to the known EC specific markers; Tie2 and VEGFR2, DESM expressed in neither hemogenic angioblasts at E 7.5 nor yolk sac vasculature. Surprisingly, the expression levels were markedly declined after the postnatal periods except retinal vessels. Null mutation of DESM developed normally, but retinal angiogenesis was significantly interfered. Remnant few DESM expression in adult organ was re-activated in pathological angiogenic endothelium. Finally, to assess the DESM role of tumor angiogenesis, B16 melanoma and Lewis lung carcinoma were subcutaneously injected at the flank of $Desm^{-/-}$ mice. Compared to wild-type control, $Desm^{-/-}$ arrested the tumor angiogenesis, thereby ~40% reduced the tumor growth.

These data suggest that our newly finding DESM obtained the unique and predominant developmental endothelium expressed patterns, which was necessary for retinal and tumor vessel growth.

PO-135

# IGF Binding Protein 2 Augments Ischaemia-Induced Neovascularisation

**Alexander-Francisco Bruns[1], Thomas A. Slater[1], Nadira Y. Yuldasheva[1], Pooja Shah[1], Michael Drozd[1], Lauren Eades[1], Katherine Paradine[1], Natalie J. North[1], Richard M. Cubbon[1], Mark T. Kearney[1], Stephen B. Wheatcroft[1*]**

[1] Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK

*s.b.wheatcroft@leeds.ac.uk*

Insulin-like growth factor binding protein 2 (IGFBP2) has been described as a regulator of insulin-like growth factor 1 (IGF1) availability. It

has, however, been suggested to have additional, IGF1-independent functions. In vascular smooth muscle cells, IGFBP2 has been proposed to regulate the IGF1-Akt signalling cascade through receptor tyrosine phosphatase inactivation. In glioma, IGFBP2 has been linked to vascular mimicry formation and to increased tumour angiogenesis via nuclear accumulation and subsequent upregulation of VEGF. Here we tested the hypothesis that IGFBP2 could stimulate angiogenic responses in endothelial cells and could thus augment ischaemia-induced neovascularisation.

HUVEC were used for all in vitro experiments. Cells were stimulated using 15nM recombinant human IGFBP2. Total and phosphorylated protein levels were analysed by immunoblotting. Nuclear accumulation was tested by cell fractionation and immunofluorescence. VEGF-levels were analysed by ELISA. A homozygous CAG-floxed-STOP-hIGFBP2/Cdh5-CreERT2 mouse line conditionally overexpressing human IGFBP2 in the endothelium (hIGFBP2iEC) was used in in vivo experiments.

Treatment of HUVEC with recombinant IGFBP2 resulted in (i) complex formation of IGFBP2 and β1 integrin, (ii) RGD-domain dependent activation of the β1 integrin-FAK-MAPK signalling pathway, (iii) increased cord-like structure formation on Matrigel, and (iv) enhanced sprouting in a 3D culture assay. Interestingly, we could neither observe nuclear accumulation of IGFBP2 nor upregulation of VEGF after IGFBP2-treatement in HUVEC. Pulmonary endothelial cells from Cre(+) hIGFBP2iEC mice showed enhanced vascular sprouting in a 3D culture assay. Furthermore, Cre(+) hIGFBP2iEC mice displayed (i) increased levels of MAPK phosphorylation in the aorta, (ii) normal vasomotor function, and (iii) enhanced reperfusion and neovascularisation after hind-limb ischaemia in comparison to Cre(-) littermate controls.

Our findings provide evidence for previously unrecognised angiomodulatory properties of IGFBP2 in endothelial cells and the ability to positively regulate neovascularisation after hind-limb ischaemia.

PO-136

# Neutralizing Antibody to proNGF Rescues Erectile Function by Regulating the Expression of Neurotrophic and Angiogenic Factors in a Mouse Model of Cavernous Nerve Injury

**Anita Limanjaya[1,2], Doo Yong Chung[2], Min-Ji Choi[1,2], Kalyan Ghatak[1,2], Jiyeon Ock[1,2], Guo Nan Yin[1,2], Mi-Hye Kwon[1,2], Jun-Kyu Suh[1,2], Ji-Kan Ryu[1,2*]**

[1] National Research Center for Sexual Medicine and Department of Urology, Inha University School of Medicine, Korea
[2] Department of Urology, Inha University Hospital, Korea

*rjko929@inha.ac.kr*

Radical prostatectomy induces some degree of cavernous nerve injury (CNI) and causes denervation-induced pathologic changes in cavernous vasculature, regardless of the advances in surgical techniques and robotic procedures. The precursor for nerve growth factor (proNGF) is known to be involved in neuronal cell apoptosis and microvascular dysfunction through its receptor p75NTR.

Age-matched 12-week-old C57BL/6 mice were distributed into three groups: sham group and bilateral CNI group treated with intracavernous injections of PBS (20 μL) or of anti-proNGF-Ab (20 μg in 20 μL of PBS) on days -3 and 0. Two weeks after treatment, erectile function was measured by electrical stimulation of cavernous nerve. Penis tissues from a sep-

arate group of animals were harvested for further analysis. We also determined the efficacy of anti-proNGF-Ab on neural preservation in major pelvic ganglion (MPG) ex vivo.

We observed increased penile expression of proNGF and p75NTR after CNI. Intracavernous administration of anti-proNGF-Ab increased nNOS and neurofilament expression probably by enhancing the production of neurotrophic factors, such as neurotrophin-3, NGF, and brain-derived neurotrophic factor. Anti-proNGF-Ab preserved the integrity of cavernous sinusoids, such as pericytes, endothelial cells, and endothelial cell to cell junctions, possibly by controlling angiogenic factors (angiopoietin-1, angiopoietin-2, and vascular endothelial growth factor); and induced endogenous eNOS phosphorylation in CNI mice. And finally, treatment with anti-proNGF-Ab rescued erectile function in CNI mice. Anti-proNGF-Ab also enhanced neurite sprouting from MPG exposed to lipopolysaccharide.

The preservation of damaged cavernous neurovasculature through inhibition of the proNGF/p75NTR pathway may be a novel strategy to treat radical prostatectomy-induced erectile dysfunction.

PO-137

# Thermostable Small Molecule Inhibitor of Angiogenesis and Vascular Permeability That Suppresses a pERK-FosB/ΔFosB-VCAM-1 Axis

**Levon Khachigian**[1*]

[1] Vascular Biology and Translational Research, School of Medical Sciences, Faculty of Medicine, University of New South Wales, Australia

*l.khachigian@unsw.edu.au*

Vascular permeability and angiogenesis underpin the pathogenesis of neovascular age-related macular degeneration and diabetic retinopathy. While anti-VEGF therapies are widely used clinically, many patients do not respond optimally, or at all, and small molecule therapies are lacking. We developed strategies to identify new inhibitors of vascular leakiness and neovascularization.

From a library of ~100,000 compounds we identified a dibenzoxazepinone BT2 that inhibits endothelial cell proliferation, migration toward VEGF-A165, wound repair after in vitro injury, endothelial network formation, and reduces angiogenesis in mice bearing Matrigel plugs.

BT2 physically interacts with MEK1, inhibits ERK phosphorylation and blocks FosB/ΔFosB and VCAM-1 expression. Using RNA-seq, BT2 inhibits FosB, VCAM-1 and a range of other genes involved in cell proliferation, migration, angiogenesis and inflammation. siRNA knockdown experiments show that VCAM-1 expression is FosB/ΔFosB-dependent. BT2 reduced retinal vascular leakage following rat choroidal laser trauma and rabbit intravitreal VEGF-A165 administration, models that

provide useful in vivo systems to test experimental therapies for nAMD/DR. BT2 suppressed retinal CD31 immunostaining induced by laser injury and inhibited pERK, VCAM-1 and VEGF-A165, which stains in a gradient relative to the wound. BT2 reduced retinal leakage in rats at least as effectively as aflibercept, first-line therapy for nAMD/DR. Remarkably, BT2 withstands boiling or autoclaving and several months' storage at 220C.

BT2 is a novel small molecule inhibitor of vascular permeability and angiogenesis.

PO-138

# SCF (Stem Cell Factor) and cKIT Modulate Pathological Ocular Neovascularization

**Songyi Seo[1], Koung Li Kim[1], Wonhee Suh[1*]**

[1] Vascular Biology & Biochemistry, Chung-Ang university, College of Pharmacy, Korea

*wonhee.suh@gmail.com*

Aberrant neovascularization is a leading cause of blindness in several eye diseases, including age-related macular degeneration and proliferative diabetic retinopathy. The identification of key regulators of pathological ocular neovascularization has been a subject of extensive research and great therapeutic interest. Here, we explored the previously unrecognized role of cKIT and its ligand, SCF (stem cell factor), in the pathological ocular neovascularization process.

Compared with normoxia, hypoxia, a crucial driver of neovascularization, caused cKIT to be highly upregulated in endothelial cells, which significantly enhanced the angiogenic response of endothelial cells to SCF. In murine models of pathological ocular neovascularization, such as oxygen-induced retinopathy and laser-induced choroidal neovascularization models, cKIT and SCF expression was significantly increased in ocular tissues, and blockade of cKIT and SCF using cKit mutant mice and anti-SCF neutralizing IgG substantially suppressed pathological ocular neovascularization. Mechanistically, SCF/cKIT signaling induced neovascularization through phosphorylation of glycogen synthase kinase-3β and enhancement of the nuclear translocation of β-catenin and the transcription of β-catenin target genes related to angiogenesis. Inhibition of β-catenin-mediated transcription using chemical inhibitors blocked SCF-induced in vitro angiogenesis in hypoxia, and injection of a β-catenin agonist into cKit mutant mice with oxygen-induced retinopathy significantly enhanced pathological neovascularization in the retina.

Our data reveal that SCF and cKIT are promising novel therapeutic targets for treating vision-threatening ocular neovascular diseases.

PO-139

# Loss of Down Syndrome Critical Region (DSCR)-1 Leads Wound Healing-Dysregulation

**Takahiro Manabe[1*], Masashi Muramatsu[1], Takashi Minami[1]**

[1] Division of Molecular and Vascular Biology, Institute of Resource Development and analysis, Kumamoto University, Japan, Japan

*205y1035@st.kumamoto-u.ac.jp*

EC activation are quickly occurred and led to inflammation and angiogenesis in wound healing. We have previously reported that EC activation via VEGF- treatments quickly induces the down syndrome critical region (Dscr)-1 gene, which modulates the EC hyper-activation by the feedback inhibition of VEGF-calcineurin-NFAT signaling. DSCR-1 encoded mainly two different isoforms; termed as DSCR-1L and DSCR-1s, and only DSCR-1s conforms the feedback circuit. Moreover, we have already reported that both Dscr-1s and L knockout (Dscr-1-DKO) mice showed dampened the host response to septic infection. However, it has not well-executed whether DSCR-1 isoform-specific differences are existed in vascular homeostasis.

To identify the Dscr-1 isoform specific function, we have generated isoform-specific knockout mice. Dscr-1L specific knockout mice were hard to breed, thus we performed wound healing study with Dscr-1-DKO and Dscr-1s specific knockout (Dscr-1s-KO) mice. Wounds were generated by an 8mm dermal biopsy punch and surgical scissors under anesthesia and measured minor and major axis by caliper every day.

Compared to wild type (WT) control, both Dscr-1-DKO and Dscr-1s-KO showed ~60 and ~30% delay in wound closure at day 7 after the dermis injury, respectively. CD45+ immune cells invasion rates were 2-fold stimulated in Dscr-1-DKO than WT. In contrast, CD31+ EC invasion rates were reduced to 60% in both Dscr-1-DKO and Dscr-1s-KO.

These data suggest that hyper-inflammatory conditions in Dscr-1-DKO and Dscr-1s-KO resulted in dysregulation of the relay to effective angiogenesis, which consequently led to delay of the tissue repair. We will proceed to verify the hypothesis of balance with inflammation/ angiogenesis in wound healing by using Dscr-1L-KO and EC-specific Dscr-1 transgenic mice.

PO-140

# Synergistic Effect of Adipose-Derived Stromal Cells and Dll4 Blockade on Fat Graft Retention

**Il-Kug Kim[1*], Choong-kun Lee[2], Dirong Wu[1], Kyoo-Ri Kwon[1]**

[1] Department of Plastic and Reconstructive Surgery, Yeungnam University College of Medicine, Korea
[2] Division of Medical Oncology, Department of Internal Medicine, Yonsei Cancer Center, Yonsei University College of Medicine, Korea

*curingyou@gmail.com*

Cell-assisted lipotransfer (CAL) is a fat graft technique supplemented with adipose-derived stromal cells (ASCs) or stromal vascular fraction cells to improve fat graft volume retention. ASCs contribute to angiogenesis and adipogenesis after the implantation of fat tissues. However, the microenvironment of grafted fat with ASCs is still hypoxic. Because the inhibition of Dll4-Notch pathway promotes angiogenesis, we speculated that it has a synergistic effect with CAL.

ASCs were isolated from DsRed C57BL/6J (B6) mice for their trace. Two hundred ul of fragmented fat from wild type B6 mice was mixed with 1.0 x 106 DsRed ASCs and grafted to recipient B6 mice. To block Dll4, 5 mg/kg anti-Dll4 antibody was intraperitoneally injected into recipient mice. Graft volume retention, proliferation and differentiation of ASCs were evaluated at 8 weeks after implantation.

Dll4 blockade promoted angiogenesis in recipient tissue and improved graft volume retention. The proliferation and differentiation into endothelial cell and adipocyte of ASCs were also increased by Dll4 blockade.

These findings identify synergistic effect of ASC supplementation and Dll4 blockade in fat graft and emphasize the therapeutic value of anti-Dll4 antibody in hypoxic tissue.

PO-141

# Desmoglein-2 Is a Novel Adhesion Molecule Important for Pancreatic Vasculature and the Development of Diabetes.

**Kay Khine Myo Min[1], Darling Rojas-Canales[2], Mark DeNichilo[1], Michaelia Cockshell[1], Toby Coates[2], My Mahoney[3], Claudine Bonder[1*]**

[1] Centre for Cancer Biology, University of South Australia & SA Pathology, Australia
[2] Adelaide Medical School, University of Adelaide, Australia
[3] Department of Cutaneous Biology, Thomas Jefferson University, USA

*claudine.bonder@unisa.edu.au*

Pancreatic islets represent the ultimate biosensor required to accurately sense blood glucose levels and rapidly release insulin for glucose metabolism. To meet these requirements, pancreatic islets are highly vascularised and receive 10% of pancreatic blood flow despite comprising only 1-2% of tissue mass. Thus, the inter-relationship between pancreatic ECs and β-cells is key to islet function. Type 1 diabetes is a chronic autoimmune disease characterized by the lack of endogenous insulin secreted from the pancreatic β-cells. We have identified a single cell surface protein, desmoglein-2 (DSG2), to be within the top 10 percent of all genes expressed by human pancreatic islets that is expressed by both the pancreatic ECs and the insulin-producing β-cells and undergoes homotypic interactions.

Using human gene expression and protein data, together with genetically modified strains of mice and mouse models of Type 1 diabetes, we have begun to unravel the unique and important role for DSG2 in islet function.

In our Dsg2 loss-of-function strain of mice (Dsg2-lo/lo), we observed a phenotypic alteration to the pancreatic vasculature with increased EC width and increased vascular permeability. These mice also exhibited a significant reduction in islet number, islet size and total insulin content. Islets isolated from Dsg2-lo/lo mice were more susceptible to cytokine-induced β-cell apoptosis and when compared to their wildtype counterparts, Dsg2-lo/lo mice demonstrated an increased susceptibility to streptozotocin-induced diabetes. We also observed that following transplantation into diabetic mice, islets isolated from Dsg2-lo/lo mice were less effective than their wildtype counterparts at curing diabetes.

Taken together, our study suggests DSG2 is an underappreciated regulator of β-cell function in pancreatic islets and that a better understanding of this adhesion molecule may provide new opportunities to combat this chronic and life-threatening disease.

PO-142

# MiR-93-5p in Systemic Lupus Erythematosus May Support Angiogenic Stability via Inhibition of EPAS1

**Nicola Edwards[1*], Alexander W.W. Langford-Smith[1], Rafia Noor[1], Michelle Barraclough[2,3], Eoghan M. McCarthy[2], Ben Parker[2,3], M. Yvonne Alexander[1], Ian N. Bruce[2,3], Fiona L. Wilkinson[1]**

[1] Translational Cardiovascular Science, Centre for Bioscience, Manchester Metropolitan University, UK
[2] NIHR Manchester Musculoskeletal Biomedical Research Centre, Manchester University Hospitals NHS Foundation Trust, Manchester Academic Health Science Centre, UK
[3] Centre for Musculoskeletal Research, Faculty of Biology, Medicine and Health, The University of Manchester, UK

*n.edwards@mmu.ac.uk*

Aberrant angiogenic signalling has been implicated in vascular damage and disease severity in Systemic Lupus Erythematosus (SLE). Endothelial microvesicles contain bioactive miRNAs, which may influence pathologies such as endothelial activation. miR-93-5p has previously been isolated from microvesicles and associated with cardiovascular disease, yet its function in SLE is unknown. This study sought to determine whether miR-93-5p has angiogenic properties within an experimental SLE context.

Controls (n=14) and patients with SLE (n=26) were assessed for clinical parameters of inflammation (MVs, IL-6, VCAM-1), cardiovascular risk (QRISK3, mean arterial pressure, pulse wave velocity) and presence of miR-93-5p in the vesicular fraction of platelet-poor plasma. MiR-93-5p constructs were generated by Gateway® cloning, integrated into lentiviral vectors and used to assess the effects of miR-93-5p overexpression in human umbilical vein endothelial cells (HUVECs; tube formation, metabolic activity, migration and viability). Empty control plasmids were generated lacking inclusion of the miRNA sequence. Bioinformatic analysis was used to identify potential targets of miR-93-5p, one of which was validated by qRT-PCR and luciferase reporter assays.

Patients with SLE demonstrated increased markers of inflammation and cardiovascular risk ($p<0.05$). Levels of endothelial microvesicles were elevated ($p<0.001$), while miR-93-5p abundance was reduced in patients ($p=0.015$). Significantly longer angiogenic tubes were detected ($p=0.038$), in HUVECS following overexpression of miR-93-5p, sustained over a 24 hour period ($p=0.036$), with no effect on HUVEC viability or migration. This was accompanied by an increase in basal glycolytic rate compared to controls ($p=0.027$). Gene ontology predicted EPAS1 as an angiogenic target of miR-93-5p and bioinformatic analysis yielded three miR-93-5p binding sites within the 3'untranslated region of EPAS1; abundance of EPAS1 was significantly reduced in HUVECs overexpressing miR-93-5p ($p=0.012$).

Reduction of miR-93-5p in SLE may support aberrant angiogenic signalling; novel therapeutic strategies for future investigation include boosting miR-93-5p abundance or targeting EPAS1 to promote vascular stability.

PO-143

# Intracavernous Delivery of Dickkopf3 Gene or Peptide Rescues Erectile Function Through Enhanced Cavernous Angiogenesis in the Diabetic Mouse

**Anita Limanjaya**[1*]

[1] School of Medicine, Inha University, Korea

*nita.limanjaya@gmail.com*

To examine whether and how Dickkopf3 (DKK3), a secreted modulator of the Wnt pathway that known to be involved in endothelial cell repair and vascular progenitor cell migration, restores erectile function in diabetic mice

Eight-week-old C57BL/6 mice received intraperitoneal injections of streptozotocin (50 mg/kg for 5 days). Eight weeks after the diabetes was induced, the efficacy of DKK3 was determined by three independent experiments: experiment 1 (DKK3 peptide [5 μg in 20 μL PBS]); experiment 2 (DKK3 plasmid DNA with electroporation [10, 40, or 100 μg in 20 μL PBS, respectively]); and experiment 3 (DKK3 adenovirus [1 × 107 , 1 × 108 , 1 × 109 virus particles per 20 μL, respectively]). Erectile function was measured by electrical stimulation of the cavernous nerve one week (for peptide) or two weeks (for genes) after treatment. The angiogenic activity of DKK3 was determined in diabetic penis in vivo and in primary cultured mouse cavernous endothelial cells (MCECs) in vitro

The cavernous expression of DKK3 protein was significantly lower in the diabetic mice than in controls. DKK3 peptide or adenovirus significantly improved erectile function in diabetic mice (70% of the control values). DKK3 adenovirus profoundly restored cavernous endothelial cell and pericyte contents and increased endothelial junction proteins in diabetic mice in vivo. DKK3 peptide induced upregulation of angiogenic factors (angiopoietin-1, vascular endothelial growth factor, and basic fibroblast growth factor) and accelerated tube formation in MCECs cultivated under the high-glucose condition in vitro

DKK3 restored cavernous vascular integrity and improved erectile function in diabetic mice. Therapeutic cavernous angiogenesis by the use of DKK3 will be a promising therapeutic strategy to treat diabetic erectile dysfunction

PO-144

# Wnt-Mediated Endothelial Transformation into Mesenchymal Stem Cell-Like Cells Forms a Chemoresistant Vascular Niche in Glioblastoma

**Menggui Huang[1], Yanqing Gong[2], Yi Fan[1*]**

[1] Radiation Oncology, University of Pennsylvania, USA
[2] Department of Medicine, University of Pennsylvania, USA

*yi.fan@pennmedicine.upenn.edu*

Malignant solid tumors exhibit high resistance to chemotherapy and radiation. This study aims to understand the mechanisms underlying vascular niche-mediated therapy resistance in glioblastoma (GBM).

GBM was genetically engineered in mice with

Cdh5-Cre;LSL-tdTomato-mediated endothelial cell (EC) lineage tracing, followed by single-cell RNA sequencing (RNA-seq) analysis. Stem cell-like transformation was determined by cell function and marker expression analyses. The molecular mechanisms that regulate Wnt activation in tumor ECs were investigated by mutagenesis and transcriptional factor activity assays. The role of Wnt activation in tumor chemoresistance was determined in EC-specific β-catenine knockdown mice. Finally, we tested experimental therapy that combines Wnt inhibition and temozolomide chemotherapy in a genetic mouse GBM model.

We reveal that endothelial cells (ECs) acquire transformation into mesenchymal stem cell (MSC)-like cells in glioblastoma (GBM), driving tumor resistance to cytotoxic treatment. Single-cell RNA-seq analysis revealed that ECs undergo mesenchymal transformation and stemness-like activation in GBM microenvironment. Furthermore, we identified a c-Met-mediated axis that induces β-catenin phosphorylation at Ser675 and Wnt signaling activation, inducing multidrug resistance-associated protein (MRP)-1 expression and leading to EC stemness-like activation and chemoresistance. Finally, genetic ablation of β-catenin in ECs overcomed GBM tumor resistance to temozolomide (TMZ) chemotherapy in vivo. Combination of Wnt inhibition and TMZ chemotherapy eliminated tumor-associated ECs, inhibited GBM growth, and increased mouse survival.

These findings identified a cell plasticity-based, vascular niche-dependent mechanism that controls tumor chemoresistance, and suggest that targeting Wnt/β-catenin-mediated EC transformation and stemness activation may overcome therapeutic resistance in GBM and possibly other solid tumors.

PO-145

# Conditioned Media Culture System Unveils an Intimate Crosstalk Between Head and Neck Cancer and Lymphatic Endothelial Cells

**Jeon Yeob Jang[1], Bok-Soon Lee[1], Chorong Seo[1], Yoo Seob Shin[1], Chul-Ho Kim[1*]**

[1] Department of Otolaryngology, Ajou University School of Medicine, Korea

*ostium@ajou.ac.kr*

Crosstalk between cancer cells and lymphatic endothelial cells (LEC) have been known to play pivotal roles in lymph node metastasis of solid cancer. However, phenotypic changes and mechanisms of the interaction between head and neck cancer (HNC) cells and lymphatic vessels are still unclear.

Here we developed conditioned media cell culture system which enabled to evaluate the interplay between HNCs and lymphatic endothelial cells (LEC) in vitro. The tumor conditioned media from FaDu cells were supplemented on the LEC-grown plates to see whether paracrine factors from tumors affect LECs. The conditioned media from stimulated LECs were added on the HNC-grown plates to see whether LECs affect tumor cells vise versa.

The tumor conditioned media significantly increased LEC proliferation in a dose-dependent manner. The pErk signaling was responsible for the increment of LEC proliferation. On the other hands, conditioned media from LECs increased the migration of HNC cells with increased levels of pSTAT1 and pSTAT3 expression while the proliferation did not changed. Chemokine array indicated CXCL5 secretion was strikingly increased from

stimulated LECs and the upregulated CXCL5 was at least partially responsible for the increments of HNC migration.

The conditioned media culture system uncovered the intimate crosstalk between HNC tumor cells and LECs. Identifying the secretory factors and responsible signaling pathways beyond these crosstalk might offer a potential therapeutic target in HNC.

PO-146

# Exercise Improves Tumor Vasculature Function via S1P Receptor Signaling

**Hannah Savage[1], Enrica Marmonti[1], Claudia Bedoya[1], Miriam Garcia[1], Jun-ichi Abe[1], Keri Schadler[1*], Riccardo Ballarò[1]**

[1] Pediatrics Research, MD Anderson Cancer Center, USA

*klschadl@mdanderson.org*

Tumor vasculature is dysfunctional and inefficient at delivering chemotherapy to tumor cells. Tumor vessels are hyper-permeable without functional endothelial cell junctions; hyper-permeability promotes high interstitial fluid pressure which prevents delivery of therapeutic agents to the tumor core. One reason for inappropriate endothelial cell junctions is likely disturbed flow within the tortuous tumor vasculature. Exercise is a well-described method for systemically increasing laminar shear stress and has been shown to increase blood flow to prostate tumors. We hypothesized that exercise can improve tumor blood vessel function via shear stress mediated improvements in endothelial cell junction.

Mice bearing ewing sarcoma or melanoma tumors were treated with moderate aerobic exercise (i.e. treadmill brisk walking) five days per week. Tumor vascular function was evaluated using lectin and high molecular weight dextran.

Exercise significantly reduced leakage of high molecular-weight dextran, suggesting improved endothelial cell barriers. Exercise also significantly increased the number of functional vessels as measured by lectin perfusion, and reduced tumor hypoxia. Further, exercise increased pericyte/vascular smooth muscle cell coverage of vessels in ewing sarcoma but not melanoma tumors. Improved tumor vasculature function after exercise correlated with increased Sphingosine-1-Phosphate Receptor 1 (S1PR1) expression and decreased S1PR2 on tumor endothelium. Treatment of tumor bearing mice with an S1PR1 agonist, SEW2871, or an S1PR2 antagonist, JTE-013, pharmacologically mimicked the effects of exercise on tumor vascular function.

Excitingly, treatment of tumor bearing mice with exercise, SEW2871, or JTE-013 in combination with chemotherapy caused a significant increase in the delivery of chemotherapy to the tumor, and significantly improved anti-tumor efficacy of chemotherapy in multiple tumor models. Ongoing work utilizing genetic models with inducible s1pr1 endothelial knock-out mice, s1pr1 reporter mice, or s1pr2-/- mice will elucidate the role of S1PR1 and S1PR2 in tumor vasculature response to exercise.

PO-147

# Focused Ultrasound-Driven Microbubbles (Sonoporation) Increases Doxorubicin Uptake in Tumor Xenografts by Enhancing Vascular Permeability

**Sonia Hernandez[1*], Aditi Bellari[2], Bianca Lec[1], Ann Marie Defnet[1], Naina Bagrodia[1], Arelly Villarreal[2], Jessica Kandel[1], Shashank Sirsi[2]**

[1] Surgery, University of Chicago, USA
[2] Bioengineering, University of Texas at Dallas, USA

*soniah@uchicago.edu*

Neuroblastoma (NB) causes 15% of cancer-related childhood deaths. Chemotherapy with doxorubicin imposes severe toxicities, and therefore the identification of reduced-toxicity strategies is urgent. Liposomal encapsulation of doxorubicin (LDOX) increases doxorubicin half-life, but tumor uptake still depends on vascular permeability. Microbubbles (MB), acoustically reactive molecules, can be targeted to a tumor when coupled with Focused Ultrasound (FUS), a process called sonoporation. We hypothesized that sonoporation could enhance vascular permeability, increasing local doxorubicin delivery to NB xenografts, and reducing systemic exposure.

1X10^6 Neuroblastoma NGP-Luciferase cells were intrarenally implanted into NCR nude mice, forming 1-2 gram tumors after 5 weeks. FUS was applied to tumors in 5 cycles during clinical ultrasound imaging during intravenous delivery of LDOX and 1x10^9MB. Controls received no treatment or no FUS. FUS+MB vs untreated controls were evaluated 30 minutes after treatment on using H&E and immunostaining for the pericyte marker alpha-smooth muscle actin (alphaSMA). 24 hours after treatment, intratumoral LDOX and the apoptosis marker TUNEL were visualized by confocal microscopy. MB measurements during sonoporation were used to calculate rates of reperfusion and relative blood volume.

H&E analysis of MB+FUS-treated tumors indicate FUS alone does not affect tumor or pericyte viability, but increases red blood cell extravasation relative to untreated controls. FUS-induced discontinuous alphaSMA coverage of EC and reduced the rate of MB reperfusion, consistent with increased vascular permeability. LDOX+MB+FUS tumors revealed >2 fold DOX and 5 fold DiD increase compared to no FUS ($p=0.01$). LDOX+MB+FUS tumors had increased TUNEL staining, suggesting increased DOX uptake, than LDOX only or untreated controls.

Sonoporation improves DOX tumor uptake by increasing vascular permeability within the tumor. These findings could be applied to other chemotherapy drugs and solid tumors; decreasing the amount of drug delivered to patients could reduce long-term toxicities.

PO-149

# Novel Antiangiogenic Therapy Targeting Biglycan in Tumor Endothelial Cell Using Liposomal-siRNA Delivery System

**Nako Maishi[1,2,3], Yu Sakurai[4,5], Hiroto Hatakeyama[4,6], Cong Li[1,2], Mohammad T. Alam1,[2,3], Hiroshi Kikuchi[2,7], Hirofumi Morimoto[2], Masahiro Morimoto[1,2,8], Kosuke Akiyama[3], Noritaka Ohga[3,8], Yasuhiro Hida[9], Hideyoshi Harashima4, Kyoko Hida[1,2,3*]**

1 Vascular Biology and Molecular Pathology, Hokkaido University Graduate School of Dental Medicine, Japan
2 Vascular Biology, Frontier Research Unit, Institute for Genetic Medicine, Hokkaido University, Japan
3 Department of Vascular Biology, Hokkaido University Graduate School of Dental Medicine, Japan
4 Laboratory of Innovative Nanomedicine, Faculty of Pharmaceutical Sciences, Hokkaido University, Japan
5 Laboratory of DDS Design and Drug Disposition, Graduate School of Pharmaceutical Sciences, Chiba University, Japan
6 Laboratory of Clinical Pharmacology and Pharmacometrics, Graduate School of Pharmaceutical Sciences, Chiba University, Japan
7 Department of Renal and Genitourinary Surgery, Hokkaido University Graduate School of Medicine, Japan
8 Department of Oral Diagnosis and Medicine, Hokkaido University Graduate School of Dental Medicine, Japan
9 Department of Cardiovascular and Thoracic Surgery, Hokkaido University Faculty of Medicine, Japan

*khida@den.hokudai.ac.jp*

Tumor blood vessels play important roles for tumor progression and metastasis. Targeting tumor endothelial cells (TECs) is one of the strategies for cancer therapy. We previously reported that biglycan, a small leucine rich proteoglycan, is highly expressed in TECs. TECs utilize biglycan in an autocrine manner for their migration and induce angiogenesis. In addition, TEC-derived biglycan stimulate tumor cell migration in a paracrine manner, which results in tumor cell intravasation and metastasis. In this study, we investigated the therapeutic effect of biglycan inhibition in TECs of renal cell carcinoma using in vivo siRNA delivery system, multifunctional envelope-type nano-device (MEND).

The MEND contains a unique pH-sensitive cationic lipid. To deliver the MEND to TECs specifically, we incorporated cyclo (Arg-Gly-Asp-D-Phe-Lys) (cRGD) into the MEND, since $\alpha v\beta 3$ integrin which is a receptor of cRGD is selectively expressed in TECs at high levels. We developed RGD-MEND encapsulating siRNA against biglycan. Firstly, we confirmed that the MEND was taken into OS-RC-2 tumor-derived TECs and induced RNAi-mediated gene silencing in vitro. Secondly, the MEND was injected intravenously into OS-RC-2 tumor bearing mice and investigated the effect of RNAi. Finally, we evaluated the therapeutic effect of biglycan silencing in TECs by MEND.

Flow cytometric analysis showed that the MEND was specifically delivered into TECs. Quantitative RT-PCR indicated that biglycan is knocked-down by biglycan siRNA-containing MEND. Tumor growth was inhibited by biglycan siRNA-containing MEND. Tumor microenvironmental factors such as fibrosis were also normalized by biglycan inhibition in TECs.

Targeting biglycan in TECs can be a novel approach for cancer treatment.

PO-150

# Increased ABCB1 Expression in Tumor Blood Vessels of Urothelial Carcinoma After Chemotherapy and Chemoresistance

**Hiroshi Kikuchi[1,2], Nako Maishi[2], Dorcas A. Annan[2], Mohammad T. Alam[2], Ryuji Matsumoto[1], Takahiro Osawa[1], Takashige Abe[1], Yasuhiro Hida[3], Toru Harabayashi[5], Kaname Ameda[6], Akira Kashiwagi[7], Yoshihiro Matsuno[4], Nobuo Shinohara[1], Kyoko Hida[2*]**

[1] Department of Renal and Genitourinary surgery, Hokkaido University Graduate School of Medicine, Japan
[2] Vascular Biology and Molecular Pathology, Hokkaido University Graduate School of Dental Medicine,, Japan
[3] Department of Cardiovascular and Thoracic Surgery, Hokkaido University Graduate School of Medicine, Japan
[4] Department of Surgical Pathology, Hokkaido University Hospital, Japan
[5] Department of Urology, Hokkaido Cancer Center, Japan
[6] Department of Urology, Hokkaido Hinyokika Kinen Hospital, Japan
[7] Department of Urology, Teine Keijinkai Hospital, Japan

*khida@den.hokudai.ac.jp*

ABCB1, ATP binding cassette transporter, one of the stem markers, causes drug resistance. We have reported that tumor endothelial cells (TECs) are resistant to paclitaxel (PTX) with ABCB1 upregulation. In clinical urology, gemcitabine / cisplatin (GC) is standard 1st line chemotherapy for metastatic urothelial carcinoma (mUC). PTX is often selected in 2nd line chemotherapy for GC resistant cases, however the therapeutic outcomes are limited. We hypothesized that TEC ABCB1 is the cause of this situation.

The ratio of ABCB1 positive (+) TECs was quantified before and after 1st line chemotherapy in UC tissues (n=66) by ABCB1 and CD31 immunostaining. The association between the ABCB1+ TECs ratio and overall survival (OS) was analyzed by Kaplan-Meier method. In vitro and in vivo assays were performed to address how endothelial cells (ECs) are affected by change of tumor microenvironment during chemotherapy. Finally, the efficacy of combination of the ABCB1 inhibitor with PTX was also verified in mouse bladder cancer model.

The ratio of ABCB1+ TECs significantly increased after 1st line chemotherapy (2.47%) compared to before 1st line chemotherapy (0.38%)($p<0.0001$). The cases with a high ratio of ABCB1+ TECs showed shorter OS compared with the cases with a low ratio of ABCB1+ TECs ($p=0.0111$). Gemcitabine and cisplatin elevated ABCB1 expression levels in ECs via increasing tumor IL-8 secretion. When the ABCB1 inhibitor was combined with PTX, tumor growth and metastasis were more reduced with anti-angiogenic effect compared to PTX alone.

Chemotherapy causes inflammatory changes in tumors, which induce ABCB1 expression in TECs and cause drug resistance. Targeting ABCB1 in TECs can be a novel strategy to overcome cancer drug resistance.

PO-151

# The Vascular Leakage Blocker Sac-1004modifies the Tumor Microenvironment and Enhances the Therapeutic Efficacy of ICB in Highly Immunogenic MC38 Tumor

**Young-Guen Kwon[1*], Songyi Park[1], Haiying Zhang[1], Minyoung Noh[1], Yeomyeong Kim[1]**

[1] Biochemistry, Yonsei University, Korea

*YGkwon@yonsei.ac.kr*

The immunosuppressive tumor microenvironment (TME) limits the vessel perfusion and pro-

motes tumor angiogenesis. Thus, TME is considered to be involved significantly in tumor growth and metastasis. Recently, immune checkpoint blockade such as anti-PD1 antibody (anti-PD1) is emerged as attractive means for treating advanced cancers, but there is still a limitation in efficacy depended on tumor types and stages.

Herein, we tested the effect of a vascular leakage blocker, Sac-1004, which normalized tumor vessels on anti-PD1 therapy in the MC38 tumor model.

Combined treatment of Sac-1004 and anti-PD1 showed a strong synergistic effect on tumor growth with vessel normalization and enhanced delivery of anti-PD1 into tumor tissues. T cell migration and specific cytotoxic CD8+T cell activity within the tumor parenchyma was prominently increased in co-treated groups compared to anti-PD1 alone. These effects were correlated with significantly enhanced expression of IFN-gamma and PDL1 in tumors of co-treated group.

Taken together, our findings suggest that Sac-1004 is a promising candidate drug capable of improving therapeutic efficacy window of anti-PD1 through beneficial changes of TME

PO-152

# The Role of LOX-1 in Tumor Endothelial Cells on Tumor Metastasis

**Takuya Tsumita[1], Dorcas Akuba-Muhyia Annan[1], Nako Maishi[1], Yasuhiro Hida[2], Kyoko Hida[1*]**

[1] Vascular Biology and Molecular Pathology, Graduate School of Dental Medicine, Hokkaido University, Japan
[2] Department of Cardiovascular and Thoracic Surgery, Graduate School of Medicine, Hokkaido University, Japan

*khida@den.hokudai.ac.jp*

It is known that tumor endothelial cells are different from normal endothelial cells in many aspects. It is also gradually revealed that there is diversity even in tumor endothelial cells. Recently, we identified that the gene expression of lectin-like oxidized LDL receptor 1 (LOX-1) is dramatically enhanced in tumor endothelial cells isolated from high metastatic melanoma than in those isolated from low metastatic melanoma. The significance of LOX-1 is well known in the field of cardiovascular disease; however, the function of LOX-1 in tumor endothelial cells and its contribution towards promoting metastasis are yet to be elucidated. In this study, we investigated the consequence of LOX-1 expression in tumor endothelial cells focusing on its role in immune cell extravasation.

We generated high metastatic and low metastatic melanoma xenografts in nude mice. LOX-1 expression and the distribution of leucocytes in each tumor were analyzed by immunohistochemistry. Additionally, LOX-1 over-expressing and LOX-1 knockdown endothelial cell models were established, respectively. The expression of genes related to leukocyte adhesion and migration were evaluated. Subsequently, the migration and adhesion abilities of leucocytes towards endothelial cells were also analyzed.

LOX-1 signals were strongly detected in the high metastatic tumors, especially around blood vessels. These high metastatic tumors had more CD11b(+) Gr-1(+) myeloid cells as compared to the low metastatic tumors. Furthermore, LOX-1 expression induced CCL2 and ICAM-1 upregulation in endothelial cells treated with the LOX-1 ligand, oxidized low-density lipoprotein. Myeloid cells migrated and adhered more to LOX-1 over-expressing endothelial cells.

LOX-1 expression in tumor endothelial cells is likely to enhance the recruitment of CD11b(+)Gr-1(+) myeloid cells into tumor, which might promote metastasis.

PO-153

# Biglycan Deficiency Normalizes Tumor Blood Vessels and Potentiates Tumor Immune Responses

**Cong Li[1], Nako Maishi[1], Dorcas Annan[1], Yasuhiro Hida[2], Kyoko Hida[1*]**

[1] Vascular Biology and Molecular Pathology, Graduate School of Dental Medicine, Hokkaido University, Japan
[2] Department of Cardiovascular and Thoracic Surgery, Graduate School of Medicine, Hokkaido University, Japan

*khida@den.hokudai.ac.jp*

Tumor vasculature is structurally and functionally abnormal. Normalization of tumor blood vessels benefits tumor microenvironment by promoting tumor blood flow, reducing hypoxia and metastasis. Biglycan (Bgn) is proteoglycan of extracellular matrix. Our previous study showed that Bgn is a novel marker of tumor endothelial cells and involved in tumor angiogenesis and metastasis. In the present study, we investigated the functional role of Biglycan in tumor vasculature in breast cancer.

Biglycan gene analysis and its prognostic values in human breast cancers were based on TCGA data. E0771 breast cancer cells were injected into WT and Bgn KO mice (offered by Dr. Young in NIH, U.S.), respectively. Blood vessel density was evaluated by CD31+ staining area. α-SMA+ CD31+ blood vessels were referred as pericyte covered blood vessels. Tumor vascular perfusion was interpreted by the lectin positivity in blood vessels. Doxorubicin+ area was used to analyze drug delivery. Accumulation of CD8+ T cell was analyzed by RT-PCR and FACS in E0771 tumors from WT and Bgn KO mice.

Biglycan was correlated with PECAM1 and ANGPT2 expression in human breast cancers. Higher biglycan mRNA and protein expressions were correlated with poorer prognosis in breast cancers patients. Vascular regression and normalization of tumor blood vessels through inhibiting Angpt2 expression in endothelial cells were observed in Bgn KO tumors. Furthermore, Bgn KO facilitates the accumulation of CD8+ T cells in tumors and decreased lung metastasis.

Targeting Bgn might normalize tumor vasculature and facilitate tumor immunotherapy.

PO-154

# Modeling 3D Human Lymphatic Vasculature on High-Throughput Microfluidic Chip to Study Tumor Immune Microenvironment

**Somin Lee[1], Habin Kang[1], Dohyun Park[2], James Yu[1], Noo Li Jeon[1,2*]**

[1] Interdisciplinary Program for Bioengineering, School of Engineering, Seoul National University, Korea
[2] Mechanical Engineering, School of Engineering, Seoul National University, Korea

*njeon@snu.ac.kr*

Lymphatic vasculature (LV) plays important role in cancer biology and has been studied as a major route for tumor metastasis. Whereas, recent onco-immunological approaches have focused its role on immune surveillance. In response to this emerging topic of lymphatic vascular biology, the appropriate human cell-based in vitro model is in need. Here, we introduce 3D human LV model recapitulating tumor immune microenvironment (TIME) based on high-throughput microfluidic chip technology. Our ultimate goal is to develop physiologically relevant model for discovering unclear roles of LV in cancer therapeutics.

We utilized 384-well plate format-based plastic microfluidic chip to reconstitute 3D lumenized human LV by robust patterning of cellular hydrogel. Through optimizing co-culture condition of human lymphatic endothelial cells, stromal cells, and various types of cancer cells with mixture of fibrin hydrogel, we could generate self-organizing LV in high-throughput manner.

3D human LV with high reproducibility could be generated within TIME. High-content profiling of LV co-culture with multiple types of cancer was conducted to quantitatively analyze how each type of cancer could affect morphological phenotype of LV. Robust screening on the effect of VEGFR3 inhibitor, SAR131675, on LV alone or in co-existence with blood microvasculature was enabled using high-throughput platform. This implies the potential of the platform to be further utilized on screening potent drug candidates regulating LV. Finally, in virtue of high perfusability of 3D LV, we designed trans-endothelial migration assay of natural killer (NK) cell. Analysis on trans-endothelial migration of NK cells depending on existence of melanoma cells in TIME was supported with the result of cytokine analysis.

We developed 3D human LV in vitro model for the first time and designed functional assays for studying multiple roles of LV in TIME. This novel high-throughput platform has powerful potential to be further utilized on investigating lymphatic-related strategies for cancer therapeutics.

PO-155

# Dynamic Changes of Tumor Blood Vessels in Tumors Resistant to Angiogenesis Inhibitors

**Yumiko Hayashi[1], Hiroyasu Kidoya[1], Fumitaka Muramatsu[1], Yohei Tsukada[1], Nobuyuki Takakura[1*]**

[1] RIMD, Osaka University, Japan

*ntakaku@biken.osaka-u.ac.jp*

The number of angiogenesis inhibitors that target the vascular endothelial growth factor (VEGF)

has been increasing over the past decade. Currently, some of these anti-angiogenic drugs were being tested in clinical trial; however, the preliminary results were not satisfying to completely suppress tumor progression. These results suggest the existence of novel mechanisms that supports tumor vessels formation independent of angiogenesis or known factors.

Here, we analyzed the dynamics of blood vessels in tumors as a response to angiogenesis inhibitors, such as Axitinib or DC101. We observed the changes of blood vessels due to administration of angiogenesis inhibitors over time by bioimaging analysis using a multiphoton confocal laser microscope with vascular imaging mice and GFP expressing tumor cells.

This analysis showed a decrease in the number of blood vessels and the regression of sprouting vessels in the tumor tissue at the early stage of administration. Interestingly, the continuous administration revealed that mature blood vessels remained and the localization of these blood vessels was dynamically changed inside tumor tissue. Furthermore, the administration of angiogenesis inhibitors markedly changed the myeloid cell subset in tumors.

These results suggest that the presence of unknown mechanism that regulates tumor vascular formation in tumors resistant to angiogenesis inhibitors. Myeloid cell groups may be involved in these changes of the tumor environment.

PO-156

# Novel Effects of the Latest Ultra-High Dose-Rate FLASH Radiotherapy on the Tumor Vasculature Through Decreased Myosin Light Chain Activation

**Young-Eun Kim[1], Seung-hee Gwak[1], Beom-Ju Hong[1], Jung-Min Oh[1], Hyung Seok Choi[1], Hoibin Jeong[1], Fangfang Zhu[2], Emil Shuler[3], Marjan Raffat[3], Cameron Koch[4], Peter G Maxim[3], Irving Weissman[2], Billy W Loo[3], G-One Ahn[5*]**

[1] Integrative biosciences and biotechnology, Pohang University of Science and Technology, Korea
[2] Stem Cell Institutes and Regenerative Medicine, Stanford University School of Medicine, USA
[3] Department of Radiation Oncology, Stanford University School of Medicine, USA
[4] Department of Radiation Oncology, University of Pennsylvania, USA
[5] College of Veterinary Medicine, Seoul National University, Korea

*goneahn@gmail.com*

We have previously reported that conventional (CONV) irradiation (IR) at 15 Gy produces a rapid but reversible vascular collapse in transplantable mouse tumors. The ultra-high dose-rate FLASH irradiation has recently been developed demonstrating a superb protection for the normal tissue toxicity although the exact mechanism is not known. In this study, we tested whether FLASH irradiation produces such vascular effects in tumors.

Lewis lung carcinomas (LLC) were subcutaneously grown in mice and irradiated by either CONV or FLASH IR at 15Gy. Tumors were harvested at 6hr and 48hr post IR and examined by immunofluorescence staining against CD31, p-MLC (phosphorylated myosin light chain) or γH2AX. Calyculin A or ML-7 was treated in cells to induce or inhibit MLC phosphorylation. In in vivo experiment, LLC bearing mice were injected with vehicle or ML-7 every

day for 7 days prior to 15Gy IR.
We found that FLASH IR did not produce the rapid and reversible vascular collapse. Immunofluorescence staining revealed that p-MLC+ or γH2AX+ cells were significantly decreased in tumors irradiated with FLASH, suggesting that FLASH IR produced less cellular contraction and DNA double strand breaks, respectively. By performing clonogenic cell survival assay using irradiated cells in vitro, we observed similar cell kill produced between FLASH and CONV IR. Mechanistically, we observed that MLC phosphorylation can regulate γH2AX disappearance kinetics in irradiated cells. Lastly, we found that the inhibition of p-MLC in tumors by ML-7 treatment prior to CONV IR mitigated the vascular collapse, the effect similar to FLASH IR.

Our results suggest that CONV IR causes vascular collapse in tumor through MLC activation, which can regulate γH2AX formation thereby affecting DNA double strand break repair kinetics. In conclusion, the inhibition of MLC activation therefore the cellular contraction in tumors can recapitulate the effect of the latest radiotherapy FLASH.

PO-157

# Blood Vessel Abnormality in Glioblastoma: Characterisation at Baseline, Recurrence and Following Radiotherapy Suggests a Therapy Induced Phenotype

**Anastasia Widyadari[1*], Teklu Egnuni[1], Michael Grant[1], Gary Shaw[1], Laura Heskin[1], Aruna Chakrabarty[2], Valerie Speirs[3], Heiko Wurdak[1], Susan Short[1], Mihaela Lorger[1], Georgia Mavria[1]**

[1] Leeds Institute of Medical Research, University of Leeds, UK
[2] Department of Medicine, Leeds Teaching Hospitals NHS Trust, UK
[3] The Institute of Medical Sciences, University of Abderdeen, UK

*umaaw@leeds.ac.uk*

Glioblastoma multiforme (GBM) is the most lethal form of brain tumour with an overall median survival of 12-15 months with current standard therapy. Histologically, GBM is characterised by high cellularity, nuclear anaplasia and microvascular proliferation. The tumour vascular network is a crucial component of the GBM and abnormalities in the vascular niche are associated with aberrant vessel function, tumour progression and therapeutic resistance, leading to poor patient outcomes. However, the precise nature of GBM vascular abnormalities and impact of radiotherapy on their development are poorly understood.

Patients with recurrent GBM present increased vascular abnormalities including high nestin positivity, larger blood vessel size and glomeruloid organisation. An experimental in vivo model of tumour regrowth after irradiation was established using CT2A murine cell line, injected intracranially to investigate the contribution of radiotherapy to vascular abnormality. Mice were treated with frac-

tionated irradiation on two consecutive days using Small Animal Radiation Research Platform (SARRP). Samples were collected at early and late time points and tumour blood vessels were characterised and analysed further using immunohistochemistry and immunofluorescence.

Analyses of tumour vasculature show that blood vessel size and overall vascularisation decrease early after radiotherapy but increase aberrantly at a later stage of tumour regrowth in comparison to non-irradiated tumours. In addition, F4/80 positive macrophages were more abundant in irradiated mice. Aberrant vessel growth post irradiation was accompanied by increased coverage by α-SMA positive perivascular cells suggesting a mature blood vessel phenotype. In contradistinction to developmental angiogenesis, vessel maturation was uncoupled from barrier function and suppression of permeability.

This study demonstrates that these vascular abnormalities observed at later stages of GBM tumour regrowth, may have a significant impact on the efficacy of therapeutic modalities administered following radiotherapy for treatment of GBM tumour recurrences.

PO-158

# Desmoglein-2, an Unsuspected Regulator of Tumor Vasculature and Immune Response in Melanoma

**Michaelia Cockshell[1]*, Lih Tan[1], Kay Khine Myo Min[1], Jeff Holst[2], Lisa Ebert[1], Claudine Bonder[1]**

[1] Centre for Cancer Biology, University of South Australia and SA Pathology, Australia
[2] School of Medical Sciences, University of New South Wales, Australia

*Minky.cockshell@gmail.com*

Formation of new blood vessels in tumours are critical for growth and metastasis. Until recently, tumor vasculature was thought to occur exclusively via angiogenesis using endothelial cells (ECs). However, there is increasing evidence that many solid tumors are capable of creating independent vascular structures using tumor cells themselves, a process known as vasculogenic mimicry (VM). VM content in solid tumors correlates with poor prognosis for cancer patients. We have identified the adhesion molecule desmoglein-2 (DSG2) as an important cadherin of the vasculature that promotes both angiogenesis and VM, where increased DSG2 expression correlates with poor outcome for patients with melanoma.

Growth of the mouse melanoma cell line B16-F10-GFP-P2A-luc is significantly reduced in mice with loss-of-function Dsg2 (Dsg2 lo/lo) when compared to control mice (WT). Histologically, melanomas from Dsg2lo/lo mice reveal restructured tumor vasculature and altered leukocyte content, with increased anti-tumorigenic CD8+ T lymphocytes and reduced pro-tumorigenic FoxP3+ Treg infiltrate.

Using the parallel plate flow chamber and transwell

migration assay, we identified that VM competent melanoma cells are capable of mediating leukocyte recruitment, particularly monocytes. Monocyte adhesion appears to be dependant on adhesion molecules classically involved in EC mediated leukocyte recruitment as demonstrated by a decrease in monocyte adhesion under flow conditions when the adhesion molecule ICAM1 is reduced through siRNA knockdown. Interestingly, an interrogation of The Cancer Genome Atlas (TCGA) melanoma cohort revealed that these adhesion molecules and chemokines are also expressed on the gene level in melanoma patients, thereby highlighting the relevance of this study in human patients.

Our results suggest that DSG2 plays an underappreciated role in regulating tumor vasculature and the infiltration of leukocytes. Ongoing investigations are underway to understand how modulating expression of DSG2 and adhesion molecules can reshape tumor vasculature for increased infiltration of cytotoxic leukocytes for a therapeutic strategy.

PO-159

# VEGF-Driven Vascular Abnormality in an Advanced GBM Mouse Model Generated by Somatic Mutations

**Eunhyeong Lee[1], Haemin Chon[1], Injune Kim[1*]**

[1] Graduate School of Medical Science and Engineering, KAIST, Korea

*injunek@kaist.ac.kr*

Glioblastoma multiforme (GBM) is a highly aggressive brain tumor with severe vascular abnormalities. Although several orthotopic high-grade glioma models have been used widely, they merely show limited characteristics of patient GBM. Thus, the pathological mechanisms of GBM onset and progression remain largely unknown. Here, we try to establish a novel GBM mouse model with somatic oncogenic mutations to better reflect vascular characteristics of human GBM.

We injected and electroporated a hybrid plasmid for both CRISPR/Cas9-mediated TP53 and PTEN inactivation and Cre recombinase induction into the lateral ventricle of mice with floxed a transcription termination cassette ahead the epidermal growth factor receptor variant III (EGFRvIII) transgene. After gene editing in neural stem cells, mice developed brain tumor spontaneously with high penetrance.

We compared histopathological feature, vascular morphology, and function among normal brain, GL261 orthotopic gliomas, and spontaneous EGFRvIII GBMs. The EGFRvIII GBM model showed the pathological characteristics of grade IV glioma, such as severe necrosis and microvascular proliferation. EGFRvIII GBM vessels also exhibited increased vessel area, enlarged vessel diameter, and decreased branching, indicating vascular dilation and remodeling as major vascular changes in GBMs. Although GBM vessels had poor contact between inner endothelium and surrounding pericytes and basement membrane, they maintained coverage by peri-endothelial components, which is opposite to the loss of peri-endothelial components in GL261 glioma vessels. As GBM vessels expressed VEGFR2 robustly, we explored whether VEGFR2 signaling is important for vascular changes in GBMs. VEGFR2 inhibition reduced vessel dilation and remodeling and restored contact between tumor endothelium and peri-endothelial compartment.

We established an advanced GBM model for better understanding on GBM pathology and developing therapeutic strategies. Our study identified vessel

dilation, vessel remodeling, and abnormally increased peri-endothelial compartment as vascular characteristics of this GBM model with grade IV features. Mechanistically, VEGF signaling drives these vascular abnormalities with intratumoral vascular heterogeneity.

PO-160

# N-Terminal Modification of the Tetrapeptide Arg-Leu-Tyr-Glu, a VEGFR-2 Antagonist, Improves Anti-Tumor Activity by Increasing Its Stability Against Serum Peptidases

**Taesam Kim[1], Wonjin Park[1], Minsik Park[1], Suji Kim[1], Young-Geun Kwon[3], Young-Myeong Kim[1,2*]**

[1] Molecular and Cellular Biochemistry, Kangwon National University, Korea
[2] Kangwon Institute of Inclusive Technology, Kangwon National University, Korea
[3] Biochemistry, Yonsei University, Korea

*ymkim@kangwon.ac.kr*

The tetrapeptide Arg-Leu-Tyr-Glu (RLYE), a vascular endothelial growth factor (VEGF) receptor-2 antagonist, has been used previously either alone or in combination with chemotherapeutic drugs for treating colorectal cancer in a mouse model.

We analyzed the half-life of the peptide and found that due to degradation by aminopeptidases B and N, it had a short half-life of 1.2 h in the serum. Therefore, to increase the stability and potency of the peptide, we designed the modified peptide, N-terminally acetylated RLYE (Ac-RLYE), which had a strongly stabilized half-life of 8.8 h in serum compared with the original parent peptide. The IC50 value of Ac-RLYE for VEGF-A-induced endothelial cell migration decreased to approximately 37.1 pM from 89.1 pM for the parent peptide.

Using a mouse xenograft tumor model, we demonstrated that Ac-RLYE was more potent than RLYE in inhibiting tumor angiogenesis and growth, improving vascular integrity and normalization through enhanced endothelial cell junctions and pericyte coverage of the tumor vasculature, and impeding the infiltration of macrophages into tumor and their polarization to the M2 phenotype. Furthermore, combined treatment of Ac-RLYE and irinotecan exhibited synergistic effects on M1-like macrophage activation and apoptosis and growth inhibition of tumor cells.

These findings provide evidence that the N-terminal acetylation augments the therapeutic effect of RLYE in solid tumors via inhibition of tumor angiogenesis, improvement of tumor vessel integrity and normalization, and enhancement of delivery and efficacy of the co-administered chemotherapeutic drugs.

PO-161

# Lack of Endothelial TGF-β Signaling Inhibits Tumor Angiogenesis In Vivo

**Kako Hanada[1*], Yuki Saito[1], Yoshiaki Kubota[2], Fumiko Itoh[1]**

[1] Laboratory of Cardiovascular Medicine, Tokyo University of Pharmacy and Life Sciences, Japan
[2] Department of Anatomy, Keio University School of Medicine, Japan

*s169083@toyaku.ac.jp*

TGF-β signaling is involved in vascular development and maintenance via type I (ALK5) and type II(TβRII) serine/threonine kinase receptors. Although TGF-β signaling regulates late stage of angiogenesis, it remains unclear how endothelial TGF-β signal affects tumorigenesis.

We have generated tamoxifen-inducible knockout mice of TGF-β type II receptor (TβRII) or type I receptor (ALK5) specifically in endothelial cells by crossing TβRII-floxed ($T\beta RII^{F/F}$) or $ALK5^{F/F}$ mice with Cdh5-BAC-CreERT2 transgenic mice; $T\beta RII^{\Delta iEC}$ or $ALK5^{\Delta iEC}$, respectively. Lewis lung carcinoma cells (LLCs) were injected subcutaneously into control, $ALK5^{\Delta iEC}$, and $T\beta RII^{\Delta iEC}$ mice which were simultaneously administrated tamoxifen for 5 days. One week after the tumor implantation, the tumor size was measured every day. Two weeks after, we analyzed blood and lymphatic vessel structures in tumors with fresh frozen sections from these tumors stained with anti-PECAM-1 and anti-VEGFR-3 antibodies.
Additionally, the effect of endothelial TGF-β signal deficiency on tumor metastasis was analyzed using experimental metastasis mice model. Three weeks after the tamoxifen administration, each group of mice were injected constitutively GFP expressing LLC (GFP-LLC) cells via tail vein and analyzed the tumor metastasis after 4 hours.

CDH5 promoter-dependent depletion of TGF-β receptors did not affect tumor growth in 14 days. However, $T\beta RII^{\Delta iEC}$ mice showed decreased vascular vessel area in tumor tissues stained with anti-PECAM-1 antibody, and $ALK5^{\Delta iEC}$ mice were detected increased tumor lymphatic angiogenesis by anti-VEGFR-3 antibody. Whereas pulmonary tumor metastasis was reduced on only in $T\beta RII^{\Delta iEC}$ mice.

These data suggest that lack of TGF-β signaling in endothelial cells did not affect tumorigenesis but the receptors might regulate angiogenesis and lymphangiogenesis in receptor dependent way.

PO-162

# Arg-Leu-Tyr-Glu Inhibits Tumor Progression by Suppressing Angiogenesis via VEGFR-2 Antagonism

**Wonjin Park[1], Sung Hwan Cho[1], Suji Kim[1], Minsik Park[1], Taesam Kim[1], Young-Guen Kwon[2], Young-Myeong Kim[1*]**

[1] Molecular and Cellular Biochemistry, Kangwon National University, Korea
[2] Biochemistry, Yonsei University, Korea

*ymkim@kangwon.ac.kr*

The tetrapeptide Arg-Leu-Tyr-Glu (RLYE) is known to inhibit vascular endothelial growth factor-A (VEGF-A)-induced angiogenesis in vitro. Herein, we examined its underlying mechanism and antitumor activity associated with vascular remodeling.

We initially examined the inhibitory effect and mechanism of RLYE on VEGF-A-induced signaling and in vitro angiogenesis in cultured HUVECs. We next assessed the inhibitory effect of RLYE in tumor angiogenesis by analyzing tumor growth and metastasis.

RLYE inhibited VEGF-A-induced angiogenesis in a mouse model and suppressed VEGF-A-induced angiogenic signal cascades in human endothelial cells. However, RLYE showed no inhibitory effect on VEGF-A-induced proliferation and migration of multiple myeloma cells expressing VEGF receptor (VEGFR)-1, but not VEGFR-2. RLYE bound specifically to VEGFR-2 at the VEGF-A binding site, thereby blocking VEGF-A-VEGFR-2 binding and VEGF-A-induced VEGFR-2 internalization. The RLYE peptide inhibited tumor growth and metastasis via suppression of tumor angiogenesis in tumor-bearing mice. Moreover, RLYE showed a synergistic effect of the cytotoxic agent irinotecan on tumor cell apoptosis and tumor progression via tumor vessel normalization due to stabilization of VE-cadherin-mediated adherens junction, improvement of pericyte coverage, and inhibition of vascular leakage in tumors.

Our results suggest that RLYE can be used as an antiangiogenic and tumor blood vessel remodeling agent for inhibition of tumor growth and metastasis by antagonizing VEGFR-2, with the synergistic anti-cancer effect via enhancement of drug delivery and therapeutic efficacy.

PO-163

# Immuno-Grab: Multi-Paratopic Decoy Receptor Targeting Tumor Microenvironment-Specific Angiogenic Factors

**Duk Ki Kim[1,3], Ho Min Kim[1,2], Keehoon Jung[3*]**

[1] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Korea
[2] Center for Biomolecular & Cellular Structure, Institute for Basic Science, Korea
[3] Department of Anatomy and Cell Biology / Biomedical Sciences, Seoul National University College of Medicine, Korea

*keehoon.jung@snu.ac.kr*

To investigate the efficacy and mechanisms of PD-1/PD-L1-targeted multi-paratopic decoy receptor (Immuno-Grab) that can simultaneously block multiple angiogenic factors (VEGF-A, PLGF) and PD-1/PD-L1 axis.

We developed a series of novel multi-paratopic VEGF decoy receptors, PD-L1-Grab and PD-1-Grab, by genetically fusing VEGF decoy receptor (VEGF-Grab) to a single chain Fv of anti-PD-L1 antibody (Atezolizumab) or anti-PD-1 antibody (Symo21), respectively. The binding affinity for each antigen was measured by ELISA. We evaluated the blocking effect of Immuno-Grab in vitro using HUVECs and PD-1/PD-L1 luciferase assay. Furthermore, we examined in vivo anti-tumor effect of the Immuno-Grab using syngeneic orthotopic pancreatic and breast cancer mouse models.

Immuno-Grab, like VEGF-Grab,has strong binding affinity for potent angiogenic factors including VEGF-A and PLGF. In vitro, Immuno-Grab effectively inhibited VEGF signaling pathway and dramatically blocked PD-1/PD-L1 axis as well. Following in vivo experiment revealed that Immuno-Grab significantly reduced the tumor size and weight.

Moreover, Immuno-Grab polarized some pro-tumoral myeloid populations to acquire anti-tumoral features. These profound effects were presumably due to specific blockade of pro-tumoral angiogenic factors, including VEGF-A and PLGF, around PD-L1 and PD-1 positive cells in the tumor microenvironment (TME). Therefore, Immuno-Grab could have efficiently modulated local gradient of VEGF-A and PLGF nearby PD-L1- or PD-1-expressing cells in the TME. This resulted in tumor vessel normalization especially where PD-L1 is highly expressed. Also, we unexpectedly observed increased influx of neutrophils into the TME over consecutive Immuno-Grab treatments.

We generated a series of novel immune-targeting multi-paratopic decoy receptors, Immuno-Grab, and demonstrated target-specific anti-tumoral effects in vitro and in vivo. Immuno-Grab may be promising therapeutics that can enhance both tumor vessel normalization and anti-tumoral myeloid polarization by target-specific modulating angiogenic molecule gradient. Further works remain to be accomplished to analyze infiltrated neutrophils upon Immuno-Grab treatment to determine whether emergent resistance can be mitigated, while preserving anti-tumor activity of Immuno-Grab.

PO-164

# Sizable Large-Volume Perfusable and Adaptable Microfluidic System to Decipher Tumor-Endothelium-Immune Cell Interactions

**Yang Lin[1], Ryan Schreiner[2], Brisa Palikuqi[1], Fuqiang Geng[1], Jesus Gomez Salinero[1], Shahin Rafii[1*]**

[1] Division of Regenerative Medicine, Ansary Stem Cell Institute, Weill Cornell Medicine, USA
[2] Department of Ophthalmology, Margaret Dyson Vision Research Institute, Weill Cornell Medicine, USA

*srafii@med.cornell.edu*

Due to the limited tubulogenic ability of adult EC (usually human umbilical vein endothelial cells, HUVEC), conventional microfluidic devices are limited by the size of microvascular beds (<1mm), which hinders the integration of tissue or tumor organoids into the system and hampers the application of trascriptome or proteome studies. ETS variant 2-transcription factor (ETV2) is crucial for the establishment of embryonic blood vessels. The transient (one week) overexpression of ETV2 confers postnatal human endothelial cells (EC) substantial adaptable tubulogenic potential, which enables us to develop an in vitro large-scale microfluidic system to investigate the interactions among tumor cells, microvascular EC and immune cells (Palikuqi B et al, Nature, In Press, 2020).

HUVEC that overexpressing ETV2 ("Reset" vascular EC, R-VEC) possess substantial vessel forming potential that could form continuous, perfusable vascular beds on 17mmX 3.8mmX0.4mm (26 microliter volume) microfluidic channels. Tumor organoids can be co-cultured with R-VEC formed vessels in vitro. R-VECs form perfusable vascular network in which Immune cells can be infused into the lumen

of R-VEC vessels irrigating tumor organoids.

To model tumor metastasis and extravasation, human bladder tumor cells were infused into established R-VEC vessels. Within 24 hours, tumor cells extravasated from the blood vessels into matrix and started to grow. The formed tumor cell clusters remodeled blood vessels and interacted with vascular endothelial cells. In R-VEC - tumor organoid co-culture model with human bladder, colon, and ovarian tumor organoids, R-VEC actively interacted and cross-adapted to tumor cells and formed perfusable microvascular network that could deliver heparinized human blood to the tumor organoids.

These in vitro models hold the promise to be adapted to investigate the interactions among tumors and their microenvironment and can be applied for cancer drug screening or CarT cell testing as well as uncovering the molecular underpinnings of tumor vascular heterogeneity.

PO-165

# Tie2-Mediated Vascular Remodeling by Ferritin-Based Protein C Nanoparticles Confers Antitumor and Anti-Metastatic Activities

**Young Sun Choi[1,2], Hyeonha Jang[1,2], Biki Gupta[1,2], Ji-Hak Jeong[1], Yun Ge[1,2], Chul Soon Yong[4], Jong Oh Kim[4], Jong Sup Bae[1,2,3], Im-Sook Song[1,2,3], In-San Kim[5], You Mie Lee[1,2,3*]**

[1] Pharmacy, Kyungpook National University, Korea
[2] BK21 Plus KNU Multi-Omics Creative Drug Research Team, Kyungpook National University, Korea
[3] Research Institute of Pharmaceutical Sciences, Kyungpook National University, Korea
[4] Pharmacy, Yeungnam University, Korea
[5] Biomedical Research Institute, Korea Institute of Science and Technology, Korea

*lym@knu.ac.kr*

Tumor-associated vascular abnormalities play crucial roles in tumor growth, metastasis and limited drug delivery. In addition, aggravated hypoxia within tumor core later on and ultimately leads to tumor angiogenesis, which provides a stimulus-response pathway to increase tumor growth and metastasis. To induce vascular normalization in tumor, we applied genetically engineered ferritin-based protein C nanoparticles (PCNs) with EPCR binding and PAR-1 activation functions to tumor vascular abnormalities.

Ferritin-based protein C nanoparticles, known as TFG and TFMG, were generated and applied in LLC allograft and MMTV-PyMT spontaneous breast cancer models. Immunohistochemical analysis was performed using tumor samples to evaluate tumor vasculature. Western blot and permeability assay were used to examine the antitumor effects of PCNs and investigate the mechanism. Target pro-

tein siRNA was used to knockdown of target gene. Statistical analysis was performed using one-way ANOVA, followed by post hoc Dunnett's multiple comparison test where appropriate.

PCNs significantly inhibited hypoxic region and increased pericyte coverage, leading to the inhibition of tumor growth, metastasis. PCNs also increased survival rate in allograft mouse LLC and spontaneous MMTV-PyMT breast cancer models. The coadministration PCNs with cisplatin induced synergistic suppression of tumor growth by increasing perfusion of blood and decreasing vascular permeability. Moreover, the PCNs altered the immune cell profiles within tumor, i.e., increasing cytotoxic T cells and M1-like macrophages with anti-tumor activity. PAR-1/PAR-3 heterodimerization through EPCR occupation by PCNs induced Gα13-RhoA-mediated-Tie2 activation and stabilized the vascular tight junctions via Akt-FoxO3a signaling axis.

Simultaneous EPCR binding and PAR-1 activation inducing tumor vascular normalization by PCNs can be utilized as a powerful option to enhance favorable anti-tumor and anti-immune responses and delivery of a chemotherapeutic agent.

PO-166

# The Role of Endothelial Cells in Immune/Inflammatory Responses Mediated Prostate Cancer Castration Resistance

**Ji-Hak Jeong[1,2], Uttam Ojha[2,3], Myo-Hyeon Park[1,2], Minhyuk Kim[2,3], So-Ra Cha[2,3], Jing Ma[2,3], You Mie Lee[1,2,3*]**

[1] Research Institute of Pharmaceutical Sciences, College of Pharmacy, Kyungpook National University, Korea
[2] National Basic Research Laboratory of Vascular Homeostasis Regulation, College of Pharmacy, Kyungpook National University, Korea
[3] BK21 Plus KNU Multi-Omics based Creative Drug Research Team, College of Pharmacy, Kyungpook National University, Korea

*lym@knu.ac.kr*

Androgen deprivation therapy (ADT), a form of castration, is a hormone therapy widely used to treat advanced and metastatic prostate cancer. Infiltrated immune cells by chemoattraction after ADT secretes chemokine and cytokine to stimulate growth of castration-resistant prostate cancer. However, the exact underlying mechanism related to recruitment of immune cells after ADT remains unknown. Here, we investigated the biological roles of endothelial cells in development of castration resistant prostate cancer.

The biological functions of endothelial cells in prostate tumor were demonstrated using in vitro and in vivo experiments. An androgen-dependent mouse prostate cancer cell line, Myc-CaP, and immune competent FVB mice were used for a prostate cancer allograft mouse model. Human androgen-dependent prostate cancer cell line, LNCaP, and human endothelial cell lines, EA.hy926 and HUVEC, were used for investigating the signaling pathway related with ADT. Quantitative real-time PCR (qRT-PCR), western blot, Immunofluorescence

(IF), and immunohistochemical (IHC) assays were employed to detect cytokines/chemokines and immune cells markers in prostate cancer cell lines and tissues. Cell proliferation assay, transwell migration assay, and co-culture assay were used to explore the function of endothelial cells in castration resistant prostate cancer.

We found that the proinflammatory chemokine interleukin-8 (IL-8) was highly increased by ADT in prostate tumor tissues, but not in androgen-dependent prostate cancer cell line. We also found that IL-8 was expressed under androgen deprivation conditions in endothelial cells through AKT/mTOR or IkBa/NFkB signaling pathway. This signaling pathway of IL-8 expression was confirmed by either treatment of inhibitors or knockdown of target genes by siRNA. The IL-8 through paracrine secretion from endothelial cells promoted growth of castration resistant prostate cancer, which was confirmed by co-culture system.

The secreted IL-8 from endothelial cells was mainly involved with recruitment of immune cells after ADT, which might facilitate prostate cancer castration resistance.

# 2. Atherosclerosis

PO-201

## Inhibitory Effects of Vernonia Amygdalina on TNF-α-Induced Oxidative Stress in Human Aortic Endothelial Cells

**Lai Yen Fong[1*], Nur Nadia Mohd Razali[1], Chin Theng Ng[2], Soek Sin Teh[3], Siau Hui Mah[4]**

[1] Department of Pre-clinical Sciences, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia
[2] Physiology Unit, Faculty of Medicine, AIMST University,Kedah, Malaysia
[3] Engineering and Processing Division, Selangor, Malaysia
[4] School of Biosciences, Faculty of Health & Medical Sciences, Taylor's University, Selangor, Malaysia

*fongly@utar.edu.my*

Oxidative stress occurs in early stage of atherosclerosis. Tumor necrosis factor alpha (TNF-α), a pro-atherogenic cytokine, causes excessive ROS production which in turn, leads to increased endothelial permeability. Therefore, attenuation of TNF-α-induced oxidative stress may serve as a promising approach to prevent early atherogenesis. Vernonia amygdalina or 'bitter leaf' is a tropical plant that grows in Malaysia and is used as folk medicine for diabetes mellitus and hypertension. V. amygdalina extracts have previously been reported to possess anti-nociceptive, anti-inflammatory, vasorelaxant, anti-hypertensive and anti-diabetic activities. This study aims to evaluate effects of V. amygdalina methanol extract on TNF-α-induced oxidative stress in human aortic endothelial cells (HAECs).

V. amygdalina methanol extract was prepared using a Soxhlet extractor. HAECs were treated with 6.25 - 800 μg/mL of V. amygdalina methanol extract for 24 h and cell viability was first examined using

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Effects of V. amygdalina on reactive oxygen species (ROS) production and endothelial permeability were then evaluated using 2',7' –dichlorofluorescin diacetate (DCFDA) and fluorescein isothiocyanate (FITC)-dextran, respectively. In these assays, HAECs were treated with 6.25 - 50 µg/mL of V. amygdalina methanol extract for 24 h followed by induction with 10 ng/mL of TNF-α for either 4 (ROS assay) or 6 h (endothelial permeability assay).

Results of cell viability assay show that methanol extract of V. amygdalina did not cause cell death at 6.25, 12.5, 25 and 50 µg/mL. Therefore, these doses were used in the subsequent assays. We report that 25 and 50 µg/mL V. amygdalina methanol extract significantly inhibited TNF-α-induced increased ROS production. Furthermore, V. amygdalina methanol extract did not attenuate endothelial hyperpermeability stimulated by TNF-α.

Our study suggests a potential anti-oxidant property of V. amygdalina, which warrants further investigation in order to reveal the endothelial protective effect of V. amygdalina.

PO-203

# Therapeutic Potential of Aegeline for the Treatment of Cardiac Dysfunction Through Cholesterol and Triglycerides Modulation: In-Vivo, In-Silico Data Analysis

**Dinesh Kumar Patel[1*], Kanika Patel[1]**

[1] Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Uttar Pradesh, India

*dinesh.patel@shiats.edu.in*

Herbal medicine is the best kinds of remedies not only to treat disorders but also have been used in the field of perfumery, nutraceuticals, fragrances, beverages, dyeing and cosmetics industry. Obesity is one of the challenging and fast growing and most dangerous yet silent diseases of all time, and the nuclear hormone receptor PPARγ play an important role in the adipocyte differentiation and development.

Various pharmacological activities of aegeline have been evaluated and presented in the various databases. Purpose of the present work is to search and develop remedies from aegeline for the treatment of cardiac dysfunction through cholesterol and triglycerides modulation. Further docking simulation have has been also performed with software programs to identified the mechanism of interaction between PPARγ and aegeline at the same active site.

Aegeline [N-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide] is the main alkaloid of Aegle marmelos. From the data analysis, it was found that aegeline had anti-diabetic, anti-dyslipidemic and anti-histamine potential. Aegeline has

also significantly decreased the plasma triglyceride levels, total cholesterol, and free fatty acids accompanied with increase in HDL-C and HDL-C/TC ratio. However docking study revealed the formation of structural bonds between aegeline and PPARγ. Further evidence based pharmacological aspects with molecular dynamics were correlated to get better results for the development of new and effective medicine for the treatment of cardiac dysfunction.

This work will be beneficial to develop drugs from aegeline for the treatment of excess cholesterol and triglycerides in all the scientific community.

PO-204

# Association Between Triglyceride Glucose-Waist Circumference and the Progression of Coronary Artery Calcification

**Yun Kyung Cho[1], Jiwoo Lee[2], Hwi Seung Kim[2], Chang Hee Jung[2], Joong-Yeol Park[2], Woo Je Lee[2*]**

[1] Endocrinology and Metabolism, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Korea
[2] Endocrinology and Metabolism, Asan Medical Center, University of Ulsan College of Medicine, Korea

*lwjatlas@gmail.com*

The triglyceride glucose (TyG) index, a product of triglyceride and fasting glucose, is a reliable marker for insulin resistance and has been reported to predict progression of coronary artery calcification (CAC). Recently, the efficiency of TyG-related markers that combine obesity markers such as waist circumference (WC) with TyG index has been studied. In this study, we aimed to investigate the association between TyG-WC and CAC progression in adult Koreans.

We enrolled 1,145 asymptomatic participants who underwent repeated CAC score measurement during routine health examinations. Progression of CAC was defined as (1) incident CAC, which indicates baseline Agatston score of zero converting to detectable CAC at the follow-up examination in a population free of CAC at baseline, or (2) increase of ≥2.5 units between the baseline and final square root of CAC scores participants who had detectable CAC at baseline examination.

According to the TyG-WC index, subjects were stratified into four groups. The prevalence of progression increased with the TyG-WC tertile (15.0%, 24.1%, 31.0% and 32.2% in the 1st, 2nd, 3rd, and 4th TyG-WC quartiles, respectively; p <0.001). In the multivariate logistic regression analysis, the odds ratio [95% confidence interval (95% CI)] for CAC score progression was 1.81 (1.15–2.85) when the highest and lowest TyG-WC index quartiles were compared. Furthermore, regarding the comparison between TyG index and TyG-WC, the predictability of TyG-WC was better than that of TyG index [area under the curve (AUC) 0.60; 95% CI 0.57–0.63 vs. AUC 0.56; 95% CI 0.53–0.59, P = 0.002].

TyG-WC is independently associated with CAC progression. TyG-WC may be a predictive marker of future coronary atherosclerosis and prognosis.

PO-205

# A Novel Small Molecule Compound SP-8356 Targeting CD147 Suppresses Plaque Progression and Promotes Plaque Stability in ApoE-Deficient Mice

**Kisoo Pahk[1], Chanmin Joung[2], Sungeun Kim[1], Won-Ki Kim[2*]**

[1] Department Of Nuclear Medicine, Korea University Anam Hospital, Korea
[2] Institute for Inflammation Control, Korea University College of Medicine, Korea

*wonki@korea.ac.kr*

Plaque vulnerability is the important therapeutic target in atherosclerosis and its related cardiovascular disease. CD147 has been suggested to play key roles in plaque vulnerability through interacting with cyclophilin A (CypA) and resultant activation of matrix metalloproteinase-9 (MMP-9). Here we report that the novel synthetic CD147 inhibitor SP-8356 ((1S,5R)-4-(3,4-dihydroxy-5-methoxystyryl)-6,6-dimethylbicyclo[3.1.1]hept-3-en-2-one) inhibits CD147/MMP-9 pathways and reduces plaque progression and stabilizes plaque vulnerability.

Advanced atherosclerotic plaque was induced in apolipoprotein E-deficient (ApoE KO) mice by partial ligation of the right carotid artery coupled with an atherogenic diet. SP-8356 (50 mg/kg) was orally given daily for 3 weeks. Histomolecular analysis was carried out on harvested carotid arteries.

Surface plasmon resonance assay showed the specific binding of SP-8356 with CD147. SP-8356 inhibited CypA-CD147 interaction and MMP-9 activation. In ApoE KO mice model, SP-8356 inhibited plaque formation, reduced the number of macrophages, increased the number of vascular smooth muscle cells, increased the fibrous cap thickness, and increased the collagen type I contents in fibrous cap. SP-8356 also reduced the apoptotic cells in the plaque lesion.

Owing to its improvement of plaque stability and inhibitory effect on plaque development, SP-8356 could be a potential therapeutic drug candidate for atherosclerosis and related cardiovascular diseases.

PO-208

# Multifunctional Peptide Micelles for Smooth Muscle Cell Targeting and MicroRNA Therapy to Prevent and Reduce Atherosclerosis

**Eunji Chung[1*]**

[1] Biomedical Engineering, University of Southern California, USA

*eunchung@usc.edu*

Although the role of smooth muscle cells (SMCs) in atherogenesis has been less studied, it is now known that synthetic SMCs transdifferentiate to multiple atherogenic cell types including macrophage-like cells and calcifying, osteoblast-like cells which contribute to inflammation and calcification and propagate disease progression. Previously, microRNA-145, the most highly expressed microRNA (miR) in SMCs, was reported to decrease the synthetic SMC phenotype and decrease total plaque area upon viral delivery. Hence, herein, we develop nanoparticles that delivery miR-145 to SMCs in atherosclerosis to provide a strategy to inhibit multiple cell types derived from SMCs for atherosclerosis therapy.

The CCR2-binding peptide motif of MCP-1, miR-145, or cy7 was conjugated onto DSPE-PEG2000 and purified by HPLC. Peptide amphiphile micelle (PAM) nanoparticles were self-assembled by dissolving DSPE-PEG2000 amphiphiles in water or PBS. The particle characteristics were measured by dynamic light scattering (DLS), transmission electron microscopy, and zeta potential measurements. Biocompatibility and therapeutic potential were assessed via Live/Dead assay and qRT-PCR on human aorta smooth muscle cells using primers against KLF-4, KLF-5, ELK-1, SMA, calponin, and myocardin. Atherosclerosis prevention and therapy was assessed in early and mid-stage ApoE knock out mice via en fas, qRT-PCR, and histological quantification. Biodistribution and half-life of the particles were also performed.

When treated with miR-145 PAMs, SMCs showed potent downregulation of athero-prone genes and enhancement of athero-protective genes in vitro. This was further verified in ApoE-/- mice with early- and mid-stage atherosclerosis via en fas and histology which demonstrated reduced plaque lesions in mice treated with miR-145 PAMs compared to non-targeting miR-145 PAMs, free miR-145, and miR-67 MCP-1 PAMs. Additionally, plaque stability was improved upon miR-145 PAM treatment, confirmed by increased collagen content and fibrous cap thickness.

To our knowledge, this is the first example of using nanoparticles to deliver miR-145 to target SMCs in atherosclerosis.

PO-212

# CD9-Induced Cellular Senescence Aggravates Atherosclerotic Plaque Formation

**Eok-Cheon Kim[1,2], Youlim Son[1,2], Jung Hee Cho[1,2], Da-Woon Lee[1,2], Yong Seop Park[1,2], Jun-Hyuk Choi[3], Kyung-Hyun Cho[4], Ki-Sun Kwon[5], Jae-Ryong Kim[1,2*]**

[1] Department of Biochemistry and Molecular Biology, College of Medicine, Yeungnam University, Daegu, Korea
[2] Smart-aging Convergence Research Center, College of Medicine, Yeungnam University, Daegu, Korea
[3] Department of Pathology, College of Medicine, Yeungnam University, Daegu, Korea
[4] School of Biotechnology, Yeungnam University, Gyeongsan, Korea
[5] Aging Research Institute, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea

*kimjrooo@gmail.com*

CD9, a 24 kDa tetraspanin membrane protein, is known to regulate cell adhesion and migration, cancer progression and metastasis, immune and allergic responses, and viral infection. CD9 is upregulated in senescent endothelial cells, neointima hyperplasia, and atherosclerotic plaques. However, its role in cellular senescence and atherosclerosis remains undefined.

We investigated the potential mechanism for CD9-mediated cellular senescence and its role in atherosclerotic plaque formation.

CD9 knockdown in senescent human umbilical vascular endothelial cells significantly rescued senescence phenotypes, while CD9 upregulation in young cells accelerated senescence. CD9 regulated cellular senescence through a phosphatidylinositide 3 kinase-AKT-mTOR-p53 signal pathway. CD9 expression increased in arterial tissues from humans and rats with age, and in atherosclerotic plaques in humans and mice. Anti-mouse CD9 antibody noticeably prevented the formation of atherosclerotic lesions in ApoE-/- mice and Ldlr-/- mice. Furthermore, CD9 ablation in ApoE-

/- mice decreased atherosclerotic lesions in aorta and aortic sinus.

These results suggest that CD9 plays critical roles in endothelial cell senescence and consequently the pathogenesis of atherosclerosis, implying that CD9 is a novel target for prevention and treatment of vascular aging and atherosclerosis.

PO-213

# Selectively Targeting The NLRP3 Inflammasome to Attenuate Diabetes-Associated Atherosclerosis

**Arpeeta Sharma[1,2], So Young Judy Choi[1], Daniel Simpson[3], James E. Vince[3], Rebecca M. Ritchie[4], Judy De Haan[1,5,6*]**

[1] Oxidative Stress, Baker Heart and Diabetes Institute, Australia
[2] Department of Diabetes, Central Clinical School, Monash University, Australia
[3] The Walter and Eliza Hall Institute of Medical Research, Department of Medical Biology, University of Melbourne, Australia
[4] Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Australia
[5] Department of Immunology and Pathology, Central Clinical School, Monash University, Australia
[6] Department of Physiology, Anatomy and Microbiology, La Trobe University, Australia

*judy.dehaan@baker.edu.au*

Diabetes is associated with an increased risk of atherosclerosis, driven by low-grade persistent inflammation. Particularly, activation of the NLRP3-inflammasome and subsequent secretion of the inflammatory cytokine, interleukin-1β (IL-1β), is closely linked to the severity of the atherosclerosis process. We investigated whether inhibiting the NLRP3-inflammasome, through the use of a specific small molecule inhibitor, MCC950, could reduce inflammation, improve endothelial function and attenuate diabetes-associated atherosclerosis.

Eight-week old ApoE knockout mice were rendered diabetic with streptozotocin. At 18-week of age, non-diabetic and diabetic mice were injected with either MCC950 (5.0mg/kg) or vehicle (3% DMSO) three-times a week for a period of 10-weeks. At termination, atherosclerotic plaque, endothelial function, oxidative stress and inflammation were analysed. Inflammasome activity was assessed in mouse bone marrow derived macrophages (BMDMs) that were cultured in high glucose (25mM) and were stimulated with LPS (0.1μg/ml) as well as in control and diabetic human aortic smooth muscle cells (HAoSMCs). The NLRP3 inhibitor, MCC950, was added to primed and/or activated BMDMs and HAoSMC prior to the second stimulus (ATP;1mM). Inflammatory gene expression (qRT-PCR) and ELISA of NLRP3-inflammasome components were assessed.

Diabetes led to a ~4-fold increase in atherosclerosis in diabetic ApoE -/- mice, which was significantly attenuated with MCC950 treatment (~49% reduction, $p<0.001$). This was associated with reduced macrophage abundance and oxidative stress. Vascular function was improved in diabetic vessels treated with MCC950 and systemic inflammation was reduced. In LPS or high glucose-treated BMDMs, as well as in control and diabetic HAoSMCs, MCC950 significantly attenuated caspase-1 and IL-1β secretion, while gene expression of NLRP3 components were unaffected.

This study demonstrated that the NLRP3-inflammasome inhibitor MCC950 reduces endothelial dysfunction and atherosclerosis in the diabetic setting. In the era of targeted therapeutics, specific NLRP3 inhibition by MCC950, may represent a novel way to improve diabetes-associated atherosclerosis.

PO-216

# A Natural Product Extract, KDE, Reduces Foam Cell Formation and Ameliorates Atherosclerosis Through Autophagy Induction

**Minjeong Ko[1], Goo Taeg Oh[2], Ho Jeong Kwon[1*]**

[1] Chemical genomics Global Research Laboratory, Department of Biotechnology, Yonsei University, Korea
[2] Immune and Vascular Cell Network Research Center, National Creative Initiatives, Ewha Womans University, Korea

*kwonhj@yonsei.ac.kr*

Traditional medicinal plants have long been widely used in Asian countries due to its various pharmacological activities. In this study, we prepared Korean medicinal plant extract, KDE, by high hydrostatic pressure (HHP) and hot water extraction to remove bacteria from raw plants and increase the extraction yield of active ingredients. Then, we confirmed the anti-atherosclerotic activity of KDE at the in vivo level.

KDE inhibited proliferation of HUVECs and induced the autophagic features of the cells including the LC3 conversion and p62 degradation in a time dependent manner. Notably, KDE suppressed foam cell formation in oxLDL-induced Raw264.7 macrophages, whereas simultaneously increased lysosomal activity that was measured by acridine orange staining.

In addition, KDE reduced atherosclerotic plaque development in apolipoprotein E knock-out (ApoE–/–) mice in vivo that were fed with high cholesterol diet.

Collectively, this study demonstrated that HHP treated-KDE exhibits anti-atherosclerotic activity via autophagy induction and provided the potential of KDE as a therapeutic agent for treating cardiovascular disease.

PO-217

# Focal Adhesion Kinase Activity is Critical for Atherosclerosis Progression through Nuclear Factor-kB Activation

**James M. Murphy[1], Kyuho Jeong[1], Donna L. Cioffi[1], Pamela Moore Campbell[2], Hanjoong Jo[2], Eun-Young Erin Ahn[4], Ssang-Taek Steve Lim[1*]**

[1] Department of Biochemistry and Molecular Biology, University of South Alabama College of Medicine, USA
[2] Department of Pathology, University of South Alabama College of Medicine, USA
[3] Department of Bioengineering, Emory University and Georgia Institute of Technology, USA
[4] Department of Pathology, O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham, USA

*stlim@southalabama.edu*

Focal adhesion kinase (FAK) activation promotes proinflammatory molecule expression in endothelial cells (ECs), but limited information is available regarding FAK mechanisms in controlling nuclear factor-κB (NF-κB) activation in ECs under chronic inflammatory conditions.

We examined if FAK inhibition alters tumor necrosis factor-α (TNF-α)-mediated NF-κB signaling in vitro and in mice using pharmacological and genetic FAK kinase-dead (KD) inhibition. NF-κB signaling was further analyzed during atherosclerosis in ApoE-/- and ApoE-/-;FAK-KD mice fed a high fat and cholesterol diet (HFD). Furthermore, we proved correlation between FAK activity and NF-κB

activation in human atherosclerotic specimen.

FAK inhibition abolished TNF-α-mediated NF-κB activity in ECs by disrupting recruitment of receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and the IκB kinase (IKK) complex to TNF-α receptor complex-I (TNFRC-I). Improper formation of TNFRC-I resulted in decreased IKK activity and elevated protein stability of IκBα. In mice given TNF-α, FAK inhibition blocked TNF-α-induced IKK-NF-κB activation in aortic ECs. In ApoE-/- mouse, FAK inhibition significantly decreased HFD-induced FAK activity, IKK-NF-κB activity and atherosclerotic lesions. In healthy arteries of mice, FAK was localized primarily within the nuclei of ECs. However, TNF-α or HFD stimulation in mice activated and redistributed FAK to the cytoplasm, causing elevated IKK-NF-κB activation. In human atherosclerotic samples, FAK exhibited significantly higher activity with increased cytoplasmic localization than controls, and FAK activity was correlated with increased NF-κB activation in ECs of atherosclerotic lesions.

FAK inhibition blocks chronic proinflammatory signaling through inhibition of IKK-NF-κB activation. FAK inhibitors may have therapeutic potential against atherosclerosis.

PO-218

# Fixed Dose Combination of Simvastatin and Fenofibrate Versus High Dose Rosuvastatin in Hypertriglyceridemia

**Jae Hyoung Park[1*], Hyo-Jung Nam[2]**

[1] Cardiology, Korea University Anam Hospital, Korea
[2] The Health screening and Promotion center, Asan Medical Center, Korea

*jhpark3992@naver.com*

Hypertriglyceridemia (triglycerides>200mg/dl) is a major cardiovascular risk factor. We want to compare between combination of simvastatin and fenofibrate and high intensity rosuvastatin (20mg) in treatment of hypertriglyceridemia.

This is a non-randomized prospective observation study. One hundred fifty four hypertriglyceridemia patients were assigned 3 groups (Group A ; rosuvastatin 20mg, Group B ; simvastatin 20mg + fenofibrate 145mg, Group C ; simvastatin 40mg + fenofibrate 145mg). The brand name of fixed dose combination is Cholib. There were significant differences of age, blood glucose, BMI and ABI between 3 groups. We checked baseline and follow up lipid profile, flow medicated dilation (FMD), pulse wave velocity (PWV), carotid intima media thickness (cIMT) and augmentation index (AIx). After 1yr, we checked above parameters again.

After treatment, significant decrease in triglyceride and total cholesterol in all groups. Compared with Group A (rosuvastatin 20mg), Group B (simvastatin 20mg + fenofibrate 145mg) and Group C (simvastatin 40mg + fenofibrate 145mg) showed better reduction in triglyceride (p=0.044, 0.001 respectively). And, compared with Group A, Group B and C showed improvements in HDL cholesterol

(p<0.001, 0.017 respectively). In contrast, Group A is better than Group B and C in LDL cholesterol reduction (p=0.025) There was no difference in changes of PWV and AIx between 3 groups except ABI of Group B and C (p=0.03). However, the difference of 2 groups was very small (0.0±0.1 vs 0.1±0.1). There was no difference in changes of IMT and FMD.

Combination treatment of simvastatin and fenofibrate was better than high intensity rosuvastatin in control of hypertriglyceridemia and improvement of HDL cholesterol.

PO-219

# Biological Potential and Therapeutic Importance of Gossypetin Against Atherosclerosis: Phytopharmaceutical Importance Through Involvement of Cholesterol and Lipid Accumulation

**Dinesh Kumar Patel**[1*]

[1] Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Uttar Pradesh, India

*dinesh.patel@shiats.edu.in*

Atherosclerosis is one of the main reasons of all type of heart related problems and occurred in the human body due to the constriction of the arteries and accumulation of various form of plaque around the different types of arteries. Flavonoidal class chemical are very important for human being as it have numerous beneficial properties and gossypetin is one of the best example of flavonoidal class chemical.

In order to know the biological potential of gossypetin in the medicine for the treatment of hyperlipidemia and other associated disorders, here in the present investigation numerous scientific databases have been searched and collected important scientific information for gossypetin. However anti-atherosclerotic and anti-hyperlipidemic potential of gossypetin have been searched in the present investigation through scientific data analysis of current scientific research work in order to know the benefit of gossypetin against atherosclerosis. Molecular docking data have been also searched from various scientific research works in order to know their potential binding mode of action against various form of ligand.

Scientific data analysis of different scientific research of the medical and allied health sector revealed the biological importance of gossypetin in the medicine. Scientific database analysis revealed the biological importance of gossypetin in the medicine due to their antioxidant activity as it belong to the important class of phytochemical called flavonoids. Data analysis revealed the therapeutic benefit of gossypetin for their anti-atherosclerotic properties. Different research works revealed the biological importance of gossypetin against various form of reactive oxygen species and it inhibit cholesterol oxidation and intracellular lipid accumulation. Molecular simulation studies revealed the level and type of interaction of target molecule with gossypetin.

Scientific data analysis of different research work revealed the biological importance and pharmacological activities of gossypetin in the medicine for the treatment of atherosclerosis and related complications.

PO-220

# Ceramide Modulation Affects Gallstone Formation

**Joo-Won Park[1*], Woo-Jae Park[2]**

[1] Department of Biochemistry, College of Medicine, Ewha Womans University, Korea
[2] Department of Biochemistry, College of Medicine, Gachon University, Korea

*joowon.park@ewha.ac.kr*

Ceramides are basic bioactive molecules, which belong to the sphingolipid group. Ceramides are composed of sphingosine and a fatty acid varying in length from C14 to C26. Ceramide synthase (CerS) enzymes determine fatty acyl chain length of ceramide. Six CerS enzymes exist in mammals. CerS2 primarily produces ceramides carrying C22-C24 acyl chain length. We investigated whether ceramides carrying C22-C24 acyl chain lengths can alter gallstone formation in vivo.

6 week-old male wild-type and CerS2 heterozygote mice were fed a lithogenic diet for 12 weeks.

CerS2 heterozygotes displayed higher gallstone formation upon high cholesterol diet. ABCG5/8 mRNA levels and ABCG8 protein levels were significantly increased in CerS2 heterozygote liver. Higher cholesterol levels were also detected in bile juice of CerS2 heterozygote gallbladder. CYP7A1, CYP7B1, CYP27A1, HMG-CoA reductase expression was not altered in CerS2 heterozygote liver.

Taken together, altered acyl chain length of ceramides may regulate ABCG5/8 gene expression and function, which affects gallstone disease progress.

PO-221

# Association Between Liver Fibrosis and Progression of Coronary Artery Calcification in Patients with Nonalcoholic Fatty Liver Disease

**Jiwoo Lee[1], Hwi Seung Kim[1], Chang Hee Jung[1], Joong-Yeol Park[1], Woo Je Lee[1*]**

[1] Endocrinology and Metabolism, Asan Medical Center, Korea

*lwjatlas@gmail.com*

Advanced liver fibrosis is known to correlate with coronary artery calcification (CAC). CAC progression is an important predictor of atherosclerosis and future cardiovascular disease, but few studies have examined its association with liver fibrosis. The aim of this study was to investigate the association between liver fibrosis and CAC progression.

The study included 1173 asymptomatic adults whose coronary artery calcium scores were measured repeatedly during routine health check-ups between 2007 and 2015. CAC progression was defined as either newly incident CAC or an increase of ≥ 2.5 units in the final square roots of CAC scores. Nonalcoholic fatty liver disease (NAFLD) was diagnosed by ultrasonography, and liver fibrosis was assessed using the fibrosis-4 index (FIB-4).

A total of 293 participants developed CAC with a mean baseline FIB-4 score significantly higher than that in patients who did not develop CAC. The prevalence of CAC progression significantly increased according to NAFLD and fibrosis statuses (20.5% in non-NAFLD, 25.3% in NAFLD plus low FIB-4, and 38.5% in NAFLD plus intermediate/high FIB-4). In multivariate logistic regression analysis, the odds ratio (95% confidence interval) for CAC

score progression was 1.76 (1.13–3.05) for subjects with NAFLD plus intermediate/high FIB-4, in comparisons with non-NAFLD subjects.

Advanced liver fibrosis assessed using a noninvasive fibrosis marker is associated with a higher risk of CAC progression in subjects with NAFLD.

PO-222

# Antibodies Against Phosphorylcholine and Malondialdehyde in 60 Years Old Cohort and Its Association with Risk of Cardiovascular Events: Differences Between Subclasses and Isotypes.

**Shailesh Kumar Samal[1], Lena Griesbaum[1], Maria Valkova[1], Max Vikström[1], Johan Frostegård[1*]**

[1] Institute of Environmental Medicine, Karolinska Institutet, Sweden

*johan.frostegard@ki.se*

Atherosclerosis is well known inflammatory disease where hardening and narrowing of arteries can be seen. IgG, IgG1, IgG2 antibodies against Phosphorylcholine (anti-PC) and against Malondialdehyde (anti-MDA) can also be used as novel protection and risk marker for CVD events in 60-year-old cohort. IgM Antibodies against PC and MDA has been previously evaluated in 60-years-old and also found to be negatively associated with cardiovascular disease. We here studied the association between IgG, IgG1 and IgG2 antibodies against anti-PC and against anti-MDA and risk of cardiovascular events such as stroke, angina and myocardial infraction in this cohort.

7-year follow-up was conducted in Stockholm County for 60-year-old men and women in which the risk factors associated in the screening of cardiovascular events for 2039 men, 2193 women along with 209 incident CVD cases were evaluated (They are defined as new events for ischemic stroke, myocardial infarction, coronary heart disease, and for angina pectoris) along with 620 age and gender matched controls were tested by In-House ELISA.

After adjusting the crude model for type 2 diabetes mellitus, hyperlipidemia, hypertension, smoking, body-mass index etc. An increased CVD risk was observed in low IgG anti-PC, low IgG anti-MDA, low IgG1 anti-PC, low IgG1 anti-MDA below 10th percentile and found to be more protective above 66th Percentile. The Stroke data came out to be very promising and after stratification by sex, the association can be found very strong among men. While IgG2 anti-PC and anti-MDA didn't show much correlated results. Combination of these antibodies found to have more protective effects at higher levels.

IgG anti-PC/IgG anti-MDA can be used as novel risk marker at lower levels while IgG1 anti-PC / IgG1 anti-MDA can be used as novel protection marker at higher levels for cardiovascular disease. This finding can have diagnostic and therapeutic implications.

PO-226

# The Deletion of GTO3, a Novel Adopokine, Ameliorates Atherosclerosis

**Sejin Jeon[1], Tae Kyeong Kim[1], Goo Taeg Oh[1*]**

[1] Department of Life Sciences, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

The progression of atherosclerosis is affected by various adipokines, produced primarily by adipose tissue. Considering the beneficial or detrimental effects of adipokines in the processes of atherosclerosis, we hypothesized that GTO3 may play a critical role in atherosclerosis caused by a metabolic defect, especially hyperlipidemia.

GTO3 expression and atherosclerosis progression were assessed in atherosclerotic aortic tissue and serum from Apolipoprotein e-deficient (Apoe-/-) mice. Apoe-/- mice lacking systemic GTO3 expression (GTO3-/-Apoe-/-) were generated to assess functional effects of GTO3. Mice were fed a Western diet (WD) for 16 weeks, and atherosclerotic lesions were investigated.

Our in vivo results conclusively showed correlation between an adipokine GTO3, which is primarily released in white adipose tissue (WAT), and the extent of atherosclerotic plaque formation. Elevated circulating GTO3 level was observed in atherosclerotic lesions of aortic apecimens from Apoe-/- mice. GTO3 deficiency decreased body weight and volume of white adipose tissue, as well as attenuated atherosclerosis progression during hyperlipidemic conditions in Apoe-/- mice. Moremover, continuous administration of GTO-P34, GTO3 antagonist peptide, alleviated atherosclerosis without altering metabolic parameters.

Our findings reveal that GTO3 deficiency and treatment of GTO3 antagonist effectively attenuate atherosclerosis progression, suggesting that secreted level of GTO3 could serve as a potential biomarker for cardiovascular diseases.

PO-228

# The Association Between Lipoprotein (a) and Peripheral Artery Disease Assessed by Ankle-Brachial Index in Patients With Type 2 Diabetes

**Ji Eun Jun[1], In-Kyung Jeong[1*], You-Cheol Hwang[1], Kyu Jeung Ahn[1], Ho Yeon Chung[1]**

[1] Endocrinology and metabolism, Kyung Hee University Hospital at Gangdong, Korea

*jik1016@naver.com*

Lipoprotein(a) [Lp(a)] is a liver-synthesized lipoprotein that is composed of an apolipoprotein B100 bound to apolipoprotein(a). Lp(a) plays an active role in vascular inflammation and atherothrombosis. Therefore, we investigated the association between serum Lp(a) levels and the risk of peripheral artery disease (PAD) in patients with type 2 diabetes.

This cross-sectional study included 649 type 2 diabetic patients (mean age 54.9 years, male 58.2%, median duration of diabetes 2.0 years) without past cardiovascular disease. Ankle-brachial index (ABI) value was classified as follows: normal, 0.90 to 1.40; abnormal, ≤0.90 (mild, 0.70 to 0.90; moderate, 0.40 to 0.69; severe, <0.40). The lower of the two ABI values was used for the analysis, and PAD was defined as ABI ≤0.90.

A total of 17 (2.6%) patients were diagnosed with PAD; 1.6 % of mild (N = 11), 0.8 % of moderate (N = 5), and 0.2% of severe PAD (N =1). Serum Lp(a) level was significantly higher in patients with PAD (median 24.2 vs. 10.6; p = 0.001) than those without. After adjustment for variables with p

Elevated Lp(a) level was significantly associated with an increased risk of PAD in patients with type 2 diabetes, even when optimal LDL-C level was achieved.

PO-229

# Lysophosphatidic Acid Receptor 4 Is a Cardiac Progenitor Stage-Specific Marker and Critical for Cardiac Repair

**Jin-Woo Lee[1], Hyun-Jai Cho[1], Choon-Soo Lee[1], Jaewon Lee[1], Hyo-Soo Kim[1*]**

[1] Department of Internal Medicine, Strategic Center of Cell & Bio Therapy, Seoul National University Hospital, Seoul, Korea, Korea

*hyosoo@snu.ac.kr*

Understanding stem cell niche and discovering a lineage-specific marker are essential processes to induce differentiation of stem cells (embryonic stem cell and induced pluripotent stem cell) and maximized differentiation efficiency. Above all processes are applicable to therapeutic purposes. We discovered a cardiac-specific marker, lysophosphatidic acid receptor 4 (LPAR4), which is G protein-coupled receptor (GPCR) and demonstrated its functional significance during cardiac differentiation.

We screened GPCR expressing on mouse cardiac progenitor cells at differentiation day 3 compared to mouse undifferentiated pluripotent stem cells (PSCs). Among candidates, we identified LPAR4.

We have found that in both mouse and human PSCs, LPAR4 has a transient expression pattern during cardiac differentiation. During in vitro differentiation of mouse and human PSCs toward cardiomyocytes, LPAR4 expression peaked for 3–5 days and then and declined immediately. Treatment with ODP (LPAR4 specific agonist) followed by p38MAPK blocker (SB203580) in the cardiac differentiation protocol significantly increased cardiac differentiation efficiency. Furthermore, there was a substantial increase in LPAR4 (+) cells in the adult mouse after myocardial infarction (MI). In vivo, sequential stimulation and inhibition of LPAR4 resulted in the reduction of infarct size and improvement of heart dysfunction after MI.

In conclusion, we demonstrated that LPAR4 is a novel cardiac progenitor cell marker and modulation of the upstream and downstream regulators shown functional significance during cardiac differentiation. Furthermore, our findings provide new insight into cell-free cardiac repair by the modulation of LPAR4 positive cells in the heart.

PO-230

# Association of Serum Omentin-1 and Visfatin Levels With Coronary Artery Disease in Patients With Type 2 Diabetes Mellitus

**Dong-Hwa Lee[1], Jang-Whan Bae[2], Sang Min Kim[2], Sang Yeub Lee[2], Hyun Jeong Jeon[1*]**

[1] Internal Medicine, Chungbuk National University Hospital, Korea
[2] Chungbuk Regional Cardiovascular Center, Chungbuk National University Hospital, Korea

*endoann@daum.net*

It is known that adiponectin plays an important role in insulin sensitivity, anti-inflammatory processes, and anti-atherogenesis. Omentin-1 and visfatin showed association with coronary artery disease (CAD). However, the link between those adipokines and CAD in patients with type 2 diabetes mellitus (T2DM) has not been adequately investigated. In this study, we aimed to investigate the alteration of serum levels of omentin-1 and visfatin in patients with CAD and T2DM.

A total of 240 patients (mean age 64.8 ± 10.4 [34–89] years, mean body mass index (BMI) 24.5 ± 3.7 kg/m2, 65.8% men) who performed coronary angiography at the Chungbuk National University Hospital were included. Patients with and without T2DM were 1:1 ratio (n = 120 in each). Number of patients with CAD was 180 and 60 patients were control subjects. Serum concentrations of omentin-1 and visfatin were measured using ELISA.

Patients with T2DM showed significantly higher BMI than those without (25.2 ± 4.2 kg/m2 vs. 23.8 ± 3.0 kg/m2, P = 0.002). Therefore, we divided patients without T2DM into two groups according to BMI cutoff 25.0 kg/m2 (obese and non-obese). In CAD patients, serum omentin-1 level was significantly decreased in obese patients without T2DM compared to patients with T2DM (343.0 ± 194.4 ng/ml vs. 474.2 ± 251.4 ng/ml, P = 0.034). In control subjects, there was no significant differences according to presence of T2DM. Significant differences were observed between non-obese and obese groups in patients without T2DM (493.3 ± 228.4 ng/ml vs. 293.4 ± 154.0 ng/ml, P = 0.026). Serum visfatin level did not show significant differences among groups.

These findings suggest that serum omentin-1 level was influenced by obesity. Presence of T2DM might affect alteration of serum omentin-1 level in CAD. Further researches are warranted to validate our results.

PO-231

# NF-kappaB-Responsive miR-155 Induces Functional Impairment of VSMCs by Downregulating Soluble Guanylyl Cyclase

**Minsik Park[1], Wonjin Park[1], Suji Kim[1], Taesam Kim[1], Young-Guen Kwon[2], Young-Myeong Kim[1*]**

[1] Biochemistry, School of Medicine, Kangwon National University, Korea
[2] Biochemistry, College of Life Science and Biotechnology, Yonsei University, Korea

*ymkim@kangwon.ac.kr*

miRNA has been implicated in endothelial dysfunction tumor progression, atherosclerosis and vascular inflammation and permeability. The sGC/cGMP

pathway plays a crucial role in altering or modulating VSMC function, the underlying mechanism involving miRNAs in inflammatory vascular diseases, including atherosclerosis and preeclampsia, remains unclear. These findings suggest that TNFα-induced miR-155 expression is a molecular risk factor for atherosclerotic intima formation and preeclamptic hypertension via impairment of the sGC/cGMP pathway.

1. Real-Time Quantitative Polymerase Chain Reaction (qRT-PCR)
2. Western blotting
3. Measurement of NO and cGMP (sGC expression and activity)
4. Wound healing assay and proliferation assay (SMC phenotype change)
5. mRNA stability assay (sGC dimerization)
6. Mouse aortic vascular tension assay (WT and miR-155 KO)

1. TNF-α-induced miR-155 inhibits sGCβ1 expression
2. Alterations in miR-155 and sGCβ1 levels in atherosclerotic and preeclamptic disease
3. TNF-α suppresses sGCβ1 expression via NF-κB-responsive miR-155 biogenesis
4. sGCβ1 is a target of miR-155
5. miR-155 induces phenotypic switching of VSMCs
6. TNF-α-responsive miR-155 impairs NO-mediated vasorelaxation
7. TNF-α does not regulate NO-mediated VSMC function in miR-155 KO aortic vessels

TNF-α-induced miR-155 inhibits sGCβ1 expression in an NF-κB-dependent manner by targeting its transcript, resulting in inhibition of the sGC/cGMP pathway. The dysfunctional sGC/cGMP axis results in phenotypic alterations of VSMCs and impairs vascular relaxation, both of which are associated with various vascular diseases. These findings offer a possible mechanistic link between NF-κB and VSMC dysfunction through miR-155-mediated downregulation of sGCβ1 during the development of atherosclerosis and preeclampsia. In combination with our previous studies the present data support that miR-155, a negative regulator of eNOS, sGCβ1, and PKG1 in the vasculature, is a common therapeutic target for the development of treatments for atherosclerosis and preeclampsia.

PO-232

# Regulation of CX3CR1 Signaling and Function by NO-Mediated Post-Translational Modification

**Hiroki Hayashi**[1*]

[1] Department of Health Development and Medicine, Osaka University, Japan

*hayashih@cgt.med.osaka-u.ac.jp*

Chemokines are known to regulate many cellular functions such as cell migration and proliferation. It is also reported that some chemokines are involved in cardiovascular disorders such as atherosclerosis, myocardial infarction through chemokine receptors, which are G protein coupled receptors (GPCR). Although some chemokines are known to share same chemokine receptor to diversify its signaling pathways, CX3CL1 (fractalkine) has been shown to have only one receptor CX3CR1, which is mainly expressed in monocytes. It is still unclear how fractalkine/ CX3CR1 signaling is modulated. The aim of this study is to evaluate whether S-nitrosylation of β-arrestin 2 affects CX3CR1 function.

Our previous study suggested that β-arrestin, known as a scaffold protein of GPCR, is S-nitrosylated, one of the post-translational modifications where NO group attaches to cysteine residues, and regulates signaling through GPCRs such as β-adrenergic

receptors in heart failure, and that β-arrestin is S-nitrosylated in LPS-induced macrophages (RAW cells) where inducible nitric oxide synthase (iNOS) is induced. We firstly examined fractalkine-induced signaling (phosphorylation of ERK) in HEK293 cells by western blot. Fractalkine-induced migration was assessed by Boyden-chamber migration assay in RAW264.7 cells overexpressing iNOS and β-arrestin 2 WT or C253S mutant.

As the result, fractalkine-induced ERK phosphorylation was decreased in HEK293 cells overexpressing β-arrestin 2 WT, compared with overexpression of β-arrestin 2 C253S mutant. In RAW264.7 cells, fractalkine-induced migration was enhanced by overexpression of iNOS and β-arrestin 2 C253S mutatant. Those result indicates that fractalkine-induced signaling is inhibited/regulated by S-nitrosylation of β-arrestin2 at Cys253 by iNOS.

In conclusion, NO-based mechanism might affect chemokine signaling through CX3CR1 to contribute to atheropathogensis.

PO-233

# Vasa Vasorum, Adventitial and Perivascular Macrophages: How Vascular Inflammation Contributes to Intimal Hyperplasia in Response to Endothelial Dysfunction and Injury

**Leo Bodganov[1], Daria Shishkova[1], Rinat Mukhamadiyarov[1], Alexander Terekhov[1], Amin Shabaev[1], Alexey Frolov[1], Anton Kutikhin[1*]**

[1] Division of Experimental Medicine, Research Institute for Complex Issues of Cardiovascular Diseases, Russian Federation

*antonkutikhin@gmail.com*

Here we aimed to study the inflammatory mechanisms within the blood vessel wall contributing to neointima formation. In particular, we investigated how adventitia and perivascular adipose tissue react to endothelial dysfunction (provoked by daily intravenous administration of calciprotein particles (CPPs) to intact rats) and endothelial denudation inflicted by a balloon injury and aggravated by the injections of CPPs as described above. We further challenged our hypothesis in the clinical setting.

Male 6-month-old Wistar rats underwent balloon angioplasty followed by daily bolus injections of either primary or secondary CPPs or innocuous magnesium phosphate particles / physiological saline (5 animals per group) during 5 days. Upon 5 weeks, all animals have been sacrificed with the subsequent excision of both injured and intact aortic segments. Saphenous veins and internal mammary arteries were harvested during the coronary artery bypass graft (CABG) surgery (n=23 patients). All vessels were

investigated by means of backscattered scanning electron microscopy. Semi-quantitative analysis was conducted using ImageJ. We quantified number and area of macrophage deposits as well as density of vasa vasorum, a surrogate marker of vascular inflammation.

Density of adventitial and perivascular vasa vasorum was higher both in intact and balloon-injured aortic segments of CPP-treated animals; the latter also contained higher quantities of adventitial and perivascular macrophages testifying to the pronounced vascular inflammation. Further, the amount of intimal hyperplasia in saphenous veins and internal mammary arteries correlated with both number and area of macrophage deposits in adventitia and perivascular adipose tissue. Immunostaining revealed a prominent expression of F4/80 and myeloperoxidase in such macrophages attesting to their functional activity.

Vasa vasorum and adventitial/perivascular macrophages are concurrently increased in response to balloon- and CPP-induced endothelial injury, reflecting the formation of neointima in animal model. Further, they correlate with the frequency and amount of intimal hyperplasia in conduits for CABG surgery.

PO-234

# HMGB1 Increases the Migration of Vascular Smooth Muscle Cells Through the Upregulated Expression of Osteopontin

**Eun Yeong Jeon[1,2], Seung Eun Baek[1,2], Ji On Kim[1,2], Jong Min Choi[1,2], Eun Jeong Jang[2], Chi Dae Kim[1,2,3*]**

[1] Pharmacology, Department of Pharmacology and BK21 Plus, School of Medicine, Pusan National University, Korea
[2] Science, Gene & Cell Therapy Research Center for Vessel-associated Diseases, Pusan National University, Korea
[3] Medicine, Research Institute for Convergence of Biomedical Science and Technology, PNU Hospital, Korea

*chidkim@pusan.ac.kr*

The migration of vascular smooth muscle cells (VSMC) is known to play a critical role in the development of vascular remodeling in the injured vasculatures. Recent studies have identified HMGB1 as a principal effector mediating biological functions in VSMC migration, however, the mechanisms involved have not been fully elucidated. Thus, this study investigated the role of HMGB1 on VSMC migration and the underlying molecular mechanisms involved.

VSMC were ex plant cultured using rat thoracic aorta, and stimulated with HMGB1 (100 ng/ml), and then cell migration were measured by wound-healing assay. The expression of osteopontin (OPN) in HMGB1-stimulated VSMC was analyzed by Western blots and RT-PCR. OPN knockdown cells were established through transfection with OPN siRNA.

In cultured rat aortic VSMC stimulated with HMGB1 (100 ng/ml), the migration of VSMC was increased in time- and dose-dependent manners. The HMGB1-induced VSMC migration was sig-

nificantly attenuated in cells treated with MPIIIB10 (100-300 ng/ml), a neutralizing monoclonal antibody for OPN as well as in cells deficient of OPN. In a separate experiment, it was demonstrated that OPN mRNA and protein levels were markedly increased in HMGB1-stimulated VSMC in association with an increased promotor activity of OPN gene.

This study provides direct in vitro evidence that the exposure of VSMC to HMGB1 increases the activity of OPN promoter, leading to higher mRNA and protein levels of OPN, and to an increased migration of VSMC. Thus, the OPN signaling axis in VSMC might serve as a potential target for future therapeutic strategies for vascular remodeling in the injured vasculatures.

PO-235

# Calciprotein Particles: A Link Between the Disturbances of Mineral Homeostasis and Vascular Disease

**Daria Shishkova[1], Elena Velikanova[1], Maxim Sinitsky[1], Anna Tsepokina[1], Anton Kutikhin[1*]**

[1] Division of Experimental Medicine, Research Institute for Complex Issues of Cardiovascular Diseases, Russian Federation

*antonkutikhin@gmail.com*

We hypothesised that calciprotein particles (CPPs), which are formed in the human blood at its supersaturation with calcium and phosphate, cause endothelial dysfunction by activating pro-inflammatory signaling and endothelial-to-mesenchymal transition.

Primary human coronary artery endothelial cells (HCAEC) and primary human thoracic artery endothelial cells (HITAEC) were cultured under the laminar flow (15 dyn/cm2) during 24 h with the subsequent 4 h exposure to primary CPPs, secondary CPPs, or innocuous magnesium phosphate particles. Alternatively, HCAEC and HITAEC were cultured in the static conditions and treated with abovementioned particles to further collect RNA, total protein, and conditioned medium. We then performed transcriptomic and proteomic profiling by means of RNA-seq and dot blot.

Despite incubation with CPPs slightly increased the proportion of dead cells, it did not disturb the integrity of an endothelial monolayer suggesting that endothelial dysfunction rather than injury is a main consequence of CPP formation. RNA-seq identified a cluster of pro-inflammatory genes significantly upregulated upon the exposure to primary and secondary CPPs which augmented release of multiple pro-inflammatory cytokines including interleukin-6, interleukin-8, macrophage migration inhibitory factor, and CXCL1. However, only interleukin-8 was raised in the cell lysate indicative of a constant cytokine release upon CPP stimulation. Further, exposure to CPPs provoked adhesion of leukocytes to endothelial cells even without TNF-α pre-treatment, probably due to the elevated levels of VCAM1, ICAM1, and E-selectin proteins and respective genes. Notably, endothelial-to-mesenchymal transition factors have also been found overexpressed as a result of CPP treatment.

CPPs cause endothelial dysfunction under flow through the pathological upregulation of pro-inflammatory paracrine signaling and endothelial-to-mesenchymal transition. This might explain increased prevalence of atherosclerotic vascular disease in patients with chronic kidney disease or bone metabolism disorders. FUNDING: This study was funded by the Russian Science Foundation, grant number 19-15-00032 “Molecular mechanisms of endothelial toxicity induced by calcium phosphate bions”.

PO-236

# Expression Landscape of the Proprotein Convertase Subtilisin/Kexin (PCSK) Family in Vascular Disease

**Bianca Suur[1*], Moritz Lindquist Liljeqvist[1], Otto Bergman[2], Eva Kärlof[1], Mariette Lengquist[1], Jacob Odeberg[2,3], Gabrielle Paulsson-Berne[2], Göran Hansson[2], Eva Hurt-Camejo[1,4], Per Eriksson[2], Ulf Hedin[1], Daniel Ketelhuth[2], Joy Roy[1], Ljubica Matic[1]**

[1] Molecular Medicine and Surgery, Karolinska Institute, Sweden
[2] Medicine, Karolinska Institute, Sweden
[3] Proteomics, KTH Royal Institute of Technology, Sweden
[4] R&D, AstraZeneca, Sweden

*bianca.suur@ki.se*

PCSKs constitute a family of 7 closely related proteases (PCSK1-7) and 2 distant members (MBTPS1, PCSK9). Their expression pattern and role in cardiovascular disease is unexplored, apart from PCSK9 that is critically involved in lipid metabolism.

We applied an integrative approach with large-scale transcriptomic and proteomic data mining from three human vascular disease tissue biobanks comprising carotid plaques, abdominal and thoracic aneurysms. Results were followed by gene expression associations with patient clinical parameters and immunohistochemistry in tissues. Knockout mice were used for functional phenotyping of selected PCSK based on the human disease data.

FURIN, PCSK5, MBTPS1 transcripts were downregulated, while PCSK6-7 were upregulated in atherosclerotic plaques vs. normal arteries and PCSK9 was expressed at borderline levels. In abdominal aneurysms, FURIN, PCSK5/7, MBTPS1 were downregulated, while PCSK6 was enriched in diseased media. In thoracic aneurysms, only FURIN was significantly upregulated. Immunohistochemistry of PCSKs indicated that protein levels correspond to mRNA expression patterns, with the exception of PCSK9 protein that was abundant in disease biopsies. Because PCSK6 protein was localised in SMA+, CD3+ and CD163+ cells in both plaques and aneurysms, we next performed immunophenotyping of Pcsk6-/- mice. We found that splenocytes from Pcsk6-/- mice secreted more cytokines than controls upon stimulation. In addition, peritoneal macrophages of Pcsk6-/- mice also secreted more cytokines compared to control mice. Bone marrow derived macrophages from Pcsk6-/- mice were more prone to take up lipids and expressed higher levels of lipid uptake receptors. Transplantation of Pcsk6-/- bone marrow to LDLr-/- mice resulted in an increased development of atherosclerosis compared to controls.

Here, we revealed FURIN enrichment in thoracic aneurysms and the presence of PCSK9 protein in vascular lesions, suggesting its accumulation from circulation. PCSK6 was the most strongly enriched protease in both plaques and abdominal aneurysms and possibly involved in immune regulation, which warrants further studies.

PO-237

# Proteoglycan 4 Is a Key Factor in Chondrogenic Smooth Muscle Cell Differentiation During Vascular Remodeling and Intimal Calcification

**Till Seime[1*], Asim C. Akbulut[2], Urszula Rykaczewska[1], Rick H. Van Gorp[2], Olivia J. Waring[3], Moritz Lindquist Liljeqvist[1], Eva Karlöf[1], Mariette Lengquist[1], Andrew J. Buckler[1], Malin Kronqvist[1], Jan H.N. Lindeman[4], Anton Razuvaev[1], Leon J. Schurgers[2], Ulf Hedin[1], Ljubica Matic[1]**

[1] Department of Molecular Medicine and Surgery, Vascular Surgery, Karolinska Institutet, Stockholm, Sweden, Sweden
[2] Department of Biochemistry, CARIM, Maastricht University, Maastricht, The Netherlands, Netherlands
[3] Department of Pathology, CARIM, Maastricht University Medical Center, Maastricht, The Netherlands, Netherlands
[4] Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands, Netherlands

*till.seime@ki.se*

The role of calcification in atherosclerotic plaque stability is poorly understood. Using a large biobank of carotid endarterectomies, we recently showed that macro-calcification is associated with a transcriptomic profile typical for stable lesions and identified Proteoglycan 4 (PRG4) as the key molecular signature of calcified human plaques, expressed in smooth muscle cell (SMC) rich regions. Here, the role of PRG4 in atherosclerosis was investigated further in functional and mechanistic studies.

In vitro, PRG4 was induced in primary human SMCs by calcifying medium, oxLDL and IGF1or TGFβ1 growth factors and it's functional effects studied via addition of full length recombinant human protein. Expression during disease progression was followed in different rodent models as well as human tissues.

PRG4 induction in calcified SMCs was coupled to increased expression of the chondrogenic master regulator SOX9, while typical contractile markers were repressed. Addition of PRG4 increased passive ectopic calcification of SMCs, arrested cell migration, suppressed expression of endogenous PRG4 and BMP2, and restored expression of contractile markers. In vivo, PRG4 was up-regulated during intimal hyperplasia and early plaque formation, correlating with chondrogenic markers. PRG4 protein was also enriched in calcified plaques of warfarin treated ApoE-/- mice, where it localized to SOX9+ cells surrounding calcified nodules. In human athero-progression in situ, PRG4 localised to SMCs in early intimal thickening, while it was deposited in the extracellular matrix in advanced lesions surrounding macro-calcifications and neo-vessels, and its expression associated with repression of SMC migration. PRG4 expression in human plaques also positively correlated to calcification volume and plaque burden as assessed by diagnostic CT-image analyses, but negatively with lipid-rich necrotic core volume.

Our results show that PRG4 influences SMC function and chondrogenic phenotype during vascular remodeling and intimal macro-calcification. PRG4 may be a key component of plaque remodeling in response to atherogenic stimuli, meriting further mechanistic studies.

PO-238

# Effect of Dioxin and AHR Pathway Activation on SMC Phenotype and Atherosclerosis

**Juyong Kim[1,2*], Quanyi Zhao[1], Ramendra Kundu[1], Paul Cheng[1,2], Robert Wirka[1], Trieu Nguyen[1], Thomas Quertermous[1,2]**

[1] Cardiovascular Medicine, Stanford University, USA
[2] Cardiovascular Institute, Stanford University, USA

*kimjb@stanford.edu*

Environmental exposure to dioxin has been linked to increased myocardial infarction. Smooth muscle cells (SMC) in the coronary vasculature play a critical role in atherosclerotic plaque remodeling due to their phenotypic plasticity, however, in-vivo mechanism linking toxic exposure to adverse SMC modulation is not clearly known.

Primary human coronary artery SMC (HCASMC) treated in culture with 10nM of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) or vehicle control were used to perform RNA-Seq, and ATAC-Seq. ChIP-Seq was performed with antibodies against Aryl-hydrocarbon receptor (AHR) and TCF21. Myh11-Cre, ROSA26tdT/WT tamoxifen inducible SMC-lineage tracing reporter mice were used to assess the in-vivo effect of TCDD on aortic SMC. After 8 weeks of high fat diet (HFD), followed by 8 weeks of TCDD injection and HFD, the aortic sinus was collected and digested to single-cell suspension. scRNA-Seq was performed using the 10X Genomics platform.

Analysis of the RNA-Seq data from HCASMC treated with TCDD showed differential enrichment of biological pathways related to cell migration, localization, and development. Further, ATAC-Seq data showed a significant enrichment for pathways regulating vascular development, cell migration, and apoptosis. There was an overall increase in the open chromatin regions suggesting transcriptional activation with TCDD. Also, there was enrichment of AHR ChIP-Seq peaks as expected, however, the TCF21 enrichment decreased significantly with TCDD treatment. The scRNA-Seq of mice treated with TCDD exposure showed differential gene expression of SMC-lineage cells enriching for extracellular matrix organization, inflammation, unfolded protein response, and apoptotic process.

Dioxin adversely remodels atherosclerotic plaque by affecting SMC-phenotypic modulation, and pathways affecting ECM organization, inflammation and unfolded protein response.

PO-239

# Blockade of Notch Ligand Delta-Like Ligand 1 Inhibits Macrophage Activation and Arteriosclerosis

**Jun-ichiro Koga[1*], Shunsuke Katsuki[1], Tetsuya Matoba[1], Hiroyuki Tsutsui[1]**

[1] The Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kyushu University, Japan

*j-koga@cardiol.med.kyushu-u.ac.jp*

Macrophage-mediated inflammation promotes various vascular diseases, but how macrophage functions are regulated has not been fully understood. Notch signaling is a fundamental pathway regulating cell differentiation in developmental stages, but its role in adult vascular diseases are obscure. We have examined the role of Notch ligand Delta-like ligand 1 (Dll1) in macrophage activation

and arteriosclerosis to explore a potential as a new therapeutic target.

1) To examine the role of Dll1, blocking antibody of Dll1 (anti-Dll1 Ab) was prepared. After pre-treatment with anti-Dll1 Ab, mouse peritoneal macrophages were stimulated with 10 ng/mL LPS + 10 ng/mL IFN-γ or 10 ng/mL IL-4 for 24 hours. mRNA expressions were quantified by real-time PCR. 2) C57BL/6J mice were subjected to femoral arterial wire injury and anti-Dll1-Ab (250 μg/injection) was intraperitoneally injected twice a week for 4 weeks, 3) Apolipoprotein E-deficient mice (Apoe-/-) were fed a high-fat diet and anti-Dll1 Ab (250 μg/injection) were intraperitoneally injected twice a week.

Blockade of Dll1 decreased mRNA expression of iNOS, TNF-α and IL-1β in macrophages treated with LPS and IFN-γ. In contrast, blockade of Dll1 increased molecules associated with alternatively activated macrophages, i.e. arginase-1 and Ym1, in IL-4 treated macrophages. Immobilized Dll1 increased mRNA expression of prototypical Notch-target Hes-1 and IL-1β. In mice subjected to femoral arterial wire injury, blockade of Dll1 attenuated intimal hyperplasia, perivascular fibrosis, and negative remodeling of injured arteries. In Apoe-/- mice, a high-fat diet feeding increased Dll1 expression in atherosclerotic plaques, especially in Mac-3 positive macrophages. Blockade of Dll1 decreased atherosclerotic plaque area and lipid content determined by oil red O staining. In addition, anti-Dll1 Ab decreased accumulation of Mac-3 positive macrophages to the plaque.

These results indicate that Dll1 promotes macrophage activation and arteriosclerosis. Dll1 may be a potential therapeutic target for vascular diseases promoted by macrophage-mediated inflammation.

PO-240

# Endothelial TKK1 Expression Ameliorates Atherosclerotic Plaque Formation

**Tae Kyeong Kim[1], Sejin Jeon[1], Goo Taeg Oh[1*]**

[1] Life Science, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

During the pathogenesis of atherosclerosis, the communication between variable cells and tight regulation of immune cell infiltration by endothelial cells (ECs) are essential. TKK1 is induced in acute inflammatory diseases for performing regulatory function and known as biomarker during the disease progression. However, little is known about the association of TKK1 with chronic inflammatory diseases like atherosclerosis.

Transcription and translational expression levels of TKK1 were confirmed in the aortas of $ApoE^{-/-}$ mice according to the severity of disease as well as primarily isolated aortic cell types. Development of atherosclerotic lesion in $ApoE^{-/-}$ mice and $ApoE^{-/-}$ mice with a genetic deletion of Tkk1 ($Tkk1^{-/-}ApoE^{-/-}$) was measured. Also, lesion development was assessed in a model of bone-marrow transplantation (BMT) using $ApoE^{-/-}$ mice and $Tkk1^{-/-}ApoE^{-/-}$mice as bone marrow (BM) recipient mice, to identify the deletion effect of Tkk1 in aortic cell types.

The expression of TKK1 was increased in the aortas of 8 weeks old $ApoE^{-/-}$ mice compared to B6/J and progressively upregulated until 48 weeks old, then decreased at later stage of atherogenesis. Among the aortic cell types, aortic ECs, but not vascular smooth muscle cells expressed murine TKK1 in atherosclerotic aorta specimen. Also, mouse aortic endothelial cells robustly expressed Tkk1 after stimulation which mimicked atherosclerotic conditions. Pres-

ence of Tkk1 only in vascular cells significantly reduced plaque formation, while absence of vascular Tkk1 accelerated plaque progression, which was in line with augmentation of lesion in whole body Tkk1 deficiency in ApoE$^{-/-}$ mice. Furthermore, endothelial deletion of Tkk1 caused elevated adhesive and transmigrated monocytes through monolayer of ECs with increased vascular permeability.

Our findings represent the in vivo experimental validation of the endothelial expression of TKK1, which possess protective effect on atherosclerotic plaque formation and has pivotal role in atherogenesis.

PO-241

# Electronic Cigarettes Activate the Unfolded Protein Response in Vascular Smooth Muscle Cells in Atherosclerosis

**Juyong Kim[1,2*], Meena Easwarian[3], Joshua Martinez[3], Paul Cheng[1,2], Elizabeth DiRenzo[3], Thomas Quertermous[1,2]**

[1] Cardiovascular Medicine, Stanford University, USA
[2] Cardiovascular Institute, Stanford University, USA
[3] Dept. of Otolaryngology/Head and Neck Surgery, Stanford University, USA

*kimjb@stanford.edu*

Electronic cigarettes pose a serious emerging risk for cardiovascular disease. Epidemiological data show a significant association between exposure to e-cigarettes and burden of coronary artery disease. The pathogenic mechanism is not well understood. The aryl-hydrocarbon receptor (Ahr) pathway has been implicated in tobacco-induced atherosclerosis but its role in e-cigarette exposure is not known.

SMC-lineage tracing mice on ApoE-/- hyperlipidemic background and SMC-specific Ahr knockout (KO) background on high fat diet for 12 weeks were exposed to pod-based electronic-cigarettes (Juul) for 2-4 weeks in a whole body chamber (inExpose, Scireq). The aortic sinus was dissected and digested, FACS was performed to sort for live single cells, then single-cell RNA-Seq and ATAC-seq were performed on the 10X Genomics platform. Analyses were completed with Seurat and Signac tools.

There were total of 20102 cells (WT Control 5282, WT exposed to Juul 9425, and KO exposed to Juul 5395). There was an increase in Cyp1b1 expression in the modulated SMC population, suggesting activation of the Ahr pathway. Also, we found a cluster of SMC that were enriched for markers of the unfolded protein response, including Hspb1, Hsp90aa1, and Cryab. The proportion of modulated SMC to quiescent SMC remained comparable following Juul exposure. The cluster enriched for the UPR pathway was nearly absent in the SMC-specific Ahr KO mice exposed to Juul. The scATAC-seq profiles showed enrichment for motifs of CCAAT/enhancer-binding proteins, and pathways of cytokine production, ECM organization, and cell migration in the clusters of UPR-activated SMC.

Using single-cell genomics, we identified the UPR pathway to be uniquely activated in the SMC-lineage population of atherosclerotic plaque in mice exposed to e-cigarettes, and that this process may be mediated by Ahr.

PO-242

# Study of Cell Composition Changing in Human Coronary Arteries During Atherogenesis

**Anton Markov[1], Diana Sharysh[1*], Olga Savelieva[2], Maria Nazarenko[1]**

[1] Laboratory of Population Genetics, Research Institute of Medical Genetics, Tomsk NRMC, Russian Federation
[2] Cancer Research Institute, Tomsk NRMC, Russian Federation

*sharysh.diana@gmail.com*

Cell composition of atherosclerotic lesions determines consequences during a human lifespan. Here we quantified contractile smooth muscle cells (C-SMCs), macrophage-like smooth muscle cells (M-SMCs), macrophages and endothelial cells (ECs) in atherosclerotic lesions on early and advanced stages by flow cytometry.

Samples of coronary arteries were obtained post-mortem from 5 donors (2 females and 3 males, aged 80±8.7), then were divided into early (up to fatty streaks) stages (N=5) and advanced plaques (N=11). A part of each sample was used for histological analyses with the evaluation of lesion type according to AHA classification. Samples of coronary lesions were enzymatically digested. Cell suspensions were stained with conjugated antibodies to CD45, CD31, CD68, aSMA. Different cell populations were evaluated by flow cytometry (MoFlo XDP, Beckman Coulter). Cell proportions presented as median and interquartile range.

Lesions at early stages were enriched with CD45+ leukocytes (15.7% (11.9; 16.1)%) more than advanced lesions (8.1%, (3.9; 8.5)%) (p=0.003). However, the relative amount of macrophages (CD45+CD68+) were equally represented at different stages and consisted on average 0.5% (0.2; 0.6)% of cells. M-SMCs (CD45-aSMA+CD68+) were discovered in both early and advanced lesions. The relative number of M-SMCs positively correlated with the histological type of lesion (r=0.8, p=0.01). Relative amount of C-SMCs (CD45-aSMA+CD68-) and ECs (CD45-CD31+) were equally represented in early and advanced lesions and made up on average 23.5% (10.3; 35.1)% and 0.6% (0.4; 2.0)% respectively. An average early lesion contained 834000 (482000;1186000) cells per 0,1 g of wet tissue, with viability in suspension after disaggregation of frozen samples 64% (60;68). And advanced plaque consisted from 312000 (52300; 470000) with viability 55% (49; 63).

The relative number of CD45+CD68- leukocytes descends, while the fraction of M-SMCs rises in coronary arteries during atherogenesis in human.

PO-243

# Deficiency of Myeloid PHD Proteins Aggravates Atherogenesis via Macrophage Apoptosis and Paracrine Fibrotic Signaling: Impact for PHD Inhibitors in the Clinic

**Kim Van Kuijk[1*], Jasper Demandt[1], Javier Perales-Patón[2], Thomas Theelen[1], Christoph Kuppe[3], Elke Marsch[1], Jenny De Bruijn[1], Han Jin[1], Marion Gijbels[1,9], Ljubica Matic[4], Barend Mees[5], Chris Reutelingsperger[6], Ulf Hedin[4], Erik Biessen[1,10], Peter Carmeliet[7], Andrew Baker[8], Rafael Kramann[3,11], Leon Schurgers[6], Julio Saez-Rodriguez[2], Judith Sluimer[1]**

[1] Department of Pathology, MUMC+, Netherlands
[2] Institute for Computational Biomedicine, Heidelberg University, Germany
[3] Division Nephrology and Clinical Immunology, RWTH Aachen, Germany

[4] Department of Molecular Medicine and Surgery, Karolinska Institute, Sweden
[5] Department of Vascular Surgery, MUMC+, Netherlands
[6] Department of Biochemistry, MUMC+, Netherlands
[7] Laboratory of Angiogenesis and Neurovascular Link, VIB Leuven, Belgium
[8] BHF Centre for Cardiovascular Sciences, University of Edinburgh, UK
[9] Department of Molecular Genetics, MUMC+, Netherlands
[10] Institute for Molecular Cardiovascular Research, RWTH Aachen, Germany
[11] Department of Internal Medicine, Nephrology and Transplantation, Erasmus Medical Center, Netherlands

*k.vankuijk@maastrichtuniversity.nl*

Atherosclerotic plaque hypoxia is detrimental for macrophage function. Prolyl hydroxylases (PHDs) initiate cellular responses to hypoxia and may therefore govern macrophage function in plaque hypoxia. PHD inhibitor, Roxadustat, is already clinically available for chronic kidney disease patients, however, consequences for cardiovascular diseases are not clear yet.
Myeloid PHD1 knock out (ko) and PHD3ko were created via bone-marrow transfer to Ldlrko mice, PHD2cko was a LysMCre conditional ko model. Mice were fed 0.25% cholesterol diet for 6-12 weeks to induce atherosclerosis.

PHD mRNA and protein levels were expressed by human and murine myeloid cells, i.e. plaque and bone-marrow derived macrophages (BMDM). Aortic root plaque size was augmented 2.6-fold in PHD2cko, and 1.4-fold in PHD3ko, but not in PHD1ko mice compared to controls. These findings were independent of plasma cholesterol levels. Macrophage apoptosis was promoted in PHDcko and PHD3ko mice in vitro and in vivo via the HIF1α-BNIP3 axis. This was shown by pathway analysis of bulk and single cell RNA data of murine BMDMs and plaque macrophages, respectively, and validated by siRNA silencing. Human plaque BNIP3 mRNA associated with carotid plaque necrotic core content, suggesting similar adverse effects in humans. Further, PHDcko plaques – not PHD1 nor PDH3ko – displayed enhanced fibrosis at two different disease stages, and across vascular beds, independent of macrophage function. PHD2cko BMDMs enhanced collagen secretion by fibroblasts in a paracrine manner, while smooth muscle cell function was unchanged. In silico analysis of macrophage-fibroblast communication predicted SPP1 signaling by PHD2cko macrophages to regulate the fibroblast transcriptome, in line with enhanced SPP1 protein content in plaques of PHD2cko mice.

Myeloid PHD2 and PHD3 deficiency enhanced murine plaque growth, macrophage apoptosis and PHDcko activated paracrine collagen secretion by fibroblasts. These adverse vascular effects of myeloid PHDs warrant detailed investigation in vascular side-effects of PHD inhibitors in humans.

PO-244

# PDGF-B Retention Motif Deletion Has Mural Cell-Independent Effects Including Increased Atherosclerotic Plaque Stability and Enhanced Extramedullary Haematopoiesis

**Renée Tillie[1*], Thomas Theelen[1], Kim Van Kuijk[1], Lieve Temmerman[1], Jenny De Bruijn[1], Marion Gijbels[1], Christer Betsholtz[2], Judith Sluimer[1]**

[1] Pathology, Maastricht University, Netherlands
[2] Immunology, Genetics and Pathology, Uppsala University, Sweden

*renee.tillie@maastrichtuniversity.nl*

Causality between microvessel hyperpermeability and atherosclerotic plaque instability remains

to be addressed. Platelet-derived growth factor B (PDGF-B) is a mitogenic, migratory and survival factor. PDGF-B recruits pericytes towards blood vessels through its retention in extracellular matrix. We hypothesized that intraplaque microvessel hyperpermeability could be mimicked with PDGF-B retention motif knockout (PDGF-Bret/ret) mice and exacerbate atherosclerosis.

PDGF-Bret/ret mice crossed with LDLR-/- mice received a 10-week 0.25% cholesterol diet (CD). Aortic root plaque size, necrotic core-, macrophage- (MOMA2), smooth muscle cell- (α-SMA) and collagen content (Sirius red), microvessel density (CD31), intraplaque hemorrhage (Perls) and apoptosis (TUNEL) were assessed. Leukocyte and haematopoietic progenitor cell proliferation was analyzed using 5-ethynyl-2'-deoxyuridine (EdU) and flow cytometry. Macrophage apoptosis susceptibility (Annexin-V) and matrix metalloproteinase (MMP) activity (OmniMMP) were analyzed.
PDGF-Bret/ret plaque size was increased (+41%, p<0.001) as were collagen content (+25%, p<0.01) and fibrous cap thickness (+49%, p<0.05), whilst necrotic core, microvessel density and intraplaque hemorrhage were unexpectedly unaffected. Plaque macrophage content was decreased (-50%, p<0.01). A 2.1-fold increase in TUNEL+ cells in PDGF-Bret/ret plaques (p=0.0149) and increased macrophage apoptosis upon incubation with 7-ketocholesterol (p<0.0001) and oxLDL (p=0.031) in vitro could explain reduced macrophage content. Moreover, decreased macrophage MMP activity (-25%, p=0.0286) could explain increased collagen content in PDGF-Bret/ret mice. In contrast to reduced plaque inflammation, systemic leukocytosis was observed in PDGF-Bret/ret mice on CD only. This was accompanied by decreased, rather than increased, circulating levels of mitogenic PDGF-B (-87%, p<0.0001). EdU injections showed increased splenic proliferation of common myeloid progenitors (CMPs), granulocytes, CD8+ T cells and Ly6Clow monocytes. Moreover, increased EdU incorporation in circulating CD4+ and CD8+ T cells was observed.
PDGF-Bret/ret results in larger but more stable plaques by affecting macrophage viability and function, unrelated to microvessel permeability. Moreover, the deletion drives extramedullary haematopoiesis and subsequent leukocytosis in hypercholesterolemia.

PO-245

# Lysophosphatidic Acid via Smad2 Linker Region Stimulates Migration of Human Vascular Smooth Muscle Cells

**Ying Zhou[1*], Danielle Kamato[1]**

[1] School of Pharmacy, The University of Queensland, Australia

*y.zhou5@uqconnect.edu.au*

Lysophosphatidic acid (LPA) generated during oxidization of low-density lipoprotein is a critical pathogenic factor in the initiation and progression of atherosclerosis. In the early stage of atherosclerosis development, the migration and proliferation of vascular smooth muscle cells (VSMCs) could progress atherosclerosis; in the late stage, VSMCs contribute to the stabilization of atherosclerotic plaques. Therefore, the first objective is to understand the role of LPA on VSMC migration. In addition, transforming growth factor â and its Smad signalling are important for initiation of atherosclerosis and the stability of plaques. Specially, our group have identified the relationship of the distinct linker region of Smad2 with the expression of enzymes involved in the initiation of atherosclerosis. Thus, the second objective is to investigate the role of Smad2 linker region in LPA mediated migration of VSMCs.

Human VSMCs were used as an in vitro model. Western blotting was used to determine the protein expression. Quantitative real time polymerase chain reaction analysis was applied to assess the gene expression.

LPA via its LPAR1 and LPAR5 significantly increased the phosphorylation of Smad2 linker region. LPAR5 stimulates linker region phosphorylation of Smad2 via cross-talking with TGFBR1 (type I transforming growth factor receptor). LPAR1 activates the Smad2 linker region independent of the TGFBR1. The Smad2 linker region phosphorylation is regulated by the PLC and ERK1/2 signalling. LPAR1 initiated mRNA expression of plasminogen activator inhibitor-1 and VSMC migration are associated with Smad2 linker region phosphorylation.

In this project, we provide evidence showing that LPA promotes the progression of atherosclerosis by initiating the migration of VSMCs. In addition, we also deciphered the underlying mechanism as LPAR1/PLC/ERK/Smad2 linker region signal axis. Therefore, we provide a new perspective of the pathological role of LPA in vascular disease settings. These underlying mechanisms may guide the development of a new targeted therapeutic reagent.

PO-246

# Endothelial PTP Mitigates Vascular Inflammation Through USF1/A20 Axis-Mediated NF-κB Inactivation

**Dong Gwang Lee[1], Min Ji Cho[1,2], Jong-Gil Park[1], Jeong-Ki Min[1,2*]**

[1] Biotherapeutics Translational Research Center, Korea Research Institute of Bioscience & Biotechnology, Korea
[2] Department of Biomolecular Science, University of Science & Technology (UST), Korea

*jekmin@kribb.re.kr*

The nuclear factor-κB (NF-κB) signaling pathway plays a critical role in the pathogenesis of numerous vascular diseases. Here, we identified endothelial cell (EC) protein tyrosine phosphatase (PTP) as a novel negative regulator of NF-κB signaling in vascular inflammation.

We established stable human aortic ECs (HUAECs) with knockdown of PTP by PTP-specific shRNA to study the role of PTP in vascular inflammation, and generated Ptp-/- mice by a CRISPR/Cas9 system to determine the pathophysiological role of PTP in atherosclerosis. To further determine the protective role of PTP in vascular inflammation, we generated HUAECs stably overexpressing PTP and transgenic (Ptp eTg) mice that express PTP under the control of the EC-specific Tie2 promoter/enhancer.

shRNA-mediated knockdown of PTP in ECs upregulated the expression of cell adhesion molecules (CAMs) and induced NF-κB-mediated gene transcription. Studies using PTP knockout or transgenic mice demonstrated the protective roles of PTP in inflammatory cytokines-induced vascular inflammation and high-fat high-cholesterol diet-mediated atherogenesis. We found that PTP increased

the transcriptional activity of upstream stimulatory factor 1 by dephosphorylating the S309 residue, inducing A20 gene transcription and inhibiting NF-κB activity.

These results demonstrate that EC PTP inhibits NF-κB-mediated transcription of inflammatory genes through the USF1-mediated A20 signaling axis and may offer a new opportunity for treating vascular inflammatory diseases including atherosclerosis and sepsis.

PO-248

# The Role of Mast Cells in Atherosclerotic Plaque Calcification

**Nikolaos-Taxiarchis Skenteris[1,2], Malin Kronqvist[1], Mariette Lengquist[1], Ilze Bot[3], Ulf Hedin[1], Erik Biessen[2], Ljubica Matic[1*]**

[1] Department of Molecular Medicine and Surgery, Karolinska Institutet, Sweden
[2] Department of Pathology; Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Netherlands
[3] Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, Netherlands

*ljubica.matic@ki.se*

Vascular calcification is a key feature of atherosclerosis and has been associated with major adverse cardiovascular events. Unstable carotid atherosclerotic plaques cause stroke and lesions from those patients are abundant with activated mast cells at the sites of rupture. Recent data from our group showed a statistically significant upregulation of activated mast cells in low calcified whereas resting mast cells were upregulated in high calcified plaques, indicating that mast cells fractions may relate with various aspects of plaque pathology. Our hypothesis is that mast cell fractions associate with key features of plaque vulnerability such as calcification, intraplaque hemorrhage and other immune cell fractions.

Biobank of Karolinska Endarterectomies (BiKE) prospectively enrolls patients (n=1300) treated for carotid atherosclerosis in Stockholm, comprising of BioBank with paraffin-embedded plaque tissues, ImageBank with quantified diagnostic CT images using VascuCap software and DataBank of 100 clinical variables as well as large-scale transcriptomic and proteomic datasets.

Correlation between VascuCAP quantifications and microarray's deconvolution data revealed that activated mast cells were negatively associated with plaque calcification but positively with extracellular matrix. Histological stainings of plaque tissue microarrays demonstrated that mast cells were systematically found in Perls+ regions and positively correlate with CD3+ cells. Deconvolution analysis denoted that activated mast cells correlate with CD4+ cells, NK cells and eosinophils, establishing possible functional cellular associations. Stratification of the results according to patient symptoms showed that activated mast cells were elevated in both symptomatic and asymptomatic patients and increased with severe symptoms of plaque instability. However, patients' medication does not impact mast cell regulation. BiKE transcriptomic and proteomic analysis showed that activated mast cell markers were unregulated in plaques from symptomatic patients.

Systematic enumeration of mast cell fractions in human plaques indicates that activated mast cells associate with increased vulnerability, both when it comes to clinical patient symptoms and morphological plaque features.

PO-249

# HMGB1/RAGE Axis Promotes ROS Generation in Monocytes via MAPK-p47phox Phosphorylation Pathway

**Seung Eun Baek[1,2], Eun Jeong Jang[2], Jong Min Choi[1,2], Eun Yeong Jeon[1,2], Ji On Kim[1,2], Chi Dae Kim[1,2,3*]**

[1] Pharmacology, Department of Pharmacology and BK21 Plus, School of Medicine, Pusan National University, Korea
[2] Science, Gene & Cell Therapy Research Center for Vessel-associated Diseases, Pusan National University, Korea
[3] Medicine, Research Institute for Convergence of Biomedical Science and Technology, PNU Hospital, Korea

*chidkim@pusan.ac.kr*

High mobility group box 1 (HMGB1) mediates oxidative stress-linked pathological processes. However, its role in the HMGB1-induced generation of reactive oxygen species (ROS) in monocytes is still unclear. Thus, this study investigated the source and mechanisms of ROS generation in monocytes stimulated with HMGB1.

Monocytes were cultured using THP-1 cells (a human monocytic leukamia cell line), and stimulated with HMGB1 (100 ng/ml), and ROS production were analyzed by flow cytometry. The expression of p47phox and mitogen-activated protein kinases in HMGB1-stimulated VSMCs was analyzed by Western blots.

Exposure of THP-1 cells to HMGB1 showed an increased production of ROS, which was attenuated by NADPH oxidase inhibitors. Linked to these results, we aimed to the signal transduction pathways involved in the activation of NADPH oxidases. In cultured human THP-1 cells stimulated with HMGB1 (100 ng/ml), ROS production was markedly increased. However, both these effects were markedly attenuated in cells pretreated with the inhibitors of ROS [N-acetyl cysteine (NAC)] and NADPH oxidase [diphenyleneiodonium chloride (DPI) and apocynin (APO)]. Moreover, HMGB1-induced expressions of p47phox phosphorylation were markedly attenuated in MAPKs inhibitors pre-treated cells, and phosphorylation of MAPKs expressions were reduced in receptors for advanced glycation end-product (RAGE)-inhibited cells. Likewise, in RAGE-inhibited cells, HMGB1-induced ROS production was significantly reduced in cells.

Overall, these results indicate that activation of the MAPKs/p47phox cascade plays a central role in HMGB1/RAGE-induced ROS generation and suggests the existence of a ROS inflammatory amplification feedback loop in monocytes. Altogether, these results suggest that RAGE plays a critical role in HMGB1-induced ROS generation in monocytes through activation of NADPH oxidase.

PO-250

# HMGB1 Induces MCP-1 Production in Vascular Smooth Muscle Cells via 5-LO-LTB4 Signaling

**Jong min Choi[1,2], Seung Eun Baek[1,2], Eun Jeong Jang[2], Eun Yeong Jeon[1,2], Ji On Kim[1,2], Chi Dae Kim[1,2,3*]**

[1] Pharmacology, Department of Pharmacology and BK21 Plus, School of Medicine, Pusan National University, Korea
[2] Science, Gene & Cell Therapy Research Center for Vessel-associated Diseases, Pusan National University, Korea
[3] Medicine, Research Institute for Convergence of Biomedical Science and Technology, PNU Hospital, Korea

*chidkim@pusan.ac.kr*

The pro-inflammatory cytokine monocyte chemoattractant protein-1 (MCP-1) plays a major role

in vascular inflammation. However, the active role of vascular smooth muscle cells (VSMCs) in MCP-1 expression in the injured vasculatures is unclear. Given the importance of high mobility group box 1 (HMGB1) in vascular injury and inflammation, this study determined MCP-1 expression in VSMCs exposed to HMGB1, and also evaluated the role of 5-lipoxygenase (5-LO) signaling pathways in HMGB1-induced MCP-1 expression.

VSMCs were ex plant cultured using rat thoracic aorta, and stimulated with HMGB1 (30 ng/ml). The expression of 5-LO and MCP-1 in HMGB1-stimulated VSMCs was analyzed by Western blots. LTB4 and MCP-1 production were determined by ELISA.

In cultured rat aortic VSMCs stimulated with HMGB1 (30 ng/ml), the expression of 5-LO was markedly increased in a dose- and time-dependent manner, as well as production of leukotriene B4. Likewise, MCP-1 expression and production in HMGB1-stimulated VSMCs were markedly increased, which was significantly attenuated in cells treated with zileuton (100 μM), a 5-LO inhibitor as well as in cells deficient of 5-LO. In response to LTB4, MCP-1 expression in VSMCs was increased dose-dependently, which was attenuated in cells treated with U75302 (10 μM), a LTB4 receptor 1 (BLTR1) inhibitor as well as in cells deficient of BLTR1. It was suggested a potential importance of LTB4 in MCP-1 expression in VSMCs.

Based on the results of this study, it was suggested that 5-LO-derived LTB4 produced by HMGB1-stimulated cells increased MCP-1 expression in VSMCs of the injured vasculatures. Thus, the 5-LO-LTB4 signaling axis in VSMCs might serve as a potential target for future therapeutic strategies for vascular inflammation in the injured vasculatures.

PO-252

# Cardioprotective Effects by Ablation of Lysozyme M-positive Cells in a Model of Noise Exposure

**Katelyn Frenis**[1*]

[1] Cardiology, Johannes Gutenberg University, Germany

*Katiefrenis@gmail.com*

Increased leukocyte extravasation in the aortic endothelium leads to a pro-oxidant and pro-inflammatory environment which facilitates the development of endothelial dysfunction and subsequently, atherosclerosis. Moderate hypertension, endothelial dysfunction, and increases in oxidative stress are associated with exposure to the novel cardiovascular risk, noise pollution. We aim to evaluate the role of LysM+ cells in the cardiovascular consequences associated with noise exposure and the possible cardioprotection offered by their ablation.

LysMCre+/-iDTR+/- mice with an inducible diphtheria toxin receptor on LysM+ cells were treated with diphtheria toxin (DTX) for a total of 10 days: 6 days of pre-treatment with toxin, and 4 days of toxin plus noise exposure. Blood pressure was determined by tail cuff method. Endothelial dysfunction was measured with isometric tension recordings of 3mm aortic segments. Protein and gene analysis were carried out using Western Blot, dot blot, and rtPCR. Oxidative stress measurements included dihydroethidium via HPLC and staining with the use of L-NAME. Inflammatory cell infiltration was assessed via FACS.

Ablation of LysM+ cells successfully normalized the blood pressure and endothelial function of mice exposed to 4 days of noise. Noise caused a significant increase in superoxide production in aortic,

cardiac, and cerebral tissue, but the DTX+Noise group was unchanged from control. Oxidative stress markers 3-nitrotyrosine, 4-hydroxynonenol, and malondialdehyde also demonstrate protection from oxidative stress in the DTX+Noise group. Expression of genes NOS3, NOX2, and VCAM-1 was correspondingly normalized in aortic tissue. FACS data confirmed the success of the ablation while protein analysis also demonstrated a reduction in cell recruitment and inflammatory markers.

Ablation of myelomonocytic cells rescued mice from cardiovascular damage, suggesting that these cells play a critical role in the damage incurred upon noise exposure. Stress and inflammation are inextricably linked and this study implies that management of both is a necessary component for cardiovascular health.

PO-255

## Effects of DHA Versus EPA on Adaptive Immunity and Associated Pathways

**Yu Ri Choi[1*], Jaeho Lee[1], Moonjong Kang[2], Dan Bi An[1], Chan Joo Lee[3], Soo-jin Ann[4], Sang-Hak Lee[3]**

[1] Graduate Program of Science for Aging, Graduate School of Yonsei University, Korea
[2] Department of Biostatistics and Computing, Graduate School of Yonsei University, Korea
[3] Division of Cardiology, Department of Internal Medicine, Yonsei University College of Medicine, Korea
[4] Integrative Research Center for Cerebrovascular and Cardiovascular Disease, Yonsei University College of Medicine, Korea

*choiyr100@yuhs.ac*

Omega-3 fatty acids have recently been shown to have beneficial effects on clinical outcomes. However, our understanding of the differential effects between these fatty acids is still incomplete. Here, we aimed to evaluate the effects of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) on T-helper type 1 (Th1) cell responses and to identify the pathways associated with these responses.

Naïve CD4+ T cells were cocultured with bone marrow-derived dendritic cells (DCs) in the presence or absence of palmitate (PA), DHA, or EPA, and the effects of these fatty acids on the differentiation, proliferation, and cytokine secretion were evaluated. These effects were also assessed in a DC-independent model. Microarray analyses were performed to identify genes differentially expressed by Th1 cells treated with DHA or EPA.

In the DC-dependent model, the number of differentiated interferon (IFN)-γ-positive cells was lower following DHA or EPA treatment. DHA and EPA inhibited the secretion of IFN-γ whereas only DHA reduced that of tumor necrosis factor (TNF)-α The two fatty acids increased interleukin-2 secretion. These effects were accompanied with reduced expression of major histocompatibility complex II on DCs. In the DC-independent model, DHA and EPA reduced Th1 cell differentiation, but only EPA markedly inhibited TNF-α secretion. PA did not have much effect on the Th1 cell responses induced by DHA or EPA. Microarray analysis identified pathways involved in inflammation, immunity, metabolism, and cell proliferation. Moreover, DHA and EPA inhibited Th1 cells through the regulation of diverse pathways and genes, including Igfi and Cpt1a.

Collectively, our results showed that DHA and EPA had similar inhibitory effects on Th1 cells. However, each of the fatty acids also had some distinct effects on specific cytokine secretion according to the presence of DCs.

PO-256

# BM-Specific Tkk1 Deficiency Alleviates Lesion Formation During Atherogenesis

**Tae Kyeong Kim[1], Sejin Jeon[1], Goo Taeg Oh[1*]**

[1] Life Science, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

Atherosclerosis is representative chronic inflammatory disease, mainly triggered by infiltration mediated pro-inflammatory responses of various leukocytes. TKK1 is known for stimulation dependent inducible expression in acute immune responses and mainly expressed by immune cells, including myeloid cells and T lymphocytes. However, the association and function of TKK1 in atherosclerosis remain unknown.

Primarily cultured bone marrow (BM)-derived macrophages (BMDM) and PBMCs were used to measure the expression of TKK1 under the various stimulation. Lesion development was assessed in a model of bone-marrow transplantation (BMT) by injecting BM of $ApoE^{-/-}$ mice and $Tkk1^{-/-}ApoE^{-/-}$ mice to $ApoE^{-/-}$ mice, to identify the deletion effect of Tkk1 only in BM-derived cell types. Mice were fed with normal chow diet for 28 weeks after BMT and whole aortas were collected for en face or flow cytometry and aortic sinus were used to analyze the characteristics of lesion.

We first found that the expression of TKK1 is remarkably upregulated in classically activated macrophages (CAMs) upon stimulation, but not in alternatively activated macrophages (AAMs), both in transcription and translation levels. Also, TKK1 was increased in $CD11c^+$ CAMs compared to unstimulated BMDMs and co-stained with $CD11c^+$ CAMs in atherosclerotic aorta specimen. Interestingly, BM-specific Tkk1 deficiency showed a tendency of reduced plaque development with decreased necrotic core formation and led to a decrease in infiltrated $CD11c^+$ CAMs in the atherosclerotic aortas. In line with this, Tkk1 deficient monocytes caused decreased adhesion on endothelial cells and further Tkk1 deficient CAMs showed less inflammatory phenotypes under the atherosclerotic conditions.

We demonstrated that TKK1 is mainly expressed by CAMs and may play an important effector role in atherogenic conditions through regulating the characteristics of lesional macrophages.

PO-257

# Apelin-13 (A13) Reduces Inflammatory Response of Endothelial Cell

**Hongryeol Park[1], Sathish Kumar Maney[1], Hyun-Woo Jeong[1], Ralf Adams[1*]**

[1] Department of Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Germany

*ralf.adams@mpi-muenster.mpg.de*

A13 is an agonist of apelin which is suggested as a potential drug for reducing blood pressure by inhibiting Angiotensin II (Ang) induced vasocontraction via allosteric trans inhibition of the receptor. However, recent single cell sequencing analysis revealed that Angiotensin II type I receptor is expressed from a mural cells rather than endothelial cells while Apelin receptor(Aplnr) is expressed in the brain and lung. Several papers report that A13 ameliorate inflammation in brain and lung. Even though the whole mark of the inflammation was assessed in these papers, the detailed mechanisms

including gene expression changes of Aplnr expressing endothelial cells are not fully addressed.

By utilizing Apln-Creert2 / Aplnr-Creert2; R26i-mT-mG mice, Apln and Aplnr expressing cells are defined. A13 effect on endothelial cells was assessed in vitro and in vivo, using HUVEC with shear stress and mice inflammation model.

Aplnr is dominantly expressed from endothelial cell especially postcapillary venule and capillary vein. Aplnr promoter derived GFP intensity show an opposite pattern with ICAM1 staining intensity during brain development. A13 treatment inhibits shear-induced inflammatory-related gene expression including ICAM1 in HUVEC flow experiment. Furthermore, A13 reduces ICAM1 expression in the inflammation mice model.

Leukocyte recruiting and infiltration to the tissue from the blood is an essential process of inflammation. The endothelial cell which forms blood vessels has an important role in these processes by secreting cytokines and expressing adhesion molecules. These inflammation-related genes are known to be upregulated by shear stress. we suggest A13 is a potential drug for inflammation which targets endothelial cell. By reducing endothelial-derived inflammatory signaling, it can prevent the development of acute inflammation to chronic.

PO-258

# Ninjurin-1 Deficient Macrophages Promote Inflammation and Atherosclerosis

**Sejin Jeon[1], Tae Kyeong Kim[1], Goo Taeg Oh[1*]**

[1] Department of Life Sciences, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

It is well known that activated macrophages produce many pro-inflammatory molecules, which play a crucial role in the development of cardiovascular diseases. In contrast, there is a paucity of knowledge regarding macrophage-derived anti-inflammatory molecules, their mechanism of action and potential as therapeutics.

Ninjurin-1 (nerve injury-induced protein [Ninj1]) expression and atherosclerosis progression were assessed in atherosclerotic aortic tissue from Apolipoprotein e-deficient (Apoe-/-) mice. Apoe-/- mice lacking systemic Ninj1 expression (Ninj1-/-Apoe-/-) were generated to assess functional effects of Ninj1. Bone marrow (BM) transplantation was also used to generate low-density lipoprotein receptor-deficient (Ldlr-/-) mice that lack Ninj1 specifically in BM-derived cells. Mice were fed a Western diet (WD) for 5 to 23 weeks, and atherosclerotic lesions were investigated.

It has been shown that Ninj1 protein is predominantly abundant in alternatively activated macrophages (AAMs) although Ninj1 transcripts have been identified in heterogeneous macrophages of atherosclerotic aortas. On the basis of these observations, we hypothesized that AAM-expressed Ninj1 affects atherosclerosis in vivo. Ninj1-deficient macrophages promoted pro-inflammatory gene

expression by activating mitogen-activated protein kinase (MAPK) and inhibiting the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway. Notably, suppression of PI3K by treatment with LY294002 has been found to promote a shift to a pro-inflammatory phenotype by enhancing phosphorylation of the MAPKs, Erk1/2 and p38. Whole-body and BM-specific Ninj1 deficiencies significantly increased monocyte recruitment and macrophage accumulation in atherosclerotic lesion through elevated macrophage-mediated inflammation.

The absence of Ninj1 exerts a pro-inflammatory effect under pathogenic conditions by enhancing MAPK signaling and blocking the PI3K/Akt pathway in macrophages. Ninj1 deficiency-mediated inflammation is accelerated the progression of atherosclerosis and results in increased macrophage accumulation in lesions.

PO-259

# Anti-Inflammatory Actions of Soluble Ninjurin-1 Protects Against Atherosclerosis

**Sejin Jeon[1], Tae Kyeong Kim[1], Goo taeg Oh[1*]**

[1] Department of Life Sciences, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

Macrophages produce many inflammation-associated molecules, released by matrix metalloproteinases (MMPs), such as adhesion molecules as well as cytokines, which play a crucial role in atherosclerosis. In this context, we investigated the relationship between Ninjurin-1 (nerve injury-induced protein [Ninj1]), a novel MMP9 substrate, expression and atherosclerosis progression.

Ninj1 expression and atherosclerosis progression were assessed in atherosclerotic aortic tissue and serum samples from coronary artery disease patients and healthy controls as well as polipoprotein e-deficient (Apoe-/-) mice. The anti-inflammatory role of Ninj1 was verified by treating macrophages and mice with the peptides Ninj11-56 (ML56) and Ninj126-37 (PN12), which mimic the soluble form of Ninj1 (sNinj1).

Our in vivo results conclusively showed correlation between soluble form fo Ninj1 (sNinj1), which is primarily released by aortic macrophages, and the extent of human and mouse atherosclerotic lesions. Macrophage Ninj1 was directly cleaved by matrix metalloproteinase 9 (MMP9) to generate a soluble form that exhibited anti-atherosclerotic effects, as assessed in vitro and in vivo. Treatment with the sNinj1-mimetic peptides, ML56 and PN12, reduced pro-inflammatory gene expression in human and mouse classically activated macrophages (CAMs), thereby attenuating monocyte transendothelial migration. Moreover, continuous administration of mPN12 alleviated atherosclerosis by inhibiting the enhanced monocyte recruitment and inflammation characteristic of this disorder in mice, regardless of the presence of Ninj1.

Ninj1 is a novel MMP9 substrate in macrophages, and sNinj1 is a secreted atheroprotective protein that regulates macrophage inflammation and monocyte recruitment in atherosclerosis. Moreover, sNinj1-mediated anti-inflammatory effects are conserved in human macrophages and likely participate in human atherosclerosis.

PO-260

# Anti-Inflammatory Effects of Bilobetin for the Treatment of Various Inflammatory Conditions: Molecular Mechanistic Study Through Data Analysis of Current

**Dinesh Kumar Patel[1*], Kanika Patel[1]**

[1] Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Uttar Pradesh, India

*dkp.iitbhu@gmail.com*

Flavonoid are important phytochemical found to be present in various natural sources and possess anti-inflammatory activity through different mechanism such as antioxidative properties, cyclooxygenase and lipoxygenase inhibition. Bilobetin is a flavonoidal compound found to be present in the male flowers of Ginkgo biloba. Phospholipase A2 (PLA2) regulates eicosanoid and platelet-activating factor production. It also plays an important role in the regulation of critical mediators in inflammatory diseases.

NO synthase (iNOS) and sPLA2 inhibitors are useful for inhibiting cytokine and NO production and could be used for various forms of inflammatory diseases. In order to know the therapeutic potential of Bilobetin for the treatment of inflammatory disorders of Human being, here in the present investigation various scientific work datas have been analyzed and presented. Detail Pharmacological activities of Bilobetin have been investigated for their anti-inflammatory potential through data analysis. Effect of Bilobetin on tumor necrosis factor-α, IL-6, PGE2 and NO molecule have been also investigated through data analysis of different scientific works. Bilobetin have been evaluated for their anti-inflammatory potential in the lipopolysaccharide-induced RAW264.7 macrophages and found to exhibited significant inhibitory effects on tumor necrosis factor-α, IL-6, PGE2 and NO. In another study, bilobetin inhibit Phospholipase A2 (PLA2) activity in lipopolysaccharide (LPS)-stimulated Raw264.7 macrophages. Bilobetin shut down the production of nitrite in the macrophages and reduced the expression of COX-2 protein. In another study, effects of flavonoids on nitric oxide production from lipopolysaccharide-induced macrophage cell line (RAW 264.7) have been also investigated and found that Bilobetin inhibit NO production.

From the data analysis of various scientific research works Bilobetin exhibited significant anti-inflammatory activity and could be used as better candidates for the development of newer and effective anti-inflammatory agents in the future.

# 3. Vascular Functions

PO-303

## Role of MMP14-Driven Tie2 Shedding in Sepsis

**Temitayo Idowu[1], Valerie Etzrodt[1], Hermann Haller[1], Sascha David[1*]**

[1] Nephrology and Hypertension, Hannover Medical School, Germany

*David.Sascha@mh-hannover.de*

Endothelial Tie2 signaling plays a pivotal role in vascular barrier maintenance. Matrix metalloprotease (MMP) 14 mediated Tie2 ectodomain shedding has recently been recognized as a possible mechanism for Tie2 downregulation in sepsis. Here, we seek to identify Tie2 ectodomain cleavage site(s) and test the therapeutic potential of selectively inhibiting MMP14 with regards to Tie2 shedding and functional consequences in vitro in endothelial cells and in vivo in murine sepsis.

Potential Tie2 cleavage sites were identified using an in silico approach followed by mass spectrometry analysis. The putative sites were further validated by site-directed mutagenesis. We carried out in vitro and in vivo, functional studies using an affinity matured exosite antibody to MMP14 termed E2C6 or IgG control. To investigate the ultimate clinical relevance, we performed a Kaplan-Meier survival analysis in a mouse model of sepsis (i.e. cecal ligation & puncture (CLP)).

Recombinant MMP14 cleaved Tie2 at three distinct sites within its Fibronectin type-III domain (FN III) and site-directed mutagenesis confirmed their relevance (up to 91% reduction in shedding). Consistently, therapeutic blockade of MMP14 (with E2C6) both in vitro and in vivo upon stimuli resulted in an 84% and 37% decrease in Tie2 shedding (p=0.0017 and 0.0006). Protection of Tie2 shedding had downstream effects on the expression of intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1. Also, E2C6 dampened endotoxemia induced cytokine release and the local infiltration of inflammatory GR1+ neutrophils (p<0.0056). Treatment with the E2C6 after the induction of CLP sepsis improved survival both in a pre-treatment (38%, p=0.0092) and a rescue approach (33%, p= 0.0264).

We identified and confirmed three distinct MMP14-driven Tie2 ectodomain cleavage sites. Blocking the pathological shedding of Tie2 using an MMP14 antibody exerts barrier protective effects making it a potential therapeutic target in sepsis.

PO-305

## Antimalarial Drug Compound 07 Blocks Endothelial Barrier Disruptions in a Model of Diabetic Retinopathy.

**Minyoung Noh[1], Young-Guen Kwon[1*], Haiying Zhang[1], Hyejeong Kim[1]**

[1] Biochemistry, Yonsei University, Korea

*ygkwon@yonsei.ac.kr*

Diabetes is a serious health problem worldwide. The prevalence of diabetes increases the risk of serious diabetes complications. Diabetic retinopathy (DR) is one of the complications of diabetes and is the leading cause of blindness in the working group. The vascular barrier required for normal retinal function is compromised in diabetic

retinopathy, causing retinal vascular leakage. Vision damage caused by DR remains a serious health problem worldwide. Recently, drug repositioning is promising as an effective strategy for finding new indications for existing drugs. Its strategy is much more efficient, cheaper and without risk than De novo drug discovery and development.

In this study, we discovered compound 07, known as an anti-malaria drug, through drug repositioning and evaluated the ability of compound 07 as a drug to assess vascular leakage.

Treatment with compound 07 demonstrated conserved barrier function by inhibiting VEGF-induced leakage in transwell permeability assays. Compound 07 inhibited the linear pattern of VEGF-induced destruction at the cell border. It also blocked the formation of VEGF-induced actin stress fibers and stabilized cortical actin rings in endothelial cells. In addition, the compounds inhibited vascular leakage in a mile assay for in vivo efficacy assessment. It also significantly reduced retinal leakage in a diabetic retinopathy mouse model.

Taken together, our findings suggest a potential therapeutic utility of Compound 07 for vascular leak disease.

PO-306

# PLVAP Is Essential for the Maturation and Maintenance of Capillaries and Large Vessels in the Choroid

**Soo Jin Kim[1,2], Sang A. Kim[1,2], Yeong A. Choi[1], Yoshiaki Kubota[3], Gou Young Koh[4], Do Young Park[1], Junyeop Lee[2*]**

[1] Department of Opthalmology, YeungNam University, College of Medicine, Korea
[2] Department of Opthalmology, Asan Medical Center, University of Ulsan, College of Medicine, Korea
[3] Department of Anatomy, Keio University School of Medicine, Japan
[4] Center for Vascular Research, Institute for Basic Science (IBS), Korea

*jleeamc@gmail.com*

Endothelial fenestration is a small opening for exchanging nutrients and metabolites. Plasmalemmal vesicle-associated protein(PLVAP) forms stomatal and fenestral diaphragms of caveolae and fenestration. Here, we investigated the structural and functional significance of PLVAP in endothelial fenestration mainly in choriocapillaris, a specialized fenestrated capillary in the eye.

We used tamoxifen-inducible endothelial cell-specific PLVAP conditional knockout mice(VE-cadherin-Cre-ERT2: PLVAPflox/flox; PLVAPiΔEC) in C57BL/6 background. We deleted PLVAP at P1-5 during maturation or deleted it at adult to evaluate the stability and maintenance. We also re-expressed PLVAP in PLVAPiΔEC. We used ultra-widefield (UWF) angiography and optical coherence tomography (OCT), electron microscopy as well as immunostaining and in vitro studies. Human choroids were harvested from donor eyes.

Although the most of choroid vasculature is normally developed before birth, the postnatal and adult PLVAPiΔEC showed abnormal endothelial ul-

trastructure and reduced number of diaphragmed fenestrations in choriocapillaris. UWF angiography and OCT also showed alteration and retardation of maturation in retinal and choroidal large vessels often with vascular leakage in PLVAPiΔEC and showed improperly remodeled and showed dilation in choriocapillaris. In addition, PLVAPiΔEC induced noticeable increase of caveolae in choriocapillaris with cloudy capillary lumen and displayed thinner choroidal layers as well as disrupted outer blood-retinal barrier (BRB). Meanwhile, in human eyes, expression of PLVAP in choroid was reduced in the aged donor compared to the young.

The loss of PLVAP not only defect the choriocapillaris fenestration but also induce abnormal remodeling of large choroidal vessels. Disrupted fenestrated structure of choriocapillaris and consequent outer BRB breakdown in PLVAP deficient mice demonstrate insufficient metabolic support to retina, which can develop to a severe visual impairment. We conclude that PLVAP is essential for the maturation and maintenance of fenestrated capillaries as well as large vessels in the choroid.

PO-307

# Dll4 Suppresses Transcytosis for Arterial Blood-Retinal Barrier Homeostasis

**Jee Myung Yang[1,2], Chan Soon Park[2], Soo Hyun Kim[2], Seoungyeol Park[2], Il-Kug Kim[4], Pilhan Kim[2], Young Seok Ju[2], Akiyoshi Uemura[3], Junyeop Lee[1], Injune Kim[2*]**

[1] Department of Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Korea
[2] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Korea
[3] Department of Retinal Vascular Biology, Nagoya City University Graduate School of Medical Sciences, Japan
[4] Department of Plastic and Reconstructive Surgery, Yeungnam University College of Medicine, Korea

*injunek@kaist.ac.kr*

While tight junction (TJ) has long been considered to be responsible for vascular barrier in central nervous system, suppressed transcytosis in endothelial cells is now emerging as a complementary mechanism. Whether transcytosis regulation is independent of TJ and its dysregulation dominantly causes diseases associated with edema remain elusive. Dll4 signaling is important for various vascular contexts, but its role in the maintenance of vascular barrier in central nervous system remains unknown. Therefore, our study aim to find a TJ-independent regulatory mechanism selective for transcytosis and identify its dysregulation as a cause of pathological leakage.

We studied transcytosis in the adult mouse retina with low vascular permeability and employed a hypertension-induced retinal edema model for its pathological implication.

Both antibody-based and genetic inactivation of Dll4 or Notch1 induce hyperpermeability by increasing transcytosis without junctional destabilization in arterial endothelial cells, leading to

nonhemorrhagic leakage predominantly in the superficial retinal layer. Endothelial Sox17 deletion represses Dll4 in retinal arteries, phenocopying Dll4 blocking-driven vascular leakage. Ang II (angiotensin II)–induced hypertension represses arterial Sox17 and Dll4, followed by transcytosis-driven retinal edema, which is rescued by a gain of Notch activity. Transcriptomic profiling of retinal endothelial cells suggests that Dll4 blocking activates SREBP1 (sterol regulatory element- binding protein 1)-mediated lipogenic transcription and enriches gene sets favorable for caveolae formation. Profiling also predicts the activation of VEGF (vascular endothelial growth factor) signaling by Dll4 blockade. Inhibition of SREBP1 or VEGF-VEGFR2 (VEGF receptor 2) signaling attenuates both Dll4 blockade–driven and hypertension-induced retinal leakage.

In the retina, Sox17-Dll4-SREBP1 signaling axis controls transcytosis independently of TJ in superficial arteries among heterogeneous regulations for the whole vessels. Uncontrolled transcytosis via dysregulated Dll4 underlies pathological leakage.

PO-308

# Superoxide Dismutase 2 Deficiency Disrupts Endothelial Barrier Function

**Atinuke Dosunmu-Ogunbi[1,2], Scott A Hahn[1], Michael Reynolds[1], Adam J Case[3], Adam C Straub[1,2*]**

[1] Pittsburgh Heart, Lung, Blood and Vascular Medicine Institute, University of Pittsburgh, USA
[2] Department of Pharmacology and Chemical Biology, University of Pittsburgh, USA
[3] Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, USA

*astraub@pitt.edu*

Superoxide dismutase 2 (SOD2) catalyzes the dismutation of superoxide to hydrogen peroxide in the mitochondria. Decreased expression of SOD2 has been associated with vascular dysfunction. The objective of this study was to investigate SOD2 deficiency and vascular permeability.

SOD2 protein expression in human lung tissue was detected using immunofluorescent staining. The morphology of endothelial cells was observed by transmission electron microscopy (TEM). In vivo permeability was measured using Evans Blue dye. siRNA was used to silence SOD2 mRNA (siSOD2) in human pulmonary microvascular endothelial cells (hPMVECs). Protein expression of TOM20 as well as Mitotracker staining was used to quantify mitochondria. Mitochondria respiration was measured using a Seahorse Analyzer. In vitro permeability was measured using electric cell impedance sensing (ECIS) and a transwell assay. L-NG-Nitro arginine methyl ester (LNAME) at a concentration of 100 μM was used for analysis of the relationship between nitric oxide synthase inhibition and permeability.

Immunofluorescent staining indicated a decrease in SOD2 protein expression in sickle cell disease patients. An endothelial cell specific SOD2 knock-

out (EC SOD2 KO) mouse model had increased Evans Blue extravasation into the lungs and kidneys. TEM also indicated disrupted pulmonary endothelial junctions in EC SOD2 KO mice. In siSOD2 hPMVECs, there was an increase in baseline and maximum respiration as well as spare capacity, but no changes in ATP-linked oxygen consumption or mitochondria quantity. Permeability was increased in siSOD2 hPMVECs. Pretreatment with LNAME reversed changes observed in permeability as well as mitochondria respiration.

In summary, these data have shown for the first time that SOD2 is functionally involved in maintaining endothelial barrier stability and that SOD2 induced changes in permeability are sensitive to nitric oxide. Future directions will be aimed towards further investigation of the consequences of endothelial SOD2 depletion in SCD and possible therapeutic treatment with LNAME using mouse models.

PO-309

# Vascular Endothelial Integrity Affects the Severity of Enterovirus-Mediated Cardiomyopathy

**Jin-Ho Park[1], Ha-Hyeon Shin[1], Eun-Seok Jeon[3], Kirk U. Knowlton[2], Byung-Kwan Lim[1*]**

[1] Department of Biomedical Science, Jungwon University, Korea
[2] Intermountain Heart Institute, Intermountain Medical Center, USA
[3] Division of Cardiology,, Samsung Medical Center, Korea

*bklim@jwu.ac.kr*

Coxsackievirus and adenovirus receptor (CAR) is present in epithelial and vascular endothelial cell junctions. We have previously shown a hemorrhagic phenotype in germ-line CAR knock-out mouse embryos; we have also found that CAR interacts with ZO-1 and β-catenin. However, the role of CAR in vascular endothelial junction permeability has not been proven.

To understand the roles of CAR in the vascular endothelial junctions, we generated endothelium-specific CAR knockout (CAR-eKO) mice. We observed the vascular permeability and virus infection in CAR-eKO mice.

In the absence of CAR, the endothelial cell layer showed an increase in transmembrane electrical resistance (TER, Ω) and coxsackievirus permeability. Evans blue dye and 70 kDa dextran-FITC were delivered by tail vein injection. We observed increased vascular permeability in the hearts of adult CAR-eKO mice compare with wild type (WT) mice. There was a marked increase in monocyte and macrophage penetration into the peritoneal cavity caused by thioglycolate-induced peritonitis. We also found that CAR ablation in endothelial cells increased

Coxsackievirus B3 (CVB3) induced myocarditis in murine model. Tissue virus titers were significantly higher in CAR-eKO mice compare with WT. Moreover, CVB3 was detected in the brain of CAR-eKO mice. Endothelial CAR deletion affects to the expression of major endothelial junction proteins, such as cadherin and PECAM-1 in the cultured endothelial cells as well as liver vessel.

We suggested that CAR expression is required for normal vascular permeability and endothelial tight junction homeostasis. Furthermore, CVB3 organ penetration and myocarditis severities were depended on the endothelial CAR level.

PO-310

# Sox17 Deficiency Promotes Hypertensive Blood-Brain Barrier Disruption

**Soo Hyun Kim[1], Tae Wook Noh[1], Chan Soon Park[1], Jee Myung Yang[1], Injune Kim[1]***

[1] Graduate School of Medical Science and Engineering, KAIST, Korea

*injunek@kaist.ac.kr*

Although hypertension is a well-known risk factor of various brain diseases associated with BBB disruption, which brain regions are affected by systemic hypertensive stress and how chronic hypertension disrupts BBB and subsequently advances to cerebrovascular diseases with brain dysfunction remain to be addressed.

A hypertensive mouse model was generated through chronic angiotensin II (Ang II) infusion. Endothelial Sox17 deletion in adult mice was achieved by tamoxifen-induced conditional genetic inactivation. BBB impairment was evaluated by assessing leakage of infused tracers and cerebral MRI. EEG recording was used to check brain function.

While 12-week Ang II infusion elicited modest cerebrovascular leakage in control mice, Ang II infusion of only 4 weeks provoked substantial vascular leakage primarily in the thalamus of mutant mice lacking endothelial Sox17. Ang II infusion induced destabilized tight junctions and increased transcytosis in Sox17-deficient thalamic endothelial cells (ECs), leading to non-hemorrhagic vascular leakage and increased theta wave activity without detectable edema, inflammation, amyloid-β accumulation, or tauopathy in the brain. When combined with endothelial Sox17 deficiency, prolonged (12-week) Ang II infusion induced persistent fluid leakage and further cerebral microbleeds in the thalamus, one of the areas most frequently affected by hypertensive intracerebral hemorrhage (ICH). Interestingly, Ang II infusion led to noticeable expression of p16INK4a, a cardinal molecule of senescence, in Sox17-deficient thalamic vessels. Moreover, p16ink4a co-deletion in ECs suppressed vascular leakage and cerebral microbleeds, suggesting premature endothelial senescence as a pathophysiological mechanism underlying hypertension-induced BBB disruption.

Our findings establish that BBB integrity in the thalamus withstands hypertensive stress in a Sox17-dependent manner. Sox17 deficiency, a genetic defect, promotes premature hypertension-driven non-hemorrhagic leakage and subsequent cerebral microbleeds, which could precede hypertensive ICH. Importantly, even a subclinical level of cerebrovascular leakage caused by hypertension could impair brain function without manifesting evidence of cerebral edema, neuroinflammation, or pathologic protein accumulation.

PO-311

# Multimodal Evaluation of IRBP-Induced Mouse Model of Experimental Autoimmune Vasculitis of the Retina

**Jee Myung Yang[1,2], KyungA Yun1, Jehwi Jeon[2], Pilhan Kim[2], Joo Yong Lee[1*]**

[1] Department of Ophthalmology, Asan Medical Center, University of Ulsan College of Medicine, Korea
[2] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Korea

*jylee.retina@gmail.com*

To characterize the anatomical and functional phenotypes of the experimental autoimmune retinal vasculitis model induced by interphotoreceptor retinoid-binding protein (IRBP).

IRBP or vehicle was systemically administered to adult C57BL/6 mice. Fundus photography, optical coherence tomography (OCT), intravital imaging using different sizes of tracers, and electroretinogram (ERG) were performed at 1, 2, and 3 weeks of the post-immunization period. Histopathologic grading of photoreceptor damage, immune cell infiltration, and retinal vasculitis were performed to characterize the degree of reaction caused by vasculitis.

IRBP-immunized mice exhibited perivascular sheathing and vitritis on fundus photography, and OCT. Intra-vital imaging with dextran tracer showed signs of obstructive vasculitis. Histopathologic grading revealed significant damage of the photoreceptor, granulomatous infiltration of the retina, and choroid accompanied by a significant degree of perivasculitis.

We characterized the histopathological and functional characteristics of the autoimmune retinal vasculitis model. Our model is a robust and reproducible tool for recapitulating human retinal vasculitis. Our data may serve as a basis for the interpretation of the results of future studies involving retinal vasculitis.

PO-313

# Nox Inhibitor Protects Primary Human Brain Microvascular Endothelial Cells Against Methamphetamine-Induced Blood-Brain Barrier Dysfunction

**Ji Hae Seo[1*], Jong Su Hwang[1]**

[1] Biochemistry, School of Medicine, Keimyung University, Korea

*seojihae7@gmail.com*

Oxidative stress is a well-known factor for vascular dysfunction under various pathological conditions associated with brain. In this study, the protective effect of GKT136901, a NOX1/4 inhibitor, against METH-induced BBB dysfunction was investigated.

Primary human brain microvascular endothelial cells (HBMECs) were used as an in vitro BBB model. HBMECs were treated with GKT136901, followed by METH exposure for 24 h. The generation of reactive oxidative species (ROS) was measured using 2′,7′–dichlorofluorescin diacetate (DCF-DA) staining. To examine the BBB function, paracellular permeability of HBMEC monolayer was measured using FITC-labeled dextran. To evaluate structural properties of BBB in HBMECs, tight junction (TJ), adherent junction (AJ), and cytoskeletal proteins

were stained and analyzed by confocal microscopy.

METH treatment rapidly increased ROS generation in HBMECs but GKT136901 treatment inhibited METH-induced ROS generation. Although METH increased the permeability of HBMEC monolayer, this effect was abolished upon GKT136901 treatment. Following METH exposure, the proteins Zonula occludens-1 (ZO-1) and vascular endothelial cadherin (VE-cadherin) were translocalized from the cell membrane to the cytoplasm, thereby destroying intercellular tight junction (TJ) and adherent junction (AJ) structures, which were ameliorated upon GKT136901 treatment. METH exposure altered the cellular morphology of HBMECs and induced stress fiber formation. However, GKT136901 prevented METH-induced morphological and cytoskeletal changes in HBMECs.

In conclusion, GKT136901 could be considered as a promising reagent for preserving BBB integrity against METH exposure and NOX1/4 might be a new therapeutic target for protecting brain function against various CNS diseases.

PO-314

## HDAC6 Selective Inhibitor, Tubastatin a Protects Blood-Brain Barrier Against the Methamphetamine Abuse.

**Ji Hae Seo[1*], Jong Su Hwang[1]**

[1] Biochemistry, School of Medicine, Keimyung University, Korea

*seojihae7@gmail.com*

Methamphetamine (METH) is the one of the most widely abused drugs with a detrimental impact on public health globally. Recent studies show that METH develops various neurodegenerative diseases. It is well documented that METH has neurotoxic effects on central nervous system (CNS) but METH-induced Blood-Brain Barrier (BBB) disruption have not been reported. Here, we found that METH disturbed endothelial function and that HDAC6 plays an important role in METH-induced BBB disruption.

Primary human brain microvascular endothelial cells (HBMECs) were used as an in vitro BBB model. HBMECs were treated with Tubastatin A, followed by METH exposure for 24 h. . To examine the BBB function, paracellular permeability of HBMEC monolayer was measured using FITC-labeled dextran. To evaluate structural properties of BBB in HBMECs, tight junction (TJ), adherent junction (AJ), and cytoskeletal proteins were stained and analyzed by confocal microscopy.

In this study, HDAC6 selective inhibitor, Tubastatin A (Tub A) alleviated the METH-induced endothelial hyper-permeability. Tub A also ameliorated the METH-induced redistribution of tight junction protein (ZO-1) and adhesion molecule (VE-cadherin), which have very significant role in maintaining BBB function. Furthermore, we demonstrated that Tub A inhibited the formation of stress fibers, leading to morphological change in endothelial cells.

Thus, our data suggest that targeting HDAC6 could be a suitable strategy to recover the METH-induced BBB disruption.

PO-315

# Reactive Species Oxygen Scavenger Protects the Blood-Brain Barrier Against Methamphetamine Exposure

**Ji Hae Seo[1*], Jong Su Hwang[1]**

[1] Biochemistry, School of Medicine, Keimyung University, Korea

*seojihae7@gmail.com*

Methamphetamine (METH) is a powerful psychostimulant that causes serious health problems worldwide due to the imprudent abuses. Recent studies have suggested that METH has deleterious effects on blood-brain barrier (BBB). Moreover, there are a few studies about mechanisms that METH-induced oxidative stress results in BBB dysfunction. Thereby, we explored whether N-tert-butyl-α-phenylnitrone (PBN) has protective effects on BBB function against METH exposure in primary human brain microvascular endothelial cells (HBMECs).

The levels of intracellular reactive oxygen species (ROS) was monitored by DCF-DA staining. BBB function/integrity were assessed by permeability assay and transendothelial electrical resistance (TEER) assay in HBMECs upon METH exposure. BBB structural properties were analyzed by immunostaining of junction proteins and cytoskeleton in HBMECs.

METH significantly increased ROS generation in HBMECs. Pretreatment of PBN decreased METH-induced ROS production. METH exposure elevated the paracellular permeability and reduced the monolayer integrity, which were reversed by PBN treatment. METH treatment changed the cellular localization of the tight junction (zonula occludens-1, ZO-1) and adherens junction (vascular endothelial cadherin, VE-cadherin) from membrane to cytoplasm. Furthermore, METH induced cytoskeletal reorganization through formation of robust stress fibers. METH-induced junctional protein redistribution and cytoskeletal reorganization were attenuated by PBN treatment.

Our results suggest that PBN might be a therapeutic reagent in METH-induced BBB dysfunction by alleviating excess ROS generation.

PO-316

# Medicinal Importance of Asiaticoside for the Treatment of Oxidative Stress Induced Vascular Disorders: A Phytotherapeutical Approach with Molecular Study

**Dinesh Kumar Patel[1*], Kanika Patel[1]**

[1] Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Uttar Pradesh, India

*dinesh.patel@shiats.edu.in*

Centella asiatica is medicinal herbs mainly known for their memory enhancing potential in the Ayurvedic medicine and commonly cultivated in the Sri Lanka and India. Asiaticoside is one of the most important phytoconstituents present in the Centella asiatica and chemically it is pentacyclic triterpenoid saponin.

In order to know the medicinal importance in the traditional and modern system of medicine, scientific data have been collected from various

literature sources and analyzed here in this present investigation for their medicinal importance and pharmacological activities of asiaticoside. Medicinal importance of asiaticoside has been investigated here in order to know the medicinal importance in the medicine. Biological importance of asiaticoside for the treatment of oxidative stress induced vascular complication has been also investigated here through data analysis. Molecular importance of various antioxidant enzymes such as SOD, Catalase and GPx in the oxidative stress have been also analyzed and reported in the present investigation to know the better molecular mechanism.

Scientific data analysis of current scientific research work signified the importance of asiaticoside in the medicine for the treatment of oxidative stress induced vascular disorders of human beings. Study also signified the importance of different enzymes in the biological systems which are directly involved in the generation of oxidative stress. However some other scientific study revealed the importance of asiaticoside in the medicine due to enhance protein levels in the body and decreased protein excretion in the urine. Importance of nitric oxide in the biological system have been also studied in some scientific research work and found that asiaticoside have inhibitory potential against production of nitric oxide.

Present study signified the biological importance of asiaticoside in the treatment of oxidative stress induced vascular disorders through different mechanism against oxidative damage and antioxidant level.

PO-317

# Time Point of Blood Pressure Drop in Patients with Orthostatic Hypotension in the Emergency Room

**Hack-Lyoung Kim[1*], Kyeongmin Jang[2], Miri Park[2], Sang-Hyun Kim[1], Myung-A Kim[1]**

[1] Division of Cardiology, Department of Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea
[2] Department of Nursing, Seoul National University Boramae Medical Center, Seoul, Korea

*khl2876@gmail.com*

In spite of the clinical importance of orthostatic hypotension (OH), time point of blood pressure (BP) drop in the diagnosis of OH is still under debate. The purpose of this study was to identify the time of BP drop of OH test, and to propose a realistic and appropriate duration in OH test.

A total of 879 consecutive patients (61-year old and 44% female) with positive on OH test in the emergency room (ER) were retrospectively reviewed. OH was defined as drop in standing systolic BP of at least 20 mm Hg or standing diastolic BP of at least 10 mm Hg from their supine values after standing for 5 minutes. BP measurement was performed at 1, 3, and 5 minutes after standing.

Six hundred eighty-four (77.8%), 152 (17.3%) and 43 (4.9%) patients had BP drop meeting OH criteria at 1, 3, and 5 minute, respectively. In multivariable analysis, older age (≥ 60 years) and higher blood urea nitrogen (BUN) (≥ 15.5 mg/dL) were independently associated with early BP drop at 1 minute even after controlling for potential confounders. Younger age (< 40 years) was independently associated with later BP drop at 5 minutes even after controlling for potential confounders.

To measure orthostatic BP for OH diagnosis at ER, older patients (≥ 60 years) with high BUN (≥ 15.5 mg/dL) should be monitored carefully because BP can drop quickly within 1 minute. On the contrary, in young people (< 40 years), BP drop occurred slowly after 3 minutes, so it is necessary to take a longer time.

PO-318

# AT1 Receptors of Angiotensin II Induce Working Memory Deficit Associated with Transient Angiogenesis, Peroxidation and Gliosis in Prelimbic Prefrontal Cortex After Amphetamine Exposure

**Osvaldo Martin Basmadjian[1,2], Natalia Andrea Marchese[1,2], Victoria Belén Occhieppo[1,2], Samanta Armonelli[1,2], Gustavo Baiardi[3,4], Claudia Bregonzio[1,2*]**

[1] Instituto de Farmacología Experimental de Córdoba (Experimental Pharmacology Institute of Córdoba), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[2] Departamento de Farmacología (Pharmacology Department), Universidad Nacional de Córdoba (National University of Córdoba), Argentina
[3] Laboratorio de Neurofarmacología, (IIBYT-CONICET), Universidad Nacional de Córdoba (National University of Córdoba), Argentina
[4] Facultad de Ciencias Químicas, Universidad Católica de Córdoba, Argentina

*claubregozio@unc.edu.ar*

Microvessels, astrocytes, microglia and neurons constitute the neurovascular unit. This is a functional coupling that allows vessels to adequately satisfy metabolic demands of neurons. Consequently, a dysfunction of neurovascular unit will result in brain function alterations. Amphetamine exposure induces noradrenergic release leading to vasoconstriction that induces ischemia. Brain cortex is particularly sensible to these changes due to locus coeroleus, the major noradrergic imput, directly innervates cortex. This transient hypoxia could contribute to reactive oxygen species release and glial activation. On the other hand, AT1 receptors of angiotensin II reduce vasoconstriction and also are present in all of neurovascular unit components.

This work aimed to study AT1 receptors involvement in working memory deficit and neurovascular unit alteration over prefrontal cortex (PFC) after amphetamine exposure. Male Wistar rats (250-300g) were used. To study the participation of AT1-R in amphetamine effects, the AT1-R blocker Candesartan (CV 3mg/kg p.o.) was administered for 10 days and Amphetamine (2.5 mg/kg i.p.) was daily administered from day 6 to 10. Lipid and protein peroxidation (LPP) was measured 30 minutes after the last amphetamine injection. On week 1 and 3 of Amph withdrawal, working memory was evaluated using Y-maze test. 24h later, the animals were perfused and the brains prepared for von Willebrand, GFAP and CD11b immunohistochemistry to evaluate angiogenesis, astrogliosis and microgliosis respectively. The results were analyzed using 2-way ANOVA followed by Bonferroni test.

It was found that repeated amphetamine exposure induce working memory deficit together with LPP and gliosis at week 1 and 3 over prelimbic prefrontal cortex. All of these alterations were prevented with the AT1 receptor blockade. Amphetamine exposure induced angiogenesis at week 1 that retracted at week 3.

We conclude that AT1 receptors participate in amphetamine-induced neurovascular alterations that alter PFC function observed as working memory deficit.

PO-319

# Cerebral Amyloid Angiopathy Disrupts Vasculature and Leads to the Impairment of Perivascular Clearance in an Alzheimer's Disease Mouse Model

**ShinHeun Kim[1,2], Ji Hoon Ahn[3], Hyunwoo Yang[1,2], Peter Lee[3], Gou Young Koh[3], Yong Jeong[1,2,3*]**

[1] Program of Brain and Cognitive Engineering, Korea Advanced Institute of Science and Technology, Korea
[2] Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Korea
[3] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Korea

*yong@kaist.ac.kr*

Cerebral amyloid angiopathy (CAA), defined as the accumulation of amyloid-beta (Aβ) on the vascular wall, is a major pathology of Alzheimer's disease (AD) and has been thought of as a failure of Aβ clearance through the perivascular space (PVS). Although two types of perivascular clearance mechanisms, called intramural periarterial drainage (iPAD) and glymphatic system, have been identified, the exact contribution of CAA on Aβ perivascular clearance is still not well understood. In this study, we investigated the effect of CAA on the structure and function of perivascular clearance systems in AD.

To investigate the alterations accompanied by CAA, changes in the perivascular basement membrane, vascular components, and vascular pulsation was evaluated in the mid-age (7-8 months) and old-age (19-21 months) AD transgenic and age-matched mice by in vivo imaging and immunofluorescence. iPAD and glymphatic system were identified and quantified by ex vivo fluorescence imaging.

Morphological changes in the perivascular basement membrane, loss of vSMCs, and augmented vascular pulsation were observed in AD mice with CAA progression. iPAD and perivascular CSF influx were impaired by AD and also by aging, but these impairments were more severe in the case of AD.

In this study, we demonstrated the disruption of PVS, vascular components, and vascular movement with CAA deposition and subsequent impairment in two types of perivascular clearance. These findings suggest that CAA is not merely a consequence of AD, but also contributors of damage to the vasculature and Aβ clearance systems, making AD more severe.

PO-320

# Neurovascular Coupling in the Neurohypophysis as a Means for the Efficient Delivery of Oxytocin Pulses

**Preethi Rajamannar[1], Gil Levkowitz[1*]**

[1] Molecular Cell Biology, Weizmann Institute of Science, Israel

*gil.levkowitz@weizmann.ac.il*

The neurohormone oxytocin(oxt) is released into the permeable vasculature of the posterior pituitary from axonal projections coming in from the hypothalamus. During the onset of physiological conditions, like osmotic stress, parturition and lactation, OXT is detected in serum as discrete pulses. The goal of this study is to understand how these pulses are propagated from the oxytocin neurons and maintained at the vascular level, thereby establishing the role of neurovascular coupling (NVC) in the hypothalamo-neurohypophyseal system (HNS) in aiding the efficient uptake and delivery of oxytocin.

We use the optical transparency of zebrafish larvae to our advantage to develop methods to visualize oxytocin release and blood flow dynamics in-vivo by two-photon microscopy at high temporal resolutions. We utilize the fluorescent serum-labelled transgenic, Tg(l-fabp:DBP-eGFP), to monitor blood flow in the HNS capillary. Oxytocin release is measured as a function of vesicle dynamics in the synapses using GFP fused synaptophysin expressed specifically in oxt neurons. Since oxytocin neurons are osmo-responsive, 50% salt stress is used to stimulate the release of oxytocin.

Under basal conditions, we see no change in vesicle dynamics, indicating almost no release of oxytocin from the termini. After osmotic stress, we see that the changes in vesicle dynamics occurs episodically as discrete pulses. Similarly, after osmotic stress, blood flow velocities in the hypophyseal capillary fluctuate at a significantly higher magnitude than of basal conditions. Furthermore, these fluctuations occur with a fixed periodicity, comparable to pulses created during oxytocin release from the axonal termini.

We show that oxytocin release into the HNS capillary is pulsatile in nature and these pulses are maintained by modulating blood flow velocities in the vasculature. Further studies are required to understand how the vasculature regulates the blood flow and hence, uptake of oxytocin.

PO-321

# Translatome Analysis of Brain Cortical Endothelial Cells Upon Neuronal Activation

**Nam-Suk Kim[1], Yan Li[1], Won-Jong Oh[1*]**

[1] Neurovascular unit research group, Korea Brain Research Institute, Korea

*ohwj@kbri.re.kr*

The reciprocal interaction between blood vessels and their adjacent neurons is critical to maintaining brain function. Both acute and chronic neuronal activation to vessels plays many roles such as hemodynamic control and energy metabolism. It has also uncovered that interference of such communications between vascular and nervous systems causes diverse neurological disorders including Alzheimer's disease as well as seizure. However, there have been few studies that have attempted to reveal the molecular mechanism in the brain endothelial cells(BECs) responded by activated neurons. For understanding the role of BECs in neurovascular interaction and its mechanism, we optimized the translatome analysis method for BECs in the cortex following neuronal activation by injection of Pentylenetetrazol(PTZ), GABAA receptor antagonist.

We were successfully able to purify brain endothelial-specific translating mRNA pools using the Tie2-Cre; Ribo-Tag mice, which is designed for expressing HA-tagged ribosomal protein in BECs.

We analyzed RNA sequencing obtained from the cortex of PBS-injected and PTZ-injected adult mice and identified 448 down-regulated genes and 518 up-regulated genes including ccl2. Furthermore, the result showed that differentially expressed genes were enriched in gene ontology terms related to response to hypoxia, blood vessel morphogene-

sis, immune response.

Taken together, we identified novel brain endothelial cell genes that are changed dynamically to the neuronal activation and are expected to improve our understanding of neurovascular interaction mechanisms.

PO-322

# Reconstruction of Acute Skin Inflammation in Human Dermal Microvascular Network in Vitro

**Habin Kang[1], Jihoon Ko[2], Yongbum Cho[3], Junsang Doh[3], Noo Li Jeon[1,2*]**

[1] Program for Bioengineering, Seoul National University, Korea
[2] Department of Mechanical Engineering, Seoul National University, Korea
[3] Department of Materials Science and Engineering, Seoul National University, Korea

*noolijeon1406@gmail.com*

Inflammation in tissue, generally caused by infectious agents or physical and chemical damages, is widely known to promote leukocyte infiltration to the inflamed site. Rising interests are upon the finding that inflammation is also related with vascular remodeling, however, not much has been studied on modulating blood vessels to alleviate inflammatory conditions. In this study, we aim to generate a 3D human dermal microvascular network within a microfluidic chip that recapitulates acute skin inflammation. Our goal is to develop a model effective for understanding roles of blood vessels in inflammatory environment and interaction with leukocytes.

We developed a 384-well plate format-based 3D cell culture platform. Manufactured through an injection molding process, the platform is used in ready-to-use form for each experiment. By designing the microstructure considering the microfluidic behavior inside the well, it is capable of patterning cell-contained hydrogel within 1 μL in a defined area. Applying this approach, co-culture of human dermal microvascular endothelial cell with fibroblasts led to the formation of robust and perfusable self-organizing microvessels.

A robust and perfusable human dermal microvascular network was formed within a microfluidic chip. LPS (lipopolysaccharide)-induced stimuli created inflammatory condition on the microvessel environment. Vessel permeability was compared between LPS-treated and non-treated models to assess for inflammatory effects expressed on the microvessels. To evaluate activation of the endothelial cells toward inflammatory stimuli, upregulation of leucocyte adhesion molecules was measured. Monocytes were introduced into the microfluidic chip and were flown into the microvessels in both inflammatory and non-inflammatory conditions to capture the differentiation of monocytes to macrophages when exposed to inflammatory stimuli.

We developed highly perfusable and reproducible human dermal microvessels on a chip that enables modeling of tissue inflammatory environment. This model can further be adopted in studying treatment options targeting vasculature normalization in skin inflammation.

PO-323

# Development of Perfusable Organ-Specific Microvasculature from hiPSC-Derived Vascular Organoids

**Yu Jung Shin[1], Ying Zheng[1*]**

[1] Bioengineering, University of Washington, USA

*yingzy@uw.edu*

We combined hiPSC-derived vascular organoid (hVO) differentiation and microvascular engineering tools to develop a perfusable organ-specific microvascular niche in vitro and understand the role of tissue parenchyma in endothelial cell (EC) fates and vessel function. Our methods aim to address the lack of controlled perfusability and organ-specific EC functions within hVOs.

hVO co-culture: hVOs were differentiated using a protocol adapted from Wimmer et al. Human renal proximal tubule epithelial cells (HPTECs) were seeded (3K) onto D21 vascular organoids and cultured for 7 days. hVOs were stained with a PVLAP antibody to assess fenestrae diaphragm expression.

hVO perfusion: A soft lithography-based micropatterning and injection-molding approach were used to fabricate a patterned vascular network (d=100um) in collagen hydrogels. During collagen injection, hVOs were inserted into the center of microvessels surrounded by a hexagon network seeded with human umbilical vein endothelial cells.

To investigate the role of tissue parenchyma on vascular tissue-specificity, hVOs were co-cultured with HPTECs. The co-cultured organoids were examined for changes in PVLAP expression, a key regulator of fenestral diaphragm formation in kidney ECs. Compared to the control vascular organoids, 7-day co-cultures with HPTECs showed enhanced expression of PVLAP, suggesting that hVOs can acquire a tissue-specific EC phenotype through either direct cellular and/or paracrine signaling from neighboring parenchymal cells.

Furthermore, we aimed to develop in vitro functional hierarchical vascular networks through perfusion of hVOs. We combined the patterned microvascular networks with self-organized capillary networks present in hVOs and cultured for 7 days to allow sprouting of hVOs towards the hexagon networks. After 7-day culture under gravity-driven perfusion, fluorescein-dextran perfusion showed particle perfusion within lumens of hVOs, suggesting anastomosis between the microchannels and hVOs.

We show that hVOs can acquire organ-specificity when co-cultured with HPTECs and that hVOs can be perfused upon integration with microvessels in vitro.

PO-324

# Renal Stromal Netrin-1 Signaling Promotes Arteriogenesis and Kidney Development

**Xiaowu Gu[1], Yadanar Htike[1], Ondine Cleaver[1*]**

[1] Molecular Biology, University of Texas Southwestern Medical Center, USA

*ondine.cleaver@utsouthwestern.edu*

The developmental steps underlying renal arterial morphogenesis are poorly defined. Building

kidney arteries requires orchestrated actions of endothelial cells (ECs) and vascular smooth muscle cells (vSMCs). In all developing organs including the kidney, vSMCs encase and stabilize arteries. vSMCs have been reported to arise from Foxd1+ stromal progenitor cells (SPs), which are themselves emerging as crucial players in kidney organogenesis. The aim of this study is to determine whether and how stromal signals govern lineage-specific stromal fate to regulate arterial and kidney formation.

Netrin-1 and Klf4 were conditionally ablated from Foxd1+ SPs in vivo by Foxd1Cre (hereafter designated as $Ntn1^{SPKO}$and $Klf4^{SPKO}$). Three-dimensional kidney arterial development was assessed by whole-mount immunostaining. Transcriptomic profiles of E13.5 $Ntn1^{SPKO}$ and control kidneys were also analyzed. Glomeruli were quantified according to a published acid maceration protocol.

We found that control kidneys develop a patent renal arterial tree at E13.5. By contrast, $Ntn1^{SPKO}$ kidneys strikingly fail to form vSMC-enwrapped arteries, with a majority of αSMA+ vSMCs accumulating on the kidney surface. Mutants also exhibit heightened renal hypoxia. In addition, reduced ureteric bud branching is evident at E13.5, and postnatal $Ntn1^{SPKO}$ mice display delayed nephrogenesis with ~30% fewer glomeruli. RNA-seq analysis revealed significant reduction of Klf4 in $Ntn1^{SPKO}$ kidneys. $Klf4^{SPKO}$ kidneys showed similar surface αSMA+ vSMC accumulation as in $Ntn1^{SPKO}$. Furthermore, $Klf4^{SPKO}$ kidney arteries are poorly developed, with limited vSMC coverage.

Our results identify critical genetic programs necessary to build a mature renal artery. Furthermore, they suggest that renal SP-derived Ntn1 and Klf4 may determine Foxd1+ SP to vSMC differentiation and/or instruct NSC-derived vSMCs to nascent arteries to initiate renal arterial assembly, thereby regulating kidney growth.

PO-326

# F-18 FDG PET/CT Can Be Used as an Imaging Biomarker for Reflecting Synthetic Vascular Smooth Muscle Cell Activity in Vascular Remodeling Disorder Rat Model

**Kisoo Pahk[1], Chanmin Joung[2], Sungeun Kim[1], Won-Ki Kim[2*]**

[1] Department Of Nuclear Medicine, Korea University Anam Hospital, Korea
[2] Institute for Inflammation Control, Korea University College of Medicine, Korea

*wonki@korea.ac.kr*

Synthetic vascular smooth muscle cells (VSMCs) play important roles in vascular remodeling disorders including atherosclerosis, restenosis, and transplant vasculopathy. 18F-fluorodeoxyglucose positron emission tomography (F-18 FDG PET/CT) is a non-invasive molecular imaging modality widely used in clinical practice for reflecting glucose metabolism. The aim of this study was to demonstrate the feasibility of measuring the activity of synthetic VSMCs using F-18 FDG PET/CT in an atherosclerotic rat model of partial carotid artery ligation

Using Sprague-Dawley rats, neointimal hyperplasia was made by right partial carotid ligation method. After 1 month, all animal models undertook F-18 FDG PET/CT images. Then the vessels were harvested and analyzed with autoradiography and histomolecular approaches.

The affected carotid artery exhibited prominent neointinal hyperplasia region with a narrowed lumen. Known surrogate marker of synthetic VSMCs

including matrix metallopeptidase-9, cyclophilin A, and collagen type III were increased in neointimal hyperplasia region. There were no immune cells such as macrophages or neutrophils. F-18 FDG PET/CT and autoradiography showed increased tracer uptake along the same affected arteries. Glucose transporter-1 was also increased in neointimal hyperplasia region.

It is feasible to use F-18 FDG PET/CT as an imaging surrogate-marker for reflecting synthetic VSMC activities in vascular remodeling disorders.

PO-327

# A Novel Drug SP-8356 Targeting CD147 Suppresses Pathological Vascular Remodeling via Affecting Vascular Smooth Muscle Cell Phenotype Expression in Atherosclerotic Rat Model

**Kisoo Pahk[1], Chanmin Joung[2], Sungeun Kim[1], Won-Ki Kim[2*]**

[1] Department Of Nuclear Medicine, Korea University Anam Hospital, Korea
[2] Institute for Inflammation Control, Korea University College of Medicine, Korea

*wonki@korea.ac.kr*

The switching of vascular smooth muscle cell (VSMC) phenotype plays key roles in neointimal hyperplasia and its related arterial stiffness. Cluster of differentiation 147 (CD147), a member of the immunoglobulin super family that induces the expression of matrix metalloproteinase-9 (MMP-9) by dimerization, may play important roles in the switching of VSMC phenotype and may therefore be an effective target for the treatment of neointimal hyperplasia. Here, we investigated whether a novel drug SP-8356 ((1S,5R)-4-(3,4-dihydroxy-5-methoxystyryl)-6,6-dimethylbicyclo[3.1.1]hept-3-en-2-one) targeting CD147 inhibitor could affects VSMC phenotype expression and inhibit neoitimal hyperplasia in atherosclerotic rat model.

The A10 VMSC cell lines were used for in-vitro experiment. Neointimal hyperplasia was generated in Sprague-Dawley rats by partial ligation of the right carotid artery combined with an atherogenic diet. Rosuvastatin was used for positive control drug. Rats were subdivided into vehicle and SP-8356 (50 mg/kg) groups. The drug was administrated via intraperitoneal injections for 4 weeks. Arterial stiffness was assessed by measuring pulse wave velocity using Doppler ultrasonography before sacrifice.

SP-8356 suppressed CD147 dimerization thereby reducing MMP-9 activity, synthetic VSMC phenotype expression, and VSMC migration. SP-8356 also reduced neointimal hyperplasia and improved arterial stiffness in animal model. SP-8356 enhanced the expression of smooth muscle myosin heavy chain, but decreased the collagen type III and MMP-9 expression in the neointimal area.

SP-8356 affects VSMC phenotype modulation thereby reducing neointimal hyperplsaisa. Thus, SP-8356 could be a promising therapeutic drug for vascular remodeling disorders related with neointimal hyperplasia and arterial stiffness.

PO-328

# FOXC1 Is a Key Transcription Factor Influencing Smooth Muscle Cell Activation in Atherosclerotic Plaques

**Urszula Rykaczewska[1], Mariette Lengquist[1], Malin Kronqvist[1], Catarina Rippe[2], Anton Razuvaev[1], Gabrielle Paulsson-Berne[3], Karl Swärd[2], Per Eriksson[3], Thomas Quertermous[4], Ulf Hedin[1], Ljubica Matic[1*]**

[1] Dept Molecular Medicine and Surgery, Karolinska Institute, Sweden
[2] Department of Experimental Medical Science, Lund University, Sweden
[3] Department of Medicine Solna, Karolinska Institute, Sweden
[4] Division of Cardiovascular Medicine, Stanford University, USA

*Ljubica.Matic@ki.se*

Rupture of atherosclerotic plaques in the carotid bifurcation is a common cause of ischemic stroke. We aimed to characterize the major transcriptional regulators of molecules dysregulated in plaques from symptomatic vs. asymptomatic patients.

Bioinformatic mapping of the predicted promotor binding motifs in genes and proteins significantly dysregulated in plaques from symptomatic vs. asymptomatic patients, identified FOXC1, LEF1, TCF4 and RUNX1 as the key upstream transcription factors, with FOXC1 being the most significantly enriched (Ptranscriptomics=1.09E-112; Pproteomics=4.06E-18).

FOXC1 transcript was downregulated in plaques from symptomatic vs. asymptomatic patients and pathway analyses linked its expression to cytoskeleton binding and heparan sulfate synthesis in normal vessels, whereas in the lesions thyroid hormone binding was the most correlated process. Transcriptomic and proteomic analyses revealed positive correlations between FOXC1 and typical SMC markers in plaques, while it was negatively associated to inflammation, extracellular matrix degradation and cell cycle progression. By immunohistochemistry, FOXC1 was abundant in SMCs in normal human arteries, but showed a broader localization in plaques. During the time-course of rat carotid intimal hyperplasia formation, FOXC1 was repressed in early phases associated with proliferating SMCs, but its expression was restored in SMCs at later stages, regaining their quiescent properties. In this model, FOXC1 was also positively correlated with the expression of thyroid hormone receptor beta. In vitro, FOXC1 was downregulated in dedifferentiating rat aortic SMCs, and stimulation of human SMCs with TGFB1 resulted in early induction of FOXC1 after 2h but downregulation later on. Functionally, silencing of FOXC1 in SMCs led to increased proliferation as determined by FACS cell cycle assessment and faster migration in a wound healing assay.

Our bioinformatic analyses identified FOXC1 as one of the master transcriptional regulators of molecules differentially regulated in symptomatic plaques, while functionally we show evidence for FOXC1-dependent regulation of SMC quiescence vs. activation in vascular disease.

PO-329

# Aligned Smooth Muscle Cells -Laden Nanofibrous Bundle Fabricated via Cell Electrospinning

**Jung Won Yoon**[1*]

[1] Physiology, School of Medicine, Pusan National University, Korea

*wjddnjssky@nate.com*

Micropatterning within a 3D structure has been proven to facilitate cellular activities especially for tissues reliant on an aligned structure for functioning.

Uniaxially arranged micropattern is important to mimic the structure of the natural extracellular matrix.

Tissue engineering demand for repairing damaged tissues or organs.

Various technologies have been introduced and applied to fabricate artificial and biocompatible scaffolds.

Electrospinning(CE) has been widely investigated to mimic the structure of extracellular matrix and provide micropattern. CE has been tested to fabricate cell-laden fibrous structures.

Various approaches have been attempted to generate functional muscle tissues in tissue engineering, yet in vitro methods to generate smooth muscles(SMC) with physiologically aligned structure remain a great challenge.

SMC maintains connections and arrangements between cells in a micro-environment, which is one of the characteristics in the tissue, and it is important to reproduce it for functional enhancement and maturation of SMC.

In order to mimic the in vivo highly aligned structure of smooth muscle cells, we used a cell micropatterning technique to control cell alignment.

SMC was differentiated from MSC and aligned by electrospinning and incubated for about 4 weeks. And markers related to cell maturation and function were confirmed by immunostatining and gene expression level.

It was confirmed that the cells were elongated and aligned in one direction by electro spinning, and the connection between cells was also observed to be close.

In aligned SMC, it was confirmed that aSMA, calponin, desmin, etc. related to SMC structural function increased and that ECM components related to SMC were also highly expressed.

SMC alignment is closely related to the maturation and function of cells.

PO-330

# Distinctive Roles of Pericytes and Smooth Muscle Cells in the Choroid

**Sang A. Kim[1,2], Soo Jin Kim[1,2], Yulim Kim[2], Yeong A. Choi[2], Do Young Park[2], Ralph H Adams[3], Yoshiaki Kubota[4], Akiyoshi Uemura[5], Gou Young Koh[6], Junyeop Lee[1*]**

[1] Department of Ophthalmology, Asan Medical Center, University of Ulsan, College of Medicine, Korea
[2] Department of Ophthalmology, YeungNam University, College of Medicine, Korea
[3] Department of Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Germany
[4] Department of Anatomy, Keio University School of Medicine, Japan
[5] Department of Ophthalmology, Nagoya City University, Japan
[6] Center for Vascular Research, Institute for Basic Science (IBS), Korea

*j.lee@amc.seoul.kr*

Perivascular mural cells (PVMC), including pericytes (PC) and smooth muscle cells (SMC), maintain

the stability of blood vessels. Blood supply of the eye mainly comes from the choroidal vessels, which is crucial for visual function. This study evaluated the regional distributions of choroidal PVMC depending on age, and investigated physiological significance of PC and SMC using cell-specific ablation models.

We used PVMC-specific cre- lines: PDGRβ-creERT2, SM22a-cre, and MYH11-creERT2. We crossed them with R26-tdTomato and evaluated distribution of PVMC using intravital imaging. We ablated specific PVMC during development and adult using DTAiΔPC or DTAiΔSMC mice, which were generated by crossing with R26-diphtheria toxin A (DTA) mice. Choroidal vessels were evaluated using ultra-widefield angiography. The phenotype of DTA lines were compared with VEcad-creERT2:PDGFB-flox/flox (Pdgfb iΔEC), and anti-PDGFRβ (APB5)-injected mice. Human choroid with various age were harvested from the donor eyes

Aged human choriocapillaris were covered with more SMC mainly at the periphery. RNA-seq revealed increased ACTA2 but decreased PDGFRβ and Angiopoietins in the aged choroid. In mice, DTAiΔSMC displayed dilated and leaky large vessels, while DTAiΔPC showed loss of choriocapillaris and large vessels. When the PC were ablated at postnatal days in DTAiΔPC, choroidal remodeling was fully recovered by increasing PDGFB expression. Both Pdgfb iΔEC and APB5-injected mice have similar phenotypes with DTAiΔPC mice, but they presented abnormal SMC recruitment along peripheral large vessels with increased TGFβ expression. DTAiΔSMC adult mice showed dilated large vessels accompanying overlying retinal pathologies mimicking human age-related macular degeneration.

PVMC are essential for the maturation and maintenance of choroidal vessels. PC and SMC play distinct roles in the regulation of structure and function of blood vessels in the choroid. Dysregulated balance of PC and SMC in the choroid are associated with aging and macular degeneration.

PO-331

# Pericyte-Derived Extracellular Vesicles-Mimetic Nanovesicles Restores Erectile Function by Enhancing Neurovascular Regeneration in Mouse Models of Cavernous Nerve Injury

**Guo Nan Yin[1], Soo-Hwan Park[2], Jiyeon Ock[1], Min-Ji Choi[1], Anita Limanjaya[1], Kalyan Ghatak[1], Kang-Moon Song[1], Mi-Hye Kwon[1], Do-Kyun Kim[3], Yong Song Gho[4], Ji-Kan Ryu[1], Jun-Kyu Suh[1*]**

[1] Department of Urology and National Research Center for Sexual Medicine, Inha University School of Medicine, Korea
[2] Department of Urology, Kosin University College of Medicine, Korea
[3] Center for Biomolecular & Cellular Structure, Institute for Basic Science (IBS), Korea
[4] Department of Life Sciences, Pohang University of Science and Technology, Korea

*jksuh@inha.ac.kr*

Pericytes play important roles in maintaining penile erection, yet no previous studies have explored the effects of pericyte-derived nanovesicles (PC-NVs) in neurovascular regeneration in the context of erectile dysfunction. To investigate the potential effect of PC-NVs in neurovascular regeneration.

PC-NVs were isolated from mouse cavernous pericytes (MCPs) and neurovascular regeneration was evaluated in an in vitro study. Twelve-week-old C57BL/6J mice were used to prepare two nerve injury models, cavernous nerve injury (CNI) and sciatic nerve injury (SNI). At the time of nerve injury creation, different doses of PC-NVs were injected into the corpus cavernosum or the area of SNI. Erectile function evaluation, histologic examination of the

penis, and western blots were assessed 2 weeks after model creation. The sciatic nerve was harvested 5 and 14 days later for immunofluorescence studies.

The PC-NVs were extracted and characterized by cryo-transmission electron microscopy and EVs positive (Alix, TSG101, CD81) and negative (GM130) markers. In the in vivo studies, PC-NVs successfully restored erectile function in CNI mice (~82% of control values) and induced sciatic nerve regeneration. Immunofluorescence staining showed significant increases in markers of pericytes, endothelial cells, and neuron contents. In the in vitro and ex vivo studies, PC-NVs significantly increased mouse cavernous endothelial cells tube formation, aortic ring sprouting, Schwann cell migration, and dorsal root ganglion and major pelvic ganglion neurite sprouting. Finally, western blot analysis revealed the PC-NVs upregulated cell survival signaling (Akt and eNOS) and induced the expression of neurotrophic factors (BDNF, NT-3, and NGF).

PC-NVs significantly restored erectile function and induced regeneration of the sciatic nerve by enhancing neurovascular regeneration. Local treatment with PC-NVs may represent a promising therapeutic strategy for the treatment of neurovascular diseases.

Keywords: Pericytes; Extracellular Vesicle; Nanovesicles; Neurovascular Regeneration; Cavernous Nerve Injury; Sciatic Nerve Injury

PO-332

# Single Cell Sequencing of Fibroblasts Reveals Transcriptional Heterogeneity in Murine and Humanvasculature

**Kim Van Kuijk[1], Ian McCracken[2], Renée Tillie[1], Ross Dobie[2], Prakash Ramachamdran[2], Barend Mees[1], Han Jin[1], Lieve Temmerman[1], Erik Biessen[1], Neill Henderson[1], Andy Baker[1,2], Judith Sluimer[1,2*]**

[1] Pathology; CARIM, Maastricht University, Netherlands
[2] CVS/CIR, Edinburgh University, UK

*judith.sluimer@maastrichtuniversity.nl*

Fibroblasts are present in healthy and diseased arteries. Their heterogeneity and role in vascular pathologies have not been fully elucidated, as current cellular markers lack precision to distinguish fibroblasts from other vascular cells.

Single cell RNA sequencing (scRNA-seq, 10x) of ~4400 CD45-/ICAM2-/PDGFRβ+ mesenchymal cells unravelled the fibroblast transcriptome and heterogeneity in healthy C57/bl6 murine adventitia.

we identified two distinct clusters of Col1a2+/Ly6a+ fibroblasts and Myh11+/Tagln+ smooth muscle cells (SMCs). In silico analysis of our dataset, and 3 independent datasets supported fibroblast specificity of six fibroblast signature genes, with validated protein expression by spindle-shaped cells in healthy adventitia. PHATE dimensionality reduction showed fibroblast heterogeneity by three differentiation trajectories, which were confirmed in an independent dataset. Gene ontology analysis supported divergent functional profiles for the three trajectories: 1) vascular development and nucleotide sugar metabolism, 2) antigen presen-

tation and cholesterol metabolism, and 3) growth factor signaling. Protein expression of trajectory specific markers, including CD55, Lifr and sFRP1 for trajectory 1, 2, and 3 respectively, was evident in healthy and atherosclerotic murine aortic roots. In human atherosclerosis, trajectory 1 and 3 correlated negatively to detrimental plaque traits, e.g. plaque size and necrotic core, while trajectory 2 showed an inverse correlation pattern. Trajectories were driven by distinct transcription factor regulons, including KLF, EGR and NFAT family members. Transcription factor and trajectory marker expression were differentially regulated by TGFB1, and oxidized low-density lipoprotein in 3T3 fibroblast in vitro.

In healthy murine adventitia, scRNA-seq provided deeper insight in the distinct genomic expression signatures of fibroblasts and SMCs. The fibroblast signature could be validated in silico and ex vivo. Three fibroblast differentiation trajectories gave rise to transcriptionally divergent fibroblast subsets, and correlated to human atherosclerotic plaque traits. The fibroblast- and trajectory signatures will be instrumental to further study fibroblast function in atherosclerosis.

PO-333

# Mechanosensor Piezo1 in Skeletal Muscle Pericytes

**Yilizila Abudushalamu[1], Hema Viswambharan[1], Romana Mughal[2], Richard Cubbon[1], Mark Kearney[1], David Beech[1], Piruthivi Sukumar[1*]**

[1] Discovery and Translational Science Department Leeds Institute of Cardiovascular & Metabolic Medicine (LICAMM), University of Leeds, UK
[2] Department of Optometry and Vision Sciences, University of Huddersfield, UK

*P.Sukumar@leeds.ac.uk*

Pericytes (PC) are a type of mural cells found in micro vessels. They play indispensable roles in angiogenesis, vascular maturity and regulation of permeability. They wrap around the endothelial cells (EC) and EC-PC communication is both via physical contact and paracrine signalling. Both EC and PC are prone to mechanical stress produced by blood flow and pressure. Shear stress sensing by endothelial mechanosensor protein Piezo1 is essential for angiogenesis and development of regular vascular architecture during embryogenesis. We hypothesise that Piezo1 may play important roles in PC too. Hence, we aim to evaluate the expression and functions of Piezo1 in PCs.

We have developed a novel transgenic mouse model – tamoxifen inducible PC-specific knockout of Piezo1 (PP-/-). We have isolated PC from mouse skeletal muscle (SkmPC) and obtained human brain vascular PC (HBVP) from commercial sources. Expression and functionality of Piezo1 were analysed by RT-PCR and Ca2+ influx assay. Using Yoda1 and Yoda1 analogues as pharmacological agents and Piezo1-specific siRNA for gene silencing, we performed functional experiments to elucidate the role of Piezo1 in PC.

Flow cytometric analysis showed that the isolated

mouse SkmPC in culture was >98% pure. We found that different populations of primary PC express functional Piezo1. RT-PCR, Ca2+ influx assay and gene-rescue experiments confirmed the PC-specific knockdown of Piezo1 in our transgenic mouse model. Transcriptomics analysis showed that chemical activation of Piezo1 leads to downregulation of interleukin-mediated signalling in PC.

Primary SkmPC and HBVP express functional Piezo1. Activation of Piezo1 alters inflammatory signalling in SkmPC and HBVP, which we hypothesise will have implications on PC pathophysiology.

PO-334

# Shc Decodes Mechanical Signals from Multiple Mechanosensors to Pull the Strings on Atherosclerosis

**Vedanta Mehta[1*], Kar-Lai Pang[1], Christopher Givens[2], Zhongming Chen[2], Daniel Sweet[2], John Reader[1], Ellie Tzima[1]**

[1] Cardiovascular Medicine, University of Oxford, UK
[2] Department of Cell and Molecular Physiology, University of North Carolina at Chapel Hill, USA

*vedanta.mehta@cardiov.ox.ac.uk*

Atherosclerosis occurs in regions of the vasculature where blood flow patterns are disturbed and endothelial cells (ECs) are characterized by upregulation of pro-inflammatory genes. ECs sense flow patterns via mechanosensors that initiate a complex array of signalling cascades, but our understanding of how these mechanosensors cooperate to ultimately drive atherosclerosis is rudimentary.Our previous work has shown that the adaptor protein Shc mediates endothelial responses to shear stress in vitro and in vivo. The objective of the current study is to examine the requirement for endothelial Shc in the amalgamation of mechanical signatures and flow-dependent atherosclerosis.

Conditional EC-specific Shc knockout mice (ShcE-CKO mice) were bred on to an ApoE-/- background and fed a high fat diet (HFD). In complementary experiments, partial carotid ligation (a model of accelerated atherosclerosis) was performed prior to feeding a HFD for 3 weeks. Plaque deposition was assessed by Oil-Red-O staining.In vitro experiments using shear stress systems and magnetic pulling experiments were performed to elucidate the mechanistic role of Shc in endothelial mechanotransduction.

Reduced plaque deposition was observed in ShcE-CKO mice compared to controls in both models of atherosclerosis. Confocal imaging of stained en face sections of aortic arches (a region of disturbed flow) revealed reduced upregulation of inflammatory markers in ShcECKO mice compared to controls. Exposure of ECs to disturbed flow in vitro also resulted in reduced upregulation of inflammatory markers in Shc knockout ECs. Mechanistically, Shc was found to associate with multiple mechanotransduction 'hotspots' in response to shear stress.Tensional force application experiments demonstrated that Shc is a critical transducer of mechanical signals from multiple established mechanosensors.

We identify Shc as an essential mechanotransducer that associates with and decodes mechanical signals from multiple mechanosensory 'hotspots' in ECs. Loss of Shc abrogates flow-induced inflammatory signals and attenuates atherosclerotic plaque deposition in regions of disturbed blood flow.

PO-335

# Endothelial Cell Rearrangements Driven by Talin and Kindlin During Vascular Morphogenesis

**Tevin CY Chau[1], Kelly A. Smith[2], Benjamin M. Hogan[3], Anne Karine Lagendijk[1*]**

[1] Cell and Developmental Biology Division, Institute for Molecular Bioscience, University of Queensland, Australia
[2] Physiology, School of Biomedical Sciences, University of Melbourne, Australia
[3] Organogenesis and Cancer Program, Peter MacCallum Cancer Centre, Australia

*a.lagendijk@imb.uq.edu.au*

Focal adhesions are dynamic adhesion sites at which cells are connected to the extracellular matrix via Integrin heterodimers. Talin and Kindlin are Integrin binding proteins that are recruited to active Integrin heterodimers to connect Integrin molecules to the actin cytoskeleton and create recruit additional proteins. Most of what we know to date on vascular morphogenesis in the absence of Integrin-actin connectivity comes from vascular specific knockout studies in mouse which have identified defects in angiogenesis, endothelial cell shape and vessel integrity. The downstream mechanism of Talin and Kindlin that underlie these phenotypes however are not fully understood.

We have previously identified distinctive cellular and junctional rearrangements that occur during maturation of the dorsal aorta using a VE-cadherin transgenic line. Here, we used this line to examine the roles of Talin1 and Kindlin2 in vascular morphogenesis using live imaging at single cell resolution in zebrafish mutant models.

Intriguingly, high resolution live imaging of VE-cadherin and F-actin at cell-cell junctions of both Talin1 and Kindlin2 deficient endothelial cells revealed that these Integrin binding proteins are required cell-autonomously for endothelial cell elongation and cell-cell junction linearization during arterial maturation. We hypothesize that mechanical signals that originate from Focal adhesions might be essential to direct changes in cellular mechanics that allow for rearrangements to occur. To test this, we inhibited actin polymerisation using a chemical Arp2/3 inhibitor (CK666) which compromised arterial maturation in wild-type embryos. Reciprocally, treatment with an actin polymerisation agonist (Jasplakinolide) rescued arterial maturation in talin1 mutant embryos. We are currently further investigating mutant F-actin dynamics at cell-cell junctions in more detail.

We have shown that Talin1 and Kindlin2 are required for endothelial cell rearrangements during arterial maturation cell-autonomously and preliminary data suggests that these cellular defects are driven by a failure to sufficiently polymerise actin.

PO-336

# Matrix Mechanotransduction Mediated by Thrombospondin-1/Integrin/YAP Signaling Pathway in Remodeling of Vessel Wall

**Yoshito Yamashiro[1*], Bui Quoc Thang[2], Karina Ramirez[1], Seung Jae Shin[1], Tomohiro Kohata[3], Shigeaki Ohata[4], Tram Anh Vu Nguyen[1], Sumio Ohtsuki[3], Kazuaki Nagayama[4], Hiromi Yanagisawa[1]**

[1] Life Science Center for Survival Dynamics of Tsukuba Advanced Research Alliance, University of Tsukuba, Japan
[2] Department of Cardiovascular Surgery, University of Tsukuba, Japan
[3] Faculty of Life Sciences, Kumamoto University, Japan
[4] Graduate School of Mechanical Systems Engineering, Ibaraki University, Japan

*yamayoshito@tara.tsukuba.ac.jp*

The extracellular matrix (ECM) initiates mechanical cues that activate intracellular signaling through matrix-cell interactions. In the blood vessels, additional mechanical cues derived from the pulsatile blood flow and pressure play a pivotal role in homeostasis and disease development. Currently, the nature of the cues from the ECM and their interaction with the mechanical microenvironment in large blood vessels to maintain the integrity of the vessel wall are not fully understood.

To search for a potential remodeling factor(s), we performed cyclic stretch experiments using rat aortic smooth muscle cells (SMCs) with high strain (1 Hz; 20% strain), mimicking the pathological condition of the aortic wall.

Here, we identified the matricellular protein thrombospondin-1 (Thbs1) as an extracellular mediator of matrix mechanotransduction that acts via integrin αvβ1 to establish focal adhesions and promotes nuclear shuttling of Yes-associated protein (YAP) in response to high strain of cyclic stretch. Thbs1-mediated YAP activation depends on the small GTPase Rap2 and Hippo pathway, and is not influenced by alteration of actin fibers. Deletion of Thbs1 in mice inhibited Thbs1/integrin β1/YAP signaling, leading to maladaptive remodeling of the aorta in response to pressure overload, and inhibition of neointima formation upon carotid artery ligation, exerting context-dependent effects on the vessel wall.

We thus propose a novel mechanism of matrix mechanotransduction centered on Thbs1, connecting mechanical stimuli to YAP signaling during vascular remodeling in vivo.

PO-338

# Shear Stress-Induced Endothelial Tenascin-X Expression Regulates Atherosclerosis Progression

**Stefan Offermanns[1*], Guozheng Liang[1]**

[1] Dept. of Pharmacology, Max-Planck-Institute for Heart and Lung Research, Germany

*stefan.offermanns@mpi-bn.mpg.de*

Atherosclerosis develops preferentially in areas of the arterial system, in which blood flow is disturbed, whereas laminar flow protects from atherogenesis. The molecular mechanisms of the flow pattern-dependent occurrence of atherosclerosis are still incompletely understood.

We performed RNAseq to identify genes which are

regulated by laminar flow in HUVECs. To assess the function of Tenascin-X (Tnx) in vivo, endothelium-specific Tnx knockout mice on a LDL-receptor-deficient background were analyzed for atherosclerosis development after feeding a high fat diet as well as after partial carotid artery ligation. In addition, smooth muscle-specific Tnx knockout mice were analysis by carotid artery ligation and in a model of angiotensin-II induced hypertension.

We found that laminar flow but not disturbed flow induced Tnx expression in HUVECs. This could be validated in vivo as areas of the aortic arch exposed to laminar flow in contrast to areas exposed to disturbed flow showed Tnx expression. Endothelial loss of Tnx expression resulted in increased endothelial inflammation and promoted the progression of atherosclerosis. These effects were accompanied by development of endothelial mesenchymal transition (EndMT) in areas exposed to laminar flow as well as by increased levels of endothelial Smad2/3 phosphorylation. Pharmacological inhibition of TGF-β rescued the phenotype in endothelium-specific Tnx-deficient mice. In in vitro experiments, Tnx inhibited TGF-β induced Smad2/3 phosphorylation in endothelial cells. In addition, we found that smooth muscle cell specific Tnx-deficiency attenuated smooth muscle cell hyperplasia during vascular injury and during hypertensive remodeling and that these effects were also sensitive to pharmacological inhibition of TGF-β.

These data indicate that Tnx inhibits TGF-β induced effects both in endothelial and vascular smooth muscle cells and that endothelial Tnx expression in vascular beds exposed to laminar flow protects from EndMT and atherosclerosis.

PO-339

# Retinal Vascular Stiffness as a Key Mediator of Retinal Vascular Pathology Associated with Diabetes

**Irene Santiago Tierno[1,2,3], Sathishkumar Chandrakumar[2,3], Haitao Liu[4], Emma M Lessieur[5,6], Yunpeng Du[5,6], Timothy S. Kern[5,6], Kaustabh Ghosh[2,3]***

[1] Molecular, Cellular, and Integrative Physiology, University of California, Los Angeles, USA
[2] Ophthalmology, University of California, Los Angeles, USA
[3] Ophthalmology, Doheny Eye Institute, USA
[4] Ophthalmology, University of Pittsburg, USA
[5] Ophthalmology, University of California, Irvine, USA
[6] Ophthalmology, Gavin Herbert Eye Institute, USA

*ghoshk@ucla.edu*

Retinal vascular inflammation contributes to the development of vascular lesions of early diabetic retinopathy (DR). We have previously shown in vitro that lysyl oxidase (LOX)-mediated retinal subendothelial matrix stiffening independently promotes Rho/ROCK and endothelial ICAM-1 upregulation, key factors inducing retinal inflammation in diabetes. Here we used a mouse model of early DR to examine the effect of LOX inhibition on Rho/ROCK-mediated retinal inflammation and downstream retinal vascular and vision defects.

Whole retina and retinal capillaries were isolated from nondiabetic (ND) and streptozotocin-induced 12-week diabetic (D) male (8-10 weeks old) C57BL/6 mice (n= 25/group) for assessment of retinal vascular stiffness (using atomic force microscopy/AFM) and LOX and ROCK protein levels. In a long-term study of 30-weeks duration of diabetes, LOX inhibitor beta-aminopropionitrile (BAPN, 3 mg/kg) was administered to one group of diabetic mice (D+BAPN). Retinas and retinal capillaries from the 30-week ND, D, and D+BAPN mice were assessed

for superoxide production, capillary degeneration (using elastase digest method and Periodic acid-Schiff staining), and vascular leakage of intravenously-injected FITC-albumin into neural retina. Visual acuity and contrast sensitivity were assessed by virtual optokinetic test. Studies are underway to assess a) the ROCK-mediated mechanotransduction pathway, b) inflammatory proteins, and c) retinal capillary stiffness.

During short-term diabetes, levels of retinal capillary stiffness, LOX and ROCK were markedly higher in D group of mice than in ND mice. Crucially, LOX inhibition in long-term diabetic mice (D+BAPN) significantly decreased the diabetes-induced increase in retinal superoxide ($p<0.05$ vs D) and capillary degeneration ($p<0.05$ vs D) but not vascular leakage. Further, the loss in contrast sensitivity seen in D group was markedly prevented in D+BAPN group.

Diabetes-induced upregulation of retinal inflammation and vascular atrophy were significantly reduced by inhibiting LOX-dependent retinal vascular stiffness and ROCK-mediated endothelial mechanotransduction, which also correlated with reduced loss in contrast sensitivity.

PO-340

# Advanced Cardiac Progenitor Cell Culture System by using ECM Motif Peptide Coated Plates

**Hye Ji Lim[1], Na Kyung Lee[1], Seung taek Ji[1], Hui-Gwan Goo[2], Sang-Mo Kwon[1*]**

[1] School of Medicine, Pusan National University, Korea
[2] Research & Development, Amogreentech, Korea

*smkwon323@pusan.ac.kr*

Cardiovascular disease continues to be a major cause of morbidity and mortality worldwide and cardiac progenitor cells (CPCs) are promising source for cell-based therapy, CPCs undergo cellular senescence and have small cell acquisition rates. To overcome these issues, we attempted to develop a priming system to enhance the function of Human CPCs (hCPCs) using bioactive peptide motif-coated plate.

We designed new ECM motif peptides from previously identified with small modification. After coating the newly engineered ECM motif peptides on a plate. To confirm the effect of ECM peptide-coated plates on the attachment and proliferation ability of CPCs performed by using CCK8. CPCs stemness evaluated by the expression of klf4 and Nanog. Further, CPCs functional ability was visualized by c-KIT expression and wound healing. deescalate of cellular senescence done by SA-β galactoisdase assay.

The engineered ECM motif peptide coated plates enhanced the activation of intracellular and extracellular processes such as attachment, proliferation, and migration thru maintain the stemness of CPCs. ECM motif peptide coated plates blocking the cellular senescence via inhibiting the expression p16,

p53 and senescence positive β-gal cells.

Overall, this study demonstrate the effectiveness of ECM motif peptide priming strategy through promoting the bioactivity of hCPCs and delayed celluar senescence.

PO-341

# Bioengineering Pluripotent Stem Cell-Derived Hemophilia A-Specific Microvascular Networks for Delivery of Full-Length Factor VIII into the Bloodstream

**Joseph Neumeyer[1], Ruei-Zeng Lin[1], Kai Wang[1], Xuechong Hong[1], Juan Melero-Martin[1*]**

[1] Cardiac Surgery, Boston Children's Hospital, USA

*Juan.MeleroMartin@childrens.harvard.edu*

Hemophilia A is a bleeding disorder caused by mutations in the F8 gene encoding coagulation factor VIII (FVIII). Current treatments are based on regular infusions of FVIII concentrates throughout a patient's life. Alternatively, viral gene therapies that directly deliver F8 in vivo have shown preliminary successes. However, hurdles remain, including lack of infection specificity and the inability to deliver the full-length version of F8 due to restricted viral cargo sizes. Here, we developed an alternative non-viral ex vivo gene therapy approach that enables the overexpression of full-length F8 in patients' endothelial cells (ECs).

We first generated hemophilia A patient-specific induced pluripotent stem cells (HA-iPSCs) from urine epithelial cells and genetically modified them using a piggyBac DNA transposon system to insert multiple copies of full-length F8. We subsequently differentiated the modified HA-iPSCs into competent ECs with high efficiency.

We demonstrated that the cells (termed HA-FLF8-iECs) were capable of producing high levels of FVIII. Importantly, following subcutaneous implantation into immunodeficient hemophilic (SCID-f8ko) mice, we demonstrated that HA-FLF8-iECs were able to self-assemble into vascular networks, and the newly-formed microvessels had the capacity to deliver functional FVIII directly into the bloodstream of the mice, effectively correcting the clotting deficiency. Moreover, our implant maintains cellular confinement, which reduces potential safety concerns and allows effective monitoring and reversibility.

In summary, our studies established the feasibility of a non-viral approach to genetically engineer hemophilia A patient-specific ECs for full-length FVIII overexpression. Moreover, we demonstrated that the modified HA-FLF8-iECs could form FVIII-secreting vascular networks within subcutaneous implants in hemophilic mice, restoring therapeutic levels of FVIII activity. We envision this proof-of-concept study could become the basis for a new autologous ex vivo gene therapy approach to treat hemophilia A.

PO-342

# Maturation of Human Embryonic Stem Cell-Derived Cardiomyocytes for Modeling of Mitochondrial Dysfunction and Heart Diseases

**Yeseul Kim[1], Jungwon Yoon[1], Dasol Kim[1], SangBin Yu[1], JaeHo Kim[1*]**

[1] Department of Physiology, School of Medicine, Pusan National University, Korea

*jhkimst@gmail.com*

Human embryonic stem cell-derived cardiomyocytes (hESC-CM) can be used for a wide range of applications such as drug screening, modeling and therapy of cardiac diseases. However, increasing evidence demonstrates that currently available hESC-CM represent immature embryonic or fetal stage, and they are structurally and functionally different from mature human cardiomyocytes. Therefore, the application of hESC-CM is largely limited by their immature phenotypes. In order to overcome the problems caused by the immature hESC-CM, it is needed to stimulate maturation of hESC-CM.

In this study, for the first time, We identified a natural compound stimulating maturation of hESC-CM. A two months treatment with Compound S from day 5 of diffrentiation increased cardiomyocyte size, density of t-tubule and mitochodnrial activity. hESC-CM

Treatment of the hESC with Compound S during cardiomyocyte differentiation stimulated expression of several cardiomyocyte-specific markers. Moreover, Compound S treatment increased the density of T-tubule and the expression of T-tubule-associated proteins. We checked that a damage of mitochondria represents cardiac irregularity of beating and make cardiomyocyte decreased beat period per minute.

In conclusion, the present study suggests that Compound S promotes maturation of hESC-CM by accelerating mitochondrial maturation and sustaining mitochondrial health, and the matured hESC-CM can be used for cardiac toxicity screening and cell therapy.

PO-343

# In Vitro Differentiation of Endothelial Cells from Porcine Epiblast Stem Cells

**Bo-Gyeong Seo[1,2], Soo-Been Jeon[3,4], Joon-Hee Lee[3,4], Cheol Hwangbo[1,2*]**

[1] Division of Applied Life Science, Gyeongsang National University, Korea
[2] Division of Life Science, Gyeongsang National University, Korea
[3] Department of Forest Environmental Resources, Gyeongsang National University, Korea
[4] Institute of Agriculture & Life Science, Gyeongsang National University, Korea

*chwangbo@gnu.ac.kr*

Pluripotent Stem Cells (PSCs) have the ability of self-renewal that can retain the characteristics of the mother cell, and of pluripotency that can differentiate into several body types. PSCs typically include embryonic stem cells (ESCs) derived from the inner cell mass (ICM) of the pre-implantation embryo, and epiblast stem cells (EpiSCs) derived from the epiblast of post-implantation embryo. Although PSCs are able to be used by differentiation into endothelial cells (ECs) as a potential treatment for vascular diseases, human ESCs and induced pluripotent stem cells (iPSCs) are followed by ethical and

safety issues. Pigs are anatomically and physiologically similar to humans.

Therefore, the goal of this study was to establish an efficient protocol that differentiates porcine epiblast stem cells (pEpiSCs) into the ECs for applying the treatment of human vascular diseases. AP-negative (-) pEpiSCs cultured in endothelial cell growth basal medium-2 (EBM-2) differentiation medium in association with 50 ng/ml of VEGF for 8 days were changed morphologically like the feature of ECs.

Expression of pluripotency-associated markers (OCT-3/4, NANOG, SOX2 and C-MYC) in porcine differentiated cells was significantly decreased ($P<0.05$). Additionally, a specific endothelial cell marker (CD31) was positively expressed in porcine differentiated cells when the pEpiSCs were cultured in EBM-2 + 50 ng/ml of VEGF.

Therefore, these results indicated that pEpiSCs cultured in EBM-2 + 50 ng/ml of VEGF culture condition were efficiently differentiated into ECs for the treatment of blood vessel diseases.

PO-344

# Centella Asiatica Attenuates Radiation Enteritis by Regulating Endothelial Secretion

**Seo Young Kwak[1], Hyosun Jang[1], Sehwan Shim[1], Min-Jung Kim[1], Won-Suk Jang[1], Sun-Joo Lee[1], Hyewon Kim[1], Sunhoo Park[1,2*]**

[1] Laboratory of Radiation Exposure & Therapeutics, Korea Institute of Radiological and Medical Sciences, Korea
[2] Department of Pathology, Korea Cancer Center Hospital, Korea

*sunhoo@kirams.re.kr*

Radiation-induced intestinal injury is observed following clinical application of radiation for pelvic cancers or occurs to radiation exposure following a nuclear accident. The endothelial cell is a key cell compartment for the response to radiation in normal tissue damage. Senescent endothelial cells product a paracrine senescence-associated secretory phenotype (SASP), which includes cytokines and growth factors. Centella asiatica (CA) has been widely used as a Chinese traditional herbal medicine and extensively researched the effects of wound healing on epithelium such as dermatitis. However, it has not yet been investigated that CA's effects in radiation-induced endothelial dysfunction and SASP that affects epithelial recovery. Here, we demonstrated the therapeutic effects of CA in radiation-induced endothelial dysfunction and communication of endothelial/epithelial cells in microenvironment.

We performed proliferative assay and SA-β-gal stain in CA treated irradiated (IR)-human umbilical vein endothelial cell (HUVEC). We also analyzed secreted cytokines from CA-treated IR-HUVEC using cytokine microarrays, ELISA, and immunofluorescence (IF). To identify the effects of secretome of

IR-HUVEC on radiation-induced epithelial damage, transepithelial electrical resistance (TEER) and paracellular permeability assay in Caco2 system were performed. Finally, we executed histological analysis of CA-treated radiation-induced enteritis mouse model.

CA-treated HUVEC was improved proliferation and reduced SA-b-gal activity compared to IR group. In the secretome analysis, CA-treated IR-HUVEC upregulated secretion of epidermal growth factor (EGF) compared to IR-HUVEC. We also identified increased EGF levels in CA-treated IR group using ELISA and IF. In addition, EGF secreted by CA enhanced TEER and inhibited paracellular permeability in radiation-induced epithelial damage. In addition, CA treatment rescued histological damage such as shortening villi length and impaired intestinal crypt and improved epithelial integrity in irradiated mouse.

Taken together, the present study suggests that CA enhanced endothelial dysfunction with secreted EGF, thereby improving epithelial damage and their efficacy in the treatment of radiation-induced enteropathy.

PO-345

# Stage Specific Transcriptome Profiles at Cardiac Lineage Commitment During Cardiomyocyte Differentiation from Pluripotent Stem Cells

**Sung Woo Cho[1*], Ji-Hee Sung[2], Hyoung Kyu Kim[2], Jin Han[2]**

[1] Department of Cardiology, Inje University College of Medicine, Ilsan Paik Hospital, Korea
[2] Cardiovascular and Metabolic Disease Center, Inje University College of Medicine, Korea

*drswcho@hanmail.net*

The cardiac specification and differentiation from pluripotent stem cells (PSCs) are quite complex and delicate; thus, each individual step of the induction, specification, and differentiation needs to be finely regulated. We investigated the stage specific transcriptome profiles and characteristics at cardiac lineage commitment during cardiomyocyte differentiation from PSCs.

We induced and sorted out Flk1+ mesodermal precursor cells (MPCs), PDGFRα+ cardiac lineage-committed cells (CLCs), PDGFRα+ cells without cardiac induction, and αMHC+ cardiomyocytes from mouse embryonic stem cells and performed microarray analysis of each cells. Genes with a p-value < 0.05 and a fold change > 3.0 were defined as differentially expressed genes.

Gene ontology analysis showed that PDGFRα+ CLCs highly expressed genes related to chemical and cytokine stimulus, cell adhesion and proliferation, and cardiovascular system development compared with Flk1+ MPCs. PDGFRα+ CLCs also significantly increased the gene expression related to heart and muscle development compared with

PDGFRα+ cells without cardiac induction. Furthermore, gene expression profiles of αMHC+ cardiomyocytes revealed a robust upregulation of genes associated with mitochondrial function and metabolism and ion channel activity compared with PDGFRα+ CLCs. We identified differently upregulated 118 genes (Il6, Cck, Fabp4, Mgst1, Zar1, Robo2, Olig3, Nell1, Saa3, Krt13, etc.) in PDGFRα+ CLCs compared with Flk1+ MPCs, PDGFRα+ cells without cardiac induction, and αMHC+ cardiomyocytes.

Collectively, PDGFRα+ CLCs can be characterized as proliferating cells, which are still in a morphologically and functionally immature state compared with differentiated cardiomyocytes. We identified differently upregulated genes at cardiac lineage specification during cardiomyocyte differentiation from PSCs.

PO-346

# Adhesion GPCR, Latrophilin-2, Specifies Cardiac Lineage Commitment Through CDK5, Src, and P38MAPK

**Choon-Soo Lee[1], Hyun-Jai Cho[2], Jin-Woo Lee[1], Hyo-Soo Kim[1,2*]**

[1] Strategic Center of Cell and Bio Therapy for Heart, Diabetes & Cancer, Seoul National University Hospital, Korea
[2] Department of Internal Medicine, Seoul National University Hospital, Korea

*hyosoo@snu.ac.kr*

Extensive studies on preclinical and clinical cell therapies for heart disease have employed several types of cells for cardiac repair. However, due to the low efficiency of cardiac differentiation from various types of stem cells, effective methods to generate homogeneous cardiac cells in sufficient amounts for clinical applications are still lacking. Therefore, we investigated a surface marker that is highly specific to cardiac progenitor cells (CPCs) and cardiomyocytes (CMCs) and that also has functional significance in both mice and humans. Here, we report a new cardiomyogenic cell surface marker latrophilin-2 (Lphn2), an adhesion G protein-coupled receptor.

When mouse and human pluripotent stem cells (PSCs) were stimulated with BMP4, Activin A, and bFGF, they differentiated into cardiac lineage cells. To examine the functional significance of Lphn2 in cardiac differentiation, we used Lphn2 knock-down or knock-out PSCs. To investigate the molecular mechanism underlying the induction of cardiac differentiation by Lphn2, we used the Phospho Explorer Antibody Array, which encompasses nearly all known signaling pathways.

Lphn2 was selectively expressed on CPCs and CMCs during the differentiation of mouse PSCs, and cell sorting with an anti-Lphn2 antibody promoted the isolation of populations highly enriched in CPCs and CMCs. In sorting experiments under cardiac differentiation condition, Lphn2+ cells derived from pluripotent stem cells strongly expressed cardiac-related genes (Mesp1, Nkx2.5, aMHC, and cTnT) as compared with Lphn2- cells. Lphn2 knock-down or knock-out PSCs did not express cardiac genes. The antibody-array analysis showed that CDK5 phosphorylation at Tyr15 increases the most during cardiac differentiation from mESCs. Analysis of the Lphn2 signaling pathway indicated that CDK5 is downstream of Lphn2 and associates with Src to induce P38MAP phosphorylation, subsequently activating cardiac-related gene transcription.

These findings provide a valuable tool for isolating cardiomyogenic progenitors and CMCs from PSCs and shed light on the still-unknown mechanisms of cardiac differentiation.

PO-347

# Vascular Regeneration Through The Reprogramming of Human Fibrobliasts by Plant Stem Cell Extracts

**Cheong-Whan Chae[1], Yoo-Wook Kwon[1*]**

[1] Cardiovascular Stem Cell Lab, Seoul National University Hospital, Korea

*ywkwon@snu.ac.kr*

We have previously established a new method to produce induced pluripotent stem cells by delivering embryonic stem cell-derived proteins into adult mouse fibroblast. However, the protocol was not optimized in human because of its difficulty to prepare sufficient yield of human ES cell extracts.

To overcome this problem, we hypothesized whether plant stem cell (callus)-derived proteins could reprogram human fibroblasts. The plant stem cell or callus, a dedifferentiated plant cell mass, can regenerate itself and differentiate into many tissues of a whole plant body. In this study, based on the dedifferentiation characteristic of plant callus, we observed reprogramming activities of plant callus extract on human dermal fibroblast.

We demonstrate molecule 'S', major component of Sequoiadendron Giganteum (SG) callus extract, reprogrammed somatic fibroblast to Mesodermal and Ectodermal precursor cells. These cells expressed neural precursor specific protein Nestin as well as Fibronectin and Vimentin and could differentiate into ectodermal and mesodermal lineage but not into endodermal lineage. These gene expression might be regulated by epigenetic modification including promoter methylation and H3K4me3.

These results indicated that the molecule 'S' could be an effective agent for direct conversion of fibroblast to Mesodermal and Ectodermal precursor cells and for vascular regeneration.

PO-348

# Re-defining Definitive Endothelial Progenitor Cells for Ischemic Cardiovascular Repair

**Yeon-Ju Kim[1], Sang-Mo Kwon[1,2*]**

[1] School of Medicine, Pusan National University, Korea
[2] 2Convergence Stem Cell Research Center, Pusan National University, Korea

*smkwon323@pusan.ac.kr*

Endothelial Progenitor Cells (EPCs) represent a promising cell source for revascularization of damaged tissue. In this study, we determined the uniform definition and hierarchy of an EPC, especially in regards to the proper molecular characterization and identifying markers, which makes therapeutic application for ischemic cardiovascular diseases.

Here, using xeno-free protocol, we developed and succeeded in the culture of EPCs (D-EPCs) with a high homogeneity and arterial specific characteristics from adult peripheral and umbilical cord blood. And then, we found that CD34+c-Kit+CXCR4+VE-cadherin+ (devoid of any mature lineage markers of hematopoietic system) phenotype could be used for identification of vascular endothelial progenitor cells. To investigate endothelial progenitor identity, we compared the genomic expression profiles and angiogenic potential of D-EPCs with different types of EPCs in vitro/in vivo.

The resulting xeno-free protocol produces cells (D-EPCs) with cobblestone shape, blood vessel-forming ability, and high clonal proliferative potential similar to Late EPCs (L-EPCs), also known as endothelial colony-forming cells (ECFCs). Besides, D-EPCs possess the stronger secretory function of angiogenic cytokines such as Early EPCs (E-EPCs). On the basis of these findings, we compared the cellular function of D-EPCs with different types of EPCs. Unlike E-EPCs with a short lifespan, we found that D-EPCs showed relatively low proliferative properties but could long-term expansion culture than L-EPCs via delayed cellular senescence. Further, D-EPCs significantly enhanced vessel formation, incorporation, and the secretion of angiogenic cytokines compared with both types of EPCs. In a murine HLI, MI model, we also demonstrated that D-EPCs improve therapeutic effects through the blood vessel differentiation and M2 macrophage polarization.

Our findings may offer important insight of EPC biology via redefining the cells and provide a clinically applicable therapeutic source for ischemic cardiovascular diseases.

# 4. Vascular Diseases

PO-403

## GNAQ-Mutant Endothelial Cells in Capillary Malformations Have Altered Response to Shear Stress and Reduced Barrier Integrity

**Colette Bichsel[1,2], Lan Huang[1,2], Sanda Alexandrescu[3], Anna Pinto[4], Joyce Bischoff[1,2*]**

[1] Vascular Biology Program, Boston Children's Hospital and Harvard Medical School, USA
[2] Department of Surgery, Boston Children's Hospital and Harvard Medical School, USA
[3] Department of Pathology, Boston Children's Hospital, USA
[4] Department of Neurology, Boston Children's Hospital, USA

*joyce.bischoff@childrens.harvard.edu*

Capillary Malformations (CM) consist of aberrant blood vessels in skin, brain leptomeninges and choroid. The identified somatic point mutation in GNAQ, p.R183Q, activates the G-protein subunit Gαq and is enriched in endothelial cells. Our aim is to uncover the mechanisms by which aberrant Gαq activation leads to CM and vessel overgrowth. Vascular beds from patients' skin and brain show abnormal vessel shapes, sprouting, stasis and red blood cell leakage, prompting us to hypothesize the mutant endothelial cells have impaired response to shear stress and barrier function.

To test this, we exposed human endothelial colony forming cells (ECFC) expressing wild-type (WT) or R183Q GNAQ to laminar shear stress (LSS) for 24h and measured barrier function with trans-endothelial electrical resistance (TEER).

WT-ECFC and R183Q-ECFCs aligned with the di-

rection of flow, but R183Q-ECFC failed to down-regulate angiopoietin-2 (ANGPT2), as expected for healthy endothelium and as seen in WT-ECFC. Further, pyruvate kinase M2 (PKM2), which contributes to endothelial barrier formation by downregulating ANGPT2, was not upregulated in LSS-exposed R183Q-ECFC. Consistent with ANGPT2's role in vessel stability, R183Q-ECFC cell-cell junctions were underdeveloped, shown by ZO-1 staining. In vivo, endothelial cells lining CM in skin and brain showed high ANGPT2 by immunofluorescence, consistent with the in vitro results. We saw lower TEER values in R183Q-ECFC, suggesting reduced barrier integrity. When challenged with thrombin, which signals through PAR1 and Gαq, R183Q-ECFC showed slower recovery of barrier integrity. R183Q-ECFCs responded more robustly than WT-ECFC to the sphingolipid S1P, but the maximum TEER achieved was still lower in the R183Q-ECFC.

Our experiments suggest elevated ANGPT2 in R183Q-ECFC, due to a faulty response to shear stress, reduces endothelial barrier integrity, which may perpetuate pro-angiogenic activities to drive CM.

PO-404

# Angiocrine FSTL1(Follistain-Like Protein 1) insufficiency Leads to Atrial and Venous Wall Fibrosis via SMAD3 Activation

**Haijuan Jiang[1], Luqing Zhang[1,2], Xuelian Liu[1], Wei Sun[2,3], Katsuhiro Kato[4,5], Chuankai Chen[1], Xiao Li[1], Taotao Li[1], Zhiliang Sun[1], Wencan Han[2], Fujing Zhang[2], Qi Xiao[2], Zhongzhou Yang[2], Junhao Hu[6], Zhihai Qin[7], Ralf H Adams[4], Xiang Gao[2], Yulong He[1*]**

[1] Cyrus Tang Hematology Center, Soochow University, China
[2] MOE Key Laboratory for Model Animal and Disease Study, Model Animal Research Institute, Nanjing University, China
[3] Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, China
[4] Max-Planck-Institute for Molecular Biomedicine, Department of Tissue Morphogenesis, University of Münster, Faculty of Medicine, Germany
[5] Department of Cardiology, Nagoya University Hospital, Japan
[6] Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China
[7] The First Affiliated Hospital of Zhengzhou University, Academy of Medical Sciences, Zhengzhou University, China

*heyulong@suda.edu.cn*

Angiocrine factors, mediating the endothelial-mural cell interaction in vascular wall construction as well as maintenance, are incompletely characterized. This study aims to investigate the role of endothelial cell-derived FSTL1 (follistatin-like protein 1) in vascular homeostasis.

Using conditional knockout mouse models and cell-specific Cre deletors, we generated the endothelial cell-specific Fstl1 knockout mouse models (Fstl1Flox/-;Tek-Cre, named Fstl1ECKO). αSMA-Cre and Vav-iCre mouse lines were used for Fstl1 deletion in smooth muscle cells (SMCKO; Fstl1Flox/-;αSMA-Cre, named Fstl1SMCKO) or hematopoietic cells (HCKO; Fstl1Flox/-;Vav-iCre, named Fstl1HCKO). In addition, an inhibitor for TGFβ

type I receptor kinase (SB431542) was employed to rescue the cardiovascular abnormality of Fstl1 mutant mice by the suppression of TGFβ-mediated signaling.

We show that loss of FSTL1 in endothelial cells (Fstl1ECKO) led to an increase of pulmonary vascular resistance, resulting in the heart regurgitation especially with tricuspid valves. However, this abnormality was not detected in mutant mice with Fstl1 deletion in smooth muscle cells or hematopoietic cells. We further showed that there was excessive αSMA associated with atrial endocardia, heart valves, veins, and microvessels after the endothelial FSTL1 deletion. There was also an increase in collagen deposition, as demonstrated in livers of Fstl1ECKO mutants. The SMAD3 phosphorylation was significantly enhanced, and pSMAD3 staining was colocalized with αSMA in vein walls, suggesting the activation of TGFβ (transforming growth factor β) signaling in vascular mural cells of Fstl1ECKO mice. Consistently, treatment with a TGFβ pathway inhibitor reduced the abnormal association of αSMA with the atria and blood vessels in Fstl1ECKO mutant mice.

The findings imply that endothelial FSTL1 is critical for the homeostasis of vascular walls, and its insufficiency may favor cardiovascular fibrosis leading to heart failure.

PO-407

# gp130 Signaling in CD4 Cells Is Important for the Pathogenesis of Pulmonary Arterial Hypertension

**Tomohiko Ishibashi[1], Takeshi Masaki[1], Tadakatsu Inagaki[1], Makoto Okazawa[1], Yoshikazu Nakaoka[1*]**

[1] Department of Vascular Physiology, National Cerebral and Cardiovascular Center Research Institute, Japan

*ynakaoka@ncvc.go.jp*

Despite the recent development of a novel therapeutic strategy, patients with advanced pulmonary arterial hypertension (PAH) have still poor prognosis. Therefore, new therapeutic agents based on the specific molecular pathogenesis of PAH are in great demand. We have reported the significant role of interleukin (IL)-6/IL-21 signaling for promoting the pathogenesis of hypoxia-induced PAH (HPH) mouse model (Hashimoto-Kataoka T, et al. PNAS. 2015;112:E2677). However, the target cells of IL-6 are poorly understood. The objective of this study is to elucidate the target cells of IL-6 in the pathogenesis of PAH.

We crossed mice harboring floxed allele of gp130, a subunit of the IL-6 receptor complex, with tissue-specific Cre mice, including Cdh5-CreER$^{T2}$ (for endothelial cells), Myh11-CreER$^{T2}$ and SM22α-Cre (for smooth muscle cells) mice. These mice were exposed to chronic hypoxia (10% oxygen) for 3 weeks, and right ventricular systolic pressure (RVSP) and right ventricular hypertrophy (RVH) were evaluated. Specificity of Cre-mediated recombination was also evaluated by crossing with mice that exhibit tdTomato fluorescence after Cre-mediated recombination.

Endothelial cell-specific gp130 deletion did not

improve the phenotype of HPH mice. Of the two smooth muscle cell-specific Cre mice, Myh11-CreER$^{T2}$ did not improved the phenotype of HPH mice either. However, gp130 deletion using SM22α-Cre mice showed improvement in RVSP and RVH. Because SM22α-Cre mice showed robust Cre recombination not only in smooth muscle cells but also in CD45-positive hematopoietic lineage cells, we assumed that the improvement of HPH phenotype in gp130$^{flox}$ / SM22α-Cre mice might be resulted in gp130 deletion in hematopoietic lineage cells. Actually, CD4 cell-specific gp130 deletion by crossing gp130$^{flox}$ mice with CD4-Cre mice ameliorate RVSP and RVH in HPH mouse.

These findings suggest that gp130 signaling in CD4 cells plays an important role in the pathogenesis of PAH.

PO-408

# Critical Roles of Interleukin-21 Signaling in the Pathogenesis of Pulmonary Arterial Hypertension

**Tadakatsu Inagaki[1], Tomohiko Ishibashi[1], Makoto Okazawa[1], Takeshi Masaki[1], Marie Mitusi[2], Kazuteru Aoki[2], Ryotaro Asano[3], Takeshi Ogo[3], Yoshikazu Nakaoka[1*]**

[1] Vascular Physiology, National Cerebral and Cardiovascular Center, Japan
[2] Discovery Research, RIBOMIC Inc., Japan
[3] Advanced Medical Research for Pulmonary Hypertension, National Cerebral and Cardiovascular Center, Japan

*ynakaoka@ncvc.go.jp*

Pulmonary arterial hypertension (PAH) is a severe disease which causes stenosis and occlusion in the pulmonary small arteries and arterioles, leading to elevation of pulmonary arterial pressure due to unknown etiology. We previously reported the importance of intelrleukin-6(IL-6)/IL-21-signaling pathway in the pathogenesis of PAH in hypoxia-induced PAH mouse model. In this study, we aimed to validate the effect of IL-21 blockade on the pathogenesis of PAH in the severe PAH rat model using IL-21 receptor (R) knockout (KO) rat, and an IL-21 aptamer for treatment of PAH.

We created IL-21RKO rats via CRISPR/Cas genome editing and subjected them to the Su5416/Hypoxia (Su/Hx) induced severe PAH rat model. Rats were treated with Sugen5416 (20 mg/kg, s.c.) followed by 3 weeks of chronic hypoxia (10%O2) and then returned to normoxia for 5 weeks. Next, we successfully developed IL-21-specific aptamers against both mice and rats, and examined the effect of pharmacological inhibition of IL-21 signaling using IL-21 aptamers on the pathogenesis of both mouse and rat PAH models.

Although WT rats show the significant elevation of right ventricular systolic pressure (RVSP) and severe vascular remodeling in the pulmonary arterioles in Su/Hx model, IL-21RKO rats show significant resistance to PAH. In addition, the pathologies of both hypoxia-induced PAH in mice and Su/Hx-induced severe PAH were suppressed by both IL-21 aptamer treatments.

In this study, we examined the effects of IL-21 signal blockade on PAH pathogenesis in severe PAH models using IL-21R deficient rats and in both mouse and rat PAH models using IL-21 aptamer. These results indicate that IL-21-signaling has a central role for the pathogenesis of PAH, and IL-21 blockade might be a promising therapeutic strategy for refractory PAH.

PO-409

# Notch-Dependent Endothelial Dysfunction in Aortic Valve of Patients with Aortic Valve Calcification

**Aleksandra Kostina[1*], Maria Bogdanova[2], Artem Kiselev[3], Daria Semenova[3], Olga Irtyuga[3], Anna Kostareva[3], Jarle Vaage[2], Anna Malashicheva[1,3]**

[1] Laboratory of Regenerative Biomedicine, Institute of Cytology RAS, Russian Federation
[2] Department of Molecular Medicine, University of Oslo, Norway
[3] Institute of Molecular Biology and Genetics, Almazov National Medical Research Centre, Russian Federation

*aleksandrakostina1991@gmail.com*

Endothelial cells ensure integrity of valve tissue regulating differentiation and functions of adjacent interstitial cells. Notch is a key signaling during cardiac valvulogenesis ensuring cross talk between different cell types. Dysregulation of Notch is involved in pathologies of aortic valve such as bicuspid aortic valve and aortic valve calcification, but the mechanisms of Notch–dependent aortic valve pathologies remain unclear.

The aim of this study was to elucidate Notch-dependent regulatory mechanisms of endothelial dysfunction in patients with aortic valve calcification.

Primary human aortic valve endothelial and interstitial cells (VEC and VIC) were isolated from patients with calcific aortic valve disease (CAVD) and healthy donors. Notch was activated by lentiviral transduction with NICD (Notch1 intercellular domain). shRNA-mediated knockdown of RPBJ was employed to inhibit Notch activity. Osteogenic differentiation was induced by osteogenic medium. Effectiveness of osteogenic differentiation was evaluated by proosteogenic genes expression and Alizarin staining.

Expression levels of NOTCH2, NOTCH3, JAG1, SNAIL and SLUG were significantly lower in VEC from the patients compared to healthy controls. VEC from CAVD patients in co-culture with healthy VIC upon osteogenic induction caused more efficient osteogenic differentiation compared to VIC monoculture. Activation of Notch pathway in endothelial cells caused upregulation of all Notch components; RBPJ knockdown resulted in inactivation of Notch. Notch activation in VIC increased efficiency of osteogenic differentiation. Knockdown of RPBJ in VIC under osteogenic induction inhibited osteogenic differentiation. Coculture of VIC and VEC with RPBJ knockdown resulted in reduced osteogenic differentiation representing the inability of VEC to enhance VIC osteogenic differentiation due to Notch inhibition.

Notch signaling pathway is dysregulated in VEC of patients with CAVD. Notch-dependent endothelial interaction causes proosteogenic signaling in VIC initiating aortic valve calcification.

PO-410

# Inhibition of p90RSK Ameliorates PDGF-BB-Induced Vascular Smooth Muscle Cell Phenotypic Change and Neointimal Hyperplasia

**Ae-Rang Hwang[1], Heejung Lee[1], Chang-Hoon Woo[1*]**

[1] Pharmacology and Smart-Ageing Convergence Research Center, Yeungnam University College of Medicine, Korea

*changhoon_woo@yu.ac.kr*

Platelet-derived growth factor type BB (PDGF-BB) has been reported to induce vascular smooth

muscle cell (VSMC) proliferation and migration, which are major events that are highly linked to vascular diseases such as atherosclerosis and restenosis. Although p90RSK have been shown to regulate multiple cellular processes including cell growth, proliferation, survival and motility through various downstream substrates in different cell types, the role of p90RSK on PDGF-BB-induced VSMC proliferation and migration, and vascular neointima formation remain unknown. In this study, we determine whether inhibition of p90RSK protects from PDGF-BB-induced cellular phenotypic change and investigate the molecular mechanisms underlying the effect of p90RSK inhibition.

VSMCs were pretreated with FMK (2 or 5uM) or BI-D1870 (2 or 5uM) and then incubated with PDGF-BB (1ug/ml). The cells were collected for levels of gene and protein expression, MTT assay, FACS analysis, and a wound scratch assay. Male C57BL/6 mice were i.p. injected with FMK (5mg/kg per day) for 3 weeks.

In cultured primary rat VSMCs, we found that pretreatment with FMK or BI-D1870, specific inhibitors of p90RSK, suppressed PDGF-BB-induced VSMC phenotypic alteration, ECM accumulation, proliferation, and migration. In addition, FMK and BI-D1870 repressed cell cycle regulatory genes cyclin D1 and Cdk4 and augmented cyclin-dependent kinase inhibitor p27 in PDGF-BB-induced VSMCs, suggesting that p90RSK induces VSMC proliferation via cell cycle regulation of G1/G0 phase. Notably, using the mouse model of partial carotid ligation model, FMK was found to attenuate the neointimal hyperplasia of carotid arteries.

These results suggest that p90RSK impedes PDGF-BB-induced VSMC phenotypic switching, proliferation, migration and neointima formation.

PO-411

# Substance P Ameliorates BAPN-Induced Thoracic Aortic Dissection Through Modulation of M2 Monocyte-Skewed Monocytopoiesis

**Jiyuan Piao[1], Hyun Sook Hong[2,3*], Youngsook Son[1]**

[1] Department Of Genetic Engineering, College Of Life Science and Graduate School Of Biotechnology, Kyung Hee University, Korea
[2] Department of Biomedical Science and Technology, Graduate School, Kyung Hee University, Korea
[3] East-West Medical Research Institute, Kyung Hee University, Korea

*hshong@khu.ac.kr*

Thoracic aortic dissection (TAD) is one of lethal cardiovascular diseases with few treatments in clinic. Although many clinical trials have been used to treat TAD, they have not resolved the fundamental problems. Substance P (SP) is well-known to provide anti-inflammatory effects and promote restoration of damaged endothelium, leading to vasculature protection and the facilitation of tissue repair. In this study, we explored the protective effects of SP on development of TAD.

To create an aortic dissection preclinical disease model, lysyl oxidase inhibitor—BAPN was administered to SD rats orally for 4 weeks and SP was injected intravenously, concurrently with BAPN treatment, twice a week for 4 weeks. The efficacy and action mechanism of SP was evaluated based on the survival rate, aorta histology, immune-cell profiles, and molecular changes in the aorta.

BAPN treatment caused the dilation of aorta with infiltration of monocyte and elevation of pro-inflammatory cytokine within 1 week and aortic dissection within 4 weeks-post treatment. However, SP clearly

attenuated BAPN-induced inflammation, smooth muscle cell (SMC) phenotype switching and SMC apoptosis, leading to inhibition of development of TAD. At the early stage of disease progression, SP treatment mitigated BAPN- activated inflammatory responses via accelerating M2-biased monocytopoiesis in the spleen thereby supplying M2-monocytes and anti-inflammatory cytokine enriched environment in the circulation within 24 hours. Although SP did not ameliorate BAPN-promoted monocyte chemoattractant protein-1 level in the blood, SP-induced M2-monocyte and its paracrine factors could provide immunosuppressive conditions under BAPN treatment. Additionally, SP-mediated immune suppression decreased the inflammation induced-VCAM-1 expression to protect endothelium and this function might contribute to the decline in infiltration of monocytes into injured aorta.

This study supports that SP blocks TAD development by modulating endothelial dysfunction and immune response, simultaneously.

PO-412

# Endothelial Sox17 Deletion Promotes Hypoxia-Induced Pulmonary Arterial Hypertension

**Chan Soon Park[1], Soo Hyun Kim[1], Hae Young Yang[1], Seung Eun Baek[1], Ho Cheol Jang[1], Injune Kim[1*]**

[1] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Korea

*injunek@kaist.ac.kr*

Pulmonary artery hypertension (PAH) is a detrimental disease characterized by pathologic vascular remodelling. Recent genome-wide studies found mutations of Sox17, an endothelial transcription factor regulating vascular homeostasis, in patients with PAH. Therefore, we aimed to establish a PAH disease model using mutant mice lacking endothelial Sox17 (Sox17iΔEC).

In this study, we assessed pathologic features of control and Sox17iΔEC mice exposed to normoxia or hypoxia.

In morphometric analyses, Sox17iΔEC/hypoxia mice showed substantially thickened distal pulmonary arteries compared to control mice, in both vascular thickness and vascular thickness/diameter ($P<0.001$, respectively). Even compared with hypoxia mice which were conventionally used as a PAH model, Sox17iΔEC/hypoxia mice demonstrated remarkable change in wall thickness. In contrast, hypoxia mice showed significant wall thickening in vascular thickness, but its significance was diminished when vascular diameter was adjusted. Sox17iΔEC/hypoxia mice also showed a significantly increased number of muscularized distal pulmonary arteries in comparison with other groups, while hypoxia mice failed to show statistical significance in post-hoc analysis. Furthermore, right ventricular hypertrophy measured as incremented fulton index were observed in Sox17iΔEC/hypoxia mice.
Based on mutations in PAH patients, we established a disease model recapitulating severe PAH, which would be useful to explore pathophysiology underlying PAH.

PO-414

# Protective Roles of miR-34c-5p in Hypoxia by Reducing Apoptosis Through BCL2

**Soyoung Kim[1], Jaeseok Han[1,2], Young-Ho Ahn[3], Chang Hoon Ha[1], Nayoung Kim[1], Jae-Joong Kim[4*]**

[1] Department of Convergence Medicine & Asan Institute for Life Sciences, Asan Medical Center, Korea
[2] Asan Medical Institute for Convergence Science and Technology, University of Ulsan College of Medicine, Korea
[3] Department of Molecular Medicine, College of Medicine, Ewha Womans University, Korea
[4] Division of Cardiology, Department of Internal Medicine, Asan Medical Center, Korea

*jjkim@amc.seoul.kr*

Cardiac allograft vasculopathy (CAV) is a major cause of death in patients after cardiac transplantation. Since the severe CAV development leads to intimal thickening of coronary vessels, early diagnosis is one of the critical factors for the long-term survival in cardiac transplant patients. Our previous data showed that miR-34c levels were up-regulated in a cardiac transplant patient with CAV, compared with that without CAV. In this study, we aimed to investigate the mechanism of miR-34c in response to hypoxia.

Human umbilical vein endothelial cells (HUVECs) were cultured in EGM-2MV media (Lonza), and incubated with hydrogen peroxide ($H_2O_2$) for 24 hours for dysoxia condition. Hindlimb ischemia was performed in mice and the hindlimb blood vessels were harvested after 3 days. The cell proliferation and caspase 3/7 activity were measured by CCK-8 assay and caspase 3/7 assay, respectively. Protein levels were assessed by western blotting.

The levels of miR-34c increased in HUVECs under dysoxia condition, and in the murine hindlimb blood vessels following ischemia. The results of luciferase assay and western blot analysis showed that miR-34c directly repressed BCL2 expression. To investigate the role of miR-34c-mediated BCL2 repression in response to dysoxia, the cell proliferation and caspase 3/7 activities were assessed in miR-34c-overexpressed or inhibited HUVECs with or without dysoxia. The data demonstrated that reduced BCL2 expression promoted cell proliferation and decreased caspase 3/7 activities. Furthermore, when miR-34c was overexpressed, the protein-level of LC3 was up-regulated, suggesting that miR-34c-mediated BCL2 repression induces autophagy signal.

We validated that the expression of miR-34c were up-regulated in dysoxia-induced HUVECs and in murine hindlimb ischemia. BCL2 expression was suppressed by miR-34c, leading to enhanced cell proliferation, reduced apoptosis, and increased autophagic activities in HUVECs. Taken together, the results suggest that miR-34c might have a protective role in hypoxia.

PO-415

# Ciliary Ift46 Is Required for Cardiovascular Development

**Seungwoon Seo[1], Ok Hee Kweon[1], Mi Bae Park[1], Jung Yun Jun[1], Goo Taeg Oh[1*]**

[1] Life Science, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

Intraflagellar transport (IFT) is the bidirectional transport of multisubunit protein complexes; retrograde IFT (IFT-A) and anterograde IFT (IFT-B). Mutations cause a wide range of genetic disorders, called ciliopathies which involve dysfunction of the cilium. IFT46, a member of IFT-B, localizes to the primary cilia and is involved in assembly and stability of cilia, however, its precise role in cardiovascular development has yet to be determined.

To specifically delete Ift46 from cardiovascular progenitors (CP), conditional knockout mice for Ift46 (CP- Ift46–/–) were generated by crossing Ift46Floxed mice with Mesp1-Cre mice, which have Cre activity in cardiovascular lineages.

Conventional Ift46–/– mutant embryos lack primary cilia and die at E10.5 due to multiple abnormalities with cardiac effusion, randomized heart looping, defective left-right patterning. CP- Ift46–/– mice have embryonic lethality around E16. 5 and show cardiac edema, vascular hemorrhage, atrial and ventricular septal defects, and persistent truncus arteriosus.

Ciliary Ift46 is essential for early cardiovascular development and vascular remodeling

PO-416

# The Single-Cell Transcriptome Analysis Reveals the Cellular Heterogeneity of Murine Aortic Valve Sclerosis

**Seung Hyun Lee[1], Nayoung Kim[2,3], Min-Kyu Kim[1], Inhee Han[1], Woong-Yang Park[3], Hae-Ock Lee[2,3], Jae-Hoon Choi[1*]**

[1] Department of Life Sciences, College of Natural Sciences, Research Institute for Natural Sciences, Hanyang University, Korea
[2] Department of Biomedicine and Health Sciences, Graduate School, The Catholic University of Korea, Korea
[3] Samsung Genome Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, Korea
[4] Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, Korea

*jchoi75@hanyang.ac.kr*

Aortic Stenosis is one of the prevalent degenerative valvular diseases with a high mortality rate. This disease is considered as irreversible, because of its trait of severe calcification. Therefore, it is important to understand the pathogenesis of Aortic Valve Sclerosis (AVS): the predisposing stage of Aortic Stenosis. Some previous studies are demonstrating the cellular diversity of the aortic valve in a normal state; however, it is still unclear to understand which cells participate in the progression of AVS.

To understand the cellular complexity, we performed Single-cell RNA Sequencing using two different AVS model mice (Apoe-/-, Ldlr-/- with high-cholesterol diet) and control mice (C57BL/6J mice with standard chow diet). To determine the localization of each cell cluster, histological techniques (RNA in situ hybridization, Immunofluorescence) were used.

Single-cell RNA Sequencing analysis elucidated the heterogeneous cellular composition of the aortic valve with AVS. Compared to the normal, AVS showed increased macrophage and other immune cell infiltration. We also found an AVS model-enriched endothelial cell cluster, which may have a key function to represent side-specific valvular lipid accumulation. By histological analysis, we identified the cluster-specific spatial distribution of each cell type including two different valvular foamy cells: Macrophage derived- and Valvular Interstitial Cell derived-foamy cells.

We expected that this study would be a guidance to understand the early mechanism of valvular disease progression.

PO-417

# Low-Density Lipoprotein, Not Very-Low-Density Lipoprotein, Triggers a Pro-Inflammatory Response in Valvular Interstitial Cells.

**Minkyu Kim[1], Seung Hyun Lee[1], Nayoung Kim[2], Inhee Han[1], Jongsuk Chung[3], Hae-Ock Lee[2], Woong-Yang Park[3], Jae-Hoon Choi[1*]**

[1] Department of Life science, College of Natural Sciences, Research Institute for Natural Sciences, Hanyang University, Seoul, Korea
[2] Department of Biomedicine and Health Sciences, Graduate School, The Catholic University of Korea, Seoul, Korea
[3] Samsung Genome Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

*jchoi75@hanyang.ac.kr*

Aortic valve stenosis is considered as most common of all valvular heart diseases. Because it is irreversible once it occurs, it is worthwhile to study the early stage of the disease. In this study we tried to identify role of Valvular interstitial cells(VICs) in hyperlipidemic conditions

To identify lipid accumulation in aortic valve Oil red O staining was performed in Apoe-/-, and Ldlr-/- mice fed western diet for 12 weeks. After identified lipid accumulation, to determine which cell type has lipid deposition, we stained lipid with BODIPY493/503 in whole-mount aortic valve. To elicit if VICs have different ability to uptake LDL, VLDL and oxLDL, in-vitro cultured VICs and ex-vivo cultured aortic valve were treated with DiI labeled Lipoprotein (LDL, VLDL, and oxLDL). Next, we performed adhesion assay with aortic valve to determine if lipid laden valve has ability to recruit monocyte.

Ldlr-/- mice fed western diet for 12 weeks showed significant amount of lipid accumulation in aortic valve compared to Apoe-/- mice. BODIPY493/503 staining in aortic valve showed major lipid-laden cell type was VICs. VICs uptake LDL more compared to oxLDL, while hardly uptake VLDL. VICs treated with oxLDL has increased level of Cx3cl1 mRNA. In adhesion assay, oxLDL treated aortic valve had more infiltrated monocyte than valve treated with PBS or LDL.

These results suggest that VICs may play an important role in immune cell recruitment to aortic valve upon aortic sclerosis which is the predisposing state of aortic stenosis.

PO-418

# Disruption of the Balance Between NUDT6 and FGF2 in Atherosclerotic Vascular Diseases

**Hong Jin[1,2], Hanna Winter[3], Ekaterina Chernogubova[2], Urszula Rykaczewska[1], Greg Winski[2], Alexandra Bäcklund[2], Lars Maegdefessel[2,3*]**

[1] Department of Molecular medicine and surgery, Karolinska Insitute, Sweden
[2] Department of Medicine, Karolinska Insitute, Sweden
[3] Department of Vascular and Endovascular Surgery, Technical University Munich, Germany

*Lars.Maegdefessel@ki.se*

Nudt6 as a natural antisense of Fgf2 was found to be significantly increased in human ruptured carotid atherscerotic plaques from our human transcriptom array database. The aim of the study was to identify the role of Nudt6 in plaque development and stability, as well as to investigate the potential

therapeutic application by using Nudt6 GapmeR inhibitor to stabalize the vulnerable lesion.

12 week old male ApoE-/- mice were used for carotid ligation and cuff model to induce plaque development the rupture.10mg/kg fluorescence labeled scrambled control oligonucleotide or NUDT6 specific GapmeR inhibitor were injected intraperitoneally to knockoukdown Nudt6 expression. In vitro study, we treated human carotid artery smooth muscle cells for Nudt6 gain and loss function study, as well as treated with different stimuli to observe FGf2 anf Nudt6 mRNA expression.

Increased Nudt6 with reduced Fgf2 were found in both human and mouse ruptured carotid artery atherosclerotic plaques. Nudt6 inhibition increased the mouse plaque stability. In vitro modulation showed decreased proliferation and enhanced apoptosis upon Nudt6 overexpression. More importantly, oxLDL can significantly increase Nudt6 mRNA while reduce Fgf2 mRNA levels, which may via epigenetic modulation.

The present study identifies NUDT6 as a novel antisense transcript of FGF2, which triggers vascular smooth muscle cell apoptosis . Therapeutic targeting of NUDT6 may serve as a novel treatment strategy to increase the athersclerotic plaque stability and prevent acute thrombotic complications.

PO-419

# Apelin-13 Inhibits Methylglyoxal-Induced Unfolded Protein Responses and Endothelial Dysfunction via Regulating AMPK Pathway

**Sujin Kim[1], Seonhui Kim[1], Changhoon Woo[1*]**

[1] Pharmacology, Yeungnam University College of Medicine, Korea

*canghoon_woo@yu.ac.kr*

The goal of study to investigate the molecular mechanism by which MGO induces endothelial dysfunction via the regulation of ER stress in endothelial cells, and to examine whether apelin-13, a cytoprotective polypeptide ligand, protects MGO-induced aortic endothelial dysfunction.

MGO-induced ER stress and apoptosis were determined by immunoblotting and MTT assay in HUVECs. For aortic endothelial dysfunction was addressed by en face immunostaining and acetylcholine-induced vasodilation analysis with aortic rings from mice treated with MGO in the presence or absence of apelin ex vivo.

TUDCA, an inhibitor of ER stress, inhibited MGO-induced apoptosis and reduction of cell viability, suggesting that MGO signaling to endothelial apoptosis is mediated via ER stress, which leads to activation of unfolded protein responses.

Apelin-13 could be a novel target for therapeutic intervention of endothelial dysfunction related to diabetic vascular complications.

PO-421

# Cardiac Microvascular Endothelial Regulation of Cardiomyocyte Relaxation and Contraction Is Impaired in Chronic Kidney Disease and Restored by Empagliflozin

**Rio Juni[1*], Rushd Al-Shama[1], Diederik Kuster[1], Jolanda Van der Velden[1], Henrike Hamer[2], Marc Vervloet[3], Etto Eringa[1], Pieter Koolwijk[1], Victor Van Hinsbergh[1]**

[1] Physiology, Amsterdam University Medical Centers, Netherlands
[2] Clinical Chemistry, Amsterdam University Medical Centers, Netherlands
[3] Nephrology, Amsterdam University Medical Centers, Netherlands

*r.juni@amsterdamumc.nl*

Endothelial dysfunction is associated with chronic kidney disease (CKD), a co-morbidity that is highly prevalent in patients with heart failure (HF), in particular HF with preserved ejection fraction (HFpEF). Whether impaired endothelial function links the development of cardiac abnormalities in CKD is unclear. It has been shown that a sodium-glucose co-transporter 2 inhibitor, empagliflozin, improves clinical outcome of patients with CKD and HF. We hypothesized that uremic serum from CKD patients impairs cardiomyocyte relaxation and contraction by inducing cardiac microvascular endothelial dysfunction, and that empagliflozin protects against this effect.

Co-culture system of human cardiac microvascular endothelial cells (CMECs) with adult rat ventricular cardiomyocytes (CMs).

We showed that CMECs promote CM relaxation and contraction. Serum from CKD patients impaired endothelial enhancement of CM function which was rescued by empagliflozin. Exposure to uremic serum reduced nitric oxide (NO) bioavailability in CMECs and increased mitochondrial reactive oxygen species (ROS) and 3-nitrotyrosine level, indicating NO scavenging by ROS. Empagliflozin restored endothelial enhancement of NO level in CMs by restoring endothelial NO bioavailability and reducing endothelial mitochondrial ROS, an effect that was largely independent of sodium-hydrogen exchanger-1.

Endothelium exposure to serum from CKD patients impairs endothelium-induced enhancement of CM relaxation and contraction through induction of endothelial dysfunction driven by an increase in mitochondrial ROS production. Empagliflozin restores the enhancement effect of CMECs on CM function by reducing mitochondrial oxidative damage, leading to reduced ROS accumulation and increased endothelial NO bioavailability.

PO-422

# Biomechanical Signaling and Non-Genetic Triggers in Cerebral Cavernous Malformations

**Salim Abdelilah-Seyfried[1*]**

[1] Institute of Biochemistry and Biology, Potsdam University, Germany

*salim.seyfried@uni-potsdam.de*

Cerebral cavernous malformations (CCM) are pathologies of the brain vasculature characterized

by capillary-venous angiomas that result in recurrent cerebral hemorrhages. Familial forms are caused by a clonal loss of any of three CCM genes in endothelial cells. The etiopathogeny of CCMs remains largely unclear. We still lack a good understanding of the molecular and physiological triggers that cause acute phases of CCM lesion growth and bleeding. Several studies suggest that a loss of CCM proteins causes pathological changes in biomechanical signaling, which has severe consequences for cardiovascular development and physiology. That blood flow may be an important factor in the etiology of the CCM pathology stems from a number of observations in human patients and diverse CCM animal models. In patients, lesions are mostly restricted to venous capillary beds, which only experience low levels of fluid shear stress.

We investigated the role of blood flow for CCM formation. In zebrafish, a complete loss of Ccm proteins causes severe cardiac defects that result in a lack of blood flow. Here, we used a genetic approach to restore cardiac function and blood flow in a zebrafish model of CCM1.

Without blood flow, these mutants exhibit vascular anomalies in major blood vessels including the lateral dorsal aorta. However, when Ccm1 was rescued specifically within the heart, blood flow was restored in ccm1 mutants. This prevented overgrowth of the lateral dorsal aorta.

This experiment suggests that blood flow has a vasoprotective role within strongly perfused blood vessels in CCM. This suggests a hypothesis that can explain the lack of vascular anomalies within major blood vessels of CCM patients.

PO-423

# Deficiency of Peroxiredoxin 2 Exacerbates Angiotensin II-Induced Abdominal Aortic Aneurysm

**Min Ji Cho[1,2], Jeong-Ki Min[1,2], Goo Taeg Oh[3], Jong-Gil Park[1*]**

[1] Biotherapeutics Translational Research Center, Korea Research Institute of Bioscience & Biotechnology, Korea
[2] Department of Biomolecular Science, University of Science & Technology (UST), Korea
[3] Immune and Vascular Cell Network Research Center, Ewha Womans University, Korea

*jonggilpark@kribb.re.kr*

Abdominal aortic aneurysm (AAA) is an inflammatory vascular disease characterised by structural deterioration of the aortas by inflammation and oxidative stress, leading to aortic dilatation and rupture. Peroxiredoxin 2 (PRDX2), an antioxidant enzyme, has been reported as a potential negative regulator in inflammatory vascular diseases and has been identified as an increased protein in ruptured AAA more so than nonruptured AAA from patients. In this study, we demonstrated that PRDX2 was a pivotal factor in the inhibition of AAA progression.

The formation of an aneurysm in the mouse model was induced through infusion of Ang II. Briefly, saline or Ang II was infused in 8 week-old mice via subcutaneous osmotic pumps at 1000 ng/kg/min for a maximum of 4 weeks.

PRDX2 was increased in AAA compared with normal aortas in both humans and mice. Ultrasound imaging revealed that the loss of PRDX2 accelerated the development of AAA at early stages and increased the AAA incidence in mice infused with angiotensin II (Ang II). Prdx2–/– mice infused with Ang II exhibited an increased aortic dilatation and maximal aortic diameter without a change in blood

pressure. Structural deterioration of the aortas from Prdx2–/– mice infused with Ang II was associated with increased degradation of elastin, intramural thrombus caused by microhemorrhages and immature neovessels, activation of matrix metalloproteinases and increment of oxidative stress compared with controls. Moreover, an increase in inflammatory responses, including cell adhesion molecules, accumulation of inflammatory cells and proinflammatory cytokines by PRDX2 deficiency accelerated the Ang II-induced AAA progression.

Our data confirm that PRDX2 plays a role as a negative regulator in the pathological process of AAA and suggest that increasing PRDX2 activity may be a novel strategy for the prevention and treatment of AAA.

PO-424

# Decreased Vascular Contractility Upon Cardiovascular Phenotyping of the APP23+/- Overexpressing Mouse Model of Alzheimer's Disease

**Jhana O. Hendrickx[1*], Sofie De Moudt[1], Debby Van Dam[2,3], Guido R. Y. De Meyer[1], Paul Fransen[1]**

[1] Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Lab of Physiopharmacology, University of Antwerp, Belgium
[2] Department of Biomedical Sciences, Laboratory of Neurochemistry and Behaviour, Institute Born-Bunge, University of Antwerp, Belgium
[3] Department of Neurology and Alzheimer Research Center, University of Groningen and University Medical Center Groningen, Netherlands

*jhana.hendrickx@uantwerpen.be*

As the most common form of dementia, Alzheimer's disease (AD) is characterized by noticeable neuropsychiatric symptoms and cognitive decline. The occurrence of cardiovascular (CV) disease in the progression of AD is confirmed by increasing epidemiological and experimental evidence. Therefore, we aimed to investigate the cardiovascular phenotype of the APP23+/- overexpressing mouse model (APP23+/-) of AD.

The current study presents the CV characterization of APP23+/- mice (male, n=10) compared to C57BL/6 (male, n=24) at the age of 6 months, including in vivo analysis of blood pressure (BP, CODA), aortic pulse wave velocity (aPWV, VEVO2100), and echocardiography (high-frequency echocardiography, VEVO2100). Serum corticosterone levels and vascular smooth muscle cell (VSMC) phenotypic markers were ascertained via ELISA and qPCR respectively. Data are presented as mean ± SEM.

CV phenotyping showed an increase in peripheral BP (systolic BP: 110 ± 5 vs. 99 ± 3 mmHg, p=0.045; diastolic BP: 81 ± 5 vs. 71 ± 3 mmHg, p=0.09) with unaltered pulse pressure. No differences were observed in systolic nor diastolic heart function upon echocardiographic analysis although in vivo aPWV was elevated in APP23+/- compared to C57BL6 mice (aPWV: 4.08 ± 0.3 vs. 2.94 ± 0.1 m/s, p=0.0004). Vascular reactivity studies revealed decreased (32%) adrenoreceptor-dependent contractions of the thoracic aorta from APP23+/- mice. Endothelial-dependent (acetylcholine) and -independent (diethylamine nonoate) relaxations were unaffected. Phenotypic markers of VSMC contractility remained unchanged. Already at the age of 6 months, APP23+/- mice experience a lower survival rate compared to C57BL/6 littermates (39% vs. 100%, p=0.001) and APP23+/- mice showed increased corticosterone levels compared to C57BL/6 mice (13.39 ± 5.15 vs. 2.30 ± 2.52 µg/mL, p=0.0025).

In conclusion, APP23+/- mice present with increased peripheral BP, elevated pulse wave velocity and elevated serum corticosterone levels in vivo and decreased vascular contractility ex vivo.

PO-425

# The Mega Aortic Syndrome -Evidence for Chronic Inflammation

**Ulrike Baranyi[1*], Nils Bukowski[1], Thomas Aschacher[2,1], Marie-Elisabeth Stelzmüller[2], Marek Ehrlich[2], David Bernhard[3], Günther Laufer[2], Barbara Messner[1]**

[1] Cardia Surgery Research Laboratory, Department of Cardiac Surgery, Medical University of Vienna, Austria
[2] Department of Surgery, Cardiac Surgery, Medical University of Vienna, Austria
[3] Division of Pathophysiology, Institute of Physiology and Pathophysiology, Johannes Kepler University Linz, Austria

*ulrike.baranyi@meduniwien.ac.at*

The mega aortic syndrome (MAS) is a dilatation of the entire aorta. The pathobiology of this rare disease is almost unknown. In a previous study we observed an increase of immune cells in the media adjacent to the adventitia in the ascending aorta. We hypothesize that the adventitia plays an important role in disease progression. In this study we quantified collagen content in the ascending aorta of patients with MAS compared to tissue of patients with spontaneous aortic aneurysm (TAA) and non-aneurysmal (NA) tissue. Additionally we determined the presence of immune cells in the adventitia and formation of novel blood vessels in the vasa vasorum.

Paraffin-embedded tissue from patients with MAS (n=9), TAA (n=9) and NA tissue (n=9) was stained with Sirius-red and the amount of collagen quantified in fluorescence microscopy. In immune-histochemistry (IHC) T cells (CD3), B cells (CD20), macrophages (CD68), and endothelial cells (CD31) were determined. To evaluate the presence of lymphatic blood vessels we performed immunofluorescence (IF) with antibodies against van Willebrand factor (vWF) and podoplanin.

We found a significant increase in collagen content in the adventitia and intima of tissue of MAS patients compared to NA controls. Aggregates of immune cells in the adventitia with high amounts of B cells are detectable in samples of MAS (9/9) patients and samples of TAV (6/9) patients compared to NA controls (1/9). Additionally an increase of lymphatic-like vessels in the adventitia of MAS tissue and TAV tissue compared to NA controls are visible.

The significant increase of collagen in tissue of MAS tissue suggests intense remodeling in aortic tissue of the ascending aorta. Clusters of immune cells and lymphatic-like blood vessels are an evidence of tertiary lymphoid organs (TLO) in the adventitia of MAS tissue in the ascending aorta suggesting chronic inflammation.

PO-426

# Latrophilin2, a Functional Marker of Heart Development, Induces Myocardial Repair After Infarction

**Choon-Soo Lee[1], Hyun-Jai Cho[2], Jin-Woo Lee[1], Hyo-Soo Kim[1,2*]**

[1] Strategic Center of Cell and Bio Therapy for Heart, Diabetes & Cancer, Seoul National University Hospital, Korea
[2] Department of Internal Medicine, Seoul National University Hospital, Korea

*hyosoo@snu.ac.kr*

Recently, there has been an increasing demand for cardiomyocyte (CMC) for research into cardiovascular disease and the toxicology of drug metabolites. Therefore, identification of lineage-specific

markers is pivotal for understanding developmental processes and developing cell therapies for CVD.

To verify whether a specific marker is expressed during heart development, we assessed its expression using the CLARITY technique. After immersion in a solution with a refractive index matching that of the CLARITY hybrid, the mouse embryo became transparent. For the purpose of cardiac regeneration, we transplanted PSC-derived Lphn2+ cells into the infarcted heart.

After immunostaining the cleared embryo sample, Lphn2 was exclusively observed in cardiac cells expressing α-sarcomeric actinin at embryonic day E9.5 and E10.5. Our clarified 3D images and movies show that four chambers of the heart are fully developed at E10.5 but not at E9.5. At E9.5, Lphn2 is observed at the ventricle and atrium, while Lphn2 is present in all chambers of the heart at E10.5. Homozygous Lphn2–/– mice were embryonically lethal and showed underdevelopment of the ventricular myocardium. The hearts of Lphn2–/– embryos revealed the disrupted conotruncal septation of ventricles at E13.5. However, Lphn2 is not required for the development of vessels, including endothelial cells and smooth muscle cells. PSC-derived Lphn2+ cells differentiated into CMCs and regenerated the myocardium when transplanted into the infarcted heart, unlike Lphn2– cells. Transplanted Lphn2+ cells improved left-ventricle systolic function and reduced infarct size. Lphn2+ cells resulted in several engrafted tissues, replacement of the LV wall, and a majority of cells expressed α-sarcomeric actinin, a cardiac muscle marker.

Our findings provide a valuable tool for identifying CPCs and CMCs differentiated from PSCs, as well as revealing novel insights into cardiac development. Furthermore, the results of this study could be of potential use in regenerative cell therapy for the restoration of cardiac muscle.

PO-427

# Low-Level Nanog Expression in the Regulation of Quiescent Endothelium

**Kishore Wary**[1*]

[1] Pharmacology and Regenerative Medicine, University of Illinois at Chicago, USA

*kkwary@uic.edu*

Nanog is expressed in adult endothelial cells at a low-level, however, its biological significance is not understood. The goal of our study was to elucidate the role of Nanog in adult ECs using a genetically engineered mouse model system.

We used human and mouse tissues and primary endothelial cells (ECs) to examine the expression of Nanog. We used chromatin immunoprecipitation (ChiP) assay, transfection experiments, and reporter assays to address the ability of Nanog to bind and transcriptionally activate EC-genes. We have performed loss- and gain-of-function experiments. Importantly, we created ROSA(mT/mG);Nanog(fl/+);Cdh5(CreERT2) mice, so that EC-Nanog gene can be deleted in adult tissues, in an inducible manner. We used immunohistochemistry and morphometric analyses, and quantified the extent of cardiovascular pathology. We determined cardiovascular physiological parameters and quantified the end-points. We used AAV-9 vector encoding a critical gene to rescue cardiovascular function.

Nanog is expressed in both adult human and mouse tissues. Primary ECs isolated from adult mice showed detectable levels of Nanog, Tert, and eNos. Wnt3a increased the expression of Nanog and hTERT in ECs and increased telomerase activity in these cells. In a ChiP experiment, Nanog directly bound to the hTERT, eNOS and FLK1-promoters/enhancers DNA elements, thereby regulating their

transcription. Administration of low-dose tamoxifen to ROSA(mT/mG);Nanog(fl/+);Cdh5(CreERT2) mice induced deletion of a single Nanog allele, simultaneously labeling ECs with green fluorescent protein and resulting in decreased Tert, eNos, and Flk1 levels. Histological and morphometric analyses of heart tissue sections prepared from these mice revealed EC apoptosis, microvascular rarefaction, and increased fibrosis in cardiac macro and microvessels, and cardiomyocyte enlargement. Importantly, these pathologies resulted in cardiac hypertrophy. Conversely, retro-orbital delivery of AAV-9 encoding hTERT-cDNA in Nanog-depleted mice, in part, restored the effect of loss of Nanog.

We showed that low-level EC-Nanog expression is required for normal EC-cardiomyocyte interactions in adult.

PO-428

# Engineered M13 Peptide Carrier Promotes Angiogenic Potential of Human Cardiac Progenitor Cells and in Vivo Engraftment

**Woong Bi Jang[1], Seung Taek Ji[1], Ji hye Park[1], Yeon-Ju Kim[1], Songhwa Kang[1], Da Yeon Kim[1], Na-Kyung Lee[1], Jin su Kim[1], Hye Ji Lim[1], Jaewoo Choi[1], Van Le Thi Hong[1], Giang Ly Thanh Truong[1], Vinoth Kumar Rethineswaran[1], Donh Hwan Kim[2], Jong Seong Ha[1], Jisoo Yun[1], Sang Hong Baek[3], Sang-Mo Kwon[1]***

[1] Department of Physiology, School of Medicine, Pusan National University, Korea
[2] Department of Neurosurgery & Medical Research Institute, Pusan National University Hospital, Korea
[3] Division of Cardiology, Seoul St. Mary's Hospital, Korea

*smkwon323@pusan.ac.kr*

Despite promising advances in stem cell-based therapy, the treatment of ischemic cardiovascular diseases remains a big challenge due to both the insufficient in vivo viability of transplanted cells and poor angiogenic potential of stem cells. The goal of this study was to develop therapeutic human cardiac progenitor cells (hCPCs) for ischemic cardiovascular diseases with a novel M13 peptide carrier.

In this study, an engineered M13 peptide carrier was successfully generated using a QuikChange Kit. The cellular function of M13 peptide carrier-treated hCPCs was assessed using a tube formation assay and scratch wound healing assay. The in vivo engraftment and cell survival bioactivities of transplanted cells were demonstrated by immunohistochemistry after hCPC transplantation into a myocardial infarction animal model.

The engineered M13RGD+SDKP peptide carrier, which expressed RGD peptide on PIII site and SDKP peptide on PVIII site, did not affect morphologic change and proliferation ability in hCPCs. In contrast, hCPCs treated with M13RGD+SDKP showed enhanced angiogenic capacity, including tube formation and migration capacity. Moreover, transplanted hCPCs with M13RGD+SDKP were engrafted into the ischemic region and promoted in vivo cell survival.

Our present data provides a promising protocol for CPC-based cell therapy via short-term cell priming of hCPCs with engineered M13RGD+SDKP before cell transplantation for treatment of cardiovascular disease.

PO-429

# Understanding Endothelial-Immune Crosstalk in Non-Alcoholic Fatty Liver Disease

**Chun Yi Ng[1], Kanxing Wu[1], Florence Chioh[1], Winston Chan[3], Yee Siang Lim[3], Yock Young Dan[4], Huck Hui Ng[3], Christine Cheung[1,2*]**

[1] Lee Kong Chian School of Medicine, Nanyang Technological University Singapore, Singapore
[2] Institute of Molecular and Cell Biology, A*STAR, Singapore
[3] Genome Institute of Singapore, A*STAR, Singapore
[4] Yong Loo Lin School of Medicine, National University of Singapore, Singapore

*ccheung@ntu.edu.sg*

Fundamental cause of deaths in non-alcoholic fatty liver disease (NAFLD) patients is often attributed to cardiovascular events. The molecular basis of endothelial dysfunction in NAFLD remains elusive.

Harnessing the replicative potential of blood outgrowth endothelial cells, we were able to establish patient-derived cellular models to investigate the underlying endothelial pathophysiology. We rendered RNA-sequencing on endothelial cells and liver biopsies derived from patients with steatosis (early stage of disease) and nonalcoholic steatohepatitis (advanced stage), as well as non-NAFLD control donors. Single-cell transcriptomics were employed to further study immune populations in order to elucidate endothelial-immune crosstalk.

Endothelial cells generated from controls and NAFLD patients demonstrated typical cobblestone cell morphology, ability to undergo angiogenic sprouting and were highly enriched for mature endothelial markers. Our RNA-sequencing data revealed that in comparison to controls, NAFLD endothelial cells had downregulated biological processes in growth factor response and cell cycle. Correspondingly, vasculature development and endothelial proliferation were significantly downregulated processes in liver transcriptomes, suggesting an impairment of vascular regenerative signals during disease progression. On the other hand, NAFLD endothelial cells had upregulated biological processes/ pathways in cell chemotaxis, immune response and cytokine-cytokine receptor interaction. In particular, several C-C and C-X-C chemokine ligands and receptor genes were within the top interactome network in NAFLD endothelial cells. Therefore, we performed single-cell RNA-sequencing on patient peripheral blood mononuclear cells, where T cell populations were intensified in NAFLD compared to controls. We carried out a mapping of chemokine-receptor interaction and found that NAFLD endothelial cells might interact most pronouncedly with cytotoxic T cell subset. Work is ongoing to study T cell-mediated cytotoxicity of endothelial cells.

Immunomodulation may serve as novel routes of vascular-protective intervention in NAFLD. Our work provide insights for translation to restore blood vessel health and potentially regenerative therapies.

PO-430

# Ccm2l Deletion Aggravates Cerebral Cavernous Malformation (CCM) Through MEKK3-KLF Signalling Pathway

**Jaesung Peter Choi**[1,2,3*]

[1] Centre for Inflammation, Centenary Institute, Australia
[2] School of Life Sciences, University of Technology Sydney, Australia
[3] Faculty of Medicine and Health, University of Sydney, Australia

*j.choi@centenary.org.au*

Ccm2-like (Ccm2l) is a paralog of Ccm2 which is selectively expressed in endothelial cells (ECs) during periods of active cardiovascular growth and angiogenesis. CCM2L competes with CCM2 for binding to CCM1 and shown to have antagonistic function to CCM2 in cultured ECs and in developing mice. CCM2L brings MEKK3 and other potential unknown factors. Our preliminary study demonstrated increased Ccm2l expression in brain ECs with CCM lesions compared to controls. The role of Ccm2l has not been studied in cerebral cavernous malformation (CCM) pathogenesis. Hence, our aim is to elucidate the role of Ccm2l in CCM and determine whether increased Ccm2l is a causal or compensatory mechanism using neonatal mouse models.

We generated CCM2L knockout mice (Ccm2l-/-) to test our aims in CCM1 (Ccm1iECKO) and CCM2 (Ccm2iECKO) deficient CCM mouse models. Brains were harvested at postnatal day 13 (P13) to measure CCM lesion burden using micro-CT. Brain ECs were isolated at P8 for gene expression studies using real-time PC.

Micro-CT analysis revealed that complete CCM2L deletion in Ccm2iECKO mice significantly increased CCM lesion volume when compared to controls, but lesion numbers did not change. Heterozygous CCM2L deletion did not affect CCM lesion burden. Klf2 and Klf4 mRNA expressions were significantly increased in Ccm2iECKOCcm2l-/- compared to controls, correlating increased lesion burden. Adamts1/4/5 expressions were not significantly affected. Hemizygote MEKK3 deletion significantly reduced lesion burden in Ccm2iECKOCcm2l-/- mouse, suggesting CCM2L regulates MEKK3-KLF signalling pathway in CCM pathogenesis. However, unlike in Ccm2iECKO, CCM2L deletion did not affect CCM lesion burden in Ccm1iECKO mice.

In summary, our study suggests increased Ccm2l expression in CCM lesions is a compensatory response. Ccm2l regulates CCM pathogenesis only in CCM2-deficient mouse through MEKK3-KLF signalling pathway. Hence, our study demonstrates a complex and selective role of Ccm2l in angiogenesis and development of CCM lesions, which warrants further studies.

PO-431

# Activation of AIM2 Inflammasome Mediates Cognitive Impairment Following Ischemic Stroke

**Hyunha Kim[1,2], Ji Seon Seo[1,2,3], Seo-Yeon Lee[4], Ki-Tae Ha[1,2,5], Byung Tae Choi[1,2,5], Young-Il Shin[6], Young Ju Yun[5], Hwa Kyoung Shin[1,2,5*]**

[1] Department of Korean Medical Science, School of Korean Medicine, Pusan National University, Korea
[2] Korean Medical Science Research Center for Healthy-Aging, Pusan National University, Korea
[3] Graduate Training Program of Korean Medicine for Healthy-Aging, Pusan National University, Korea
[4] Department of Pharmacology, School of Medicine, Wonkwang University, Korea
[5] Department of Korean Medicine, School of Korean Medicine, Pusan National University, Korea
[6] Department of Rehabilitation Medicine, School of Medicine, Pusan National University, Korea

*julie@pusan.ac.kr*

Although over one-third of stroke patients may develop post-stroke cognitive impairment (PSCI), the mechanisms underlying PSCI remain unclear. We explored here, the involvement of post-stroke inflammasomes in long-term PSCI development.
We used to induce a 45 min-middle cerebral artery occlusion (MCAO)/reperfusion for ischemic brain injury model. Histological assessment was performed at 1, 3, or 7 days and cognitive function test at 28 days post-stroke.
Evaluation of inflammasome sensor gene expression in aged mouse brains showed dominant expression of absent in melanoma 2 (Aim2) in 6-, 12-, and 18-month-old mouse brains. AIM2 mRNA and protein increased until 7 days post-stroke. Ischemic injured mice decreased anxiety in elevated plus maze test and impaired spatial learning and memory functions in Morris water maze test 28 days post-stroke. AIM2 and other inflammasome subunit immunoreactivities, including those for caspase-1, interleukin (IL)-1β, and IL-18, were higher in the hippocampus and cortex of the PSCI than in those of the sham group 7 days post-stroke. AIM2 immunoreactivity of the PSCI group was primarily co-localized with Iba-1 (microglial marker) and CD31 (endothelial cell marker) immunoreactivities but not NeuN (neuronal marker) and GFAP (astrocyte marker) immunoreactivities, suggesting that microglia or endothelial cell-induced AIM2 production mediated PSCI pathogenesis. Additionally, inflammasome-induced pyroptosis might contribute to acute and chronic neuronal death after stroke. AIM2 knockout (KO) and Ac-YVAD-CMK-induced caspase-1 inhibition in mice significantly improved cognitive function and reversed brain volume in the hippocampus relative to those in stroke mice.

Our results suggest that AIM2 inflammasome-mediated inflammation and pyroptosis likely aggravated PSCI. These results could expand previous understanding of the pathophysiology of secondary brain damage, and thus create a new avenue for the development of therapeutic strategies for PSCI.

PO-433

# Neuroprotective and Anti-Inflammatory Effect of Peroxiredoxin1 Following Ischemic Brain Injury

**Sinai Kim[1], Goo Taeg Oh[1*]**

[1] Life Science Ewha Womans Univ., National Creative Research Center for Immune and Vascular Cell Network, Korea

*gootae@ewha.ac.kr*

Inflammatory response is an essential cause of ischemia stroke damage. Reactive oxygen species

(ROS) play a pivotal role in the induction disorder of inflammatory response. Under the inflammatory conditions, oxidative stress leads to infiltrate inflammatory cells across the blood brain barrier (BBB). Several studies suggested that immune responses induced by macrophages and DCs have essential roles in the ischemic stroke. However, there are no study that Peroxiredoxin 1 (Prdx1) antioxidant protein can initiate the antioxidant response and inhibit the inflammatory brain damage. This study demonstrated that deficiency of Prdx1 induced more severe damage at the ischemic stroke.

To explore the role of Prdx1 in the development of acute ischemic brain injury, Prdx1 deficient and WT mice to 1hr of middle cerebral artery occlusion (MCAO) followed by time based of 12hr, 24hr, 72hr reperfusion. Prdx1 deficient exhibited increased neurological deficits and enlarged brain infarction compared with Prdx1 WT mice. FACS results show that infiltrated inflammatory cells in the Prdx1 KO brain are increased dramatically through time lapse and also compared to Prdx1 WT brain.

The immune responses shown different pattern that infiltrated macrophages were increased at the severe damaged Prdx1 KO brain compared to Prdx1 WT. Expression pattern of inflammatory cytokines also supports this results.

In conclusion, Prdx1 has protective role in the ischemic stroke through regulating immune system.

PO-434

# Effect of Cerebral Endothelial Glycocalyx on Capillary Stalling

**Jin-Hui Yoon[1,3], Paul Shin[2,3], Jongyoon Ju[2,3], Gaon Kim[1,3], Wang-Yuhl Oh[2,3], Jeong Yong[1,3]***

[1] Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology, Korea
[2] Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology, Korea
[3] KI for Health Science and Technology, Korea Advanced Institute of Science and Technology, Korea

*yong@kaist.ac.kr*

Healthy brain is maintained by appropriate cerebral microcirculation. However, detailed study on microcirculation is still elusive including capillary stalling. Capillary stalling is a brief interruption of blood flow in capillary segments mainly by leukocytes. This has been observed not only in normal physiology but also in several pathological conditions such as Alzheimer's disease. The underlying mechanism of capillary stalling remains elusive. Meanwhile, cerebral capillary has endothelial glycocalyx (EG) but the exact understanding of EG in cerebral vessels is lacking. It is important to reveal underlying mechanism of capillary stalling and we investigated the EG as a possible candidate.

Longitudinal imaging with optical coherence tomography and two photon microscopy in vivo imaging was utilized. In addition, immunohistochemistry was performed to reveal possible relation between capillary stalling and histopathological features of SVaD. To further investigate casual role of EG in capillaries to stalling, we degraded EG with hyaluronidase.

Capillary stalling increased in SVaD mouse model with disease progression. In addition, the capillary stalling incidence was correlated with pathological features such as gliosis and blood brain barrier

leakage. There were certain capillaries susceptible to stalling. We found that the extent of endothelial glycocalyx was smaller in stalled capillaries. We found increased capillary stalling after enzymatic shedding.

We conclude with suggesting EG as one of the underlying mechanisms of capillary stalling and that it can be a promising therapeutic target of impaired microcirculation in SVaD.

PO-435

# Determining the Effects of a Novel Medium-Cut-Off Haemodialysis Membrane on Biomarkers of Vascular Health in Kidney Disease Patients Undergoing Dialysis

**Annie Herring[1*], Liliana Shalamanova[1], Fiona Wilkinson[1], Yvonne Alexander[1], Kunaal Kharbanda[2], Sandip Mitra[2]**

[1] Center for Bioscience, Manchester Metropolitan University, UK
[2] Manchester Institute of Nephrology and Transplantation, Manchester Royal Infirmary, UK

*18060802@stu.mmu.ac.uk*

Healthy kidneys filter metabolic waste molecules of up to 60kDa from the blood, whereas conventional haemodialysis membranes can only clear molecules of up to 20kDa. Retention of larger molecules in dialysis patients is linked to retention of toxic uremic compounds and endothelial dysfunction, leading to increased cardiovascular disease (CVD) risk. Novel medium cut-off dialysis membranes (MCO) have been developed that can more effectively clear middle-sized molecules (≤45kDa) and decrease the levels of uraemic toxins.
The aim of this project are to determine if the MCO dialysis membrane is more effective at reducing biomarkers of inflammation and endothelial dysfunction in patients compared to the standard haemodialysis membrane (HDF).

This pilot clinical trial includes 51 patients randomised into two groups; dialysis using the novel MCO versus the HDF membrane. Blood samples were taken at 0, 3 and 6 months and the levels of endothelial microvesicles (EMVs) were measured as a marker of endothelial dysfunction using flow cytometry. Vascular health-related cytokines and clinical parameters were also measured at each time point. The effect of patient serum on endothelial cell viability was measured using HUVEC cells in-vitro.

EMV levels decreased significantly over 6 months in the MCO treatment group compared to the patients that underwent 6 months of HDF treatment ($p<0.05$). EMVs were significantly increased ($p<0.05$) in a single session of HDF dialysis. There was no significant difference in the viability of HUVEC cells exposed to serum from either of the treatment groups.

These data suggest that the MCO membrane is superior to standard HDF dialysis in terms of reducing EMVs and thus, endothelial dysfunction, suggesting a potential to improve clinical management of vascular health and assist prevention of CVD-related mortality in kidney disease patients. Ongoing experiments to measure additional biomarkers of inflammation, endothelial activation and clinical measures aim to corroborate these data.

PO-436

# Sustained Cognitive Decline Alleviation After Intermittent Fasting Is Paralleled with Hippocampal Neuroprotection and Astrocytosis in a Subcortical Vascular Dementia Mouse Model

**Faris Rizky Andika[1,2], Jin-Hui Yoon[1,2], Gaon Sandy Kim[1,2], Yong Jeong[1,2*]**

[1] Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Korea
[2] KI for Health Science and Technology, Korea Advanced Institute of Science and Technology (KAIST), Korea

*yong@kaist.ac.kr*

Intermittent fasting (IF) emerges as a dietary intervention that was found to exhibit neuroprotection across various cerebrovascular insults including ischemia, but IF intervention has been mainly applied before the insults occur. Whether IF implementation after an established condition can prevent long-term detrimental effects of the disease remains unknown. In this study, we investigated how IF affects cognitive impairments and cerebrovascular pathologies manifested in a mouse model of subcortical vascular dementia (SVaD).

The SVaD model was developed by inducing hypoperfusion and hyperlipidemia to apolipoprotein E-deficient mice. These mice were subjected to 1-month IF, in a form of time-restricted feeding for 6 h per day, or ad libitum feeding at 8 weeks post-surgery. Assessments on recognition and spatial working memories were performed during the 1-month IF treatment prior to histopathology.

IF significantly restored the impaired recognition and spatial working memory performances at 15 days post-IF, and these alleviations were maintained after 30 days of IF. These sustained enhancements were paralleled by the preservation of hippocampal neuronal density in the SVaD model mice, but with further increase in activated astrocytes and unaltered cerebral microcirculation damages. The cognitive performances of IF-fed mice were found to be correlated with the extent of hippocampal neuroprotection observed in respective mice.

Our findings suggested that IF could be a potential non-pharmacological therapy for ameliorating SVaD conditions. This study may stimulate future investigations on the possible neuroprotective effects of fasting for remediation across many neurovascular diseases.

PO-438

# Understanding the Role of CCM3 in Endothelial Development and Disease

**Tvisha Misra[1*], James Knight[2], Anne Claude Gingras[2], Ian Scott[1]**

[1] Department of Stem Cell and Developmental Biology, Hospital for Sick Children, PGCRL, Canada
[2] Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Canada

*tvisha.misra@gmail.com*

Cerebral cavernous malformations (CCMs) are focal dilations in the cerebral vasculature leading to haemorrhaging, strokes and in extreme cases death. Of the three proteins associated with CCMs, CCM1/2/3, CCM3, a scaffold protein highly conserved through species, is the least understood and proposed to have the most detrimental effects.

Though various models have been used to study endpoint vascular defects, not much is known about the earliest cellular events which eventually lead to CCMs. We use the zebrafish as a vertebrate model to understand the role of Ccm3 in early vascular development and disease progression.

Methods: zebrafish cardio vascular development CRISPr/Cas9 mutants Confocal time lapse imaging BioID

With CRISPR/CAS9 we generated a ccm3a/b double mutant to knockout the two copies of zebrafish Ccm3. ccm3a/b(-/-) embryos exhibit cardiac edemas, loss of blood flow, and are lethal. Time lapse imaging was used to characterise defects in endothelial cell migration, lumen formation, blood flow, and membrane dynamics. To explore the mechanism of Ccm3 function, BioID was used to determine the potential interactome of Ccm3. Cellular Ccm3 resides mostly in the striatin interacting phosphatases and kinase (STRIPAK) complex. We generated CRISPR/CAS9 mutants of these components of the STRIPAK complex, consisting of largely unstudied genes, to assess their role in vascular development and their relationship to Ccm3. We also know that CCM disease progression is linked to RhoGTPase activity. We determined Cdc42 is implicated in Ccm3 function: ccm3a/b KO embryos show aberrant Cdc42 activity and KO/KD of cdc42 leads to transient cerebral haemorrhages in embryos. A

Altogether, we have established a zebrafish model to study the role of Ccm3 and its interaction partners of the STRIPAK complex i early endothelial development.

PO-439

# Coronary Circulatory Indices in Non-Infarct Related Vascular Territories in a Porcine Acute Myocardial Infarction Model

**Hyun Kuk Kim[1], Joo Myung Lee[2*], Seung Hun Lee[2], Kyung Seob Lim[3]**

[1] Cardiology, Chosun University Hospital, Korea
[2] Cardiology, Samsung Medical Center, Korea
[3] Futuristic Animal Resource & Research Center, Korea Research Institute of Bioscience and Biotechnology, Korea

*drone80@hanmail.net*

This study aimed to evaluate temporal changes of coronary hemodynamic and physiologic indices in non-infarct related artery (non-IRA), which might be affected by adjacent infarcted myocardium, using an experimental animal model of acute myocardial infarction (AMI).

In Yorkshire swine, acute myocardial infarction was simulated with selective balloon occlusion at left circumflex artery (LCX) as IRA for 30 minutes. Non-IRA stenosis was created using bare metal stent implantation in left anterior descending artery (LAD) 4 weeks before the experiments. Serial changes of systemic hemodynamics, coronary pressure, and Doppler-derived coronary flow velocity were measured in a non-occluded LAD as non-IRA from baseline, balloon occlusion of LCX, and 15 minutes after reperfusion of LCX.

Among the 6 experimental subjects, the median diameter stenosis of non-IRA was 33.9% (Q1-Q3: 21.7%-46.1%). During balloon occlusion of IRA, there were transient significant changes in both resting and hyperemic aortic pressure (Pa), distal coronary pressure (Pd), averaged peak velocity (APV),

trans-stenotic pressure gradient (PG), and microvascular resistance of non-IRA (all P values<0.020). After reperfusion of IRA, the resting APV (P=0.002) and resting trans-stenotic PG (P=0.004) were significantly increased and resting microvascular resistance (P=0.004) was significantly decreased than those in baseline phase. However, the hyperemic APV (P=0.479), hyperemic trans-stenotic PG (P=0.778), and hyperemic microvascular resistance (P=0.816) were not significantly different compared with those in the baseline phase. After reperfusion, the FFR in non-IRA was not significantly different (0.94±0.01 vs. 0.93±0.01, P=0.353), while the CFR (1.93±0.07 vs. 1.36±0.07, P=0.025) and resting Pd/Pa (0.98±0.01 vs. 0.94±0.01, P=0.017) were significantly lower than baseline values.

In a porcine AMI model, occlusion of IRA induced significant changes of systemic hemodynamics and coronary circulatory indices of non-IRA. However, after reperfusion of IRA, FFR did not change significantly, while resting Pd/Pa and CFR showed significant changes compared with the baseline values.

PO-441

# Stengthening of VE-Cadherin-Mediated Endothelial Junction Improves Cardiac Function and Remodeling by Suppressing Edema and Inflammation in Cardiac Ischemia-Reperfusion Injury

**Hae Young Chang[2], Hyeok Kim[1], Hun-Jun Park[1], Young-Guen Kwon[2*]**

[1] Cardiology, Seoul St. Mary's Hospital, The Catholic University of Korea, Korea
[2] Biochemistry, College of Life Science and Biotechnology, Yonsei University, Korea

*ygkwon@yonsei.ac.kr*

Vascular hyperpermeability caused by distorted endothelial cell-cell junctions is an early event of the no-reflow phenomenon after opening of the occluded vessels in patients with acute myocardial infarction (AMI), which is associated with a worse prognosis at follow-up and a higher incidence of death. Thus, preservation of vascular integrity is a promising therapeutic strategy to prevent no-reflow phenomenon and reduce ischemia-reperfusion (I/R) injury.

We already demonstrated that CU06-1004, a vascular leakage blocker, prevented vascular hyperpermeability in I/R injury, which might be associated with decreased infarct size, edema, and inflammation. To investigate the therapeutic effects of CU06-1004 on I/R hearts, the drug was diluted with PBS (500 μl) and intravenously delivered just before reperfusion. The rats were randomly divided into three groups as following; 1) Vehicle with PBS, 2) low-

CU06-1004 (1 mg/kg, twice at 24 hours interval), 3) high-CU06-1004 (5 mg/kg, once before reperfusion).

Serial echocardiography showed that injections of low-CU06-1004 and high-CU06-1004 significantly improved cardiac function compared to MI control. Interestingly, compared with low-CU06-1004, injection of high-CU06-1004 prior to reperfusion exerted greater cardiac function from 1 week after I/R injury and continued until 8 weeks post I/R. In addition, hearts receiving high-CU06-1004 exhibited significantly lower cardiac fibrosis and higher capillary density. These findings suggest that the early intervention with effective dose of CU06-1004 reduce vascular damage in cardiac I/R injury.

These data showed that enhancing vascular integrity with CU06-1004, a vascular leakage blocker, might constitute a relevant target for preventing no-reflow and conferring secondary cardioprotection during AMI.

PO-443

# Protective Effects of PCSK9 Through Ly6C Monocyte-Macrophage Axis After Acute Myocardial Infarction

**Shin Hye Moon[1], Jing Jin[1], Goo Taeg Oh[1*]**

[1] Department of Life Science, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

PCSK9 regulates cholesterol homeostasis by determining low-density lipoprotein receptor (LDLR) fate and has emerged as a novel therapeutic option to cardiovascular diseases. Myocardial infarction (MI) elicits enormous inflammatory cell recruitment to the heart. However, the effect of PCSK9-deficient macrophages on ischemic heart has yet to be investigated.

We generated PCSK9-deficient (PCSK9-/-) mice and macrophage-specific PCSK9 deficient (Lyz2-cre; PCSK9F/F) mice. Conventional PCSK9-/- and Lyz2-cre; PCSK9F/F mice were subjected to myocardial infarction using left anterior descending artery (LAD) occlusion.

We analyzed the infarcted hearts of PCSK9-/- and Lyz2-cre; PCSK9F/F post-MI. PCSK9 deficiency increases the Ly6Clow monocytes, but reduces macrophage in the infarcted heart at 7 days post-MI. When bone marrow-derived macrophages isolated from Lyz2-cre; PCSK9F/F mice were treated for 24hrs with 200ng/ml LPS, anti-inflammatory cytokines IL-4 and IL-10 were increased, while pro-inflammatory cytokine IFN-γ was decreased. PCSK9-deficient Ly6C monocytes can be differentiated into wound healing or anti-inflammatory macrophages.

Our results indicate that PCSK9 deficiency in macrophages improves cardiac function through Ly6C monocyte-macrophage axis following MI.

PO-444

# Sex Differences in the Incidence of Ischemic Heart Disease According to Health Care Utilization of Newly Treated Hypertensive Patients Among Koreans

**Jiae Shin[1], Dongwoo Ham[2], Sangah Shin[3], Hee-Young Paik[4], Hyojee Joung[1,2*]**

[1] Department of Public Health Science, Graduate School of Public Health, Seoul National University, Korea
[2] Institute of Health and Environment, Seoul National University, Korea
[3] Department of Food and Nutrition, School of Food Science and Technology, Chung-Ang University, Korea
[4] Centered for Gendered Innovation in Science and Technology Research (GISTeR), Korea Federation of Women's Science & Technology Associations, Korea

*hjjoung@snu.ac.kr*

This study aimed to investigate sex differences in the incidence of ischemic heart disease (IHD) according to health care utilization among newly treated hypertensive patients using a nationwide data.

We analyzed the National Sample Cohort version 2.0 of the National Health Insurance Service. Newly treated hypertensive patients aged ≥20 were extracted from those who received health examinations during 2003-2006 without pre-existing IHD. Total 23,135 patients diagnosed as hypertension with medication were selected for analyses and followed for 10 years. Person-year was defined as the period from the prescription of hypertension medication to the incidence of IHD. Cox proportional hazards multivariate regression model was used to investigate the associations between IHD and health care utilization such as out-of-pocket expense, frequency of visits, location and type of health care providers, and medication adherence after adjusting for known confounders for IHD.

Incidence rates (per 1,000 person-years) of IHD were 34.8 in men and 36.5 in women. Women patients were less likely to increase the risk of IHD than men (hazard ratio (HR)=0.93, 95% confidential intervals (CI) 0.876–0.995). Both men and women showed an increased risk of IHD when they were visiting tertiary health care exclusively (HR=1.76, 95% CI 1.61–1.92), consulting physicians over 12 times (HR=2.97, 95% CI 2.79–3.17), in the highest tertile group of out-of-pocket expense per person-year (HR=1.55, 95% CI 1.41–1.69), and with non-adherence (HR=1.67, 95% CI 1.58–1.77). However, the risk of IHD decreased when patients visited health care providers in both urban and rural areas (HR=0.75, 95% CI 0.67-0.84) and used more than two types of health care providers (HR=0.93, 95% CI 0.88-0.99).

Both men and women patients were associated with the risk of incident IHD according to health care utilization and men showed a greater risk of developing IHD than women patients.

PO-445

# High Sensitivity of Coronary Calcium Scoring As Additional Diagnosis Protocol on Coronary Artery Disease

**Danan Budi Primadi[1], Rifqi Akhdan Pradipta[2], Eka Nurdina Inayatussholeha[3], Sekar Salma Putri[4], Mochamad Afifudin[1*]**

[1] Undergraduate Program of Medical Education, Universitas Islam Indonesia, Indonesia
[2] Undergraduate Program of Chemical Engineering, Universitas Islam Indonesia, Indonesia
[3] Undergraduate Program of Chemical Analysis, Universitas Islam Indonesia, Indonesia
[4] Undergraduate Program of Statistic, Universitas Islam Indonesia, Indonesia

*17711025@students.uii.ac.id*

Coronary artery disease (CAD) is the most common type of heart disease caused by plaque that narrows the coronary artery. People with overweight, smoking, and historical of metabolic disease have a high risk for CAD. Data from CDC and AHA stated that 18.2 million American have CAD. There are no specific or pathognomonic symptom of CAD. The diagnosis tool for early detection need to be provided. The most of post-mortem case due to CAD shown increase calcium formation in coronary artery and the researcher have developed the coronary calcium scoring (CCS) that already use in clinical setting. The aim of this study is to evaluate CCS as diagnostic scoring to predict CAD patients.

Clinical research literature collected and study performed from January 2018 to 2020 were selected. Forty-six studies have extracted and total 4 were included from Google Scholar, Science Direct, PubMed, and Nature with the keyword: Coronary Calcium Scoring AND Sensitivity AND Performance AND Significance. Data were analyzed and synthesized.

Addition of CCS in myocardium imaging shown reduction the false negative test, increased the sensitivity from 84% to 96%, and negative predictive value (78% to 89%). Patient who had calcium plaque in mitral annulus and aortic valve (stage≥2) presented a high correlation with high CAD frequencies, with 82% sensitivity and 87% specificity, 95% positive predictive value, and 60% negative predictive value. Contrary, another study also shown the ability of CCS combined with CTA to exclude CAD with 100% sensitivity, 42% specificity, 48% positive predictive value and 100% negative predictive value. There's also new discovered study about protocol and algorithm improvement to make better CCS scoring.

Addition of CCS in clinical assessment improve the diagnosis accuracy to determine or exclude CAD. The diagnosis algorithm needs to be developed as main protocol for combination method of CAD diagnosis.

PO-447

# Patients with Vasospastic Angina Are Associated With Worse Long-Term Outcomes: Results From VA-Korea Registry, a Prospective Multi-Center Cohort

**Sang-Ho Jo[1*]**

[1] Internal Medicine, Hallym University Sacred Heart Hospital, Korea

*sophi5neo@gmail.com*

The long–term prognosis of patients with vasospastic angina (VA) has been controversial.

We investigated clinical outcomes of VA patients as compared to those with non-VA using large scale nation-wide cohort.

We enrolled 2960 patients who received coronary angiography (CAG) and ergonovine provocation test prospectively in 11 university hospitals in Korea. Among them, 1838 patients were diagnosed with VA and 867 with non-VA.We performed 1:1 propensity score matching and 630 patients was allocated in each group. Primary outcome defined as composite of death, acute coronary syndrome (ACS) and symptomatic arrhythmia during 3-year follow-up was compared between 2 groups.

Primary composite outcome occurred significantly more common in patients with VA, 3.8% vs. 1.4% (HR, CI P=0.008). Individual outcome of ACS (2.7% vs. 0.1%)and the rate of emergency room visit or re-hospitalization (14.8% vs. 8.4%) was also significantly higher in VA group, but not different in total death (0.3% vs. 0.3%), arrhythmia (1.0% vs. 0.3%) and stroke (0.3% vs. 0%).Survival analysis indicated the poor outcomes in patients with VA.Multivariate Cox-proportional hazard regression analysis demonstrated VA is an independent risk for composite endpoint (HR 3.304, 95% CI 1.262-8.648, P=0.015) along with BMI, dyslipidemia and current smoking.Worse outcomes of VA group sustained across all subgroups.

VA patients had worse clinical outcome at 5-years, mainly attributed to higher rate of ACS and emergency room visit/re-hospitalizations.

PO-450

# Modelling Vascular Responses in Pulmonary Arterial Hypertension in a Microfluidic Pulmonary Artery-on-a-Chip Platform

**Alexander Ainscough**[1], **Beata Wojciak-Stothard**[1*]

[1] National Heart and Lung Institute, Imperial College London, UK

*b.wojciak-stothard@imperial.ac.uk*

Pulmonary arterial hypertension (PAH) is a debilitating disease for which there is currently no cure. Inhibition of bone morphogenetic protein receptor 2 (BMPR2) contributes to endothelial dysfunction and vascular remodelling in PAH. At present, no single animal model of PAH fully reflects human disease. To address this issue we have created a microfluidic model of the human pulmonary vascular wall, the pulmonary artery-on-a-chip, where HPAEC and HPASMC are co-cultured under physiological haemodynamic conditions in distinct microfluidic chambers corresponding to the size of the human peripheral lung arterioles affected by the disease.

Primary HPAEC were transduced with adenoviruses containing short hairpin RNA against BMPR2. Blood-derived endothelial cells (BOECs) were obtained from healthy controls and PAH patients with BMPR2 haploinsufficiency. Endothelial permeability measurements were conducted using 40kDa FITC-Dextran perfused over the endothelial layer. Proliferation of endothelial and smooth muscle cells was assessed through fluorescent EdU staining. Cell alignment was studied with fluorescent confocal microscopy and ImageJ.

HPAEC exhibited preferential alignment to the direction of shear stress, whilst HPASMC exhibited perpendicular alignment to the direction of

flow, resembling the orientation of arterial cells in vivo. Endothelial cells grown in the chip under physiological flow conditions showed significantly enhanced barrier function compared with the cells grown in static conditions and showed an increased sensitivity to stimulation with thrombin. Endothelial BMPR2 knockdown reduced endothelial cell adaptation to flow, and increased proliferation of HPASMCs, likely to reflect cell responses in the remodelled PAH lung. BOECs from PAH patients displayed abnormal alignment and proliferation under flow, compared with healthy controls.

The pulmonary artery-on-a-chip displays arteriole-like features, recapitulates aspects of vascular dysfunction in PAH and therefore can serve as a tool for modelling changes seen in PAH. This approach is a step towards development of a novel precision medicine platform for patients with PAH and other cardiovascular diseases.

PO-452

# The Calcium Sensing Receptor (CaSR) Is a Bi-Phasic Regulator of Vascular Reactivity and Can Be Modulated by the Cardiovascular Risk Factor Asymmetric Dimethylarginine (ADMA)

**Laura Dowsett[1*], Erin Higgins[1], James Leiper[1]**

[1] Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK

*laura.dowsett@glasgow.ac.uk*

The Calcium sensing receptor (CaSR) is expressed in the vasculature by both endothelial cells (ECs) and vascular smooth muscle cells (vSMCs). CaSR stimulation in ECs is pro-vasodilatory, while in contrast CaSR activation in vSMCs is pro-contractile. We have recently discovered that the cardiovascular risk factor asymmetric dimethylarginine (ADMA) is a positive allosteric modulator of CaSR. Elevated plasma ADMA has been extensively associated with cardiovascular diseases including hypertension, stroke and atherosclerosis. The aim of this study was to confirm the bi-phasic nature of the vascular CaSR and to determine the effect of ADMA on vascular CaSR signalling.

Wire myography of aortic rings from C57/BL6 mice was used to assess vascular reactivity. In vitro human lung microvascular cells (HuLMVECs) were used to assess intracellular Ca2+ mobilisation using the Ca2+ -sensitive dye Cal520 and NO production by the NO-sensitive dye DAF-AM. Ca2+ dose response curves was used to stimulate CaSR. NPS-2143 (1μM) is a CaSR antagonist. ADMA and phenylalanine (1mM) were utilised to modulate CaSR activity.

Treatment of aortic rings with NPS-2143 increased maximum contraction to phenylephrine (Ctrl 4.92mN, NPS 7.684mN, $p<0.0001$). In contrast in endothelial denuded vessels NPS-2143 reduced contraction (Ctrl 6.75mN, NPS 6.072mN, $p=0.0151$). Phenylalanine reduced maximal contraction (52%), while 3μM ADMA mimicked this reduction (48.7%, $p<0.0001$). In contrast 30μM ADMA increased contraction to phenylephrine (115%, $P<0.0001$) similar to NO blockade (L-NAME, 145%). In HuLMVECs ADMA increased intracellular Ca2+ release (F/Fo Ctrl 2.264, ADMA 3.023, $p=0.022$) and left-shifted the EC50. NO release was increased by 10μM ADMA (140%) whereas 100μM ADMA deceased NO production (63%, $p=0.023$).

We have confirmed previous data that CaSR acts opposingly in EC and vSMCs to regulate vascular reactivity. We have also shown that that CV risk factor ADMA has a bi-phasic effect through both CaSR activation and NO blockade.

PO-453

# Study of Antihyperglycemic, Antihypertensive Effects of Cucurbita Ficifolia in Human Type 2 Diabetes

**Dhananjay Yadav**[1*]

[1] Department of Medical Biotechnology, Yeungnam University, Korea

*dhanyadav16481@gmail.com*

The present study was to design to analyze the antihyperglycemic, antihypertensive effects of Cucurbita ficifolia juice in type 2 diabetic subjects

A total 34 subjects were recruited for the study out of which 14 subjects were with type 2 diabetes mellitus, 10 subjects were selected as a normal control group and 10 subjects were selected as a normal treated group. The patients mean age was less than 45 years, and subjects were advocated for Cucurbita ficifolia juice starting at day 0 and continuing up to 40 day both for diabetic and normal subjects. Fasting blood glucose, blood pressure levels were measured at 0 days and at 40 days after Cucurbita ficifolia therapy. Results: This study compared the measurement of glucose levels and blood pressure in all three groups [normal control normal treated and type 2 diabetic patients treated], at 0 days and after therapy of 40 days with Cucurbita ficifolia juice.

After 40 days of Cucurbita ficifolia juice therapy there was 17.62 % (P<0.001) decline in fasting blood glucose levels in case of diabetic subjects, while there was slight increase in normal treated group. The blood glucose levels of normal control group remained constant during the course of the study (1.72% decline). Blood pressure measurement after 40 days of Cucurbita ficifolia juice therapy there was a decline in systolic blood pressure by 13.31% and 2.38% for diabetic (P<0.001) and normal subjects respectively. Diastolic blood pressure also alleviated by 19.67% and 8.75% for diabetic (P<0.001) and normal treated subjects.

The results suggest that the Cucurbita ficifolia juice therapy in type 2 diabetes may have the potential to modulate hyperglycemia and hypertension.

PO-454

# Exacerbation of Adverse Cardiovascular Effects of Aircraft Noise in an Animal Model of Arterial Hypertension

**Katie Frenis[1*], Sebastian Steven[1,2], Sanela Kalinovic[1], Miroslava Kvandova[1], Matthias Oelze[1], Johanna Helmstädter[1], Omar Hahad[1], Konstantina Filippou[1], Kamil Kus[3], Chiara Trevisan[4], Klaus-Dieter Schlüter[5], Kerstin Boengler[5], Stefan Chlopicki[3,6], Katrin Frauenknecht[4], Rainer Schulz[5], Mette Sorensen[7,8], Andreas Daiber[1,9], Swenja Kröller-Schön[1], Thomas Münzel[1,9]**

[1] Laboratory for Molecular Cardiology, Laboratory of Molecular Cardiology, University Medical Center of the Johannes Gutenberg-University, Germany
[2] Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany, Germany
[3] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagellonian University, Poland
[4] Institute of Neuropathology, University Hospital Zurich, Switzerland
[5] Department of Physiology, University of Giessen, Germany
[6] Pharmacology, Jagellonian University, Poland
[7] Department of Environment and Cancer, Danish Cancer Society, Denmark
[8] Department of Natural Science and Environment, Roskilde University, Denmark
[9] Deutsches Zentrum für Herz-Kreislauf-Forschung, German Center for Cardiovascular Research (DZHK), Germany

*katiefrenis@gmail.com*

A direct linear relationship between increases in blood pressure and risk of cardiovascular mor-

tality make hypertension a major contributor to the cardiovascular disease burden. Noise pollution is a recently identified cardiovascular risk associated with increased oxidative stress and inflammation and resultant moderate hypertension and endothelial dysfunction. We looked to evaluate the additivity between these traditional and novel cardiovascular risk factors through the induction of arterial hypertension with simultaneous noise exposure.

To induce hypertension, C57Bl/6J mice were implanted with minipumps delivering angiotensin II (ATII) and exposed to aircraft noise for 7 days at a mean SPL of 72 dB(A). Non-invasive plethysmography was used to evaluate changes in blood pressure. Endothelial dysfunction was measured with isometric tension recordings of 3mm aortic segments. Protein and gene analysis were carried out using Western Blot and rtPCR, respectively. Oxidative stress measurements used dihydroethidium via HPLC and staining. Inflammatory cell infiltration was assessed via FACS.

Blood pressure measurements of ATII+Noise mice revealed a small but significant increase over ATII-only mice. There was a corresponding increase in endothelial dysfunction and endothelin-1 expression, along with a deficit in NO signaling, exemplified by the lowest plasma NO2-, aortic p-eNOSSer1177 and p-VASP protein in the double-exposure group. There were significant changes in levels of reactive oxygen species in the ATII+Noise vs ATII mice. Corresponding levels of aortic inflammatory cell infiltrates (inflammatory monocytes, neutrophils, and T-cells) were found as well as highest gene expression of inflammatory and cell recruitment markers in both aortic and brain tissue.

We demonstrate a mechanistic interaction between the onset of a traditional risk factor, hypertension, and the exposure to a novel risk factor, noise. In the hypertensive setting, noise exacerbated the cardiovascular and cerebrovascular inflammatory and pro-oxidant phenotype, forging an important link between environmental stressors and cardiovascular health.

PO-455

# Shades Study: Secondary Hypertension in India and Its Relation with Anthropometric, Demographic, Environmental and Socioeconomic Factors

**Smriti Rastogi[1*], Narsingh Verma[2], Munnalal Patel[3]**

[1] Physiology, King George's Medical University, India
[2] Physiology, King George's Medical University, India
[3] Medicine, King George's Medical University, India

*smriti198719@gmail.com*

1.Relation & effect of anthropometric, demographic, environmental and socio-economic factors on secondary hypertensives
2. Effect of early and late night meal intake on blood pressure in secondary hypertension patients

1200 secondary hypertensive patients aged (18-60 years) were enrolled from Medicine OPD, KGMU, Lucknow. They were studied for anthropometric measurements, 3 readings of blood pressure (average)were taken in each arm(right and left) from OMRON HEM CR-24 BP monitor . Patients with Systolic Blood Pressure (SBP) >= 140 mm Hg, Diastolic Blood Pressure (DBP) >= 90 mmHg were enrolled. A proforma with anthropometric, demographic, environmental and socioeconomic was filled for each subject. Place of residence (low/high population density), village/city, colony/individual house/apartment/multi storeyed building, park nearby/not, living with family/alone, vegetarian/not, meal timings, alcohol & or tobacco intake, physical activity, meditation/yoga, office hours, self-driving/not, marital status, time spent on social media, exposure to artificial light, online gaming, frequency of fasting, pets, leisure hours ,per month

income, medical history, total duration and quality of deep, light, REM sleep (3 days) was recorded for each patient.

Out of 1200 patients, 68.25 % were hypertensive males and hypertensive females were 31.75% 93.6 % (1124 out of 1200) of the secondary hypertensives were living with their families and 76 patients were living alone. 32 % ( 300 patients) were found to living in a joint family set up whereas 68 percent were living with their immediate family. All respondents were married, 19% were widowed.

Age > 40 years for males > 49.8 years for females. BMI > 22.4 for males and > 24.6 for females, less than four hours per week of physical activity (walking), having late night dinner (after 9 pm ), having an education less than standard 10 were significantly associated with hypertension.

PO-456

# To Evaluate the Effect of Add-on Yogic Asana and Relaxation Techniques in Management of Hypertension Associated with Osteoarthritis Knee

**Vandana Awasthi[1*], Narsingh Verma[1], Ajai Singh[2]**

[1] Physiology, George's Medical University, India
[2] Orthopedic Surgery, King George's Medical University, India

*awasthi.vandna@gmail.com*

To assess the effect of yoga on pain, mobility, and quality of life in management of hypertension associated with osteoarthritis knee

Patients with HTN and OA Knee were randomized into two groups -group A with conventional treatment of HTN, OA knee + add on Yoga. Group B with conventional treatment antihypertensive drug treatment only. BP and WOMAC score for clinical parameters of severity of Osteoarthritis knee were assessed before and after giving the treatment (follow up at the interval of 3 months).

Statistically significant fall was present in mean systolic and diastolic pressure of both groups. Significant reduction occurred in doses of antihypertensive drugs, of group A. In few subjects (Group A) blood pressure was controlled with Yoga & RT. Blood pressure rose significantly to pre- Yoga levels in patients who left practicing yoga. With the use of yoga & RT in therapy of HTN, requirement of antihypertensive drugs may be significantly decreased and in some cases may be totally dispensed with. Assessment by WOMAC has shown that severity of pain was 3.267 times lower (p=0.001), physical functions were 3.414 times improved (p=0.001), score of WOMAC functions was 3.410 times lower (p=0.001) and blood glucose level decreased after giving treatment with Yoga & RT for 3 months as compared to the group B.

Yoga &RT is beneficial as a useful adjunct in treatment of HTN when combined with the conventional therapy.

PO-457

# Adolescent Hypertension Status Across Schools of North India: A Cross-Sectional Study

**Vandana Awasthi[1*], Narsingh Verma[2], Abhinav Verma[3]**

[1] Physiology, King George's Medical University, India
[2] Physiology, King George's Medical University, India
[3] Medicine, Hind Institute of Medical Sciences, India

*awasthi.vandna@gmail.com*

To find the current status of prevalence of adolescent hypertension in 25 schools in North India

A cross-sectional study was conducted in 25 schools in and around Lucknow, Uttar Pradesh, India from January 2018 to April 2019. A proforma with detailed medical history was filled of 800 students of standard 7th to standard 12th age (11 to 17 years of age). Anthropometric measurements of height, weight, waist size, hip size were done. Heart rate and Blood pressure both systolic, diastolic was taken thrice each in left and right arm with Omron Blood pressure monitor and average was noted.

62% (n= 496) were boys and 38% (n = 304) were girls. Mean age was 14.26 ± 0.8 years. Boys were found to be more obese as compared to girls. 92 students (11.5%) were found to have systolic hypertension while 29 (3.62%) were found to have (DBP) diastolic hypertension. Mean body weight was 46.44 kgs. Average height of 800 students was 145.94 centimetres. Overall prevalence of hypertension was 15.12% in Indian school going adolescents. Mean (SBP)systolic blood pressure for 800 students was 115.13 mm of Hg and mean diastolic blood pressure was 68.17 mm of Hg. Mean heart rate was 86.17. Waist size and hip size were significantly associated with a greater chance of hypertension. Gender were found to be independent predictor for systolic hypertension.

Adolescent HTN is now not rare in developing countries like India, this may be attributed to family history and other social factors including dietary habits and stress levels.

PO-458

# Fibroblast Growth Factor 12 Inhibits Vascular Smooth Muscle Cell Remodeling in Pulmonary Arterial Hypertension

**Yeong ju Yeo[1], Wonhee Suh[1*]**

[1] Vascular Biology and Biochemistry, Chung-Ang University Pharmacy, Korea

*wonhee.suh@gmail.com*

Loss of bone morphogenic protein (BMP) signaling induces the phenotype switch of pulmonary arterial smooth muscle cells (PASMCs), which is the pathological basis of pulmonary vascular remodeling in pulmonary arterial hypertension (PAH). Here, we identified fibroblast growth factor 12 (FGF12) as a novel regulator of the BMP-induced phenotype change in PASMC and elucidated its role in pulmonary vascular remodeling during PAH development.

Using murine models of PAH, we observed that FGF12 expression was significantly reduced in PASMCs. In human PASMCs, FGF12 expression was increased by canonical BMP signaling. FGF12 knockdown blocked the anti-proliferative and pro-differentiation effect of BMP on human PASMCs, suggesting that FGF12 was required for

the BMP-mediated acquisition of quiescent and differentiated phenotype of PASMCs. Mechanistically, FGF12 regulated this phenotype change by inducing myocyte enhancer factor 2a (MEF2a) phosphorylation via p38MAPK, thereby modulating the MEF2a target gene expression involved in cell proliferation and differentiation. Furthermore, we observed that transgenic mice with SMC-specific FGF12 overexpression were protected from chronic hypoxia-induced PAH development, pulmonary vascular remodeling, and right ventricular hypertrophy. Consistent with in vitro data using human PASMC, FGF12 transgenic mice showed high levels of MEF2a phosphorylation and a substantial change in MEF2a target gene expression compared to that in wild type controls.

Overall, our findings suggested FGF12 as a potential molecular target for the development of therapeutics directed toward pulmonary vascular remodeling in PAH.

FGF12 is necessary for BMP-induced acquisition of the quiescent and differentiated phenotype of PASMCs; ectopic overexpression of FGF12 prevents pulmonary vascular remodeling during PAH development in mice.

PO-459

# The Relationship Between Age, Gender, and Hypertension Staging in Karangpandan Public Health Care, Indonesia: A Single Centre Study

**Indah Sagitaisna Putri[1*], Mohamad Yusuf Habibi[1], Sumardiyono[2]**

[1] Faculty of Medicine, Faculty of Medicine Sebelas Maret University, Indonesia
[2] Public Health Departement, Faculty of Medicine Sebelas Maret University, Indonesia

*indahsagitaisna18@gmail.com*

Based on data from World Health Organization (WHO), premature death due to non-communicable diseases is more experienced by men. But specifically, hypertension insidence in Indonesia occurs more in wome group. Based on the age group, hypertension affects more in the older population. The epidemiology of a disease related to sex and age needs to be studied further because it can have an impact on the selection of prevention and control strategies for a disease based on the characteristics of a population group.

This study was an observational analytic study with a cross-sectional approach based on secondary data obtained from the Puskesmas information management system (SIMPUS) of Karangpandan Puskesmas in Karanganyar Regency in 2019. The sampling technique used in this study was quota sampling. The inclusion criteria in this study were hypertension patients using JKN-PBI facilities, domiciled in Doplang village, and examinations at the Karangpandan main health center. Exclusion criteria were secondary hypertension patients, and hypertensive patients with complications. The classification of hypertension is based on the Eight Joint National

Committee (JNC VIII). In this study, researchers conducted a descriptive analysis for univariate data, while bivariate data were analyzed with chi-square to assess the relationship between dependent and independent variables.

The results of the study showed that the relationship between age and stage of hypertension was not significant (p = 0.126). Then the relationship between sex with stage hypertension was also not significant (p = 0.543)

Age and sex were not significantly related to the stage of hypertension of patients diagnosed with essential hypertension who live in the village of Doplang and were examined at the Karangpandan main health center.

PO-460

# PKN2 Mediates eNOS Activation and NO Production in Response to Fluid Shear Stress

**Young-June Jin**[1*]

[1] Pharmacology, Max Planck Institute for Heart and Lung Research, Germany

*Young-June.Jin@mpi-bn.mpg.de*

Endothelial nitric-oxide synthase (eNOS) is an important enzyme in the cardiovascular system producing nitric oxide (NO), a key regulator of blood pressure and vessel remodeling. Phosphorylation of eNOS at different regulatory sites serves different roles in the regulation of enzyme activation in response to laminar flow. Although phosphorylation of eNOS has been suggested to regulate enzyme activity, the mechanism of eNOS activation is still unclear. PKN is the first identified Serine–Threonine protein kinase, which can bind to and be activated by small GTPase Rho. There are at least three different isoforms of PKN (PKN1, PKN2, and PKN3). PKN2 promotes the activation of several kinases involved in regulation of the actin cytoskeleton, cell cycle, and apoptosis. Global loss of PKN2 in mice results in growth, morphogenetic, and cardiovascular defects and is embryonically lethal by day E10.5. However, the function of PKN2 in endothelial cell is unclear.

In a mass spectrometry-based phosphoproteomic analysis of HUAECs exposed to flow, we found that eNOS is phosphorylated in response to laminar flow not only at serine 1177 but also at serine 1179. To test whether PKN2-mediated endothelial eNOS activation is physiologically relevant, we generated mice with tamoxifen-inducible endothelium-specific PKN2 deficiency.

Laminar flow-induced PDK1-dependent PKN2 phosphorylation and activation induces eNOS phosphorylation at S1177 and S1179. Endothelial PKN2 deficiency results in hypertension and loss of flow-induced vasodilation.

PKN2 is a central mediator of flow-induced eNOS activation and vascular regulation

PO-461

# HMGB1 Emerges as a Key Molecule Mediating Vascular Inflammation in Hypertension

**Ji On Kim[1,2], Seung Eun Baek[1,2], Eun Yeong Jeon[1,2], Jong Min Choi[1,2], Eun Jeong Jang[2], Chi Dae Kim[1,2,3*]**

[1] Pharmacology, Department of Pharmacology and BK21 Plus, School of Medicine, Pusan National University, Korea
[2] Science, Gene & Cell Therapy Research Center for Vessel-associated Disease, Pusan National University, Korea
[3] Medicine, Research Institute for Convergence of Biomedical Science and Technology, PNU Hospital, Korea

*chidkim@pusan.ac.kr*

Hypertension is associated with vascular inflammation and increased mechanical stretch (MS). The interplay between inflammation and MS in hypertension remains undefined. Several reports showed that vascular inflammation and mediated high mobility group box 1 (HMGB1) appears to be critical for the development of hypertension the underlying mechanism, however, the secretion of HMGB1 particularly on vascular smooth muscle cells (VSMCs) still remain unknown. Given the potential importance of vascular inflammation in cellular responses mediated by MS, this study investigated the role for HMGB1 in VSMCs in the development of hypertension.

VSMCs were ex plant cultured using rat thoracic aorta, and stimulated with 0-5% MS. HMGB1 production was determined by ELISA. The protein level of HMGB1 in MS-stimulated VSMCs was analyzed by Western blots.

In cultured rat aortic VSMCs stimulated with MS (0-5% strain, 60 cycles/min) for 0-12 hrs, HMGB1 secretion from media of cell culture was markedly increased. HMGB1 protein levels in cytosolic/nuclear fractions were measured by western blot, which was accompanied by an increased cytosolic translocation of nuclear HMGB1.

Taken together, MS mediates the translocation and release of HMGB1 in VSMCs. The translocation of the protein HMGB1 from the nucleus to the cytoplasm and its secretion or passive release through the permeabilized plasma membrane, constitutes a major cellular danger signal. Thus, targeting secretion of HMGB1 in MS-stimulated VSMCs offers a promising therapeutic strategy for hypertension including vascular inflammation.

PO-462

# NF-kB Responsive miR-31-5p Elicits Endothelial Dysfunction Associated with Preeclampsia via Downregulation of Endothelial Nitric Oxide Synthase

**Suji Kim[1], Wonjin Park[1], Minsik Park[1], Taesam Kim[1], Kwon-Soo Ha[1], Young-Guen Kwon[3], Young-Myeong Kim[1,2*]**

[1] School of Medicine, Kangwon National University, Korea
[2] Kangwon Institute of Inclusive Technology, Kangwon National University, Korea
[3] College of Life Science and Biotechnology, Yonsei University, Korea

*ymkim@kangwon.ac.kr*

Inflammatory cytokines, including TNF-α, were elevated in patients with cardiovascular diseases and are also considered as crucial factors in the pathogenesis of preeclampsia; however, the underlying pathogenic mechanism has not been clearly elucidated. The present study provides novel evi-

dence that TNF-α leads to endothelial dysfunction associated with hypertension and vascular remodeling in preeclampsia through downregulation of eNOS by NF-kappaB-dependent biogenesis of miR-31-5p, which targets eNOS mRNA.

Human specimens were obtained from normal and preeclamptic women after full-term normal deliveries and informed consent was obtained from women with normal and preeclamptic pregnancies. Diverse factors and miRNAs were quantified by ELISA, qRT-PCR and Western blotting.

The treatment of human endothelial cells with TNF-α or miR-31-5p mimic decreased eNOS mRNA stability without affecting eNOS promoter activity, resulting in inhibition of eNOS expression and NO/cGMP production. Moreover, TNF-α and miR-31-5p mimic evoked endothelial dysfunction associated with defects in angiogenesis, trophoblastic invasion, and vasorelaxation in an ex vivo cultured model of human placental arterial vessels, which are typical features of preeclampsia.

These results suggest that NF-kappaB-responsive miR-31-5p elicits endothelial dysfunction, hypertension, and vascular remodeling via post-transcriptional downregulation of eNOS and is a molecular risk factor in the pathogenesis and development of preeclampsia.

PO-463

# The Renin-Angiotensin-Aldosterone System (RAAS) is One of the Effectors used by VEGF/anti-VEGF to Control the Endothelial Cell Barrier

**Yueru Li[1], Andrius Kazlauskas[1*]**

[1] Department of Ophthalmology and Visual Science, University of Illinois at Chicago, USA

*ak20@uic.edu*

Leakage of retinal blood vessels, which is an essential element of diabetic retinopathy (DR), is driven by chronic elevation of vascular endothelial growth factor (VEGF). VEGF quickly relaxes the endothelial cell barrier by triggering signaling events that post-translationally modify preexisting components of intercellular junctions. VEGF also changes expression of genes, which are known to regulate barrier function. This project's goal was to identify effectors by which VEGF and anti-VEGF control the endothelial cell barrier in cells that were chronically exposed to VEGF (hours instead of minutes).

Electric cell-substrate impedance sensing (ECIS)- and FITC dextran-based approaches were used to quantify barrier function of high glucose-treated primary human retinal endothelial cells. RNA-seq and qRT-PCR were used to identify VEGF/anti-VEGF-regulated genes. Pharmacological approaches were used to activate or antagonize the renin-angiotensin-aldosterone system (RAAS).

We discovered that the duration of VEGF exposure influenced both barrier relaxation, and anti-VEGF-mediated closure. Most VEGF-induced changes in gene expression were not reversed by anti-VEGF. Those that were constitute VEGF ef-

fectors that are targets of anti-VEGF. Pursuit of such candidates revealed that VEGF used multiple, non-redundant effectors to relax the barrier in cells that were chronically exposed to VEGF. One such effectors was ACE (angiotensin converting enzyme), which is a member of the renin-angiotensin aldosterone system (RAAS). Pharmacologically antagonizing either ACE, or the receptor for angiotensin II, attenuated VEGF-mediated relaxation of the barrier. Finally, activating the RAAS reduced the efficacy of anti-VEGF.

RAAS is one of the effectors that relax the endothelial barrier in cells that are chronically exposed to VEGF. These discoveries provide a plausible mechanistic explanation for the long-standing appreciation that antagonizing RAAS is beneficial for patients with DR.

PO-465

# Circular RNA Regulates Vascular Calcification in Vascular Smooth Muscle Cells

**Juhee Ryu[1,2], Nakwon Choe[1], Duk-Hwa Kwon[1], Sera Shin[1], Anna Jeong[1], Yun-Gyeong Lee[1], Yeong-Hwan Lim[2], Young-Kook Kim[2], Hyun Kook[1*]**

[1] Pharmacology, Chonnam National Medical School, Korea
[2] Biochemistry, Chonnam National Medical School, Korea

*kookhyun@chonnam.ac.kr*

Vascular calcification (VC) is one of the major risk factor for cardiovascular disease (CVD). VC is commonly observed in patients with chronic kidney disease, diabetes mellitus, and CVD. Circular RNAs (circRNAs) are one of the non-coding RNAs which has circular form and are mostly produced by back-splicing events. Previously, we identified circRNAs that are highly expressed in vascular smooth muscle cells (VSMCs) with inorganic phosphate (Pi)-induced calcification. Since circRNA can play an important role in VC, we decided to investigate circ-VC1, one of the differentially regulated circRNA in Pi-induced VSMCs.

The expression of circ-VC1 in the RNA-sequencing was experimentally assessed by reverse transcription polymerase reaction (RT-PCR). The circular form was tested by treating circ-VC1 with exonucleases. Then subcellular localization of circ-VC1 was evaluated. Additionally, to explore the role of circ-VC1 in VC, the method of gain- and loss-of-function was utilized. Since circRNAs are usually localized in cytoplasm, we hypothesized that it may act as miRNA sponges. Thus, bioinformatics databases and miRNA microarray of Pi-induced VSMCs were used to predict the possibility of circ-VC1 as miRNA sponges.

The expression of circ-VC1 was found to be down-regulated after VC induction as shown in RNA-seq analysis. Circ-VC1 was resistant to exonuclease digestion and was predominantly localized in cytoplasm. Overexpression of circ-VC1 reduced VC, whereas knockdown of circ-VC1 promoted VC in VSMCs. Furthermore, we identified potential target miRNAs that may bind with circ-VC1 by using bioinformatics databases and analyzing miRNA microarray.

To conclude, circ-VC1 regulates VC and may act as miRNA sponges.

PO-466

# Peptide Micelle Nanoparticles for the Reduction of Atherosclerotic Calcification Using Targeted microRNA-145 Therapy

**Deborah Chin[1], Christopher Poon[1], Jackson Cook[1], Kayley Cheng[1], Julia Lee[1], Eun Ji Chung[1,2,3*]**

[1] Biomedical Engineering, University of Southern California, USA
[2] Division of Vascular Surgery and Endovascular Repair, Keck School of Medicine University of Southern California, USA
[3] Division of Nephrology and Hypertension, Keck School of Medicine University of Southern California, USA

*eunchung@usc.edu*

Atherosclerotic calcification, which consists primarily of a calcium phosphate mineral called hydroxyapatite (HA), is strongly correlated with cardiovascular disease progression and increased susceptibility to myocardial infarction. During diseased conditions, smooth muscle cells (SMCs), dictated in part by reduction in microRNA-145 levels, proliferate and differentiate from a quiescent, contractile state into a diseased synthetic phenotype. While in the synthetic phenotype, SMCs increasingly release extracellular vesicles containing HA that propagate into HA crystals in the arterial wall. Small HA microcalcifications (5-65 μm) found in the fibrous cap of atherosclerotic plaques decrease plaque stability leading to plaque rupture while bulk calcifications cause vascular stiffening. As such, a possible therapeutic intervention is through targeted delivery of miR-145 to atherosclerotic plaques to prevent detrimental SMC phenotypic change.

To effectively deliver microRNAs that normally get degraded in serum conditions, we have developed a peptide amphiphile micelle system that utilizes peptides to target calcified plaques while protecting the microRNA (miR) cargo from degradation. Efficacy of miR-145 micelles in reducing SMC-induced calcification and plaque calcification were verified via calcification quantification and gene expression in vitro and in ApoE-/- mouse models.

We demonstrate the stability of miR-145 micelles in serum conditions and their ability to reduce calcification deposits produced by calcifying SMCs. We verify miR-145 micelles are able to decrease expression levels of pro-calcific factors such as RUNX2, MSX2, and BMP2 in calcifying SMCs through qRT-PCR. Fluorescence analysis of intravenously injected miR-145 micelles in ApoE-/- mice validate that nanoparticles are successfully shuttled into atherosclerotic lesions in the aorta. In late-stage atherosclerotic models, miR-145 micelles reduced RUNX2, and MSX2 gene expression in the aortic root and heart tissue. Additionally, histological analyses of the aorta also demonstrate miR-145 micelles reduce calcification in plaques.

MiR-145 micelles are effective miR delivery platforms that show potential in mitigating harmful calcification growths in atherosclerosis.

PO-467

# miR-KO Targets OK to Reduce Calcium Deposition in Vascular Smooth Muscle Cells

**Nakwon Choe[1*]**

[1] School of Parmacology, Chonnam National University Medical School, Korea

*69supercheese@gmail.com*

Investigating roles of micro RNAs in vascular calcification.

To investigate changes in levels of micro RNA (miR) and messenger RNA (mRNA) in Pi-treated rat vascular smooth muscle cells (RVSMCs), miR & mRNA microarray were performed and the results were confirmed by quantitative reverse transcript polymerase chain reaction (qRT-PCR). miR-KO was selected for further investigation, and its effect on putative target, searched on http://www.targetscan.org/, http://www.microrna.org, and http://mirdb.org/, was confirmed by Luciferase assay. The calcification in A10 cells, a vascular smooth muscle cell line derived from rat, was checked by Alizarin Red staining and calcium assay. The effect of miR-KO and OK were analyzed by transfecting miR-KO mimic/inibitor and mammalian expression vector/small interfering RNA of OK . In vivo calcification was induced in mouse aorta by vitamine D, and the result was analyzed by Alizarin Red staining, in situ hybridization, and immunohystochemisy.

In both A10 cells and mouse aorta, induction of calcification down-regulated miR-KO and up-regulated OK.. On the contrary, miR-KO mimic reduced calcification in A10 cells while miR-KO inhibitor increased it, while over-expressing and knocking down of OK showed reverse effect. Furthermore, miR-KO-induced reduction in calcium deposition is recovered by OK. The effect on calcification were accompanied by changes in expression of calcification markers including runt-related transcription factor 2 (Runx2) and alkaline phosphatase (Alp).

Our results suggest that reduction of miR-KO may contribute to the development of vascular calcification by de-repression of OK.

PO-468

# miR-134-5p Induces Calcium Deposition by Inhibiting Histone Deacetylase 5 in Vascular Smooth Muscle Cells

**Duk-hwa Kwon[1], Nakwon Choe[1], Sera Shin[1], Hosouk Joung[1], Juhee Ryu[1], Yongwoon Lim[1], Hyun Kook[1*]**

[1] Pharmacology, Chonnam National University, Medical Center, Korea

*kookhyun@chonnam.ac.kr*

Calcium deposition in vascular smooth muscle cells (VSMCs) is a form of ectopic ossification in blood vessels. It can result in rigidity of the vasculature and an increase in cardiac events. Here, we report that the microRNA miR-134-5p potentiates inorganic phosphate (Pi)-induced calcium deposition in VSMCs by inhibiting histone deacetylase 5 (HDAC5).

Using miRNA microarray analysis of Pi-treated rat VSMCs, we first selected miR-134-5p for further evaluation. Quantitative RT-PCR confirmed that miR-134-5p was increased in Pi-treated A10 cells, a rat VSMC line. Transfection of miR-134-5p mimic potentiated the Pi-induced increase in calcium contents.

miR-134-5p increased the amounts of bone runt-related transcription factor 2 (RUNX2) protein and bone morphogenic protein 2 (BMP2) mRNA in the presence of Pi but decreased the expression of osteoprotegerin (OPG). Bioinformatic analysis showed that the HDAC5 3'untranlated region (3'UTR) was one of the targets of miR-134-5p.

The luciferase construct containing the 3'UTR of HDAC5 was downregulated by miR-134-5p mimic in

a dose-dependent manner in VSMCs. Overexpression of HDAC5 mitigated the calcium deposition induced by miR-134-5p. Our results suggest that a Pi-induced increase of miR-134-5p may cause vascular calcification through repression of HDAC5.

PO-469

# miR-223-3p Regulates TLR4-Dependent Endothelial Senescence by Targeting HDAC2

**Hyo-Jin Kim[1,2], Seo-bo Gyeong[1,2], Patty J. Lee[3], Cheol Hwangbo[1,2*]**

[1] Division of Life Science, Gyeongsang National University, Korea
[2] Division of Applied Life Science (BK21 Plus), PMBBRC, Korea
[3] Pulmonary, Critical Care and Sleep Medicine, Department of Internal Medicine Yale University School of Medicine, USA

*chwangbo@gnu.ac.kr*

We had previously reported that innate immune receptor toll-like receptor 4 (TLR4) is a critical regulator of endothelial cell senescence through modification of histone acetylation by HDAC2 in age-induced emphysema. However, the molecular mechanism of HDAC2 in regulating aging and cellular senescence remained unclear.

Using cigarette smoke extract (CSE), the main cause of emphysema, we demonstrate that TLR4 and HDAC2 expression was reduced, and cellular senescence was induced in response to CSE in HUVECs like as emphysematous endothelial cells (Ec) or aged cells. The expression level of microRNAs in COPD patient and normal lung tissues were analyzed using bioinformatical analysis, miR-223 expression was found to be the most increased in COPD patient lung compared to normal.

miR-223-3p mimic is increasing p16INK4A expression and subsequently cell cycle arrest and cellular senescence, whereas miR-223-3p silencing decreased HDAC2 and p16INK4a, and prevent cellular senescence in context of Ec-aging.

These findings demonstrate that miR-223-3p act as an important regulator of cellular senescence by targeting HDAC2; thus providing a novel function into previously unrecognized links between microRNA and cellular senescence in emphysema.

PO-471

# Aortic Stiffness Precedes Peripheral Blood Pressure Alterations and Cardiac Disease in Spontaneous Ageing of C57Bl6 Mice

**Sofie De Moudt[1*], Jhana O. Hendrickx[1], Dorien G. De Munck[1], Arthur J. Leloup[1], Wim Martinet[1], Guido R.Y. De Meyer[1], Paul Fransen[1]**

[1] Laboratory of Physiopharmacology, University of Antwerp, Belgium

*sofie.demoudt@uantwerpen.be*

The present study aims to investigate the temporal development of aortic stiffening and related peripheral blood pressure (BP) alterations and cardiac disease in spontaneously ageing mice.

MA longitudinal cardiovascular characterization of spontaneously ageing C57Bl6 mice (2, 4, 6, 9, 12 and 24-month old) (male, n>8) was performed using in

vivo analysis of peripheral BP (Coda), aortic pulse wave velocity (aPWV, Vevo2100), and echocardiography (Vevo2100), as well as ex vivo aortic studies of isometric reactivity (organ baths) and aortic stiffness measurements (Peterson modulus, Ep) in the Rodent Oscillatory Tension set-up for Arterial Compliance (ROTSAC). Data are represented as mean ± SEM, * P<0.05, ** P<0.01, *** P<0.001.

In vivo and ex vivo characterisation confirms that aortic stiffness precedes peripheral BP alterations in spontaneously ageing C57Bl6 mice, with significantly increased aortic stiffness from 6 month of age onward (aPWV: 2.0±0.1, 2.6±0.2, 2.9±0.1**, 3.9±0.3***, 3.5±0.2***, 4.9±0.3*** m/s in 2,4,6,9,12,24 month old mice respectively). Thereafter, cardiac hypertrophy was observed at 9-months of age (interventricular septum thickness: 1.0±0.0, 1.1±0.1, 1.1±0.0, 1.3±0.1*, 1.2±0.0, 1.7±0.1*** mm), and peripheral BP measurement reveals elevated pulse pressure only in 24-month old mice (30% increase vs. all other ages, ***). Ex vivo investigation of the thoracic aorta shows both passive, contraction-independent aortic stiffness (isobaric Ep: 290±7, 307±4, 319±4**, 322±8**, 327±4***, 368±6*** mmHg), as well as active aortic stiffening resulting from increased contractions to phenylephrine (PE contraction: 5.7±0.5, 3.6±1.0, 8.0±0.7**, 7.9±1.0*, 8.4±1.0**, 7.1±1.5 mN).

Spontaneously ageing C57Bl6 mice present with significant aortic stiffness by 6-months of age, which is both passive and active in origin. Aortic stiffness thereby precedes the development of cardiac hypertrophy by 3 to 6 months, and peripheral BP alterations by 18 months.

PO-472

# Aortic Stiffness in L-NAME Treated C57Bl6 Mice Displays a Shift from Early Endothelial Dysfunction to Late-Term Vascular Smooth Muscle Cell Disease

**Sofie De Moudt[1*], Jhana O. Hendrickx[1], Dorien G. De Munck[1], Arthur J. Leloup[1], Wim Martinet[1], Guido R.Y. De Meyer[1], Paul Fransen[1]**

[1] Laboratory of Physiopharmacology, University of Antwerp, Belgium

*sofie.demoudt@uantwerpen.be*

Since endothelial dysfunction acts as a common player in most cardiovascular risk factors (e.g. hypertension, smoking), this study aims to characterize the longitudinal ex vivo aortic function changes in Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) treated mice, which underlie its cardiovascular disease phenotype.

C57Bl6 mice (male, n=10) received 0.5 mg/mL L-NAME through the drinking water for 1, 2, 4, 8, and 16 weeks, followed by in-depth ex vivo studies of thoracic aorta isometric reactivity and arterial stiffness (Peterson modulus, Ep) measurements in the Rodent Oscillatory Tension set-up for Arterial Compliance (ROTSAC). Data are represented as mean ± SEM, * P<0.05, ** P<0.01, *** P<0.001.

L-NAME treated mice display fast-onset aortic stiffening after 1-week L-NAME (Ep: + 24±8, + 30±10, + 28±10, + 42±11, + 40±11 mmHg vs. control after 1,2,4,8,16 weeks L-NAME respectively, ***), and aortic reactivity studies reveal different stages of disease. Early on (1-4 weeks), mice show elevated phenylephrine contractions (30% increase vs. con-

trol, *) and impaired acetylcholine relaxations (10% decrease vs. control, *), consistent with L-NAME related endothelial dysfunction. After 8 weeks however, isometric reactivity normalizes. Nonetheless, stiffness analysis in the ROTSAC reveals a constant slight reduction in basal NO levels (4% decrease vs. control, *), and a late-term shift towards vascular smooth muscle cell disease. This involves basal cytoplasmic calcium loading (16 weeks: - 12±4 mN when calcium is removed from extracellular environment, *) and a sharply increasing contribution of voltage-gated calcium channels (diltiazem-induced relaxation: + 15.9±3.6, + 7.2±6.4, + 12.8±5.3, + 16.0±5.3, + 24.0±5.1 % vs. control after 1,2,4,8,16 weeks L-NAME respectively, ***).

NOS inhibition by L-NAME treatment causes a distinct time-dependent aortic disease phenotype, underlying its fast-onset aortic stiffness phenotype.

PO-473

# Role of Gastrin-Releasing Peptide in Vascular Calcification

**Hyun-Joo Park[1], Moon-Kyoung Bae[1*]**

[1] School of Dentistry, Pusan National University, Korea

*mkbae@pusan.ac.kr*

Vascular calcification is the pathological deposition of calcium/phosphate in the vascular system and is closely associated with cardiovascular morbidity and mortality. Here, we investigated the role of gastrin-releasing peptide (GRP) in phosphate-induced vascular calcification and its potential regulatory mechanism.

We induced calcification of vascular smooth muscle cells by stimulating them with inorganic phosphate. We used an ex vivo organ culture of a rat aorta and mouse chronic kidney disease models to mimic in vivo vascular calcification.

We found that the silencing of GRP gene, and treatment with the GRP receptor antagonist, RC-3095, attenuated the inorganic phosphate-induced calcification of vascular smooth muscle cells (VSMCs). This attenuation was caused by inhibiting phenotype change, apoptosis, and matrix vesicle release in VSMCs. Moreover, the treatment with RC-3095 effectively ameliorated phosphate-induced calcium deposition in rat aortas ex vivo and aortas of chronic kidney disease in mice in vivo.

Therefore, the regulation of the GRP-GRP receptor axis may be a potential strategy for treatment of diseases associated with excessive vascular calcification.

PO-474

# Understanding the Molecular Mechanisms of Endothelial Senescence in COPD – the Role of the Renin-Angiotensin System

**Koralia Paschalaki[1,2*], Justin C. Mason[1], Peter J. Barnes[2], Anna M. Randi[1]**

[1] Vascular Sciences, NHLI, Imperial College London, UK
[2] Airway Disease Section, NHLI, Imperial College London, UK

*k.paschalaki@imperial.ac.uk*

Cardiovascular disease (CVD) is a major cause of death in smokers and patients with chronic

obstructive pulmonary disease (COPD). DNA damage response (DDR) and endothelial senescence, regulated by ataxia-telangiectasia-mutated (ATM) protein kinase, promote vascular ageing. Endothelial colony forming cells (ECFC) is a unique tool providing non-invasive access to endothelial cells in patients. The renin-angiotensin system (RAS) and particularly Angiotensin(Ang)-II are implicated in COPD pathogenesis and in CVD. Our aim is to investigate the role of AngII in endothelial senescence and vascular ageing in COPD, and test possible pharmacological interventions.

ECFC were isolated and expanded from peripheral blood samples of healthy non-smokers, healthy smokers and COPD patients. The mononuclear fraction was placed in culture in the presence of endothelial growth factors and ECFC colonies appeared between days 7 and 24. Senescence was measured by senescence-associated β-galactosidase (SA-β-Gal) activity. Additional markers of senescence (p16, p21) and DNA damage (γ-H2AX, 53BP1) were measured by immunofluorescence confocal microscopy. We have recently used a high-throughput 'organ-on-a-chip' microfluidic platform called OrganoPlate from MIMETAS company that allows the long-term culture of endothelial cells and formation of microvessels for functional and immunofluorescent analysis.

We have previously shown that ECFC from COPD patients exhibit increased DDR and senescence due to epigenetic dysfunction involving the histone deacetylase sirtuin-1(SIRT1) linked to ATM protein kinase. We have optimised in vitro 2D and 3D models of premature endothelial senescence caused by AngII. Pharmacological treatment with SIRT1 activators, ATM inhibitors and AngII receptor blockers could inhibit the increased senescence in ECFC from COPD patients.

We demonstrate that ECFC from smokers and COPD patients exhibit increased senescence possibly due to aberrant activation of the RAS system and could be amenable to treatment. These defects may contribute to endothelial dysfunction and CVD in COPD and could potentially constitute therapeutic targets for intervention.

PO-475

# Endothelial-Pericyte Cross Talk via Tie2 and Pdgfb Affect Capillary Rarefaction and Tubulointerstitial Fibrosis in Chronic Kidney Disease

**Riikka Pietilä[2], Samrand Nanavazadeh[2], Gou Young Koh[3], Susan E Quaggin[4], Christer Betsholtz[1,2], Marie Jeansson[1,2*]**

[1] Department for Medicine, Huddinge, Karolinska Institutet, Sweden
[2] Immunology, Genetics, and Pathology, Uppsala University, Sweden
[3] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Korea
[4] Feinberg Cardiovascular Research Institute, Northwestern University, USA

*marie.jeansson@ki.se*

Progressive renal diseases are associated with loss of peritubular capillaries, capillary rarefaction, but the underlying mechanisms are not well described. We hypothesized that renal blood vessels through loss of Tie2 signaling upregulates Pdgfb that in turn act as a mitogen to activate pericytes and fibroblasts.

To investigate this, we utilized floxed alleles for Tie2 and Pdgfb together with inducible endothelial specific Cre and lineage reporter.

Our results show that loss of Tie2 results in increased injury to peritubular capillaries and tubulointerstitial fibrosis in an experimental model of chronic kidney disease. Further studies revealed a Tie2 dependent regulation of endothelial Pdgfb, and endothelial knockout of Pdgfb decreased activation of fibroblasts and pericytes, hence decreased tubulointerstitial fibrosis in chronic kidney disease. In conjunction with this, attenuated disease

progression could be seen by antibody mediated increase in Tie2 signaling. Data from patients was used to confirm that the same pathway is activated in human chronic kidney disease.

Taken together we suggest that endothelial – pericyte crosstalk is a novel pathway in both capillary rarefaction and tubulointerstitial fibrosis in progressive renal disease.

PO-478

# Gut Microbial Colonization Restricts NETosis in Acute Mesenteric Ischemia-Reperfusion Injury

**Stefanie Ascher[1], Franziska Bayer[1], Giulia Pontarollo[1], Eivor Wilms[1], Henning Formes[1], Frano Malinarich[1], Christoph Reinhardt[1*]**

[1] University Medical Center Mainz, Johannes Gutenberg-University Mainz, Germany

*christoph.reinhardt@unimedizin-mainz.de*

Acute mesenteric ischemia is threatening bowel viability by the reduction of blood flow, leading to bowel necrosis and multiple organ failure. During ischemia and reperfusion, the recruitment of neutrophils and the formation of neutrophil extracellular traps (NETs) contribute to the lethality in acute mesenteric infarction. To investigate the impact of the gut microbiota in acute mesenteric infarction, gnotobiotic mouse models were studied to explore if the commensal gut microbiota affects the recruitment and reactivity of neutrophils towards NETosis.

Mesenteric ischemia was induced by ligating the Arteria mesenterica cranialis with a small vascular clamp. One hour after ischemia, reperfusion was allowed by releasing the clamp. Adherent leukocytes (stained with Acridine Orange) and NETs (dyed with the extracellular DNA stain SYTOX Orange) were visualized and quantified in vivo with a high-speed epifluorescence intravital microscope before and immediately after ischemia-reperfusion.

To investigate if the adherence of leukocytes and the formation of NETs in acute mesenteric infarction is influenced by the host colonization status, germ-free (GF) and colonized C57BL/6J mice were compared. GF mice, which lack a commensal gut microbiota, showed reduced neutrophil accumulation in ischemia-reperfusion-injured mesenteric venules. In contrast to conventionally raised (CONV-R) SPF mice, the neutrophils of GF mice were prone to in vivo NETosis. Interestingly, colonization of GF mice with the minimal microbial consortium altered Schaedler flora (ASF) suppressed NETosis. In line, neutrophils from CONV-R mice that were treated with an antibiotic cocktail to deplete colonizing gut bacteria showed increased NET formation in ischemia-reperfusion-injured mesenteric venules.

Demonstrating a protective role for the gut microbiota in mesenteric ischemia-reperfusion injury, our results revealed that the presence of the gut microbiota or colonization with a defined microbial consortium suppresses NETing neutrophil hyperreactivity.

PO-479

# Angiopoietin-2 Increases Over Time in COVID-19 ICU Patients

**Michael Hultström[2,3], Anders Larsson[5], Christer Betsholtz[2,3], Robert Frithiof[2], Miklos Lipcsey[2], Marie Jeansson[1,4*]**

[1] Department for Medicine, Huddinge, Karolinska Institutet, Sweden
[2] Surgical Sciences, Anesthesia and Intensive Care Medicine, Uppsala University, Sweden
[3] Medical Cell Biology, Uppsala University, Sweden
[4] Immunology, Genetics, and Pathology, Uppsala University, Sweden
[5] Medical Sciences, Clinical Chemistry, Uppsala University, Sweden

*marie.jeansson@ki.se*

SARS-CoV-2 infection can be paucisymptomatic or lead to the coronavirus disease-2019 (COVID-19), which has a broad range of disease severity, in particular in patients with cardiovascular comorbidities. COVID-19 is associated with coagulopathy characterized by an increase in procoagulant factors including fibrinogen, together with a strong elevation of D-dimers that have been associated with higher mortality. Circulating Angiopoietin-2 is increased in many diseases characterized by vascular dysfunction and correlates with disseminated intravascular coagulopathy in septic patients.

To test the hypothesis that COVID-19 patients have elevated Angiopoietin-2 levels that may correlate with coagulopathy, we investigated plasma from 20 COVID-19 patients from the intensive care unit (ICU) at Uppsala University Hospital. Angiopoietin-2 and Angiopoietin-1 were measured from plasma taken at admission and after two weeks at the ICU. Healthy controls were blood donors matched for age and gender.

Already at admission to the ICU, COVID-19 patients had significantly elevated Angiopoietin-2 levels compared to healthy controls, from 1451 pg/mL (1185-1776, 95% CI) in controls to 2997 pg/ml (2398-3746, 95% CI) in COVID-19 patients ($p<0.0001$). Two weeks after admission, Angiopoietin-2 was further increased to 4804 pg/ml (3716-6209, 95%CI; $p<0.01$ compared to admission) in these patients. Angiopoietin-1 levels were 7231 pg/ml (5440-9612, 95% CI), 5412 pg/ml (4145-7066, 95% CI), and 6049 pg/ml (4915-7445, 95% CI) for controls, patients at admission, and patients 14 days after admission, respectively.

Our study clearly shows that Angiopoietin-2 is regulated over time in critically ill COVID-19 patients. Ongoing studies aim at analyzing clinical chemistry data to investigate possible correlations to coagulopathy factors, including D-dimer.

PO-480

# Cell-Specific Function of Fibulin-4 in Progression of Ascending Aortic Aneurysm in Mice

**Tram Anh Vu Nguyen[1*]**

[1] University of Tsukuba, Life Science Center for Survival Dynamics, Tsukuba Advanced Research Alliance, Japan

*nvtramanh137@gmail.com*

Fibulin-4 (Fbln4) is an extracellular matrix (ECM) protein expressed in all layers of the aortic wall and is essential in elastic fiber formation. We previously showed that smooth muscle cell (SMC)-specific deletion of fibulin-4 (Fbln4SMKO;

SMKO) led to ascending aortic aneurysm. However, the function of Fbln4 in endothelial cells (EC)s and whether it affects aneurysm formation are not well investigated. Therefore, this study aims to elucidate the role of endothelial Fbln4 in aortic aneurysm formation.

EC-specific knockout mice for Fbln4 (Fbln4loxP/KO; Tie2-Cre; ECKO) were generated and crossed with SMKO mice to generate double knockout mice (Fbln4SMKO/ECKO; DKO). Animals were sacrificed at postnatal day 7 and 2 months old and morphological analysis was performed. The endothelial barrier function of DKO aneurysmal wall was also evaluated by the injection of 1% Evans blue in the tail vein and then immunostaining.

ECKO mice appeared normal and did not develop any aortic aneurysms. However, DKO mice showed exacerbated aneurysms compared to SMKO mice, expanding from the aortic root to the aortic arch. Aortic valves in DKO mice were much more thickened compared to SMKO and control mice at postnatal day 7 and 2 months old, and the blood flow velocity measured by Doppler echocardiography revealed a turbulent flow in the DKO ascending aorta. Immunostaining showed Evans blue leakage in the medial layer of DKO aortas, indicating the compromised EC barrier function.

The deletion of Fbln4 in ECs and SMCs results in severe aortic aneurysms with thickening of the aortic valves and disturbed flow in the ascending aorta. In addition, endothelial barrier dysfunction may have contributed to the worsening of the aneurysm phenotype in DKO mice. The detailed molecular mechanism is currently investigated through the proteomic analysis of Fbln4-interacting proteins in ECs using proximity labeling methods (BioID and TurboID).

# 5. Vascular Metabolism

PO-503

## PTX3 Deficiency Ameliorates Streptozotocin-Induced Hyperglycemia and Pancreatic β-Cell Apoptosis

**Sun-Hee Kim[1], Suji Kim [1], Chang-Hoon Woo[1*]**

[1] Pharmacology, Yeungnam University, Korea

*changhoon_woo@yu.ac.kr*

Diabetes is a group of metabolic disease characterized by elevated level of blood glucose for prolonged periods of time. Low-grade chronic inflammation is a common feature in diabetes. Pentraxin-3 (PTX3) acts as an important mediator of innate immunity and is involved in the regulation of inflammation and tissue remodeling. However, little is known about its role in diabetes. Herein, we investigated the role of PTX3 in the regulation of Streptozotocin (STZ)-induced hyperglycemia and pancreatic β-cell apoptosis.

PTX3 knockdown INS-1 cells were generated using siRNA for Ptx3 gene and treated with STZ (1 mM). INS-1 cells were collected for gene and protein expression by qPCR and western blotting, respectively. For the in vivo studies, WT and PTX3 knockout mice were i.p. injected with STZ (50 mg/kg body weight daily of 5 days). The mice were sacrificed at 2 weeks after the first injection of STZ to measure hyperglycemia and protein expression.

Hyperglycemia was inhibited by treating PTX3 deficient mice with STZ as compared with C57BL/6J mice administered with STZ. In addition, TUNEL assay and immunoblotting data revealed that

STZ-induced pancreatic β-cell apoptosis was inhibited in PTX3 deficeint mice. In vitro, depletion of PTX3 with siRNA-PTX3 blocked STZ-induced apoptosis in INS-1 cells. Moreover, STZ-induced pancreatic β-cell apoptosis was exacerbated by treating recombinant PTX3.

These results suggest that PTX3 plays a critical role in STZ-induced pancreatic β-cell apoptosis and hyperglycemia.

PO-504

# Identification of Small Molecule Modulators of the Insulin Receptor/Insulin-Like Growth Factor 1-Receptor Hybrid Dimer as a Future Treatment for Type 2 Diabetes

**Chloe Wilkinson[1], Martin McPhillie[2], Mark Kearney[1], Katie Simmons[1*]**

[1] Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK
[2] School of Chemistry, University of Leeds, UK

*K.J.Simmons@leeds.ac.uk*

Type-2 diabetes is a highly degenerative and increasingly prevalent disease, phenotyped as either insufficient production of insulin or a non-response in the presence of insulin. In 2019, 463 million adults globally were affected by diabetes, with diabetes listed as the causal factor for around 4.2 million deaths. Several markers of premature diabetes can be identified prior to the development of the condition. One of these, insulin resistance, is also an independent risk factor in the progression of premature cardiovascular disease. It is well documented that the insulin receptor (IR) and insulin-like growth factor 1 receptor (IGF1-R) can form hybrid dimers, consisting of a single monomer of each respective receptor. Hybrid stoichiometry is a critical determinant in endothelial cell insulin sensitivity, NO bioavailability and vascular repair.

Using an in-house homology model of the hybrid ectodomain and virtual high-throughput screening cascade, commercially available small molecules predicted to specifically modulate hybrid formation were identified. Inhibition was determined using a Bioluminescence Resonance Energy Transfer assay and confirmed by co-immunoprecipitation and western blotting to quantify levels of hybrid and homodimer formation. Angiogenic properties, NO bioavailability and off-target effects in the presence of the inhibitor were analysed. Molecular dynamic simulations have been used to develop models simulating binding of insulin and IGF1 to hybrid dimers.

Molecules which reduce hybrid formation in endothelial cells have been identified. In addition to studying their effect on IR and IGF1-R homodimer formation, effects on downstream signalling for both IR and IGF1-R were ascertained as well as their ultimate effect on NO bioavailability. Molecular dynamic simulations were able to rationalise the observed specificity of hybrids for binding of IGF1 over insulin.

Hybrid stoichiometry can be controlled by introducing small molecule inhibitors in vitro and ex vivo, this can increase endothelial cell signalling and therefore improve vascular function.

PO-505

# Serum Triglycerides Are a Predictive Factor for the Development of Diabetes Nephropathy in Patients with Type 2 Diabetes

**Sangmo Hong[1*], Sung Hoon Yu[1], Chang Beom Lee[1]**

[1] Endocrinology and Metabolism, Hanyang University Guri Hospital, Korea

*lanugo35@gmail.com*

There is controversial that serum lipids affect the development of microvascular complications in patients with type 2 diabetes(T2D). This observational retrospective study investigated whether dyslipidemia contributes to develop diabetes nephropathy.

We collected 2302 patients with T2D with albumin-to-creatinine ratio (ACR) < 30 mg/g or eGFR > 90 ml/min per 1.73m2. The development of diabetes nephropathy was defined as ACR ≥ 30 mg/g or eGFR ≤ 60 ml/min per 1.73m2.

After 3.8±1.8 years mean follow up, 468 patties developed the diabetes nephropathy (160/1000 person-year). In bassline lipid profile, only serum triglyceride was significantly different between patients with and without new diabetes nephropathy(p=0.05). In cox-regression model, higher triglyceride level associated with 1.512 times higher risk for new diabetes nephropathy (95% CI: 1.237-1.848).

In this study, higher triglyceride level is one of independent risk factor in the development of diabetes nephropathy.

PO-506

# Intravital Imaging of Blood Flow Changes in Diabetic Retinopathy Mouse Models

**Jehwi Jeon[1], Philhan Kim[1*]**

[1] GSMSE(Graduate school of medical science and engineering), KAIST (Korea Advanced Institute of Science and Technology), Korea

*pilhan.kim@kaist.ac.kr*

To evaluate retinal blood flow velocity and vascular changes in early stage diabetic retinopathy (DMR) models and to standardize blood velocity changing curve of normal mouse with aging.

By using custom-built video-rate confocal microscopy, we imaged fluorescently labelled red blood cell (RBC) streams in vessels of retina in vivo. In normal mice with different ages of 3, 4, 8, 14, 18, 22, 32, 42, 62 weeks, we measured RBC velocity with follow-up ex vivo validation of in vivo magnification ratio. Additionally, we serially followed same mouse for 22 weeks in B6 and balb/c strains. As DMR models, we used APB5 (PDGFR-beta antibody) induced pericyte depletion model and STZ (streptozotocin) induced beta cell depletion model. For pericyte depletion, 40μg of APB5 was injected to newborn mouse at postnatal day 1. For beta cell depletion, STZ (150mg/kg) was injected to 6 weeks mouse. We chose high glucose level mouse above 200 mg/dL by 1 week follow-up BST screening.

Two strains showed similar tendency in blood flow velocity change with aging, which gradually increased until around 18 to 22 weeks and remain unchanged up to 62 weeks. In both DMR models, blood flow velocity was decreased in comparison with normal mice. RBC velocity in artery, vein and capillary was decreased in APB5 models. Similarly, RBC velocity in artery and vein velocity was

decreased in STZ models (0.05>p-value). With histologic analysis we observed sustained decreased pericyte to endothelial cell ratio even in 29 weeks old STZ mouse.

We successfully monitored blood velocity changes in normal aging mice as a standard baseline for further quantitative analysis. We observed blood velocity decreases and decline of pericyte number around vessel walls in early stage of DMR.

PO-509

# Anti-Hypercholesterolemic Effect of Berbamine in High Fat Diet (HFD) and Streptozotocin (STZ) Induced Diabetes: Phytotherapeutical Importance Through Data Analysis of Current Scientific Research Works

**Dinesh Kumar Patel[1*], Kanika Patel[1]**

[1] Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, India

*dkp.iitbhu@gmail.com*

Diabetes is the disorders of carbohydrate and protein metabolism which produce elevated level of glucose and lipid level in the Human body. Role of various type of enzymes in the carbohydrate and lipid metabolism have been well documented in the scientific work which are one of the main factors of diabetes mellitus. Berbamine is bisbenzylisoquinoline class alkaloidal compound found to be present in the various species of Berberis including Berberis amurensis.

Medicinal importance and Pharmacological activities of Berbamine have been investigated in the present study through data analysis of various research works of current scientific literature. Importance of Berbamine in high fat diet (HFD) and streptozotocin (STZ) induced diabetic rats have been investigated in the present investigation through scientific data analysis to know the anti-hypercholesterolemic effect of Berbamine. Effect of Berbamine on TC, TG, LDL-c, HMGCR and MTP has been also investigated through data analysis for their molecular mechanism. All the data have been analyzed statistically to get better molecular mechanism.

Data analysis of the current scientific research work revealed the importance of Berbamine in various form of diabetes mellitus. In the scientific work, effect of berbamine on various enzymes of carbohydrate and lipid metabolism in high fat diet and STZ- induced diabetic animal have been investigated and found that berbamine improved insulin secretion. However berbamine also have positive role in the carbohydrate metabolic enzymes as it improved glycogen content in the tissues. In another scientific study, anti-hypercholesterolemic effect of berbamine has been studied in zebrafish and revealed significant effect on TC, TG and LDL-c. However mRNA expression of HMGCR and MTP in liver was also down-regulated.

These data analysis revealed that berbamine has anti-hypercholesterolemic effects mainly through up-regulation of cholesterol transport and inhibition of cholesterol synthesis.

PO-510

# Blood Glucose Lowering Potential of Bavachinin Through Interaction of Peroxisome Proliferator-Activated Receptor γ (PPARγ): Importance of Natural Molecule in Diabetes Treatment Through Data Analysis

**Dinesh Kumar Patel[1*], Kanika Patel[1]**

[1] Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, India

*dkp.iitbhu@gmail.com*

Herbal medicine have numerous health benefits as it have different pharmacological activities and could be used for the treatment of Human disorders. Peroxisome proliferator-activated receptor γ (PPARγ) play an important role in the diabetes and related complications mainly used against diabetes and related complication. PPARγ have effectiveness due to their blood glucose lowering effect and effectiveness on insulin sensitivity. Flavonoidal compounds have important role in the diabetes and showed effectiveness against PPARγ activity.

Bavachinin is found to be present in the various plants including Chinese herb Fructus Psoraleae and used for the treatment of asthma in the medical field. In the present investigation, beneficial effect of bavachinin has been evaluated through data analysis of current scientific works. Medicinal importance of bavachinin for their blood glucose lowering potential has been investigated in the present investigation through scientific data analysis of potential research works. Importance of Peroxisome proliferator-activated receptor γ (PPARγ) in the treatment of diabetes and related disorders has been also evaluated through data analysis of current scientific work. All the data analysis has been done statistically to get the better result in the present investigation for their blood glucose lowering potential.

From the analysis of the current scientific research work it was found that synthetic analogues of bavachinin have been found to show higher PPARγ agonist activities compared to the bavachinin. Another scientific work data analysis revealed the importance of Bavachinin for their glucose-lowering activity without affecting body weight gain and any form of toxicity in the liver. Further it also showed synergistic effect with thiazolidinediones to treat blood disorders through act as PPAR-γ agonists.

From the analysis of the scientific works it was found that bavachinin is better molecules for the treatment of diabetes and related complication due to its glucose and lipid-lowering potential synergistically acting through PPAR-γ.

PO-512

# HNF1A-MODY Disease Modeling-in-a-Dish Reveals Increased Vascular Permeability Related to Alterations in Endothelial Cell Junctions

**Neli Kachamakova-Trojanowska[1*], Jacek Stepniewski[2], Dawid Skoczek[1,2], Jozef Dulak[2]**

[1] Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland
[2] Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Poland

*neli.kachamakova-trojanowska@uj.edu.pl*

Maturity onset diabetes of the young (MODY) is an autosomal dominant monogenic diabetic disease typically affecting individuals before the age of 25. The most common form of the disease is caused by mutation in hepatocyte nuclear factor 1A (HNF1A) gene. Patients with HNF1A-MODY were associated with abnormalities in endothelial function, microvascular complications like retinopathy, and increased risk factor for cardiovascular diseases. However, up to date there is no clear relation between mutation in HNF1A gene and endothelial dysfunction.

Patient-specific induced pluripotent stem cells (hiPSCs), upon differentiation toward endothelium, could be a promising tool for analysis of disease-specific mechanisms, responsible for vascular function impairment. For proper disease modeling we generated isogenic hiPSCs lines through CRISPR/Cas9, which differed only at the gene of interest.

Using these isogenic lines, we have shown that the endothelial cells (ECs), derived from hiPSCs, with mono- or biallelic mutation in HNF1A gene, have increased vascular permeability in response to pro-inflammatory cytokine. Moreover, the increase was more prominent in cells with biallelic mutation, suggesting dose-dependent effect and potential impairment of cell-cell contacts of these ECs related to HNF1A mutation. Importantly, RNA-seq results of hiPSC-EC with and without HNF1A mutation showed differential expression of genes involved in processes like cell communication, adhesion, and cellular components like cell junction and cytoskeleton. Cumulatively, these results suggest certain predisposition of HNF1A-mutated ECs to increased vascular permeability through alteration in cell-cell communication, which is the basis of diabetic-related complications like retinopathy. Moreover, increased reactive oxygen species (ROS) production was detected in HNF1A-mutated ECs under basic conditions, further supporting the putative susceptibility to endothelial-related complications in HNF1A-MODY patients.

Summarizing, heterozygous mutation of HNF1A gene in ECs lead to increased vascular permeability most probably through alterations in ROS production and cell junction proteins. The work was supported by Opus grant from NCN 2016/23/B/NZ1/01804.

PO-514

# Triglyceride and Glucose (TyG) Index as a Predictor of Gestational Diabetes Mellitus

**Kyung-Soo Kim[1], Kyungdo Han[2], Cheol-Young Park[3*]**

[1] Department of Internal Medicine, CHA Bundang Medical Center, CHA University School of Medicine, Korea
[2] Department of Statistics and Actuarial Science, Soongsil University, Korea
[3] Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Korea

*cydoctor@chol.com*

Insulin resistance is associated with gestational diabetes mellitus (GDM). Triglyceride and glucose (TyG) index is a useful marker of insulin resistance but there are few studies about association between TyG index and GDM. The aim of this study was to investigate the association between pre-pregnancy TyG index and GDM.

We analyzed 413,370 women who gave birth between 2011 and 2015 using the Korean National Health Information Database. They underwent the Korean National Health Screening Program within one year before pregnancy and were not prescribed drugs for diabetes or dyslipidemia and were not diagnosed diabetes mellitus before antepartum 280 days. The TyG index was calculated as the Ln[fasting triglyceride (mg/dL) x fasting plasma glucose (mg/dL)/2].

The prevalence of GDM was 12.64% (n = 52,243). GDM was increased with increasing quartiles of TyG index (Q1 10.26%, Q2 11.40%, Q3 12.64%, Q4 16.25%; P for trend < 0.001). After adjustment for confounding factors, women with the highest quartile of TyG index were 1.392 times (95% CI 1.348 – 1.437) higher risk for GDM than those with the lowest quartile. Cut-off value of TyG index for GDM is 8.07 and the area under the receiver-operating characteristic curve was 0.6335 (0.6306-0.6364). Multivariable logistic regression analyses showed that the odds of having GDM was increased with every 1 increase of TyG index (OR = 1.335, P < 0.001) and was the highest among other risk factors including age, body mass index, regular physical activity, and family history of diabetes.

In conclusion, a high pre-pregnancy TyG index was associated with GDM and the pre-pregnancy TyG index might be useful to predict GDM.

PO-515

# Comparative Efficacy of Lobeglitazone Versus Pioglitazone in Patients with Type 2 Diabetes Mellitus on Albuminuria

**Kyung-Soo Kim[1], Hong-Yup Ahn[2], Cheol-Young Park[3*]**

[1] Department of Internal Medicine, CHA Bundang Medical Center, CHA University School of Medicine, Korea
[2] Department of Statistics, Dongguk University-Seoul, Korea
[3] Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Korea

*cydoctor@chol.com*

The aim of this analysis was to evaluate the efficacy of lobeglitazone on albuminuria for 24 weeks in patients with type 2 diabetes mellitus (T2DM) compared with pioglitazone using data from a randomized, double-blinded phase III trial.

In the phase III trial, patients who were inadequately controlled with metformin received 0.5 mg of

lobeglitazone or 15 mg of pioglitazone for 24 weeks. Post hoc, exploratory analysis was used to investigate mean changes from baseline in urinary albumin creatinine ratio (UACR) between lobeglitazone (N = 104) and pioglitazone (N = 101).

After 24 weeks of treatment, UACR was slightly decreased in the lobeglitazone group (-4.3 mg/g Cr) and increased in the pioglitazone group (5.2 mg/g Cr) with no change in estimated glomerular filtration rate in either group even though it was not statistically significant (P=0.476). The incidence of new-onset microalbuminuria (2.4%) and the progression of albuminuria by > 1 stage (2.9%) in the lobeglitazone group were lower compared to the pioglitazone group (6.8% and 6.1%, respectively). Half of the patients in the lobeglitazone group exhibited regression to normoalbuminuria, whereas 39.3% of the patients in the pioglitazone group had regressed. In the lobeglitazone group with micro- and macroalbuminuria, UACR tended to be more decreased and HbA1c was more reduced compared to patients with normoalbuminuria (P = 0.014).

Lobeglitazone had a tendency to improve albuminuria in patients with T2DM and had comparable effects on albuminuria compared with pioglitazone which has demonstrated beneficial effects.

PO-516

# Biological Importance of Sciadopitysin Against Diabetes and Related Complication Through Protection of Pancreatic β-Cells and Oxidative Stress Induced Cellular Damage

**Dinesh Kumar Patel**[1*]

[1] Shalom Institute of Health and Allied Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, India

*dinesh.patel@shiats.edu.in*

Plants and their derive plant material are responsible for pharmacological activities of natural products including the basic role in the plants such as development of, colors, flavor and defense mechanism. Plant derived secondary metabolites are well known for their beneficial potential in the treatment of various form of cancerous disorders. Diabetes is a metabolic disorder of carbohydrate, lipid and fat which affect many person lives in the form of physical and physiological aspects. Sciadopitysin is a phytochemical found to be present in the various types of medicinal plants belongs to the flavonoidal class chemical.

In order to know the biological importance of sciadopitysin in the medicine, here in the present investigation numerous scientific data have been collected from different literature sources. Pharmacological activities of sciadopitysin have been investigated through various scientific databases. Biological potential of Sciadopitysin for the treatment of diabetes and its related complication have been investigated through scientific databases

analysis. Effect of Sciadopitysin on the different cell lines such as pancreatic β-cell line and RIN-m5F cells have been investigated through scientific data analysis of various literature databases.

Scientific databases analysis of different research work of the scientific field revealed the medicinal importance and biological activities of sciadopitysin in the medicine for the treatment of diabetes and related complications. Scientific study revealed the biological importance of sciadopitysin against the protection of RIN-m5F cells and produce impact on insulin secretion. Other scientific research work data analysis revealed that sciadopitysin decreased the level of reactive oxygen species (ROS) and mitochondrial superoxide in the cellular level which signified their importance in the diabetes and related complications.

Scientific database analysis of current scientific research work revealed the biological importance of sciadopitysin against diabetes and related disorders through protection of pancreatic β-cells.

PO-518

# Mitochondrial ATP Production Drives Endothelial Fatty Acid Uptake and Transport

**Ayon Ibrahim[1], Nora Yucel[1], Boa Kim[1], Zoltan Arany[1*]**

[1] Cardiovascular Institute, University of Pennsylvania Perelman School of Medicine, USA

*zarany@pennmedicine.upenn.edu*

Most organs use fatty acids (FAs) as a key nutrient, but it is largely unknown how bloodborne FAs traverse the endothelium to reach underlying tissues. We wish to elucidate the underlying mechanisms regulating endothelial FA uptake and transport.

We conducted a high-throughput small-molecule screen to discover chemical regulators of FA uptake in cultured primary human endothelial cells. This screen employed a luminescent FA uptake readout system. Outside of the screen, we used an orthogonal system that measured the uptake of a fluorescent FA analog known as BODIPY-C12. Seahorse oxygen consumption assays were used to measure ATP production. Lastly, confocal Airyscan microscopy was used for the final portion of this study.

Our chemical screen identified niclosamide as a suppressor of endothelial FA uptake and transport. Structure/activity relationship studies demonstrated that niclosamide acts through mitochondrial uncoupling. Inhibition of oxidative phosphorylation also suppressed FA uptake, as did inhibition of mitochondrial ATP/ADP shuttling, indicating a key role for ATP production. Decreasing total cellular ATP by blocking glycolysis had no effect, indicating that specifically mitochondrial ATP is required. This ATP is likely being used by proteins such as FATP4, a putative FA transporter that we have found to strongly promote endothelial fatty acid uptake – but only when its ATP-dependent acyl-CoA synthetase domain is intact. Finally, confocal microscopy revealed that FATP4 resides in the endoplasmic reticulum (ER), and that endothelial ER is intimately juxtaposed to mitochondria.

Together, these data indicate that mitochondrial ATP production promotes endothelial FA uptake and transport via ATP-dependent acyl-CoA formation in mitochondrial/ER microdomains.

PO-519

# The Endothelial Dysfunction Blocker CU06-1004 Ameliorates Choline-Deficient L-Amino Acid Diet-Induced Non-Alcoholic Steatohepatitis in Mice

**Cho-Rong Bae[1], Jeong Eun Park[1], Young-Guen Kwon[1*]**

[1] Biochemistry, Yonsei University, Korea

*ygkwon@yonsei.ac.kr*

Non-alcoholic steatohepatitis (NASH) is a severe progressed form of NAFLD (non-alsocholic fatty liver disease) associated with all features of the metabolic syndrome, which is charicterized by hepatic steatosis, inflammation and fibrosis. Also, NAFLD is associated with endothelial dysfunction within the hepatic vasculature. Our recently reported that CU06-1004, a vascular leakage blocker suppressed the expression of adhesion molecules and inflammatory response in ischemia reperfusion injury. However, the effect of CU06-1004 on NASH model is not known.

In this study, we investigated the protective effects of CU06-1004 in a choline-deficient L-amino acid (CDAA)-induced mouse model of NASH for 3 or 6 weeks.

Specifically, we evaluated the effects of CU06-1004 on lipid accumulation, inflammation, hepatic fibrosis, and liver sinusoidal endothelial cell (LSEC) capillarization through biochemical analyses, immunohistochemistry, and real-time PCR. We found that the administration of CU06-1004 to mice improved liver triglyceride (TG) and serum alanine aminotransferase (ALT) in this CDAA-induced model of NASH for 6 weeks. In groups of NASH induced mice for both 3 and 6 weeks, CU06-1004 significantly reduced the hepatic expression of genes related to lipogenesis, inflammation, and cell adhesion. However, expression of genes related to hepatic fibrosis and vascular endothelial changes were only decreased in animals with mild NASH.

These results suggest that the administration of CU06-1004 suppresses hepatic steatosis, inflammation, fibrosis, and LSEC capillarization in a CDAA-induced mouse model of NASH . This suggests that CU06-1004 has therapeutic potential for the treatment of mild NASH.

PO-520

# Carbonic Anhydrase 2 (CAII) Supports Tumor Blood Endothelial Cell Survival Under Lactic Acidosis in the Tumor Microenvironment

**Dorcas Akuba Muhyia Annan[1], Nako Maishi[1], Tomoyoshi Soga[2], Randa Dawood[1], Cong Li[1], Hiroshi Kikuchi[3], Takayuki Hojo[4], Masahiro Morimoto[1], Tetsuya Kitamura[1], Mohammad Towfik Alam[1,6], Kazuyuki Minowa[6], Nobuo Shinohara[3], Jin-Min Nam[7], Yasuhiro Hida[5], Kyoko Hida[1*]**

[1] Vascular Biology and Molecular Pathology, Hokkaido University, Japan
[2] Institute for Advanced Biosciences, Keio University, Japan
[3] Department of Renal and Genitourinary Surgery, Hokkaido University, Japan
[4] Department of Dental Anesthesiology, Hokkaido University, Japan
[5] Department of Cardiovascular Thoracic Surgery, Hokkaido University, Japan
[6] Department of Dental Radiology, Hokkaido University, Japan
[7] Global Institution for Collaborative Research and Education (GI-CoRE), Hokkaido University, Japan

*khida@den.hokudai.ac.jp*

Tumor endothelial cells (TECs) are critical players in the angiogenesis process that supports tumor growth and metastasis. As tumors grow, their cel-

lular glycolysis contributes to tumor lactic acidosis through high lactic acid production. Lactic acidosis adversely affects all cells; however, the adaptive mechanisms by which TECs survive this harsh condition in tumors remain unknown. Moreover, current angiogenesis inhibitors are designed to target proangiogenic growth factors and their receptors on endothelial cells. Unfortunately, drug resistance and other undesirable side effects arise from using these angiogenesis inhibitors. We elucidated how TECs survived in lactic acidosis and identified a new target for cancer antiangiogenic therapy.

The capillary electrophoresis time-of-flight mass spectrometry (CE/TOFMS) was used to analyze the metabolome of the cells. Gene expression was analyzed by quantitative reverse transcription-PCR. Western blotting and ELISA were used to validate protein expression, and cell proliferation was measured by the MTS assay. Melanoma tumor xenograft models were used in evaluating the effect of carbonic anhydrase inhibition on tumor angiogenesis in vivo. Tissue CAII expression, tumor microvessel density, microvessel pericyte coverage, and hypoxia were analyzed by immunohistochemistry.

Intracellular levels of glycolytic metabolites, lactate, and ATP were higher in TECs than in NECs. Unlike NECs, TECs proliferated when exposed to lactic acid. Furthermore, pH regulators, including the endothelium-associated carbonic anhydrase II (CAII), were upregulated in TECs. CAII expression was confirmed in murine and human tumor endothelium. Functionally, CAII knockdown decreased TEC survival under lactic acidosis and nutrient-replete conditions. VEGF/VEGFR2 signaling amplified CAII expression in NECs. CAII inhibition with acetazolamide minimally reduced tumor angiogenesis in vivo. However, the number of matured blood vessels increased, and the acetazolamide-treated mice also showed decreased lung metastasis.

These findings suggest that due to their effect on blood vessel maturity, pH regulators like CAII are promising targets of antiangiogenic therapy.

PO-521

# Global Endothelial Histone Acetylation Does Not Respond to Extracellular Nutrient Availability

**Ioana Soaita[1*], Boa Kim[1], Erika Varner[2], Eliana Von Krusenstiern[2], Nathaniel Snyder[2], Zoltan Arany[1]**

[1] Cardiovascular Institute, Department of Medicine, University of Pennsylvania Perelman School of Medicine, USA
[2] Center for Metabolic Disease Research and the Department of Microbiology and Immunology, Temple University Lewis Katz School of Medicine, USA

*isoaita@pennmedicine.upenn.edu*

An emerging hallmark of cellular nutrient sensing is that intracellular concentrations of acetyl-CoA can oscillate in response to extracellular nutrient availability. Acetyl-CoA is the sole known acetyl group donor for protein acetylation, and bulk histone acetylation has been shown to respond to nutrient availability in a variety of cell types. The resulting changes in chromatin structure can directly impact gene expression and cell proliferation or migration. ECs are continually exposed to changing nutrient levels, since they come in direct contact with the blood and are thus first to be impacted by postprandial fluctuating blood nutrient levels. The regulation of histone acetylation and acetyl-CoA metabolism, however, remain unexplored in ECs. Thus, we aimed to determine if extracellular nutrient availability could modulate histone acetylation and acetyl-CoA availability in ECs.

We used primary human ECs and acid-based histone extractions to measure histone acetylation; stable isotope carbon tracing coupled with liquid-chromatography mass spectrometry to measure relative substrate contribution to acetyl-CoA and EdU labeling/ scratch assays to measure EC angiogenic potential.

We show that neither extracellular availability of glucose, glutamine, acetate nor long-chain fatty acids alter global histone acetylation levels in ECs, despite there being turnover of histone acetylation. We find that glucose, acetate and fatty acids contribute about equally to the acetyl-CoA pool. Furthermore, upon loss of enzymes responsible for production of glucose- or acetate-derived acetyl-CoA, there is significant compensation from other substrates to maintain acetyl-CoA levels, suggesting that ECs are metabolically plastic in order to maintain the acetyl-CoA pool. Finally, as a result of this metabolic plasticity, loss of acetyl-CoA producing enzymes leads to minor functional effects on EC angiogenic potential.

Our data indicate that EC histone acetylation levels are remarkably stable in response to shifts in nutrient availability, suggesting the presence of mechanisms that protect EC histone acetylation from external metabolic fluctuations.

PO-523

# VEGF-B Ablation in Pancreatic β-Cells Upregulates Insulin Expression Without Affecting Glucose Homeostasis or Islet Lipid Uptake

**Frank Chenfei Ning**[1*]

[1] Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden

*frank.chenfei.ning@ki.se*

Type 2 diabetes mellitus (T2DM) affects millions of people and is linked with obesity and lipid accumulation in peripheral tissues. Increased lipid handling and lipotoxicity in insulin producing β-cells may contribute to β-cell dysfunction in T2DM. The vascular endothelial growth factor (VEGF)-B regulates uptake and transcytosis of long-chain fatty acids over the endothelium to tissues such as heart and skeletal muscle. Systemic inhibition of VEGF-B signaling prevents tissue lipid accumulation, improves insulin sensitivity and glucose tolerance, as well as reduces pancreatic islet triglyceride content, under T2DM conditions. To date, the role of local VEGF-B signaling in pancreatic islet physiology and in the regulation of fatty acid trans-endothelial transport in pancreatic islet is unknown.

To address these questions, we have generated a mouse strain where VEGF-B is selectively depleted in β-cells, and assessed glucose homeostasis, β-cell function and islet lipid content under both normal and high-fat diet feeding conditions.

We found that Vegfb was ubiquitously expressed throughout the pancreas, and that β-cell Vegfb deletion resulted in increased insulin gene expression. However, glucose homeostasis and islet lipid uptake remained unaffected by β-cell VEGF-B deficiency.

In conclusion, our data indicates that local paracrine VEGF-B signaling in pancreatic islets, in contrast to a systemic VEGF-B targeting approach, shows limited therapeutic effect on overall systemic glucose homeostasis.

PO-524

# Effect of Add On Sitagliptin-A Dipeptidyl Peptidase Inhibitor-IV On Lipid and Adipokine Level in Patients With Type 2 Diabetes Mellitus-A Randomized Controlled Trial

**Pradeep Dwivedi[1*], Suraj Singh Yadav[2], Sartaj Hussain[2], Sanjay Khattri[2], Kausar Usman[3,2]**

[1] Pharmacology, All India Institute of Medlcal Sciences Jodhpur, India
[2] Pharmacology, Kgmu Lucknow, India
[3] Medicine, Kgmu Lucknow, India

*dr.prad99@gmail.com*

Sitagliptin, a Dipeptidy peptidase-IV inhibitor inhibits metabolism of glucagon like peptide-1 (GLP-1) that stimulates insulin and suppresses glucagon secretion apart from inhibiting gastric emptying, and reducing appetite. GLP-1 also increases adipocytokine (adiponectin) level directly through protein kinase A pathway. Data are lacking on the level of lipid and adiponectin on addition of Sitagliptin, to usual care in type 2 diabetes mellitus patients of north Indian population.

In this randomized, open label, placebo controlled (Add On) parallel group study, we screened 239 patients with type 2 diabetes, and randomly assigned 193 to treatment; 98 to metformin 500mg twice daily (standard care/monotherapy group) and 95 to sitagliptin 100mg once daily plus metformin 500mg twice daily (Add On group). Both groups were followed for a period of 12 weeks. Baseline and at the end of 12 weeks data on Lipid profile-Triglycerides, Low Density Lipoprotein, High Density Lipoprotein & Total Cholesterol (TG, LDL, HDL & TC), glycated hemoglobin (HbA1c) and adiponectin level were recorded.

At 12 week, there was a significant difference between metformin monotherapy (standard care) and sitagliptin Add On group in mean difference of TG level of -19.34 (95% CI, -28.34 to-16.34) ($p<0.001$); mean difference of HDL level of -6.07 (95% CI, -8.49 to -5.65) ($p<0.01$);mean difference of LDL level of 10.88 (95% CI, 7.15 to 18.61) ($p<0.001$); mean difference of HbA1c level of 0.67 (95% CI, 0.38 to 8.56) ($p<0.001$); and mean difference of Adiponectin level of -0.37 (95% CI, -0.64 to 0.07) ($p=0.01$) while comparable in mean difference of TC level of -6.10 (95% CI, -6.98 to 5.75) ($p=0.85$).

Among patients with type 2 diabetes mellitus, add on sitagliptin to usual care appears better in altering lipid/glycemic level and adiponectin level during study period. (Clinical Trial Registry of India, CTRI/2018/01/011284.) Acknowledgement: Indian Council of Medical Research, India.

PO-525

# Anti-Obesity Effect of Eriocitrin on the Obesity and Hyperglycemia Through Glucose Utilization, Lipogenesis and Fecal Lipid Excretion: Phytopharmaceutical Importance Through Research Data Analysis

**Dinesh Kumar Patel[1*], Kanika Patel[1]**

[1] Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, India

*dkp.iitbhu@gmail.com*

Eriocitrin is an important flavonoidal class phytochemical found to be present in the lemons and have been isolated from Japanese tea plant i.e. Satsuma mandarin and know for their antioxidant potential. Enzymes inhibitions through natural product including pure phytoconstituents play an important role in the management of obesity and hyperglycemia and inhibition of α-glucosidase activity is the best examples which prevent hyperglycemic condition.

Eriocitrin have been used in the medicine due to their potential role against treatment of numerous Human disorders; here in the present investigation anti-obesity effect of eriocitrin have been analyzed through data analysis of various scientific researches. Effect against hyperglycemic condition has been also investigated in this study through data analysis. Role of various enzymes such as α-glucosidase and carbonic anhydrase in obesity and hyperglycemic condition has been also investigated through scientific data analysis of different scientific research works to know the potential role of eriocitrin against obesity and hyperglycemia.

Effect of eriocitrin for their anti-obesity effect in high-fat diet (HFD)-fed obese mice have been investigated in the scientific works and data analysis of these research work revealed the importance for improving adiposity, energy expenditure, and mRNA expression in brown adipose tissue and skeletal muscle. Further eriocitrin prevent hepatic steatosis, enhancing FA oxidation, fecal lipid excretion and improve insulin sensitivity. In another scientific study, effects of eriocitrin on enzyme inhibition have been investigated through molecular docking study found that it interact with α-glucosidase which further revealed the α-glucosidase inhibitory potential. Other scientific research work revealed their potent free radical scavenging potential and inhibitory activity against human carbonic anhydrase.

Data analysis of scientific works revealed the importance of eriocitrin against diet-induced adiposity and related secondary metabolic complications and could be used for the development of better drugs against diabetes and obesity.

PO-526

# Cyclase-Associated protein1 (CAP1) Is a Binding Partner of Proprotein Convertase Subtilisin/Kexin Type-9 (PCSK9) and Is Required for the Degradation of Low-Density Lipoprotein Receptors by PCSK9

**Dasom Shin[1,2,5], Hyun-Duk Jang[1,2,3], Hyun-Chae Lee[1,2,3], Hwan Lee[1,2,5], Jaewon Lee[1,2,3], Soungchan Kim[1,2,5], Hyo-Soo Kim[1,2,4*]**

[1] National Leading Laboratory for Stem Cell Research, SNUH, Korea
[2] Korea Research-Driven Hospital, SNUH, Korea
[3] Strategic Center of Cell & Bio Therapy, Seoul National University Hospital, SNUH, Korea
[4] Department of Cardiology, SNUH, Korea
[5] 5 Department of Molecular Medicine and Biopharmaceutical Sciences, SNUH, Korea

*hyosoo@snu.ac.kr*

Proprotein convertase subtilisin/kexin type-9 (PCSK9), a molecular determinant of low-density lipoprotein (LDL) receptor (LDLR) fate, has emerged as a promising therapeutic target for atherosclerotic cardiovascular diseases. However, the precise mechanism by which PCSK9 regulates the internalization and lysosomal degradation of LDLR is unknown. Recently, we identified adenylyl cyclase-associated protein 1 (CAP1) as a receptor for human resistin whose globular C-terminus is structurally similar to the C-terminal cysteine-rich domain (CRD) of PCSK9. Herein, we investigated the role of CAP1 in PCSK9-mediated lysosomal degradation of LDLR and plasma LDL cholesterol (LDL-C) levels.

The direct binding between PCSK9 and CAP1 was confirmed by immunoprecipitation assay, far-western blot, bio-molecular fluorescence complementation, and surface plasmon resonance assay. Fine mapping revealed that the CRD of PCSK9 binds with the Src homology 3 binding domain (SH3BD) of CAP1. Two loss-of-function polymorphisms found in human PCSK9 (S668R and G670E in CRD) were attributed to a defective interaction with CAP1.

siRNA against CAP1 reduced the PCSK9-mediated degradation of LDLR in vitro. We generated CAP1 knock-out mice and found that the viable heterozygous CAP1 knock-out mice had higher protein levels of LDLR and lower LDL-C levels in the liver and plasma, respectively, than the control mice. Mechanistic analysis revealed that PCSK9-induced endocytosis and lysosomal degradation of LDLR were mediated by caveolin but not by clathrin, and they were dependent on binding between CAP1 and caveolin-1.

We identified CAP1 as a new binding partner of PCSK9 and a key mediator of caveolae-dependent endocytosis and lysosomal degradation of LDLR.

PO-527

# Comparative Effect of Statin Types and Omega-3 Supplement on Cardiovascular Events: Meta-Analysis and Network Meta-Analysis of Randomized Controlled Trials Including 267,346 Participants

**Tung Hoang[1], Jeongseon Kim[1*]**

[1] Cancer Biomedical Science, National Cancer Center Graduate School of Cancer Science and Policy, Korea

*jskim@ncc.re.kr*

Statins and omega-3 supplement have been recommended for cardiovascular disease (CVD) prevention, but the comparative effects have not been investigated. This study aimed to summarize current evidence of the effect of statin types and omega-3 supplement on cardiovascular events.

We searched Pubmed for systematic reviews and meta-analyses which were published until January 2020. We included randomized controlled trials (RCTs) comparing statins versus controls or omega-3 versus controls with at least 1 year of follow-up. A meta-analysis was used to calculate the pooled relative risks (RRs) and 95% confidence intervals (CIs) for the effects of specific statins and omega-3 supplement compared with controls. The pairwise effects among statins and omega-3 supplement were additionally examined with a network meta-analysis. Total CVD, coronary heart disease (CHD), myocardial infarction (MI), and stroke.

We included 63 RCTs in the final analysis. Overall, the statin group showed significant risk reductions in total CVD, CHD, MI, and stroke, while omega-3 supplement significantly decreased the risks of CHD and MI only, in the comparison with the control group. In comparison with omega-3 supplement, pravastatin significantly reduced the risks of total CVD (RR=0.80, 95% CI=0.71-0.90), CHD (RR=0.75, 95% CI=0.60-0.94), and MI (RR=0.67, 95% CI=0.51-0.89). The risks of total CVD, CHD, and MI in the omega-3 group were statistically higher than those in the atorvastatin group, with RRs and 95% CIs of 1.26 (1.15-1.38), 1.56 (1.22-1.98), and 1.43 (1.14-1.80), respectively.

The findings of this study suggest that pravastatin and atorvastatin may be more beneficial in reducing the risk of some cardiovascular events than omega-3 supplement.

PO-528

# Tilting Time In Our Favour: A Comparative Study of The Effect of Time Restricted Meal Intake on Anthropometric and Biochemical Parameters in Patient

**Smriti Rastogi[1*], Shiv Srivastav[1], Narsingh Verma[1]**

[1] Physiology, King George's Medical University, India

*smriti198719@gmail.com*

1. To study the effect of time restricted meal intake on various anthropometric and biochemical parameters in Type 2 diabetes patients. 2. To study whether time restricted meal intake can act as an adjunct alongwith the standard conventional treatment of diabetes in patients of Type 2 diabetes mellitus.

400 Type 2 diabetes patients from OPD Endocrinology, were randomly divided based on whether the patients consented to having early dinner TRM (time restricted meal) group (200 patients) or not (control group) (200 patients). Follow up was done at 6 and 12 months for anthropometric measurement, height, weight, waist hip ratio, neck size , fasting, post prandial blood sugar, HbA1c, serum urea, serum creatinine and lipid profile. Patient was given a clear understanding about chronomedicine. A single intervention was done - timing of dinner was at or around 7 pm in the evening for TRM group. Both groups were given standard conventional treatment of diabetes.

65% (TRM group) 40 % (non- TRM) had normal HbA1c after 12 months. BMI, Hip size, systolic blood pressure, HbA1c, blood sugar (fasting and post-prandial) were significantly different ($p < 0.05$) in TRM Group. p value <0.0167 observed in values of blood sugar (fasting) in TRM Group, p value < 0.8113 (control group). Blood sugar (post- prandial) (TRM Group ) p value <0.0001 (highly significant), control group p value<0.6938. Hip size (TRM Group) p value < 0.0012 (Significant) (Difference between means=2.762 ± 1.261). BMI (TRM Group) p value = 0.0030, significant. Neck Size (TRM Group) p value= 0.9697, not significant. Waist Size (TRM Group)p value= 0.2832, not significant. Hip Size (TRM Group) p value = 0.0012, significant, correlation coefficient=0.8380. Systolic Blood Pressure (TRM Group) p value = 0.0211, significant. Diastolic Blood Pressure (TRM Group) p value = 0.536, not significant.

Novel, pathbreaking findings.

PO-530

# Association Between Histological Severity of Nonalcoholic Fatty Liver Disease and Arterial Stiffness

**Hack-Lyoung Kim[1], Bo Kyung Koo[2], Sae Kyung Joo[3], Won Kim[3*]**

[1] Division of Cardiology, Department of Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea
[2] Division of Endocrinology, Department of Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea
[3] Division of Gastroenterology and Hepatology, Department of Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea

*wonshiri@yahoo.com*

Although arterial stiffness has been reported to be associated with nonalcoholic liver disease (NAFLD), previous studies only relied on non-invasive assessments for diagnosis of NAFLD. This study

attempted to investigate the association between arterial stiffness and histological severity of NAFLD.

This study included patients ≥18 years old with wave reflections of central aorta data between 2013 and 2019 within a biopsy-evaluated prospective NAFLD cohort. Non-invasively quantified wave reflections [expressed as augmentation index corrected for heart rate of 75 b.p.m. (AI75)] was obtained using applanation tonometry of the radial artery.

A total of 179 patients with biopsy-proven NAFLD (mean, 55 years and 54% female) were included in this study. Patients with NASH (n=88) were older and had more cardiovascular risk factors than patients with NAFL (n=91). The AI 75 value was significantly higher in patients with NASH than patients with NAFL (78.7±14.2% vs. 71.0±13.2%, P=0.001). Patients with high AI 75 (≥76%, median value) showed more severe grades of lobular and portal inflammation, and hepatocellular ballooning, and more advanced stages of fibrosis, compared to those with low AI 75 (<76%) (P <0.05 for each). The presence of NASH [adjusted odds ratio (aOR), 3.14; 95% confidence interval (CI), 1.50–6.58; P=0.002], lobular inflammation ≥grade 2 (aOR, 2.17; 95% CI, 1.20–3.90; P=0.010), hepatocellular ballooning ≥grade 1 (aOR, 2.97; 95% CI, 1.37–6.44; P=0.006), and significant fibrosis (≥F2) (aOR, 3.83; 95% CI, 1.75–9.36; P=0.001) were independently associated with higher AI 75 (≥76%) even after adjusted for confounding variables, including age, sex, height, hypertension, diabetes, and smoking.

High AI 75 is significantly associated with NASH and significant fibrosis in patients with biopsy-proven NAFLD. Therefore, patients with NASH or significant fibrosis are at risk of an increased arterial stiffness, a surrogate marker of cardiovascular disease.

PO-532

# Ambient Temperature and Physical Activity Are Associated with Metabolic Syndrome in Ecuadorian Adults: A National Cross-Sectional Study

**Christian F. Juna[1], Yoon Hee Cho[2], Dongwoo Ham[1,3], Hyojee Joung[1,3]***

[1] Graduate School of Public Health, Seoul National University, Korea
[2] Department of Biomedical and Pharmaceutical Sciences, The University of Montana, USA
[3] Institute of Health and Environment, Seoul National University, Korea

*hjjoung@snu.ac.kr*

The present study aimed to determine the associations among ambient temperature at residence, health-related lifestyles, and the risk of Metabolic Syndrome (MetS) in an Ecuadorian adult population.

This cross-sectional study was conducted utilizing secondary data from the 2012 Ecuador National Health and Nutrition Survey (ENSANUT-ECU). A total of 6,024 adults (1,964 men and 4,060 women) 20 to 60 years old were included in the study. Ambient temperature was obtained from the Ecuadorian Institute for Meteorology and Hydrology (INAMHI), whereas dietary intake was measured using a single 24-hour recall and health-related lifestyle via risk and physical activity standardized questionnaires. MetS was defined on the basis of the National Cholesterol Education Program Adult Treatment Panel III and the Latin American Diabetes Association criteria. Multiple logistic regression analyses were used to examine whether ambient temperature of residence and health-related lifestyles can increase risk of MetS.

Residing in an ambient temperature over 23°C augmented the risk of increased waist circumference (1.77; 95% CI, 1.29-2.42 p for trend 0.0003) in men and (1.49; 95% CI, 1.15-1.92 p for trend 0.0014) in women and the risk of elevated fasting glucose in both men (3.15; 95% CI, 2.01-1.92 p for trend

Our findings suggest that higher ambient temperature and physical inactivity may play important roles in development of MetS in Ecuadorian adults.

# 6. Lymphangiogenesis and Inflammation

PO-602

## Distinct Fibroblast Subsets Regulate Lacteal Integrity Through YAP/TAZ-Induced VEGF-C in Intestinal Villi

**Seon Pyo Hong[1], Myung Jin Yang[1], Hosung Bae[1], Ralf Adams[2], Kari Alitalo[3], Gou Young Koh[1*]**

[1] Center for Vascular Research, Institute for Basic Science, Korea
[2] Department of Tissue Morphogenesis, Max Planck Institute for Molecular Biomedicine, Germany
[3] Translational Cancer Biology, University of Helsinki, Finland

*gykoh@kaist.ac.kr*

Emerging evidences suggest that intestinal stromal cells (IntSCs) play essential roles in maintaining intestinal homeostasis. However, the extent of heterogeneity within the villi stromal compartment and how IntSCs regulate the structure and function of specialized intestinal lymphatic capillary called lacteal remain elusive.

We explored the role of intestinal villi stromal cells by generating PDGFRβ-specific YAP/TAZ hyperactivation or depletion mouse models.

We show that selective hyperactivation or depletion of YAP/TAZ in PDGFRβ$^+$ IntSCs leads to lacteal sprouting or regression with junctional disintegration and impaired dietary fat uptake. Indeed, mechanical or osmotic stress regulates IntSC secretion of VEGF-C mediated by YAP/TAZ. Single-cell RNA sequencing delineated novel subtypes of villi fibroblasts that upregulate Vegfc upon YAP/TAZ activation. These populations of fibroblasts were distributed in proximity to lacteal, suggesting that

they constitute a peri-lacteal microenvironment.

Our findings demonstrate the heterogeneity of IntSCs and reveal that distinct subsets of villi fibroblasts regulate lacteal integrity through YAP/TAZ–induced VEGF-C secretion, providing new insights into the dynamic regulatory mechanisms behind lymphangiogenesis and lymphatic remodelling.

PO-603

# Angiopoietin 2-Induced, Tie2-Independent, Lymphatic Endothelial Cell Migration Is Mediated by Formins

**Racheal Akwii**[1*]

[1] Pharmaceutical Sciences, Texas Tech University Health Science Center, USA

*racheal.akwii@ttuhsc.edu*

To we investigate Ang2-driven functions and signaling pathways in lymphangiogenesis in vitro and in vivo

Immunoblotting by western blot
Immunoprecipitation
Loss of function by siRNA knockdown
RhoA Pulldown
Proteomics
Immunofloresence
Ear Sponge Assay with C57BL6 mice RhoA wildtype and RhoA knockout

Ang2 activated RhoA in a time- and dose-dependent manner in Human Dermal Lymphatic Endothelial Cells (HDLECs). RhoA inhibition or knockdown abrogated Ang2-induced HDLEC migration. Ang2-induced migration was independent of the Tie2 receptor, whereas Tie1 knockdown induced basal migration levels and no higher effect was observed with Ang2 stimulation. Ang2 interacted with beta-1 integrin in HDLECs, inducing GEF-H1- and PDZ-RhoGEF-mediated RhoA activation and cell migration. Although Ang2 treatment induced MLC activation along with the classical integrin signaling, ROCK inhibition did not abrogate Ang2-induced HDLEC migration: instead knockdown experiments demonstrated that the combined effect of formins, Diap1 and FHOD1 regulated HDLEC migration, with FHOD1 playing a dominant role. The biological significance of the RhoA signaling pathway on Ang2-induced lymphangiogenesis was demonstrated in vivo, using conditionally deficient mice for RhoA in the lymphatic endothelium.

In conclusion, Ang2 induces HDLEC migration through integrin-mediated RhoA and formin activation, inducing actin filamentation/polymerization and leading to cell migration. This study elucidates a novel pathway of Ang2-induced lymphangiogenesis, providing possible drug targets for many inflammatory pathological conditions.

PO-604

## ERK5 Regulates Lymphatic Development and Homeostasis

**Woosoung Choi[1], Ah-Ra Kim[1], Suk-Won Jin[1*]**

[1] School of Life Sciences, Gwangju Institute of Science and Technology, Korea

*sukwonjin@gmail.com*

The current study was conducted to delineate the role of ERK5 signaling in lymphatic development and homeostasis.

erk5 mutant line was generated by CRISPR/Cas9 genome editing system in Tg(fli1a:eGFP), Tg(prox1a:Gal4-UAS:RFP) background. Fluorescence signal from transgenic zebrafish were detected by confocal microscope. Pregnant mice were intravenously injected with XMD8-92 dissolved in dimethyl sulfoxide at E14.5. Po mice were sacrificed and dissected. Diaphragms were collected and stained for LYVE-1 and CD31 antibody, followed by observed with fluorescence microscope.
Acquired images were analyzed using LSM imaging software and Image J.

erk5 morphant and mutant zebrafish showed severe edema and reduced length of thoracic duct. We found that these erk5 deficient zebrafish show abnormal localization of lymphatic endothelial cells due to migration defects. Impaired lymphatic development was also observed in erk5 deficient mice. Erk5 mutant mice showed decreased lymphatic vascular area in diaphragm.

Our study shows the role of ERK5 during lymphatic development by promoting migration of lymphatic endothelial cell in zebrafishes and mice.

# 7. Technical Advances of Vascular Biology

PO-703

## Ezh2 as an Epigenetic Checkpoint During Monocyte Differentiation: A Potential Target for Cardiac Recovery After Myocardial Infarction

**Julie Rondeaux[1], Sylvanie Renet[1], Anaïs Dumesnil[1], Jean-Paul Henry[1], Eric Durand[2], Vincent Richard[1], Ebba Brakenhielm[1], Sylvain Fraineau[1*]**

[1] Normandie Univ, UNIROUEN, INSERM U1096 EnVI & FHU REMOD-VHF, France
[2] Department of Cardiology, Rouen University Hospital, FHU REMOD- VHF, France

*sylvain.fraineau@univ-rouen.fr*

Monocytes-macrophages play an important role in cardiac repair after Myocardial Infarction (MI). Specifically, M2-like macrophages (M2) modulate inflammation and fibrosis, and promote angiogenesis during cardiac repair post-MI. Interestingly, an epigenetic histone modification: JMJD3-dependent H3K27 demethylation, has been shown to promote M2 polarization.
We hypothesized that JMJD3 antagonistic enzyme EZH2, responsible for H3K27 methylation, could act as an epigenetic checkpoint during monocyte to M2 differentiation regulating cardiac repair post-MI.

Human monocytes were selected from patients' peripheral blood by negative selection before treatment with EZH2 inhibitor, GSK-343. We took advantage of ChIP-Sequencing already published data to establish the full list of bivalent genes. EZH2 di-

rect targets were identified by RNA-Sequencing and the bivalent status of selected genes was confirmed by ChIP qPCR.

We demonstrate for the first time that while Ezh2 is localized in the nucleus of monocytes as well as Mo and M1 macrophages, unexpectedly it translocates to the cytoplasm in M2 polarized cells in both healthy and post-MI mouse hearts in vivo. We reproduced this phenomenon in vitro in differentiating monocytes. At the chromatin level, ChIP-Sequencing in monocytes revealed that bivalent genes, known to be regulated by EZH2, are implicated in angiogenesis and cardiac repair processes. Gene expression profiling by RNA-Seq performed on monocytes treated with GSK-343, allowed identification EZH2-dependent bivalent genes including DLL1 and VEGFA. EZH2 inhibition decreased H3K27me3 level at the promoter of these genes to promote their expression. mRNA profiling revealed that pharmacological EZH2 targeted de-repression of bivalents genes brings monocytes closer to M2 macrophage profile than to any other myeloid cell lineage.

Altogether, our data argue for a critical role of EZH2 as an epigenetic checkpoint during monocyte to M2 macrophage differentiation and highlights the potential therapeutic use of GSK-343 to promote this process in the aim to stimulate angiogenesis and cardiac repair post-MI.

PO-704

# In Vivo Function of Flow-Responsive Cis-DNA Elements in the Endothelial Nitric Oxide Synthase Gene: A Role for Chromatin-Based Mechanisms

**Kyung Ha Ku**[1,2], **Philip Marsden**[1,2,3*]

[1] Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada
[2] Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Canada
[3] Department of Medicine, St. Michael's Hospital, Canada

*p.marsden@utoronto.ca*

Endothelial nitric oxide synthase (eNOS) is an endothelial cell (EC)-specific gene predominantly expressed in medium- to large-sized arteries with laminar flow and high shear stress. Disturbed flow with lower average shear stress reduces eNOS transcription, leading to atherogenesis especially at bifurcations and curvatures of arteries. Intriguingly, the eNOS promoter contains two distinct flow-responsive cis-DNA elements, the Shear Stress Response Element (SSRE) and the Krüppel-Like Factor (KLF) element. However, the in vivo function of these elements remains unknown.

Insertional transgenic mice with a mutation at each flow-responsive cis-DNA element were generated using a murine eNOS promoter-β-galactosidase reporter via linker-scanning mutagenesis and compared with episomal-based mutations in vitro. DNA methylation at the eNOS proximal promoter was assessed by bisulfite sequencing or pyrosequencing

Wildtype mice with a functional eNOS promoter-reporter transgene exhibited reduced endothelial reporter expression in the atheroprone regions.

Surprisingly, the SSRE mutation abolished the eNOS transcription with aberrant hypermethylation at the eNOS proximal promoter, demonstrating that the SSRE is critical for eNOS transcription. The KLF mutation evidenced integration site-specific decreases in eNOS transcription with marked eNOS promoter methylation, suggesting that the KLF element alone is not sufficient for eNOS transcription. In contrast, episomal-based mutations had no effects on eNOS promoter activity in vitro. This indicates that these cis-DNA elements are chromatin-based flow sensors which regulate eNOS transcription epigenetically. In wildtype mice, the native eNOS proximal promoter was significantly hypermethylated in aortic ECs from the atheroprone regions where eNOS expression was markedly suppressed due to chronic disturbed flow, highlighting a functional role for flow-dependent DNA methylation in transcriptional regulation of eNOS in vivo.

This is the first in vivo study to demonstrate that both the SSRE and the KLF element are necessary for eNOS transcription. Furthermore, it provides a novel mechanism between the function of flow-responsive cis-DNA elements and chromatin-based transcriptional processes.

PO-708

# Long Non-Coding RNA TERRA Influences DNA Damage and Survival of Endothelial Cells and Cardiomyocytes

**Diewertje Bink[1*], Tan Phát Pham[1], Patrick Hofmann[2], Stefanie Dimmeler[2], Reinier Boon[1,2]**

[1] Physiology, Amsterdam UMC, Vrije Universiteit Amsterdam, Physiology, Amsterdam Cardiovascular Sciences, Amsterdam, Netherlands
[2] Institute for Cardiovascular Regeneration, Centre of Molecular Medicine, Goethe University, Frankfurt, Germany

*d.bink1@vumc.nl*

Ageing is the major risk factor for cardiovascular disease. Current therapies are mainly based on proteins, while targeting long non-coding RNAs (lncRNAs) is largely unexplored. Although telomeres are heterochromatic regions, non-coding transcripts called Telomeric repeat-containing RNA (TERRA) are transcribed from the telomeres of most chromosomes. The transcription of TERRA starts at the subtelomere and ends in the telomere, leading to 0.2-10kb molecules. This study aims to characterize the role of TERRA in the cardiovascular system.

TERRA transcription was measured in aging and disease models. LNA GapmeRs were used to knockdown the TERRA molecule transcribed from chromosome 20 (h20q-TERRA).

TERRA was upregulated in hearts of old compared to young mice. Increased TERRA expression was also shown in heart tissue of patients with ischemic heart disease compared to donor heart tissue. H20q-TERRA was found increased in old passage human umbilical vein endothelial cells (HUVECs) compared to young HUVECs. IPSC-derived cardiomyocytes also increased the expression of

h20q-TERRA with increasing passage. H20q-TERRA knockdown in HUVECs show less sprout formation in a spheroid assay compared to negative control transfected HUVECs, without showing a change in migration or proliferation. H20q-TERRA knockdown revealed an increase in apoptosis, DNA damage and γH2AX levels and an increase in γH2AX-telomere colocalization. The amount of phosphorylated P53 was also increased after knockdown, while it was decreased after TERRA overexpression. Silencing the m18-TERRA molecule in mouse endothelial H5V cells led to an increase in caspase activity similar to what was shown in HUVECs. In addition, increased caspase activity, γH2AX levels and γH2AX-telomere colocalization was also shown in human cardiomyocytes after h20q-TERRA knockdown.

In summary, our data demonstrates that TERRA is upregulated with ageing and plays a role in endothelial and cardiomyocyte function and survival. These data show that TERRA transcripts are induced in cardiovascular ageing and are essential for endothelial cell function.

PO-709

# The Role of Long Noncoding RNA MEG8 in Endothelial Cell Function

**Veerle Kremer[1*], Reinier Boon[1,2]**

[1] Physiology, Amsterdam UMC, Netherlands
[2] Institute of Cardiovascular regeneration, Goethe University, Germany

*v.kremer@amsterdamumc.nl*

A large portion of the genome, known as long non-coding RNA, is transcribed but does not encode protein. Many lncRNAs have been shown to be involved in important regulatory processes such as genomic imprinting and chromatin modification. The 14q32 locus contains many non-coding RNAs such as Maternally Expressed 8 (MEG8). We investigate the role of MEG8 in endothelial function and the underlying mechanism. We hypothesize that MEG8 plays an important role in cardiovascular disease via epigenetic regulation of gene expression.

Experiments are performed in human umbilical venous endothelial cells (HUVECs). We compared low to high passage HUVECs to investigate MEG8 expression during aging. A 5-fold increase in MEG8 expression was observed in high passage cells, suggesting MEG8 is induced by aging. LNA-GapmeRs (Exiqon) were used to silence MEG8 expression.

Interestingly, downregulation of MEG8 resulted in a 1.5-fold increase in senescence as shown by beta-gal staining. This suggests MEG8 could play a role in inhibiting senescence. Furthermore, we investigate how MEG8 affects angiogenesis. Total sprout length was reduced in MEG8 silenced cells in a sprouting angiogenesis assay. Proliferation was shown to be reduced. We performed RNA sequencing to assess changes in gene expression after loss of MEG8. Interestingly, Tissue Factor Pathway Inhibitor 2 (TFPI2) was shown to be induced following MEG8 silencing. TFPI2 was shown in literature to be an inhibitor of angiogenesis. A possible explanation for the upregulation of TFPI2 could be the observation that MEG8 silencing resulted in a reduction of the inhibitory histone modification H3K27me3 at the TFPI2 promoter.

Aging induces expression of MEG8. Silencing of MEG8 impairs endothelial function, suggesting a potential beneficial role during aging. Loss of MEG8 was shown to result in impaired angiogenic sprouting and proliferation. In addition, silencing of MEG8 resulted in increased expression of TFPI2, thought to regulate angiogenesis in vivo.

PO-710

# DNA Methylation Analysis of MIR10B and TWIST1 Genes in Atherosclerosis

**Anton Markov[1*], Maria Golubenko[1], Nadezhda Babushkina[1], Ramil Salakhov[1], Diana Sharysh[1], Aleksei Zarubin[1], Elvira Muslimova[2], Sergey Afanasiev[2], Maria Nazarenko[1]**

[1] Research Institute of Medical Genetics, Tomsk NRMC, Russian Federation
[2] Cardiology Research Institute, Tomsk NRMC, Russian Federation

*anton.markov@medgenetics.ru*

MicroRNA miR-10b is involved in atherosclerotic plaque formation and is known to be regulated by TWIST transcription factors, which play a role in maintaining endothelial phenotype and functioning. Inhibition of miR-10b caused the development of atherosclerosis-resistant phenotype and stabilized advanced atherosclerotic lesions in mice. But epigenetic regulation of miR-10b is poorly researched. The study aimed to investigate DNA methylation within MIR10B and TWIST1 genes in blood vessels and peripheral blood cells to understand the epigenetic state of these loci in atherosclerosis.

Patient-matched samples of carotid atherosclerotic plaques (CAP), adjacent intact tissues of the carotid artery (CIT), great saphenous veins (GSV) and peripheral blood leukocytes (PBL) from patients with carotid atherosclerosis (n=22, 65±7 years), as well as blood leukocytes (HBLs) from healthy donors (n=14, 66±8 years) were used as a material. DNA methylation patterns within MIR10B (23 CpG-sites), TWIST1 promoter (29 CpG-sites), and LINE1 (19 CpG-sites, used as a proxy for global DNA methylation) were assessed using targeted bisulfite next-generation sequencing.

Global DNA methylation level equally varied across samples: 59±6% in blood vessels and 62±5% in PBL. However, CpG methylation level within MIR10B was high in GSV (65±8% at average) and significantly lower in CAP (24±7%) and PBL (23±9%) of patients, and similarly in HBL (23±6%). Interestingly, CIT samples were intermediately methylated (40±17%) and clustered into groups with GSV-like and PBL-like CpG methylation. TWIST1 methylation had a tissue-specific manner and varied only between blood vessels (28-30%) and blood leukocytes (9-10%, adjusted-p<0.01).

MIR10B is hypomethylated in blood leukocytes and atherosclerotic plaques compared to intact arteries at the nearly equal global DNA methylation background, which is in agreement with published up-regulation of miR-10b in advanced atherosclerotic lesions and can be associated with underlying inflammation process. DNA methylation pattern of TWIST1, a known mechanosensitive MIR10B regulator, remains highly tissue-specific regardless of atherosclerotic lesion development.

PO-711

# Human Peripheral Blood-Derived Endothelial Colony-Forming Cells Represent a Transitional Cell Population in the Endothelial Hierarchy

**Anton Kutikhin[1*], Alexey Tupikin[2], Vera Matveeva[1], Daria Shishkova[1], Larisa Antonova[1], Marsel Kabilov[2], Elena Velikanova[1]**

[1] Division of Experimental Medicine, Research Institute for Complex Issues of Cardiovascular Diseases, Russian Federation
[2] Genomics Core Facility, Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Russian Federation

*antonkutikhin@gmail.com*

Endothelial colony-forming cells (ECFCs) are currently considered as a promising cell population for pre-endothelialisation of tissue-engineered constructs, including small-diameter biodegradable vascular grafts. However, the extent of heterogeneity between ECFCs and mature vascular endothelial cells (ECs) is unclear.

Here we performed a transcriptome-wide study to compare gene expression profiles of ECFC derived from peripheral blood mononuclear cells (PBMCs), human coronary artery endothelial cells (HCAEC), and human umbilical vein endothelial cells (HUVEC). Characterisation of abovementioned cell populations was carried out by immunophenotyping, tube formation assay, and evaluation of proliferation capability while global gene expression profiling was conducted by means of RNA-seq.

ECFCs had a canonical immunophenotype (CD31+vWF+KDR+CD146+CD34-CD133-CD45-CD90-), considerable tube formation activity and high proliferative potential. HCAEC and HUVEC were generally similar to ECFCs with regards to their global gene expression profile; nevertheless, ECFC overexpressed the markers specific for all endothelial lineages (endothelial progenitor cell marker CD34, venous endothelial lineage marker NRP2, arterial endothelial lineage markers NOTCH4 and DLL2, and lymphatic endothelial lineage markers FLT4 and LYVE1) as well as extracellular matrix (ECM) proteins including basement membrane components (COL1A1, COL1A2, COL4A1, COL4A2). Therefore, ECFCs overexpress venous and lymphatic endothelial markers and major ECM components when compared to HCAEC (also showing reduced HEY2 expression) whilst overexpressing arterial and also lymphatic endothelial markers and ECM components in comparison with HUVEC.

Gene expression profile and behaviour of PBMC-derived ECFCs show sufficient extent of similarity to vascular ECs for using them for pre-endothelialisation of bioartificial vascular grafts, whereas in terms of developmental biology they significantly differ from HCAEC and HUVEC in the expression of certain gene categories relevant for endothelial biology (endothelial specification markers and synthesis of ECM/basement membrane components).
FUNDING: This study was funded by the Russian Science Foundation, grant number 17-75-20004 "Development of personalized tissue-engineered, small-diameter vascular graft in vitro under pulsatile flow conditions".

PO-712

# Regulation of Endothelial Cell Homeostatic Functionality and Organotypic Adaptability

**Jesus Maria Salinero[1], Franco Izzo[2], David Redmond[1], Tomer Itkin[1], Yang Lin[1], Sean Houghton[1], Balvir Kunar[1], Jenny Zhaoying Xiang[3], Sina Rabbany[1,4], Dan A. Landau[2], Robert Schwartz[5], Anna M. Randi[6], Shahin Rafii[1]***

[1] Medicine, Weill Cornell Medicine, USA
[2] Division of Hematology and Medical Oncology, Weill Cornell Medicine, USA
[3] Genomics Resources Core Facility, Weill Cornell Medicine, USA
[4] Bioengineering Program, Hofstra University, USA
[5] Division of Gastroenterology and Hepatology, Weill Cornell Medicine, USA
[6] National Heart and Lung Institute, Imperial College London, UK

*srafii@med.cornell.edu*

Endothelial cells (ECs) orchestrate diverse physiological functions across the body. However, ECs from each organ are instructed with specific programs that allows the regulation of specific functions such as barrier in the brain or filtering in the liver. Understanding how ECs maintain their physiological functions, while they acquire their organotypic attributes is a fundamental question in vascular biology.

We have identified that the ETS transcription factors Erg and Fli1 in the adult regulating the global identity and homeostatic chores ECs. To uncover the organotypic transcription factors downstream of the Fli1/Erg, we studied liver vasculature as it serves as a prototypical hierarchical vascular model.

Using single-cell RNAseq and immunofluorescence analysis at multiple developmental timepoints, we show how different liver EC compartments emerge during fetal development and establish sinusoidal zonation postnatally. Further spatial-RNAseq analysis have shown that liver hepatocytes follow a similar pater of postanal zonation. Lineage-tracing analysis validated that Portal Vein ECs contribute to liver vasculature during development. Our analysis identified the transcription factor c-Maf in the endothelial cell compartment as an important molecular regulator establishing liver-specific transcriptional program. Endothelial-restricted deletion of c-Maf disrupts sinusoidal specialization and zonation, and increases liver vulnerability to injury. Moreover, c-Maf overexpression generates induced liver sinusoidal ECs that are capable of maintaining functioning hepatocytes in vitro.

While these results infer that organotypic attributes are regulated by specific transcription factors, such as c-Maf in the liver, these are not involved in the regulation of adaptive endothelial physiological functions. Therefore, while cooperative function of Fli1/Erg mastermind multi-organ vascular functions, the organotypic attributes of ECs are specified during development, by the expression of distinct transcription factors such as c-Maf.s, These studies lay foundation for unraveling the molecular regulation of intra- and inter- organ EC identity, as well as potential extrinsic signals that dictates organotypic adaptation.

PO-714

# In Vivo Longitudinal Imaging of Glioblastoma Driving Vascular Remodeling

**Eunji Kong[1], Eunhyeong Lee[1], Haemin Chon[1], Injune Kim[1], Pilhan Kim[1,2*]**

[1] Graduate School of Medical Science and Engineering, KAIST, Korea
[2] Graduate School of Nanoscience and Technology, KAIST, Korea

*pilhan.kim@kaist.ac.kr*

Glioblastoma (GBM) is an aggressive brain tumor with poor survival and high mortality rates. Given that glioma cells' invasive property and related vascular alternation can accelerate the disease severity, previous studies have struggled to dissect how the individual glioma cells propagate and how they progressively modulate blood vessels. Yet, these key questions could not be tackled with conventional methods lacking proper level of spatial and/or temporal resolutions. Technical advances enabling a real-time subcellular-resolution imaging of tumor microenvironment (TME) in deep brain tissue are highly required to advance our understanding of cellular and vascular mechanism of GBM progression.

To cater to these, we newly established an intravital deep brain tissue imaging method that permits a direct in vivo visualization of cellular- and vascular-dynamics as well as their interplay in spontaneously induced GBM mouse models. This was achieved by implementing a custom-built video-rate laser-scanning confocal microscopy system integrated with a tissue-implantable window chamber; which provides longitudinal imaging access to glioma-initiating and proliferating regions in living animal models over weeks.

Repetitive and longitudinal multi-color in vivo imaging of deep brain tissue of GBM model enabled the imaging-based quantitative analysis of GBM tumorigenesis in different stages with distinct cellular- and vascular- changes over times. With multi-dimensional analysis, GBM vascular co-option was identified in vivo by statistically comparing co-opting zone (COZ) and non-COZ within invasive tumor areas. Additionally, highly dynamic vascular remodeling (e.g. vascular dilation and regression) during active GBM proliferation was clearly visualized.

We established a deep brain tissue imaging method carefully optimized to visualize TME of GBM model in vivo. It can serve as a versatile tool to investigate structural and functional dynamics of cell-vessel interaction in GBM tumorigenesis, which can provide new insights to improve our understanding of GBM.

PO-715

# Longitudinal in Vivo Visualization of Dynamic Hepatic Microenvironment in NAFLD Mouse Model

**Jieun Moon[1,2], Eunji Kong[2,3], Jehwi Jeon[2,3], Pilhan Kim[1,2,3*]**

[1] Graduate School of Nanoscience and Technology, Korea Advanced Institute of Science and Technology, Korea
[2] KI for Health Science and Technology (KIHST), Korea Advanced Institute of Science and Technology, Korea
[3] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Korea

*pilhan.kim@kaist.ac.kr*

Nonalcoholic fatty liver disease (NAFLD) is a rapidly increasing chronic liver disease with es-

timated global prevalence of 25 %. Yet, the exact pathogenic pathways of NAFLD is mostly remained incomplete and effective treatment strategy is not well established. To clarify complex cellular mechanisms in NAFLD progression, a longitudinal intravital visualization of hepatic microenvironment is highly desirable. Thus, we implemented a longitudinal in vivo visualization technique to analyze dynamic alterations of vessel and infiltration of immune cells simultaneously during the NAFLD progression in mouse model.

CX3CR1-GFP transgenic mice expressing GFP in hepatic monocyte-derived macrophages and circulating monocytes were used. NAFLD was induced by feeding methionine and choline-deficient (MCD) diet for 8 weeks. To observe the vessel structure, liver sinusoidal endothelial cells were labeled in vivo by intravenous injection of anti-CD31 antibody conjugated with near far-red fluorescence dye. Using a custom-built intravital confocal microscopy, we visualized the changes of vascular structure and recruitment of macrophages at different stages of NAFLD in vivo.

We identified the alteration in vessel architecture, reduction in vascular volume and vessel diameter with continued MCD diet. Relatively homogeneous structure of vessel was maintained during 2 weeks of MCD feeding. After 3 weeks of MCD feeding, the vascular structure was disorganized with reduction of vessel density around inflamed area with apoptotic bodies. Additionally, the number of infiltrated CX3CR1-GFP cells was greatly increased at the inflamed area. Recruited CX3CR1-GFP cells protruded their dendrites to apoptotic hepatocytes and engulfed apoptotic bodies generated from hepatocytes.

We established a longitudinal visualization method for the analysis of the dynamic cellular-level events in NAFLD progression. It can be highly useful to improve comprehensive understanding on unknown cellular mechanism in the pathogenesis of NAFLD.

PO-716

# Real Time Visualisation of Endothelial Erk Signalling During Angiogenesis and Vessel Wounding in Zebrafish

**Kazuhide S Okuda[1,2,3], Mikaela Keyser[2], David B Gurevich[4], Caterina Sturtzel[5], Scott Patterson[1,2,3], Huijun Chen[2], Mark Scott[2], Nicholas Condon[2], Paul Martin[4], Martin Distel[5], Benjamin M Hogan[1,2,6*]**

[1] Organogenesis and Cancer Program, Peter MacCallum Cancer Centre, Australia
[2] Institute for Molecular Bioscience, The University of Queensland, Australia
[3] Sir Peter MacCallum Department of Oncology, University of Melbourne, Australia
[4] School of Biochemistry, University of Bristol, UK
[5] Innovative Cancer Models, Children's Cancer Research Institute, Austria
[6] Department of Anatomy and Neuroscience, University of Melbourne, Australia

—

*Ben.Hogan@petermac.org*

A major challenge in vascular biology is understanding how signalling events occur dynamically and are regulated in real time to drive cellular responses. Erk is a central downstream effector of Vegfa signalling and reports the signalling that drives angiogenesis.

We generated an Erk biosensor zebrafish transgenic line by driving the expression of a kinase translocation reporter (KTR) from an endothelial specific promoter (Tg(fli1aep:ERK-KTR-Clover)uq39bh). The ERK-KTR-Clover construct enables real-time visualisation of ERK phosphorylation as it translates phosphorylation events into a nucleo-cytoplasmic shuttling event of the synthetic reporter.

Pharmacological inhibition of the Kdr/Kdrl/Mek/Erk pathway resulted in quantifiable translocation of ERK-KTR-Clover expression from the cytoplasm to the nucleus, validating the utility of the sensor.

High-speed imaging identified a rapid asymmetric Erk-signalling event immediately following tip-cell divisions that drive embryonic angiogenesis. Finally, we revealed dynamic blood endothelial cell Erk activation following vessel wounding that is dependent on Ca2+ signalling but is surprisingly neither Vegfr- nor macrophage-dependent. Following this initial response, regeneration is maintained over a longer timeframe by an independent Vegfr-dependent mechanism. This reveals that the vessel wounding response involves multiple sequential signalling events that fine-tunes Erk activity at the regenerative site.

Altogether, this study demonstrates the utility of a unique biosensor strain for analysing dynamic endothelial Erk signalling events and uncovers new biology in vascular regeneration.

PO-717

# Mouse Retinal Cell Behaviour in Space and Time Using Light Sheet Fluorescence Microscopy

**Claudia Prahst[2], Parham Ashrafzadeh[3], Thomas Mead[1], Lena Claesson-Welsh[3], Claudio A Franco[4], Katie Bentley[1*]**

[1] Cellular Adaptive Behaviour Lab, Francis Crick Institute, UK
[2] Center for Vascular Biology Research and Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, USA
[3] The Beijer Laboratory, Department of Immunology, Genetics and Pathology, Uppsala University, Sweden
[4] Vascular Morphogenesis Lab, Instituto de Medicina Molecular, Portugal

*katie.bentley@crick.ac.uk*

Mouse retinas are a well-established system for studying angiogenesis during development and are often used to model eye diseases characterised by abnormal retinal vasculature, such as diabetic retinopathy. However, by flatmounting retinas for imaging with confocal microscopy, crucial 3D information vital for understanding the progression of these diseases can be lost. In this talk, we hope to demonstrate that rapid, quantitative 3D and 4D (time lapse) imaging of cellular and subcellular processes of the mouse eye vasculature is feasible, with and without tissue clearing, using light-sheet fluorescent microscopy (LSFM).

We used a correlative approach to imaging, whereby both LSFM and Confocal were used to track the behaviour of mouse retinal vasculature in both normal and oxygen-deprived conditions. We then verified the 3D morphologies seen using micro-CT.

Flat-mounting retinas for confocal microscopy significantly distorts tissue morphology, confirmed by quantitative correlative LSFM-Confocal imaging of vessels. Additionally, LSFM readily reveals new features of even well-studied eye disease mouse models, such as the oxygen-induced retinopathy (OIR) model, including a previously unappreciated 'knotted' morphology to pathological vascular tufts, abnormal cell motility and altered filopodia dynamics when live-imaged.

We conclude that quantitative 3D/4D LSFM imaging and analysis has the potential to advance our understanding of the eye, in particular pathological, neurovascular, degenerative processes.

# 8. Others

PO-801

## Mitochondrial MsrB2 Serves as a Switch and Transducer for Mitophagy

**Seung Hee Lee[1,2], Won-Ho Kim[1], John Hwa[2*]**

[1] Division of Cardiovascular Diseases, Korea National Institute of Health (KNIH), Korea
[2] Cardiovascular Research Center, Yale University, USA

*John.hwa@yale.edu*

The molecular spatial–temporal mechanisms governing autophagosomal selection of reactive oxygen species (ROS)-damaged mitochondria, particularly in a diabetic platelet

1. Isolation of diabetic human platelets
2. Identification of MsrB2 in diabetic platelets as LC3 interaction protein
3. Platelet specific MsrB2 KO mice
4. LC-mass analysis of Parkin oxidation

We now report that the mitochondrial matrix protein MsrB2 plays an important role in switching on mitophagy by reducing Parkin methionine oxidation (MetO), and transducing mitophagy through ubiquitination by Parkin and interacting with LC3. This biochemical signaling only occurs at damaged mitochon- dria where MsrB2 is released from the mitochondrial matrix. MsrB2 platelet-specific knockout and in vivo peptide inhibition of the MsrB2/LC3 interaction lead to reduced mitophagy and increased platelet apoptosis. Pathophysiological importance is highlighted in human subjects, where increased MsrB2 expression in diabetes mellitus leads to increased platelet mitophagy, and in platelets from Parkinson's disease patients, where reduced MsrB2 expression is associated with reduced mitophagy. Moreover, Parkin mutations at Met192 are associated with Parkinson's disease, high- lighting the structural sensitivity at the Met192 position.

Release of the enzyme MsrB2 from damaged mitochondria, initiating autophagosome formation, represents a novel regulatory mecha- nism for oxidative stress-induced mitophagy.

PO-803

## The Relationship Between Age with Hypertension in Primary Health Care at Jatiyoso Indonesia: Epidemiology Study

**Bastomy Eka Rezkita[1*]**

[1] Pathology Anatomy, Sebelas Maret University, Indonesia

*bastomyeka@gmail.com*

This research is to know relation between age and hypertension in Puskesmas (Primary Health Care) Jatiyoso.

This was an analytic observation research with cross sectional design. The study was conducted in the Puskesmas Jatiyoso (Primary Health Care) in April 2019. The sample came from 112 patients who examined themselves at the Puskesmas (Primary Health Care) Jatiyoso with a total sampling technique. In this study, most of the respondents were male, namely 33 people and 79 women. Data analysis using chi square test.

Age is made into two classifications, more than 45 years and less than 45 years. Statistical analysis showed that there was a relationship between age and the incidence of hypertension with a p value of 0.01 (p> 0.05) and the result of OR = 22,667, CI 95% = 2,792 – 8,751.

There was significant correlation between age and hypertension in Primary health care Jatiyoso Indonesia.

PO-804

## Effect of Statin and Ezetimibe on Insulin Resistance with Prediabetes

**Jun Hwa Hong**[1*]

[1] Division of Endocrinology, Eulji University Hospital, Daejeon, Korea

*lammoth@naver.com*

LDL lowering treatment is essential to prevent atherosclerotic cardiovascular disease. Although statin is first choice to lower LDL cholesterol, new onset diabetes is nettlesome, especially to prediabetes. Ezetimibe shows additive LDL lowering effect in combination with statin and also improves insulin resistance. In this study, we investigated the change of insulin resistance with atorvastatin and ezetimibe in prediabetic patients.

The participants are people of prediabetes and dyslipidemia with LDL over 130 mg/dl. We analyzed the differences of the metabolic parameters and HOMA-IR before treatment and 3 months after treatment of atorvastatin and ezetimibe.

113 patients with prediabetes and dyslipidemia were enrolled. Baseline LDL cholesterol level was 159.97 ± 21.90 mg/dl. After 3 months with atorvastatin and ezetimibe combination therapy, LDL cholesterol level was lowered to 72.65 ± 23.24 mg/dl (54.58%). HbA1c level didn't show significant change (5.69 ± 0.27%, 5.67 ± 0.33, p=0.556). Fasting glucose level was lowered from 104.05 ± 9.83 mg/dl to 101.08 ± 11.75 mg/dl (p=0.014). HOMA-IR was also lowered from 2.54 ± 2.23 to 1.95 ± 1.74 (p=0.017). Additionally, the improvement of insulin resistance was more prominent to patients with good responder of LDL cholesterol lowering (> 50%) and patients with higher insulin resistance (HOMA-IR > 2).

The combination therapy of atorvastatin and ezetimibe showed high LDL cholesterol lowering effect and improvement of insulin resistance. This treatment modality may be helpful to overcome the side effect of new onset diabetes with statin.

PO-805

## Comprehensive Evaluation of Circadian Rhythmicity in Cardiovascular System

**Tao Zhang[1], Xiaojiao Du[2], Yue Gu[1], Yingying Dong[1], Ling Yang[3], Ying Xu[1], Fei Zhou[1*]**

[1] CAM-SU Genomic Resource Center, Medical College of Soochow University, China
[2] Division of Cardiology, the First Affiliated Hospital of Soochow University, China
[3] School of Mathematical Sciences, Soochow University, China

*fzhou@suda.edu.cn*

Circadian factor is becoming an integral part of the occurrence, development, therapy and prognosis of cardiovascular diseases (CVDs). However,

whether the diurnal parameters of heart rate (HR) are the predictive factors for CVD risk remains unknown. We aimed to determine whether the HR rhythmicity could be used for evaluation of cardiac function or CVD risks.

An integrated analytical strategy was developed to detect diurnal rhythms of HR using longitudinal data collected by clinically used Holter monitors and wearable devices. By combining in-house developed algorithms with existing analytical tools, multiple dimensions of diurnal parameters of HR can be obtained for different purposes.

The analytical strategy is robust and also sensitive enough to identify variations in HR rhythms influenced by multiple effectors such as jet lag, geological location, and age from a total of 211 volunteers. HR phase identified by this strategy is correlated to personal chronotype and dim-light melatonin onset (DLMO). When applying this strategy to 10,095 sets of 24-hour Holter ECG data, the significant risk factors including arrhythmic pattern, anti-phase pattern, trough phase with less than 1 (extremely advanced) or more than 5 (extremely delayed) were identified.

The HR-based analytical strategy provides useful information for monitoring personalized diurnal changes in cardiovascular system. Our findings have important implications for understanding how a regular heart chronotype benefits cardiac function and raising the possibility of non-pharmacological intervention against circadian disruption related CVDs.

PO-806

# Vascular Flow Enhances On-Chip Cancer Metastatic Intravasation

**Sanshiro Hanada[1], Peter Friedl[2], Takashi Miura[3], Ryuji Yokokawa[4], Koichi Nishiyama[1*]**

[1] International Research Center for Medical Sciences, Kumamoto University, Japan
[2] Nijmegen Medical Center, Radboud University, Netherlands
[3] Department of Anatomy and Cell Biology, Kyushu University, Japan
[4] Department of Micro Engineering, Kyoto University, Japan

*nkanako@kumamoto-u.ac.jp*

Cancer metastasis is the most life-threatening aspect of human cancer, which is responsible for vast majority of motility in patients with refractory solid cancer. To realize effective therapy targeting cancer metastasis, it is necessary to have a deep understanding of the nature of cancer metastasis. In hematogenous metastasis, cancer cells from a primary tumor invade the surrounding interstitial tissue and enter the circulation by directly crossing the vascular wall barrier (intravasation). Intravasation is a key step for hematogenous metastasis. However, the underling mechanisms still remain not fully understood.

To dissect the issue, we firstly developed an on-chip model for cancer metastatic intravasation with perfusable vascular network. In the model, 3D self-organized vasculature of human umbilical vein endothelial cells (HUVECs), which was surrounded by basement membrane, was generated in extracellular matrix (ECM) on microfluidic device. Interstitial invasion and the following intravasation of cancer cells were induced by embedding a metastatic sarcoma cell (HT1080) spheroid in an avascular region of ECM. Continuous microvascular flow (307±219 μm/sec) was realized by perfusing culture media into the reconstructed vasculature using hydrostatic pressure differences.

Using the system, we analyzed effects of vascular flow on cancer intravasation. During 3 days culture, the cancer cells invaded into the ECM, some cells were attracted to the vascular wall and sometimes they were incorporated in it irrespective of the presence or absence of vascular flow. However, we found that these cancer cell migrations were enhanced in the presence of vascular flow and, especially, the cells tended to move against vascular flow. More interestingly, the frequency of cancer intravasation apparently increased under vascular flow, although the underlying mechanisms are under investigation.

Our on chip model would be useful for dissecting cancer intravasation mechanisms, especially the involvement of blood flow.

PO-807

# MAPK Phosphatase 5 Deficiency Protects Against Cardiac Fibrosis

**Chao Zhong[1,2], Zhiqiang Zhao[1], Cheng Zhang[1], Erhe Gao[3,4], Ying Shao[1,4], Rajika Roy[3,4], Walter J. Koch[3,4], Xiao-feng Yang[1,4], Anton Bennett[5], Jun Yu[1*]**

[1] Center for Metabolic Disease Research and Department of physiology, Temple University Lewis Katz School of Medicine, USA
[2] Key Laboratory for Pharmacology and Translational Research of TCM of Nanchang, Jiangxi University of Traditional Chinese Medicine, China
[3] Center for Translational Medicine, Lewis Katz School of Medicine, Temple University, USA
[4] Department of Pharmacology, Lewis Katz School of Medicine, Temple University, USA
[5] Department of Pharmacology and Department of Comparative Medicine, Yale University School of Medicine, USA

*jun.yu@temple.edu*

Cardiac fibrosis is characterized by excessive accumulation of extracellular matrix in heart tissues and leads to profound impairment of heart function. The molecular control of fibrogenesis is not fully defined. Mitogen-activated protein kinase (MAPK) phosphatase 5 (MKP-5) is a member of the dual specificity phosphatases family proteins and specifically targets and dephosphorylates p38 MAPK and JNK. In this study, we investigate the role of MKP-5 in cardiac fibrosis.

Male and female, 8-weeks-old MKP-5 knock-out (MKP-5-/-) and wild-type (WT) control mice were subjected to transverse aortic constriction (TAC) surgery. Cardiac functions were accessed by echocardiography and, cardiac fibrosis was examined by histology and RT-PCR. Cardiac Ly6C-high and Ly6C-low macrophages were quantified and sorted out by FACS. Unbiased RT-PCR array of 91 fibrotic genes were analyzed in WT and MKP-5-/- cardiac Ly6C-low macrophages.

MKP-5 expression was upregulated in the fibrotic tissue after TAC. MKP-5-/- attenuated adverse cardiac remodeling, as demonstrated by a significant improvement of cardiac function and decreased extracellular matrix deposition in the TAC hearts. Ly6C-low and Ly6C-high macrophages are previously implicated in regulating cardiac remodeling. FACS analysis showed that MKP-5 deficiency significantly inhibited the accumulation of inflammatory Ly6C-high macrophages infiltration without affecting Ly6C-low cardiac macrophage number. PCR array reviewed significant alteration of an array of fibrosis-related genes including MMP-13, MMP-9, Dcn, Plau, Vegfa and Thbs1 in Ly6C-low cardiac macrophages isolated from MKP-5-/- compared to those from WT. Our mechanistic study in vitro further confirmed that collagen-degrading MMP-13 as a key downstream target negatively regulated by MKP-5 via p38 MAPK in Ly6C-low cardiac macrophages. MKP-5 deficiency upregulated MMP-13 and promoted the resolution of cardiac fibrosis after TAC in vivo.

Our study indicates, for the first time, that MKP-5 deficiency protects pressure overload induced

heart failure and attenuates cardiac fibrosis by promoting Ly6C-low cardiac macrophage-mediated, MMP-13-dependent collagen degradation during cardiac remodeling.

PO-808

# The Molecular Mechanism of a Partial RANKL Peptide, MHP1-AcN as an Anti-Inflammatory Agent

**Nan Ju[1], Hiroki Hayashi[1], Munehisa Shimamura[1,2], Hironori Nakagami[1*]**

[1] Department of Health Development and Medicine, Osaka University Graduate School of Medicine, Japan
[2] Department of Neurology, Osaka University Graduate School of Medicine, Japan

*nakagami@gts.med.osaka-u.ac.jp*

RANKL/RANK signaling showed neuroprotective effects in LPS-stimulated neuron-glia mixed culture (Proc Natl Acad Sci U S A. 2014). Because systemic administration of recombinant RANKL induces osteoporosis, we developed a novel partial RANKL peptide, microglia healing peptide-1 (MHP1), and modified it with N-terminal acetylation and C-terminal amidation (MHP1-AcN) with increased stability and strengthened inhibitory effects on TLR-mediated inflammation in microglia/macrophages without inducing osteoclast differentiation. Moreover, systemic administration of MHP1 and MHP1-AcN has been shown to be effective for the treatment of ischemic stroke in mice. However, relatively little is known about the molecular mechanism of MHP1-AcN as an anti-inflammation agent.

RAW 264.7 cells were incubated with TLR ligands and MHP1-AcN. Biotinylated MHP1-AcN was pulled down by avidin agarose and its binding partner was identified. Cell lysate was examined for NF-κB and IRF3 activation, and mRNA was isolated for cDNA synthesize and checking proinflammatory cytokine expressions. In peritonitis model, LPS and MHP1-AcN were injected into the mouse abdomen at the same time for 6 hours. Serum was collected for proinflammatory cytokine assay.

MHP1-AcN dose-dependently competed with LPS for CD14 binding (C-terminus). LPS-induced TLR4 internalization, NF-κB and IRF3 nuclear translocation, and proinflammation cytokine expressions (Il6, Il1b, Tnfa and Nos2) were reduced by MHP1-AcN in macrophages. TLR3 or TLR7 ligand-induced IL-6 production was also inhibited by MHP1-AcN. Moreover, lower serum IL-6 and TNF-α levels were observed in MHP1-AcN groups in LPS-induced peritonitis model.

MHP1-AcN competes with LPS for CD14 binding and inhibits subsequent TLRs-mediated inflammation. This novel mechanism provide the possible indication of MHP1 for the treatment of septic shock, chronic infection, autoimmune disorders, and other inflammatory diseases by targeting CD14.

PO-809

# Entelon® (Vitis Vinifera Seed Extract) Reduces Inflammation and Calcification in a Beagle Dog Model of Intravascular Bovine Pericardium Implantation

**Hong Ju Shin[1*], Jae Seung Shin[1]**

[1] Cardiovascular Surgery, Korea University Ansan Hospital, Korea

*babymedi@naver.com*

Inflammation and calcification are major factors responsible for failure of bioprosthetic valve and other substitute heart valve implantations. The objective of this study was to evaluate the anti-inflammatory and anti-calcification effects of Entelon150® (consisting of grape-seed extract) in a beagle dog model of intravascular bovine pericardium implantation.

In total, 8 healthy male beagle dogs were implanted with a bovine pericardium bilaterally in the external jugular veins and divided into two groups. Animals in the Entelon150® group (n = 4) were treated with 150 mg of Entelon150® twice daily for six weeks after surgery. The negative control (NC) group (n = 4) was treated with 5 ml of saline using the same method. After six weeks, we measured the calcium content, performed histological examination, and performed molecular analysis.

The calcium content of implanted tissue in the Entelon150® group (0.56±0.14 mg/g) was significantly lower than that in the NC group (1.48±0.57 mg/g) ($p < 0.05$). Histopathological examination showed that infiltration of chronic inflammatory cells, such as fibroblasts and macrophages, occurred around the graft in all groups; however, the inflammation level of the implanted tissue in the Entelon150® group was significantly lower than that in the NC group. Both immunohistochemical and western blot analyses revealed that bone morphogenetic protein 2 expression was significantly attenuated in the Entelon150® group.

Our results indicate that Entelon150® significantly attenuates post-implantation inflammation and degenerative calcification of the bovine pericardium in dogs. Therefore, Entelon150® may increase the longevity of the bovine pericardium after intravascular implantation.

PO-810

# Prostaglandin D2 Inducible Epithelial-to-Mesenchymal Transition (EMT) in Non-Small Cell Lung Carcinoma A549 Cells

**Farzaneh Vafaeinik[1], Seo Yeon Jin[1], Hye Jin Kum[1], Sun Sik Bae[1*]**

[1] Pharmacology, Pusan National University School of Medicine, Korea

*sunsik@pusan.ac.kr*

Despite the rapid advances in diagnosis and treatment, lung cancer remains the leading cause of cancer-related death in the United States and worldwide. Mounting evidence has demonstrated involvement of epithelial-mesenchymal transition (EMT) in drug resistance and metastasis of cancers. So in this study we are going to find the role of EMT in lung cancer by studying effect of PGD2 in EMT, since PGD2 is a mediator in various patho-

physiological process, including inflammation and tumorigenesis.

In the present study, A549 cells, were treated with PGD2 to induce EMT in A549 epithelial cells. Our results indicate that efficacy of PGD2 mediated a significant reduction in A549 cells proliferation and promoting migration and invasion of these cells. Furthermore, protein analysis by immunofluorescence results and western blot analysis confirms that well-known markers of EMT i.e, E-cadherin was downregulated while Vimentin and Collagen expression was upregulated in A549 cells post treatment with PGD2.

Our findings may have important implication for PGD2 as a possible therapeutic agent for targeting cancer.

Additionally, PGD2 receptors may be considered as a possible therapeutic targets in lung cancer prevention or to identify drug that target the COX-enzymes.

PO-811

# LTF Promotes Cell Proliferation in CRPC by Replacing the Role of the Androgen Receptor

**Hye Jin Kum[1], Seo Yeon Jin[1], Farzaneh Vafaeinik[1], Sun Sik Bae[1*]**

[1] Pharmacology, Pusan National University (Medicine), Korea

*sunsik@pusan.ac.kr*

Androgen deprivation therapy (ADT) is the mainstay of advanced prostate cancer treatment. But almost all the hormone-sensitive prostate cancer (HSPC) patients progress to castration-resistant prostate cancer (CRPC). We identified several genes that shows differential expression between HSPC and CRPC. A significant gene of them was chosen that lactotransferrin (LTF) was highly expressed in samples from patients with CRPC as well as androgen-insensitive PC3 cells.

Silencing of LTF expression in PC3 cells showed low proliferation rates. We also identified two LTF forms present in PCa and knockdown and overexpression each isoform to determine what effect two isoforms had on PC3 cell. We speculated that LTF would increase proliferation by replacing the role of androgen receptors because there is no androgen receptor in PC3 cells. We verified effect of LTF expression on the DHT-induced proliferation.

DHT enhanced the expression of LTF as well as proliferation of LNCaP cells. Silencing of LTF in LNCaP cells decreased DHT-induced proliferation. Overexpression of LTF in LNCaP cells resulted in the enhancement of proliferation even in the absence of DHT.

Therefore, we suggest that LTF may plays an essential role in regulating the proliferation of prostate cancer cells.

PO-812

# Effect of Five-Day Residential Smoking-Cessation Therapy on 24-Week Smoking-Cessation Rate: A Retrospective Cohort Study

**Yu Hyeon Yi**[1*]

[1] Department of Family Medicine and Busan Tobacco Control Center, Pusan National University Hospital, Korea

*eeugus@hanmail.net*

We provided a 5-day residential smoking-cessation therapy since 2015 in South Korea. We conducted a retrospective cohort study to evaluate its effectiveness that performed at Busan tobacco control center.

We recruited the participants who had failed previous quit attempts and whocame to seek intensive smoking cessation services between 2015 and 2016. A 5-day residential program of activities were arranged for intensive individual and group counseling. Participants were hospitalized in a supportive, smoke-free ward for five days. Pharmacotherapy was also provided nicotine withdrawal. This was followed by an outpatient clinic during six months. Seven-day point prevalence of smoking abstinence was determined through participants' self-reporting of zero cigarettes smoked, which was verified through urinary cotinine and exhaled carbon monoxide tests.

Overall, 166 patients, all males, were analyzed in this study. Of these, 155 reported quitting smoking at four weeks; this fell to 140 at 12 weeks, and at 24 weeks the number was 118. Further, 110 quitters and 42 non-quitters received medication. There was no difference between the groups regarding prescription of medication (varenicline 66.4%, bupropion 33.3%). The duration of medication was 76 days for the quitters and 61 days for the non-quitters. It was not statistically significant.

This study demonstrated that a 5-day residential smoking-cessation therapy is effective for helping smokers who have been smoking for more than 20 years and want to quit smoking.

PO-813

# Sac-1004 Alleviates Experimental Colitis by Modulating Colonic Vessel Dysfunction

**Young-Guen Kwon**[1*], **Yeseul Kim**[1], **Haiying Zhang**[1]

[1] Biochemistry, Yonsei University, Korea

*ygkwon@yonsei.ac.kr*

Inflammatory bowel disease (IBD) is an autoimmune disease that causes inflammation of chronic gastrointestinal tract. Endothelial dysfunction consists of decreased endothelial barrier, and increased expression of adhesion molecules is a pathological process as related to IBD. In this study, we showed the therapeutic effect of CU06-1004 in the experimental mouse colitis.

Acute colitis induced by providing 3% dextran sodium sulfate (DSS) in drinking water for 7 days. 24h after DSS supply, drugs are treated by oral administration in every day.

CU06-1004, endothelial dysfunction blocker has been investigated its therapeutic effect by reducing vascular hyper-permeability and inflammatory activity through protecting the endothelium. Oral admin-

istration of CU06-1004 significantly reduces clinical manifestation (losing weight, diarrhea, bloody stool), and histological alteration (epithelial loss, inflammatory cell infiltration, crypt destruction) induced by DSS. And pro-inflammatory cytokines reduced, which indicated that inflammation ameliorated. In a vascular aspect, CU06-1004 attenuated interrupted and tortured vessels. Furthermore expression of junction proteins enhanced, and inflammatory adhesion molecules reduced. These suggest that the whole marker of endothelial dysfunction was decreased by CU06-1004 administration. By endothelium protection, epithelial barrier restoration, and down-regulation of epithelial inflammation followed.

Our results suggest that directly blockage of endothelial dysfunction significantly ameliorated the progression of IBD, and thus, CU06-1004 could become a potential therapeutic means for the treatment of IBD and other inflammatory diseases.

PO-814

# Therapeutic Effect of Bleomycin Induced Scleroderma Mice Model Using W-Peptide Through Immune Regulation and Anti Fibrotic Effects

**Gyu Tae Park[1], Yang Woo Kwon[1], Young-Cheol Song[1], Jae Ho Kim[1*]**

[1] Physiology, School of Medicine, Pusan National University, Korea

*jhkimst@pusan.ac.kr*

Systemic sclerosis is autoimmune disease mediated by overproduction of extracellular matrix. It is deadly disease by autoimmunity. Pathology of this disease is inflammatory cell activation, such as macrophage, and fibroblast differentiation to myofibroblast. FPR2 is a G-protein coupled receptor that modulate inflammatory response by activation of macrophage. However, the role of FPR2 in scleroderma is still unclear. This study was under-taken to investigate the therapeutic effect of scleroderma by activation of FPR2 with W peptide.

To establish scleroderma mice model, C57BL6/J wild-type and Fpr2 knockout mice were subcutaneously injected with 100 μL BLM solution (1mg/ml) for three weeks, and administration of 100 μL PBS containing both BLM (1 mg/mL) and WKYMVm (1 μM) for an additional three weeks. The therapeutic effects of WKYMVm on scleroderma were examined by histological analysis of the scleroderma skin tissues, and inflammatory cytokine expression in mouse serum of mice by ELISA assay.

W peptide has thera-peutic effect in bleomycin induced scleroderma through dermal thickness decreasion and collagen fiber down-regulation. And fibroblast activation through differetiation by myofibroblast and inflammatory response in sclero-derma, such as Inflammatory cell infltration and cytokine expression, is down-regulated by W peptide. But, thera-peutic effect by agonistic peptide in FPR2 knock-out mice has no-effect through dermal thick-ness downregulation and collagen fiber than Wild type mice, and they didn't regulated fibroblast activation and inflammatory response by peptide.

These results suggest that FPR2 regulation that binding with W peptide leads to inhibition of sclerosis by attenuating of inflammatory response.

PO-815

# Identification of Novel Ribonuclease Regulating Self-Renewal and Quiescence of Hematopoietic Stem Cells

**Hiroyasu Kidoya**[1*]

[1] Department of Signal Transduction, Research Institute for Microbial Diseases, Osaka University, Japan

*kidoya@biken.osaka-u.ac.jp*

Acute myelogenous leukemia (AML) is the most common adult acute leukaemia, and it develops through regulatory defects of cellular processes of hematopoietic stem cell (HSC). The balance between self-renewal and quiescence capacity of HSC are tightly controlled and that disorder can lead the generation of pre-leukemic stem cells. Several regulatory molecules that are working to keep the balance of self-renewal and quiescent of HSC have been identified. However, the key regulator that decides cells fate to be normal HSC or abnormal LSC is still unknown.

We searched the molecule that is contributing normal self-renewal and quiescence fate of HSC and identified Regnase-1 (Reg1) by bioinformatics analysis. Reg1 is a novel ribonuclease witch destabilize target mRNAs. To analyze the function of Reg1 in HSC, we generated hematopoietic cell specific Reg1 deficient mice based on Reg1 floxed mice and Vav1-Cre transgenic mice.

Although hematopoietic cell specific Reg1 deficient mice were born without overt defects, Reg1 deletion in HSC impaired the balance of self-renewal and quiescence, and markedly increase the number of immature long-term HSC. Additionally, Reg1 deficiency leads the development of aggressive AML phenotype results in the accumulation of transcriptional factor Gata2 and Tal1. Transcriptome and functional studies elucidated the marked molecular similarities between the disease in Reg1 deficient mice and human AML.

Thus, Reg1-regulated pathways are responsible for AML development, thereby providing potential therapeutic targets.

PO-816

# Thymosin b4 – A Novel Therapeutic in Protection Against Aortic Aneurysm

**Sonali Munshaw**[1*]

[1] Department of Physiology Anatomy and Genetics, University Of Oxford, UK

*sonali.munshaw@dpag.ox.ac.uk*

Aortic aneurysm (AA) is a degenerative vascular disease and a leading cause of mortality. Dysregulation of smooth muscle cell (VSMC) phenotype critically impairs vascular stability. Low density lipoprotein receptor related protein 1 (LRP1), an endocytic regulator of VSMC PDGFRβ signalling, is associated by GWAS with AA risk. Thymosin β4 (Tβ4) is an actin binding peptide required for embryonic VSMC differentiation. We validated a novel interaction between Tβ4 and LRP1 in VSMCs. As a regulator of VSMC differentiation, we hypothesise that Tβ4 interacts with LRP1, to maintain healthy vasculature postnatally and protect against disease.
Objectives

- To determine whether Tβ4 knockout mice display increased susceptibility to AA.
- To elucidate the molecular mechanism of growth factor signalling via Tβ4 –LRP1.

• To explore the therapeutic potential of exogenous Tβ4 in vascular protection.

Global and VSMC-specific Tβ4KO mice were infused, alongside controls, with 1mg/kg/day Angiotensin II. For rescue experiments, C57BL/6 mice additionally received 12mg/kg/day Tβ4 or saline. VSMC phenotype, signalling, elastin integrity, ECM composition and inflammation were analysed using FACS, histology, immunostaining and immunoblotting. Using primary VSMCs and MOVAS-1 cells, endocytosis of the LRP1-PDGFRβ complex was tracked by surface biotinylation and proximity ligation assays, following PDGF-B stimulation.

Global and VSMC-specific Tβ4KO mice, like LRP1 KO, demonstrated predisposition to AA, with aortic dilatation and rupture in <5 days. Accelerated disease progression was not caused by exacerbated inflammation, rather by enhanced VSMC phenotypic switching and dysregulated LRP1/PDGFRβ signalling. Tβ4-depleted VSMCs demonstrated increased recycling of LRP1-PDGFRβ to the plasma membrane and reduced lysosomal targeting, following PDGF-B stimulation. Exogenous Tβ4 significantly reduced aortic dilatation and rupture, with preserved VSMC and elastin phenotype and normalised PDGFRβ signalling.

We identify Tβ4 as a key regulator of LRP1-PDGFRβ endocytic signalling, for maintaining VSMC differentiation and vascular health. Tβ4 is a promising candidate for treatment of vascular disease.

PO-817

# Visualization of Vasculature and CD11c+ Cells in Dentin-Pulp Complex with Optical Clearing Method

**Sujung Hong[1,2], Jingu Lee[1,2], Pilhan Kim[1,2,3*]**

[1] Graduate School of Nanoscience and Technology, KAIST, Korea
[2] KI for Health Science and Technology (KIHST), KAIST, Korea
[3] Graduate School of Medical Sciecne and Engineering, KAIST, Korea

*pilhan.kim@kaist.ac.kr*

There are various immune cells in dental pulp, and it has been suggested that the CD11c+ pulpal dendritic cells might have a role of sensing pathogens and functioning as sentinel cells in a pathological condition. However, immune modulation and vascular changes in teeth has not yet been well examined and understanding of underlying molecular and cellular mechanisms is very limited. To explore dynamic roles of CD11c+ cells in pupal inflammation, we observed pulpitis induced teeth of CD11c-YFP transgenic mouse in 3D cellular-resolution using optical clearing techniques.

For 3D cellular-level visualization of intact mouse teeth, we utilized tooth optical clearing method based on modified Murray's clear and a custom-built laser scanning confocal microscopy.

We newly observed a few CD11c+ cells extending cytoplasmic tails into dentinal tubules. In pulpitis induced irritated teeth, CD11c+ cells were densely recruited near the lesion with regressed vessels forming a line seemingly as a barrier and CD11c+ odontoblast-like cells significantly increased in comparison with the normal teeth. Histological analysis with DMP-1 and DSPP, typical marker for odontoblast, confirmed that the CD11c+ odontoblast-like cells

were not odontoblasts and might have a previously unknown role in responding to acute pulpitis.

We newly identified CD11c+ odontoblast-like cells those might be involved in immunological response in dentin-pulp complex. In pulpitis induced teeth, densely recruited CD11c+ immune cells formed a barrier near the irritated site with vessel regression.

PO-818

## Longitudinal Intravital Imaging of Neurovascular Unit Alteration at the Onset of Microinfarction

**Jingu Lee[1], Sujung Hong[1], Soyeon Ahn[1], Pilhan Kim[1,2*]**

[1] Graduate School of Nanoscience and Technology, Korea Advanced Institute of Science and Technology, Korea
[2] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Korea

*pilhan.kim@kaist.ac.kr*

Microinfarctions, blood vascular occlusion with inflamed vascular walls, exists in normal personnel and also neurodegenerative diseases patients. Most of microinfarctions were detected in post-mortem studies of patients. Previous studies identified that Alzheimer's disease (AD) patients have more microinfarctions by using a 7-T magnetic resonance (MR) imaging. Additionally, some researchers established microinfarction model in a rat using photosensitizer, Rose Bengal, that showed progressive cognitive decline. However, it has not yet been reported how neurovascular unit (NVU) changes at onset of microinfarction by in vivo visualization technique.

We established double transgenic mice for NVU visualization using NG2-DsRed pericyte-reporter mouse and Aldh1l1-GFP astrocyte-reporter mouse. Microinfarction in the brain were induced by 561nm laser illumination after intravenous injection of Rose Bengal. For 30 days following the induction of microinfarction, we longitudinally observed the NVU and brain microenvironment using customized intravital confocal microscopy.

Brain tissue showed expansion and shrinkage after the onset of microinfarction. Vascular leakage, blockage and reflow of capillaries and cellular alteration were detected. Pericyte coverage was significantly decreased for 3 days and then recovered over 2 days Astrocytes' morphology and distribution were greatly changed and might play key roles in scar formation followed by microinfarction.

Our results indicate that NVU undergoes highly dynamic alteration at onset of microinfarction, and each cell types of NVU showed different behaviors in long-term response.

PO-819

## CD137 Signaling Controls Acute Colitis via RALDH2-Expressing CD11b-CD103+ DCs

**Jing Jin[1], Shin Hye Moon[1], Goo Taeg Oh[1*]**

[1] Department of Life Science, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

CD137, a potent costimulatory receptor for CD8+ T cells, is also expressed in a variety of non-T

cells. However, limited information is available on the precise role of CD137 in the development of colitis in experimental model.

To answer this, we generated CD137 deficient (CD137-/-) mice and dendritic cell (DC) specific CD137 deficient (CD11c-cre CD137F/F) mice. To determine the molecular mechanisms of CD137 in CD11b-CD103+ DCs, we assessed in bone marrow derived CD11b-CD103+ DCs.

We show that CD137 signaling in intestinal CD11b-CD103+ dendritic cells (DCs) restricts the progression of acute colitis. Specific deletion of the CD137 gene in DCs results in a reduction in CD11b-CD103+ DCs and regulatory T cells of the lamina propria and mesenteric lymph nodes during acute colitis. Mechanistically, CD137-mediated activation of TAK1 in CD11b-CD103+ DCs results in inhibiting degradation of the anti-apoptotic Bcl-2 and Bcl-xL and upregulating retinaldehyde dehydrogenase 2 (RALDH2) expression. The quantitative and qualitative changes in DCs are linked to induction of Foxp3+ regulatory T cells and suppression of pathogenic IL-23 production by intestinal CD11b+CD103- DCs.

Our results indicate that CD137 signaling in intestinal CD11b-CD103+ DCs is an important immune checkpoint for acute colon inflammation induced by epithelial barrier disruption.

PO-820

# The Role of SOD1 in Mouse Acute Colitis

**Jing Jin[1], Jiyoung Hwang[2], Goo Taeg Oh[1*]**

[1] Department of Life Science, Ewha Womans University, Korea
[2] Department of Nutritional Science and Food Management, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

Superoxide dismutase 1 (SOD1) binds copper and zinc ions and is one of three superoxide dismutase responsible for destroying free superoxide radicals in the body. Reactive oxygen species (ROS), including free superoxide radicals, play an important role in colitis. However, the role of SOD1 in oxidative stress under colitis remains unclear.

To determine this, we examined the in vivo role of SOD1 in the DSS-induced mouse model of colitis. SOD1+/+ and SOD1-/- mice were randomly divided into five groups: normal control group (NC), DSS-treated SOD1+/+ group, DSS-treated sod1-/- group, DSS + B. amyloliquefaciens SOD (BA SOD) treated SOD1-/- group and DSS + BA SOD + spore treated sod1-/- group. BA SOD and placebo was administered by gavage for 17 days. For induction of acute colitis, mice received 2% of DSS dissolved in drinking water for last 9 days.

SOD1 deficiency resulted in severe oxidative stress with body weight loss, epithelial barrier disruption and decreased antioxidant enzyme activities. The levels of neutrophils, monocytes, pro-inflammatory CD11c+ macrophages and CD11b+CD103- dendritic cells (DCs) were increased, while anti-inflammatory CD206+ macrophages and CD11b-CD103+ DCs were decreased, in DSS-treated SOD1-/- mice compared to DSS-treated wild-type mice. Furthermore, rescue of SOD activity in SOD1-/- mice by oral gavage of BA SOD significantly ameliorated enhanced DSS-induced colitis seen in these mice by

suppressing pro-inflammatory immune responses.

Taken together, our results suggest that SOD1-mediated inhibitory responses play a crucial role in limiting the development of DSS-induced colitis, and that BA SOD is a promising candidate for treating colitis.

PO-821

## CXCL11 from Senescent Endothelial Cells Promotes Aggressiveness of Breast Cancer Cells via the ERK1/2 Signaling Pathway

**Hyunjung Hwang[1], Ye-Rim Lee[1], Donghee Kang[1], Jae-Seon Lee[1*]**

[1] Molecular Medicine, Inha University, Korea

*jaeslee@inha.ac.kr*

The effects of senescence associated secretory phenotype (SASP) from therapy-induced senescent endothelial cells on tumor microenvironment (TME) remains to be clarified. Here, we investigated effects of ionizing radiation (IR)- and doxorubicin-induced senescent HUVEC on TME.

When we treated HUVEC with a neutralizing anti-CXCL11 antibody or CXCL11 SiRNA, or treated MDA-MB-231 cells with CXCR3 SiRNA, we observed synergistic diminution of the ability of the HUVEC SASP to alter the migration and spheroid invasion of cancer cells. ERK activation was involved in the HUVEC SASP-induced aggressive activity of MDA-MB-231 cells. Finally, we observed the in vivo effect of CXCL11 from the senescent HUVEC in tumor-bearing mice.

MDA-MB-231 cancer cells treated with conditioned medium (CM) from senescent HUVEC or co-cultured with senescent HUVEC significantly increased cancer cell proliferation, migration, and invasion. We found that CXCL11 plays a principal role in the senescent CM-induced aggressive activities of MDA-MB-231 cells.

Our results demonstrate that SASP from therapy-induced senescent endothelial cells promotes cancer aggressive features and CXCL11 could be the principal target to prevent adverse effects of therapy-induced senescent endothelial cells on tumor microenvironment.

PO-822

## On-Chip Tumor Vasculatures Reconstitute Endothelial Immune Barrier Expressing FasL

**Seunggyu Kim[1], Joonha Park[1], Jessie Jeon[1*]**

[1] Department of Mechanical Engineering, KAIST, Korea

*jsjeon@kaist.ac.kr*

Here we propose a microfluidic tumor vasculature model that recreates physiologically relevant tumor microenvironments, inducing apoptosis in immune cells through FasL-Fas ligation.

A perfusable tumor microvascular network (MVN) was constructed in 3D hydrogel inside a microfluidic chip. Human umbilical vein endothelial

cells transfected with red fluorescence protein (RFP-HUVEC) and hepatocarcinoma cells (HepG2) were mixed in fibrin gel (10:1 ratio) and were injected into a central gel region of the chip. With 4-day culture and additional 20-hour treatment in the humidified incubator, FasL-expressed MVN with HepG2 was successfully established.

We quantitatively measured on-chip endothelial FasL expression levels under TME-specific conditions using immuno-fluorescently labeled antibodies. In particular, replacement of oxygen level from 18% (Control condition) to 1.5% increased endothelial FasL intensity by 1.9-fold in the MVN w/o HepG2 under EBM culture. We further investigated whether the cell-to-cell interaction between HepG2 and HUVECs influences endothelial FasL expression. Notably, co-culture with HepG2 under 18% O2 EBM significantly increased the relative HUVEC FasL intensity by 1.5-fold. Hypoxic microenvironment amplified the tendency that the relative HUVEC FasL intensity increased 1.4- and 2.1-fold, to the MVN w/ HepG2 under 18% O2 EBM and to the control condition, respectively. We further investigated whether the upregulated endothelial FasL in TME-specific conditions promotes apoptosis of target Jurkat cells via Fas-FasL binding. As expected, the percentage of Jurkat apoptosis significantly increased in the MVN w/ HepG2 chips where oxygen level was maintained at 1.5% instead of 18%, suggesting the possible correlation between the increased apoptosis rates and the FasL enhancement (55.0±8.5% v.s. 12.5±5.6%, p-value<0.001).

Taken together, these results support that our microfluidic assay could be utilized in evaluating anti-tumor immunity of patient-derived T cells and screening of novel therapeutic blockades.

PO-823

# Multicellular Tumor Spheroid on a Chip with Functional Vessel Perfusion

**Joonha Park[1], Seunggyu Kim[1], Jessie Jeon[1*]**

[1] Mechanical Engineering, KAIST, Korea

*jsjeon@kaist.ac.kr*

Cancer is one of the brutal cause of death worldwide. Fortunately, the rise of the microfluidic system sheds light on the in-vitro platform recapitulating the in-vivo microenvironment. Here, we present an on-chip vascularized multicellular tumor spheroid(MCTS) model composed of tumor cells, stromal cells, and vascular endothelial cells that are constructed for mimicking pathophysiological relevant in-vitro 3D microtumor model.

Three different cell types including human liver cancer cell (HepG2), human umbilical vascular endothelial cell (HUVEC), human fibroblast (IMR90) are assembled together forming MCTS within 24 hours. The cellular ratio in the MCTS is assessed by fluorescent imaging as each cell types are pre-labeled with different colors. The PDMS microfluidic device is obtained from 3D printed PC-like mold. The MCTSs are introduced to microfluidic channel along with endothelial cells and co-cultured for 4 days.

Vascular endothelial cells anastomosed and formed a vascular network together with MCTS inside the microfluidic device within 4 days. Perfusion of the microbeads shows a lumenized functional vascular bed has formed. Immunofluorescence staining of vascular endothelial marker CD31 displayed the positive formation of cell-to-cell junctions. We also compare vascular permeability between the MCTS of distinct cell types for explor-

ing the impaired barrier function that is considered as a unique feature of tumor vasculature. Notably, we have shown that MCTS containing all three cell types resulted in the most prominent growth pattern while vascular permeability and basement membrane intensity were comparable with different cases.

The microfluidic tumor vasculature model, capable of monitoring dynamic drug delivery, have potential to suggesting personalized medical strategies according to the implementation of patient-specific microenvironment.

PO-824

# The Impact of Oligomerization and Enzymatic Activity of Peroxidasin on Endothelial Cell Function

**Kyung Ah Ham[1,2,3], Hong Seok Choi[1,2,3], Young Ae Joe[1,2,3]***

[1] Cancer Research Institute, College of Medicine, The Catholic University of Korea, Korea
[2] Department of Medical Life sciences, College of Medicine, The Catholic University of Korea, Korea
[3] Department of Biomedicine & Health Sciences, The Catholic University of Korea, Korea

*youngjoe@catholic.ac.kr*

Peroxidasin (PXDN), a multidomain heme peroxidase including extracellular matrix motifs as well as catalytic peroxidase domain, catalyzes sulfilimine crosslinking of collagen IV to reinforce the collagen IV scaffolds. We previously reported that PXDN is essential for endothelial cell survival and growth signaling by sulfilimine crosslink-dependent matrix assembly. However, it is unclear whether multimerization and enzymatic activity of PXDN is necessary for PXDN function in endothelial cells (ECs). In this work we performed the structural and functional analysis of mutation variants of PXDN to address this issue.

We constructed truncation or replacement variants of PXDN with deletion of free cysteines (M1c), deletion of von Willebrand factor type C (M2v) or point mutation of peroxidase domain (M3p). After isolation of HEK 293 clones stably expressing these mutant proteins, conditioned medium (CM) was obtained after incubating the cells in serum free media for 24 h and evaluated.

First, we examined oligomerization pattern of the mutant proteins by western blot analysis under non-reducing condition. M1c mutant protein showed mainly monomeric form whereas other mutant proteins formed trimers. Next, we tested the peroxidase activity of the mutant proteins by TMB oxidation assay. Most of PXDN mutant proteins showed normal enzymatic activity compared to wild type PXDN except M3p mutant protein. In addition, we performed complementation assay of PXDN-depleted (by siRNA knockdown) human umbilical vascular endothelial cells using the CMs. CM containing wild-type (WT) PXDN restored the proliferation of PXDN-depleted cells, whereas M3p mutant protein failed to restore it. CM containing mutant PXDN M1c or M2v showed a decreased recovery compared to WT CM, but with a considerable level.

We concluded that peroxidase activity of PXDN is critical for EC function, and oligomerization of PXDN also contributes to EC function in some degree.

PO-825

# Protective Role of Secretory Ref-1 Against Lipopolysaccharide-Induced Vascular Inflammation in Septic Mice

**Hee Kyoung Joo[1], Yu Ran Lee[1], Eun-Ok Lee[1], Sung Min Kim[1], Hao Jin[1], Byeong Hwa Jeon[1*]**

[1] Department of physiology, School of Medicine, Chungnam National University, Korea

*bhjeon@cnu.ac.kr*

Redox factor-1 is a multifunctional protein identified as a DNA base excision repair enzyme and redox modulator for several transcriptional factors. The aim of this study is to evaluate the role of secreted Ref-1 on lipopolysaccharide-induced vascular inflammation in cultured cells and in vivo.

We generated a secretory Ref-1 adenoviral vector system, AdPPT-LS-Ref-1, by conjugation of pre-protrypsin leading sequence with full-length Ref-1 sequences.

Expression of tumor necrosis factor-α (TNF-α)-induced vascular cell adhesion molecule-1 (VCAM-1) in endothelial cells and lipopolysaccharide (LPS)-induced cyclooxygenase-2 in Raw264.7 cells was inhibited by secretory Ref-1, and this inhibitory effect was abrogated following neutralization of Ref-1 with anti-Ref-1 antibody. Treatment with LPS markedly increased VCAM-1 expression, cathepsin or myeloperoxidase activity, which were significantly suppressed by treatment with AdPPT-LS-Ref-1. Furthermore, LPS-induced cytokines, such as TNF-α, interleukin (IL)-1β, IL-6, and monocyte chemoattractant protein 1, were significantly inhibited in AdPPT-LS-Ref-1-treated mice. However, LPS-induced myeloperoxidase activities were not suppressed by treatment with the redox mutant of secretory Ref-1, AdPPT-LS-Ref-1(C65A/C93A), or wild-type AdRef-1.

Collectively, these results suggest that secreted Ref-1 has anti-inflammatory properties and that its redox cysteine residue is associated with the anti-inflammatory activity in vivo. Our data highlight secretory Ref-1 as potential candidate for the development of new strategies for the treatment of systemic inflammation.

PO-826

# Role of KAP1 in the Regulation of LIN28 Stability in Embryonic Stem Cells

**HyeJi Moon[1], Eunkyoung Do[1], JaeHo Kim[1*]**

[1] Department of Physiology, Pusan National University, Korea

*jhkimst@pusan.ac.kr*

Lin28 has been implicated in mammalian development and maintenance of the pluripotency of embryonic stem cells (ESCs). Post-translational modification (PTM) of proteins plays critical roles in various biological processes, including proliferation and differentiation. However, the role of PTM in the regulation of expression and function of Lin28 in ESCs is poorly understood.

Using affinity purification and mass spectrometry, in this study, we identified KAP1 (KRAB-associated protein 1) as a novel Lin28 binding protein. Immunoprecipitation analysis showed the interaction domain between KAP1 and Lin28. We showed direct interaction through in vitro pull-down assay using recombinant purified proteins.

KAP1 specifically interacted with Lin28 in ESCs and the interaction was mediated through coiled-coil domain of KAP1. Induced overexpression of KAP1 in ESCs stimulated self-renewal and suppressed differentiation of ESCs. KAP1 overexpression led to increased protein level, but not mRNA level, of Lin28 protein, suggesting an involvement of PTM in the regulation of Lin28 expression. KAP1 overexpression significantly abolished Lin28 ubiquitination in ESCs. In contrast, short interfering RNA mediated knockdown of KAP1 promotes Lin28 ubiquitination, leading to proteasomal degradation of Lin28 protein in NIH3T3 cells. KAP1 overexpression interfered ubiquitination of Lin28 mediated by Trim71, an E3 ubiquitin ligase for Lin28.

These results suggest that KAP1 plays a key role in the regulation of stability of Lin28 in ESCs by modulating Trim71-meidated ubiquitination and subsequent degradation of Lin28 protein.

PO-827

# Krüppel -Like Factor 4 Regulates the Alteration of Drug Resistance in Anaplastic Thyroid Cancer

**Su In Lee[1], Dae Kyoung Kim[1], Seoyul Lee[1], Jae Ho Kim[1*], Min Joo Shin[1], Ye Eun Kim[1]**

[1] School of Medicine, Pusan National University, Korea

*jhkimst@pusan.ac.kr*

The objective of this study was to evaluate the involvement of Kru¨ppel-like factor 4 (KLF4), a stemness-associated transcription factor, in the undifferentiated phenotype and drug resistance of ATC.

ATC cells were compared to thyroid cancer cells in drug resistance and gene expression. The effects of KLF4 knockdown in ATC cells on in vitro and in vivo drug resistance were measured. The effects of KLF4 overexpression and knockdown on ABC transporter activity were determined.

ATC cells, such as HTH83, 8505C, and SW1736, exhibited higher resistance to the anticancer drug paclitaxel and higher expression of KLF4 than TPC-1 papillary thyroid cancer cells. Knockdown of KLF4 expression in ATC cells increased the expression of the thyroid-specific differentiation genes, such as thyrotropin receptor, thyroid peroxidase, thyroglobulin, and sodium–iodide symporter. Knockdown of KLF4 expression in ATC cells decreased the resistance to doxorubicin and paclitaxel, and reduced ABC transporter expression. Luciferase reporter assay results showed that KLF4 overexpression increased ABCG2 promoter activity, which was abolished by KLF4 knockdown. A tumorigenicity assay showed that the combination of paclitaxel treatment and KLF4 knockdown significantly decreased tumor mass originated from HTH83 cells in mice.

ATC cells show high expression of KLF4, and KLF4 expression is necessary for maintaining the undifferentiated phenotype and drug resistance in vitro and in vivo. The present study identifies KLF4 as a potential therapeutic target for eliminating ATC cells.

PO-828

# Cancer Stem Cells Targeted Therapy Through Inhibition of LPA Pathway in Ovarian Cancer Stem Cells

**Min Joo Shin[1], Dae Kyoung Kim[1], Su In Lee[1], Seo Yul Lee[1], Ye Eun Kim[1], Jae Ho Kim[1*]**

[1] Department of Physiology, School of Medicine, Pusan National University, Korea

*jhkimst@gmail.com*

Ovarian cancer shows high mortality due to development of resistance to chemotherapy and relapse. Cancer stem cells are a subpopulation of cancer cells characterized by self-renewal ability, tumorigenesis and drug resistance. In previous studies, we have shown that autotaxin and lysophosphatidic acid (LPA) play an important role in cancer stem cell characterization such as migration, sphere forming ability and drug resistance in ovarian cancer. So in this study, we found a drug that could target cancer stem cells by inhibiting the LPA pathway.

Western blot, PCR, Flow cytometry analysis, Cell viability analysis, Tumor xenograft model.

When the LPA inhibitor was treated with A2780-SP, sphere-forming ability was significantly reduced compared to the treatment with KI16425 and Paclitaxel. When ovarian cancer stem cells and paclitaxel were treated with ovarian cancer cells, cancer cells showed low survival rate even at low concentration and cancer stem cells showed high survival rate even at high concentration. However, when LPA inhibitor was treated with ovarian cancer stem cells and ovarian cancer cells, survival rate was much lower in cancer stem cells than in cancer cells. It was confirmed that ovarian cancer stem cells isolated from ovarian cancer patients had the same results as cell lines. In addition, after processing LPA inhibitor at different concentrations was confirmed that a concentration-dependent manner the expression is reduced when the check ABC transporter such as ABCB1 and ABCG2 by western blot. When the cell death was confirmed by FACS analysis, it was confirmed that the cells were killed more by the lpa inhibitor treatment as in the previous results.

These results suggest that the LPA inhibitor may be an effective drug for the treatment of ovarian cancer stem cells.

PO-829

# Roles of N-α-Acetyltransferase 10 (Naa10) 235 in Tumor Growth

**Mi-Ni Lee[1], Hyae Yon Kweon[1], Goo Taeg Oh[1*]**

[1] Department of Life Science, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

Acetylation is one of the most common protein modifications that regulate normal cell functions and affect tumor development. N-α-acetyltransferase 10 (Naa10) is a subunit of Nα-terminal protein acetyltransferase that plays a role in many biological processes. Recently, Human NAA10 homolog ($NAA10^{235}$) was associated with cell growth and differentiation that has emerged recently as a critical molecule in cancer progression. However, the regulation of biological activities of $NAA10^{235}$ at present in tumor development is poorly understood.

We established a mouse ES cell line with the $Naa10^{-/-}$ (homozygous null) genotype. Naa10 targeted ES cells were injected into mice and tumor growth was mea-

sured. Also, we generated $Apc^{Min/+}$ /$Naa10^{235}$ transgenic mice and quantified growth of intestinal polyps. The number of cells with overexpression or deficiency of Naa10 genes ($Naa10^{225}$ and $Naa10^{235}$) was measured. All statistical tests were two-sided, and P values less than .05 were considered statistically significant.

The growth of transplanted tumors in mice injected with Naa10 targeted ES cells was statistically significantly reduced than in mice injected with control cells ($P < .01$). $Apc^{Min/+}$ /$Naa10^{235}$ transgenic mice (n=18) had significantly more intestinal polyps than $Apc^{Min/+}$ mice (n=10) ($P < .01$). Also, cell growth was increased in $Naa10^{235}$ –overexpressing 293T cells. Moreover, Naa10 targeted ES cells and $Naa10^{225}$ -expressing Naa10 targeted ES cells showed decreased cell proliferation compared to control ES cells. On the other hand, $Naa10^{235}$-expressing Naa10 targeted ES cells proliferated at a rate similar to control ES cells.

These findings collectively implicate that $NAA10^{235}$ affects a tumor activation via up-regulation of cell proliferation.

PO-830

# Naa10 Deficient Mice Show Developmental Defects As in Human Syndromes

**Hyae Yon Kweon[1], Mi-Ni Lee[1], Goo Taeg Oh[1*]**

[1] Life Science, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

N-α-acetyltransferase 10 (Naa10), which is conserved from yeast to humans, modulates diverse biological processes including cell cycle regulation, DNA damage, apoptosis, cancer progress, development and disease. Naa10 regulates protein functions by acetylating the first α-amino group of a protein (N-α-acetylation) or the internal lysine residues of mature proteins (N-ε-acetylation). It also plays a role independent of its acetylation functions by binding its partners to regulate their activities. Recent findings revealed that depletion of Naa10 in few species resulted in embryonal defects and point mutations of human NAA10 caused developmental defects. However, the effect of Naa10 on mice is poorly known.

Naa10-knockout mice were generated based on a standard gene-targeting in E14 embryonic stem (ES) cells (129/Sv). The targeting vector was constructed to delete exons 1–4 in the Naa10 gene. Genotyping was performed by PCR and Southern blot analyses of genomic DNAs obtained from the tails. Perinatal lethality was monitored daily at P0 to P3. All statistical tests were two-sided, and P values less than .05 were considered statistically significant.

We report that mice lacking Naa10 displayed common clinical features seen in human diseases such as developmental delays, growth retardation, hydrocephalus, cardiac defects and urogenital abnormalities. In particular, the postnatal lethality was increased, which is likely to be associated with structure anomalies in heart and hydrocephalus.

In conclusion, our data demonstrates that Naa10 has essential roles for viability, development and diseases.

PO-831

# ATP Binding Cassette Transporter A1 Is Involved in Extracellular Secretion of Acetylated APE1/Ref-1

**Yu Ran Lee[1], Hee Kyoung Joo[1], Eun Ok Lee[1], Sungmin Kim[1], Hao Jin[1], Byeong Hwa Jeon[1*]**

[1] Department of Physiology, School of Medicine, Chungnam National University, Korea

*bhjeon@cnu.ac.kr*

The apurinic/apyrimidinic endonuclease-1/redox factor-1 (APE1/Ref-1) secretion showed remarkable regulatory effects on inflammatory cytokine-stimulated cells both in vitro and in vivo. The different subcellular distribution of APE1/Ref-1 and the evidence of its extracellular secretion together suggest that its distribution and translocation are controlled not only by stimulatory agents like chemicals or hormones but also by plasma membrane transporters. Although APE1/Ref-1 has been demonstrated to accumulate along the plasma membrane in the presence of a deacetylase inhibitor, trichostatin A (TSA), the mechanism of its translocation across the plasma membrane remains unknown.

Human embryonic kidney 293T (HEK293T) cells were treated with the inhibitor for 2 h, followed by TSA treatment for 1 h, as indicated. Cell viability was analyzed using the RealTime-GloTM MT luminescent kit. The supernatant was used for detection of secretory APE1/Ref-1. The amount of APE1/Ref-1 in each sample was determined by ELISA analysis. The plasma membrane of HEK293T cells, which had been transfected with ABCA1 siRNA, was prepared by sucrose density gradient centrifugation. The membrane pellet was analyzed by immunoblotting using anti-ABCA1 and anti-N-cadherin antibodies. Interaction of ABCA1 and APE1/Ref-1 was visualized using a Duolink II fluorescence kit.

While APE1/Ref-1 targeting was not affected by inhibition of the endoplasmic reticulum/Golgi-dependent secretion, its secretion was reduced by inhibitors of ATP-binding cassette (ABC) transporters, and siRNA-mediated down regulation of ABC transporter A1. TheassociationbetweenAPE1/Ref-1 and ABCA1 transporter was confirmed by proximal ligation assay (PLA) and immunoprecipitation experiments. An APE1/Ref-1 construct with mutated acetylation sites (K6/K7R) showed reduced co-localization with ABC transporter A1. Exposure of trichostatin A (TSA) induced the acetylation of APE1/Ref-1, which translocated into membrane fraction.

Acetylation of APE1/Ref-1 is considered to be necessary for its extracellular targeting via non-classical secretory pathway using the ABCA1 transporter.

PO-832

# The Role of ICAM1 in Leukocyte Recruitment in Breast Cancer

**Anahita Fouladzadeh[1*], Emma Thompson[1], Michaelia Cockshell[1], Michael Samuel[1], Angel Lopez[1], Claudine Bonder[1]**

[1] Centre for Cancer Biology, University of South Australia and SA Pathology, Australia

*fouay003@mymail.unisa.edu.au*

Angiogenesis is a process driven by the endothelial cells (ECs) and plays a significant role in cancer progression, metastasis, and recurrence. Although anti-angiogenic drugs can specifically

target the ECs, tumour mass can undergo an adaptive, independent process known as vasculogenic mimicry (VM, whereby the cancer cells themselves form vascular structures) to avoid the shortage of nutrient and oxygen inside the tumour. This process has been shown in triple negative breast cancer (TNBC) which is a highly vascularised and the most aggressive subtype of breast cancers. In EC-lined blood vessels, ECs directly control leukocyte entry into the tumour via cell-cell adhesion. Intercellular adhesion molecule-1 (ICAM1/CD54) is a transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily and mediates the capture of circulating leukocytes via the ICAM1 ligand lymphocyte function-associated antigen-1 (LFA-1). Whether VM structures (like their angiogenic counterpart) express adhesion molecules such as ICAM-1 to mediate leukocyte recruitment remains unknown. Here we test the hypothesis that VM-competent TNBC cells express ICAM1 for the selective recruitment of pro-tumourigenic myeloid cells.

We have new data suggesting that while VM-competent breast cancer cell lines (MDA-MB231, SUM159, HCC70) express high levels of ICAM1, VM-incompetent breast cancer cells (MCF7, T47D) do not express ICAM1. In vitro testing of cell adhesion via the flow chamber assay allowed us to examine different leukocyte subsets as they are syringe pumped over a monolayer of VM-competent cancer cells at a physiologically relevant shear rate.

Results support our hypothesis that ICAM1 supports leukocyte recruitment (particularly CD14+ monocytes) by VM-competent TNBC cells as determined by ICAM1 targeting siRNA knockdown and the flow chamber assays.

Identification of ICAM-1 as a TNBC target and biomarker can potentially lead to the formation of a novel platform and strategy to address a critical gap in TNBC patient care.

PO-833

# Increased p32 Ubiqitination Mediated by Parkin via Arginase II Donw-Regulation

**Bon Hyeock Koo[1], Sung Woo Ryoo[2*]**

[1] BIT Bio-medical Convergence, Kangwon National University, Korea
[2] Biological Science, Kangwon National University, Korea

*ryooswo8@kangwon.ac.kr*

The p32 protein has a crucial role in the regulation of Ca2+ between cytosol and mitochondria, which plays important roles in vascular endothelial function. Our previous studies shown that increased p32 level in the vascular endothelial cells increase the influx of Ca2+ from the cytosol to the mitochondria, which reduces the Ca2+-dependent eNOS activation and decreased acetylcholine induced vasorelaxation responses throughout reduced NO production. So, regulation of p32 level is important to maintain vascular homeostasis, but the mechanism of regulating p32 level is still unknown. Therefore, we investigated the stability of p32 which can be modified post-translation such as ubiquitination by arginase II down-regulation.

siRNA against the arginase II mRNA (siArgII) was shown abolished the total p32 level and increased ubiquitination of p32. Furthermore, to confirm whether p32 ubiquitination is induced by siArgII, we used the pCMV6-XL5-p32 plasmid (WT p32) and mutated (K154R, K220R) p32 plasmid (MT p32).

In results, MT p32 was abolished the ubiquitination of p32 compared with WT p32 and MT p32 also didn't recover the Ca2+-dependent eNOS activation compared with WT p32. Then, Ubiquitin E3-ligase involved ubiquitination of p32, we aimed parkin which was well known as E3-liagase targeted mitochondrial protein. According to our expectation,

siRNA against the parkin mRNA (siparkin) transfected groups were prevented ubiquitination of p32 and decreased Ca2+-dependent eNOS activation axis by increasing total p32 level.

In conclusion, stability regulation mechanism of p32 is crucial in the vascular endothelial cells and p32 is anticipated to be a new therapeutic target for various vascular disorders.

PO-834

## Endothelial Cell-Specific Knockout of p32 Can Improve Vascular Function in Ca2+-Dependent Manner

**Byeong Jun Yoon[1], Sung Woo Ryoo[2*]**

[1] BIT Medical Convergence, Kangwon National University, Korea
[2] Biological science, Kangwon National University, Korea

*ryooswo8@kangwon.ac.kr*

$Ca^{2+}$ as a secondary messenger performs a very important function in cells and can induce various cellular responses. In endothelial cells, $Ca^{2+}$ is closely related to the activity of endothelial nitric oxide synthase (eNOS), and activated eNOS produces nitric oxide (NO) using L-arginine as a substrate. NO plays a major role in maintaining vascular homeostasis by inhibiting platelet aggregation, proliferation and migration of smooth muscle cells. p32 protein is mainly located in the mitochondria and has a donut-shaped homotrimer structure with a pore in the center.

Recent studies show that p32 can regulate cytosolic $Ca^{2+}$ level, suggesting that p32 will act as a $Ca^{2+}$ channel. The cytosolic $Ca^{2+}$ can regulate the activity of eNOS, so p32 may be related to the activity of eNOS. Therefore, we investigated whether p32 can modulate eNOS activity in a $Ca^{2+}$-dependent manner.

First, it was confirmed that p32 was reduced in $p32^{f/f}_{Cre+}$ mice. Reduced expression of p32 increased the activity of eNOS in a $Ca^{2+}$-dependent manner, which increased NO production. In the aorta of $p32^{f/f}_{Cre+}$ mice, the acetylcholine response was increased, and the phenylephrine response was decreased. Also, Blood pressure tended to decrease, but there was no significance.

In conclusion, p32 can regulate the cytosolic $Ca^{2+}$, which can induce eNOS activity. However, further research is needed on the mechanism by which p32 regulates cytosolic $Ca^{2+}$.

PO-835

## Overexpressed p32 Localized in the Endoplasmic Reticulum and Mitochondria Negatively Regulates Calcium-Dependent Endothelial Nitric Oxide Synthase Activity

**Bon Hyeock Koo[1], Sung Woo Ryoo[2*]**

[1] BIT Bio-medical Convergence, Kangwon National University, Korea
[2] Biological Science, Kangwon National University, Korea

*ryooswo8@kangwon.ac.kr*

The p32 protein plays a crucial role in the regulation of cytosolic Ca2+ concentrations ([Ca2+]c) that contributes to the Ca2+-dependent signaling cas-

cade. Using an adenovirus and plasmid p32-overexpression system, the aim of the study was to evaluate the role of p32 in the regulation of [Ca2+] and its potential associated with Ca2+-dependent endothelial nitric oxide synthase (eNOS) activation in target organelles from endothelial cells.

Using electron and confocal microscopic analysis, p32 overexpression was observed to be localized to mitochondria and the endoplasmic reticulum and played an important role in Ca2+ translocation, resulting in increased [Ca2+] in these organelles and reducing cytosolic [Ca2+] ([Ca2+]c). This decreased [Ca2+]c following p32 overexpression attenuated the Ca2+-dependent signaling cascade of calcium/calmodulin dependent protein kinase II (CaMKII)/AKT/eNOS phosphorylation.

Moreover, in aortic endothelia of wild-type mice intravenously administered adenovirus encoding the p32 gene, increased p32 levels reduced NO production and accelerated reactive oxygen species (ROS) generation. In a vascular tension assay, p32 overexpression decreased acetylcholine (Ach)-induced vasorelaxation and augmented phenylephrine (PE)-dependent vasoconstriction. Notably, decreased levels of arginase II (ArgII) protein using siArgII were associated with downregulation of overexpressed p32 protein, which contributed to CaMKII-dependent eNOS phosphorylation at Ser1177.

These results indicated that increased protein levels of p32 caused endothelial dysfunction through attenuation of the Ca2+-dependent signaling cascade and that ArgII protein participated in the stability of p32. Therefore, p32 may be a novel target for the treatment of vascular diseases associated with endothelial disorders.

PO-836

# 3, 4, 5–Trihydroxycinnamic Acid (THC), an Arginase Inhibitor, Improves Vascular Function via Ca2+/CaMKII/eNOS Axis

**Byeong Jun Yoon[1], Sung Woo Ryoo[2*]**

[1] BIT Medical Convergence, Kangwon National University, Korea
[2] Biological science, Kangwon National University, Korea

*ryooswo8@kangwon.ac.kr*

Cardiovascular diseases, such as atherosclerosis, is one of the leading causes of death worldwide. Among many factors, the reduction of nitric oxide (NO) is one of the main causes of atherosclerosis. NO plays a major role in maintaining vascular homeostasis by preventing platelet and leukocyte adhesion, and proliferation and migration of smooth muscle cells. Endothelial NO synthase (eNOS) is an enzyme that produces NO with L-arginine as a substrate. Thus, endothelial dysfunction refers to the reduction of NO produced by eNOS, which is considered an indicator of most vascular diseases. Arginase hydrolyzes L-arginine to produce L-ornithine and urea, and two isoforms exist. Arginase ll (Argll), the extrahepatic isoform of arginase, is the principal form in human and mouse endothelial cells.

Previous studies have shown that argll activity has increased in various vascular diseases such as atherosclerosis. Therefore, the purpose of this study is to find out how arginase inhibition can improve endothelial function.

In this study used 3, 4, 5–Trihydroxycinnamic acid (THC) to check a function as an arginase inhibitor. THC decreased arginase activity in mice tissue ly-

sates and human umbilical vein endothelial cells (HUVECs) in a concentration-dependent manner. THC increased cytosolic $Ca^{2+}$ levels and $Ca^{2+}$/calmodulin dependent protein kinase ll (CaMKll) and eNOS phosphorylation in HUVECs. THC also increased NO production and endothelium-dependent vasodilation response to acetylcholine (ACh) in mice aortas.

In conclusion, arginase inhibiton with THC improved vascular function by activating CaMKll / eNOS signaling cascade in a $Ca^{2+}$-dependent manner.

PO-837

## The Absence of Ninjurin-1 Causes Multiple Neural Crest-Related Defects

**Sejin Jeon[1], Tae Kyeong Kim[1], Goo Taeg Oh[1*]**

[1] Department of Life Sciences, Ewha Womans University, Korea

*gootaeg@ewha.ac.kr*

Ninjurin-1 (nerve injury-induced protein [Ninj1]) is known to be an adhesion molecule participating in the process of cell migration, which in turn is a major aspect of life activity throughout life. However, the role of Ninj1 still has not been studied in vivo clearly.

In order to get a better understanding of the in vivo functions of Ninj1, we generated a conventional Ninj1-/- mice and analyzed its phenotype. Ninj1 caused several problems in the process of embryogenesis, which led to various deformities.

Here we showed that Ninj1 deficiency results in not only defective embryonic development, but also abnormal phenotypes. Some Ninj1-/- embryos showed small body size and craniofacial malformations, while others had cardiac developmental defects. Furthermore, we identified that those abnormal appearances caused by Ninj1-/- occurred in embryonic stages, and further in severe cases, the animals died immediately after birth or during growth, before reaching adulthood.

In conclusion, Ninj1 deficiency causes fetal or postnatal lethality continued from embryonic developmental defects as well as craniofacial and cardiac malformations. Thus, we predict that Ninj1 may have a role in neural crest cell migration.

PO-838

## High-Throughput Tumor Vasculature Model for NK Cell Cytotoxicity Assay and Its Further Application as a Drug Screening Platform

**Hyeri Choi[1], Dohyun Park[2], Jiyoung Song[2], Noo Li Jeon[1,2*]**

[1] Program for Bioengineering, Seoul National University, Korea
[2] Mechanical Engineering, Seoul National University, Korea

*noolijeon1406@gmail.com*

Natural killer (NK) cells play a vital role with their anti-tumor activity, inducing apoptosis of tumor cells during tumor progression. Improving NK cell-mediated cytotoxicity has become a new strategy for immunotherapy, while a lack of in vitro model for studying NK cell activity still remains to be addressed. We aim to develop a high-throughput

tumor vasculature model by co-culture of different cell types and introduce NK cells into perfusable vessels for cytotoxicity assays. Our model is expected to be further used as a drug screening platform to enhance NK cell cytotoxicity.

Our injection-molded microfluidic chip comprises 28 wells with the size of 384-well formats. Endothelial cells were co-cultured with tumor cells, and fibroblasts in hydrogels, and after blood vessel formation, NK cells were introduced through side channels for evaluating the cytotoxic activity of NK cells using live-cell imaging.

Vascular networks formed by different tumor cell types showed slightly distinct characteristics compared to the control condition without tumor cells. Fluorescein isothiocyanate-dextran was used to ensure that vascular networks were fully perfusable as in molecules or cells were allowed to flow through the vessels. NK cells introduced into vessels tended to recruit at the site of tumor cells, and the ability of NK cells to kill tumors cells varied with the type of tumor cells, confirming that colorectal cancer cells were more resistant to NK cells.

The cytotoxic activity of NK cells can be observed within vascular networks, and vessels co-cultured with diverse tumor cell types showed morphological differences and varied NK cell cytotoxicity. The next step of this project is to test various drugs to enhance the activity of NK cells or reduce the resistance of cancer cells. Our platform has the potential as a drug screening system with high-throughput experiments and image analysis.

PO-839

# VEGF Diversity in the Animal Kingdom

**Michael Jeltsch**[1,2,3*], **Khushbu Rauniyar**[1,3]

[1] Research Programs Unit, University of Helsinki, Finland
[2] Wihuri Research Institute, Finland
[3] Faculty of Pharmacy, University of Helsinki, Finland

*michael@jeltsch.org*

The vascular endothelial growth factor (VEGF) family comprises in vertebrates 3-6 members, depending on the species: VEGF(-A), PlGF, VEGF-B, VEGF-C, VEGF-D, and VEGF-F. Despite the absence of blood and lymphatic vascular systems, PDGF/VEGF-like growth factors have also been found in invertebrate species like Drosophila and C. elegans. We wanted to comprehensively address the evolutionary relationships between the PDGF/VEGF growth factors.

We search all animal phyla for the occurrence of VEGF-like growth factors using a BioPython workflow programmatically identifying paralogous and orthologous relationships. We analyze the coding sequences of VEGFs for conservation and adaptation. Overlaying this data with established evolutionary relationships and the full genome duplications, we propose a phylogenetic tree.

Whole genome duplications contribute to the expansion of VEGF diversity, but several individual gene duplications are necessary to account for the temporal pattern of emergence. Some VEGF family members appear completely absent in some classes: Functional genes coding for VEGF-B appear to be absent from the phylum Archosauria, which includes crocodiles, birds, and, by inference, also non-avian dinosaurs. Furthermore, the phylogenetically oldest VEGFs likely featured a C-terminus with BR3P-homology repeats, which is a hallmark of the modern-day lymphangiogenic VEGF-C/

VEGF-D. When analyzing conservation and diversification, significant differences can be found a) between different parts of the VEGF molecules and b) between the different VEGF paralogs. The sequence conservation of the VEGF homology domain is remarkable over large evolutionary distances. However, the junctional sequences that are crucial for proteolytic activation show signs of adaptive evolution.

There is not only heterogeneity of proteolytic VEGF-C activation within an organism (i.e. several different proteases are able to activate VEGF-C/VEGF-D under different physiological settings), but also between animal classes (i.e. deploying different VEGFs and different activating proteases for the same physiological task). Such heterogeneity has potentially consequences when interpreting data generated with disparate animal models.

PO-841

# Hepatic Stellate Cell–Specific Knockout of Transcriptional Intermediary Factor 1γ Aggravates Liver Fibrosis

**Eun Ju Lee[2], Injoo Hwang[1], Ji Yeon Lee[1], Jong Nam Park[1], Keun Cheon Kim[1], Irene Kim[1], Dodam Moon[1], Hyomin Park[1], Seo-Yeon Lee[2], Hong Sug Kim[6], Dae Won Jun[3], Sung-Hye Park[4], Hyo-Soo Kim[1,5*]**

[1] Department of Molecular Medicine and Biopharmaceutical Sciences, Seoul National University, Korea
[2] Biomedical Research Institute, Seoul National University Hospital, Korea
[3] Department of Internal Medicine, Hanyang University School of Medicine, Korea
[4] Department of Pathology, Seoul National University College of Medicine, Korea
[5] Department of Internal Medicine, Seoul National University College of Medicine, Korea
[6] Division of Genome application, Macrogen Inc, Korea

*hyosoo@snu.ac.kr*

Transforming growth factor β (TGFβ) is a crucial factor in fibrosis, and transcriptional intermediary factor 1γ (TIF1γ) is a negative regulator of the TGFβ pathway in HSCs; however, its role in liver fibrosis is unknown.

In this study, mesenchymal stem cells derived from human embryonic stem cells(hE-MSCs), which secrete hepatocyte growth factor (HGF) as the most abundant growth factor, were used as a strategic tool to screen for a target for repairing thioacetamide (TAA)-induced liver fibrosis and in vitro. The role of TIF1γ in liver fibrosis was corroborated by experiments in vitro using LX2 HSCs and in vivo using site- and time-specific TIF1γ gene targeting in transgenic (TG) mice.

Our results showed that TIF1γ was significantly decreased in LX2 cells when exposed to TGFβ1. Such

a decrease of TIF1γ was prevented considerably by co-culture with hE-MSCs. Transplantation of hE-MSCs prevents TAA-induced liver fibrosis in nude mice.

Interaction of TIF1γ with SMAD2/3 and binding to the promoter of the α-smooth muscle actin gene (αSMA) suppressed αSMA expression. Mechanism of TIF1γ up-regulation by HGF: Phosphorylation of cAMP response element–binding protein (CREB) and binding on the TIF1γ promoter region induced TIF1γ expression. Generation of TG mice with inducible, HSC-specific knockout of TIF1γ shows that Knockout of TIF1γ accelerates liver fibrosis in mice.

We suggest the following mechanisms of TIF1γ in HSCs during fibrotic stimulation and restoration. Suppression of CREB phosphorylation reduces TIF1γ expression and the interaction with SMAD2/3, whereas the up-regulation of TIF1γ enhances the interaction with SMAD2/3 and inhibits αSMA expression. Experiments in TG mice with inducible, HSC specific knockout of TIF1γ demonstrated that TIF1γ has potential as a novel therapeutic approach for the prevention of liver fibrosis. In addition to cell therapy using hE-MSCs, gene therapy would be feasible to directly increase or activate TIF1γ directly.

PO-842

# A Vaccine Targeting Aged Cells Mitigates Metabolic Disorders in Obese Mice

**Hironori Nakagami**[1*]

[1] Department of Health Development and Medicine, Osaka University, Japan

*nakagami@gts.med.osaka-u.ac.jp*

Aged, or senescent, cells are known to harm their surrounding younger cells by creating an inflammatory environment. A specific type of immune cell, called T cell, can accumulate in fat tissues in obese individuals in senescence, causing chronic inflammation, metabolic disorders and heart disease.

To achieve their goal, the researchers developed a novel vaccine targeting the surface protein CD153 that is present on senescent T cells populating fat tissues, thereby ensuring that normal T cells are not affected.

To achieve their goal, the researchers developed a novel vaccine targeting the surface protein CD153 that is present on senescent T cells populating fat tissues, thereby ensuring that normal T cells are not affected. To test the effects of their vaccine, the researchers fed mice with a high-fat diet to make them obese and ultimately to mimic the metabolic changes seen in diabetes. These include insulin resistance and an improperly functioning glucose metabolism, both of which can facilitate a deterioration of the eyes, kidneys, nerves and the heart. When they vaccinated these mice against CD153, the researchers observed a sharp decline of senescent T cells in the fat tissues of the mice, demonstrating the success of their approach.

Our findings provide new insights into removing

specific senescent cells using specific vaccines and could potentially be used as a novel therapeutic tool for controlling glucose metabolism in obese individuals

PO-843

# Resistin as a Therapeutic Target of Breast Cancer Metastasis

**Hwan Lee[1], Hyun-Woo Park[1], Gyurae No[1], Hyun-Duk Jang[1], Hyo-Soo Kim[1*]**

[1] Internal Medicine, SNUH, Korea

*hyosoo@snu.ac.kr*

Both obesity and inflammation are important in breast cancer development and progression. Yet the role of an adipokine resistin, which is related to both obesity and inflammation, is unclear in cancer biology. Here, we identified resistin as a novel therapeutic target of breast cancer metastasis.

We compared serum resistin levels of breast cancer patients and healthy controls in a case-control study and observed that serum resistin was higher in breast cancer patients than in controls (17.2±10.6 ng/mL vs 11.3±6.1 ng/mL, P=0.011).

Resistin was expressed by both infiltrative monocytes in cancer tissue and circulating monocytes. Then, we evaluated its prognostic significance in a cohort of patients with lymph node positive breast cancer, which showed that baseline serum resistin level was a strong predictor of distant recurrence of breast cancer (hazard ratio 1.047, 95% confidence interval 1.020-1.074). Resistin promoted cancer cell migration through Src activation in vitro. Resistin also increased metastasis of breast cancer cells in a xenograft mouse model after tail vein injection, which was significantly attenuated by a resistin blocking antibody and Src inhibitor.

Our results show that resistin, which promotes breast cancer metastasis through Src activation and cancer cell migration, can be a novel therapeutic target to prevent breast cancer metastasis.

PO-844

# Ablation of Sirt1 in the Non-Hematopoietic Bone Marrow Microenvironment Is Not Required for Hematopoietic Stem Cell Function in the Adult Mice

**Suyeon Woo[1], Jayoung Kim[1], Hee Seon Choi[1], Dongjun Lee[1*]**

[1] Department of Convergence Medicine, Pusan National University, Korea

*ldj319@gmail.com*

SIRT1 is known as a histone deacetylase, performs a wide variety of function in biological systems. It has been reported that SIRT1 is relevant to stem cell homeostasis including cell proliferation, differentiation, apoptosis, and inflammatory responses. Also this SIRT1 plays a important role in delay aging, extending life span and prevent aging-related in response to mitochondrial metabolic. The previous study demonstrated that loss of Sirt1 in the hematopoietic stem and progenitor system regulates the expansion of hematopoietic stem and progenitor cell population under stress conditions.

In addition, SIRT1 activator regulates hematopoeitic stem and progenitor cell and changed the number fo hematopoietic stem cell. This study investigated the role of SIRT1 in the non-hematopoietic bone marrow microenvironment.

Ocn-cre transgenic mice were used for the investigation of effects in bone marrow niches. Sirt1floxed/floxed mice were crossed with Ocn-Cre to know the role of hematopoietic stem cell maturation of Sirt1 in the bone marrow niche. Control and Sirt1Δ/Δ mice accepted lethal doses of irradiation previous the transplantation of 1x106 bone marrow cells by retro-orbital injection. After 4 months, The bone marrow cells were collected femurs and tibias of mice. The extracted mouse bone marrow cells were stained with antibodies and performed FACS analysis.

This study investigated the role of SIRT1 in the non-hematopoietic bone marrow microenvironment. Ablation of Sirt1 in the non-hematopoietic bone marrow microenvironment demonstrated that the production of mature blood cells and frequencies of hematopoietic stem and progenitor cell population attained similar results to those of controls. Moreover the ablation of Sirt1 in the non-hematopoietic bone marrow microenvironment had no influence on stem cell function under stress conditions.

Thus, SIRT1 is dispensable for a physiological role for non-cell-autonomous function in the maintenance of the adult hematopoietic stem cell compartment in the adult mice.

PO-845

# Role of Microglial STAT3 Activation in Diabetic Brains

**Jang-Hyuk Yun[2], Da-Hye Lee[1], Chung-Hyun Cho[1*]**

[1] Department of Biomedical Sciences and Pharmacology, Seoul National University, Korea
[2] Department of Veterinary Pharmacology, Kangwon National University, Korea

*iamhyun@snu.ac.kr*

Diabetes mellitus (DM) characterized by hyperglycemia leads to a variety of complications, including cognitive impairment or memory loss. The hippocampus is a key brain area for learning and memory and is one of the regions that is most sensitive to diabetes. However, the pathogenesis of diabetic neuronal lesion is not yet completely understood. In the present study, we focused on the association of microglia activation and brain lesions in diabetes because that diabetes can cause the activation of microglia, and an increase in cytokines is associated with cognitive decline in the diabetic brain.

We investigated hippocampal neuronal cell death and STAT3 activation using a streptozotocin-induced type 1 DM model. In vitro, we assessed mouse and human cell lines to confirm the effect of microlial STAT3 activation on neuronal cells. Also, we used myeloid-specific STAT3-deficient mice and WP1066, JAK2 inhibitor, to block microglial STAT3.

We demonstrated that STAT3 activation in microglia is associated with neuronal apoptosis in a diabetic hippocampus. We also took advantage of mice lacking STAT3 in microglia and found that depletion of microglial STAT3 reduced neuronal apoptosis in the diabetic hippocampus.

These results demonstrate a role for microglial STAT3 in mediating diabetic neuronal lesions and thus provide a potential therapeutic target (This

work is supported by the Seoul National University Research Grant in 2019).

PO-846

## Shockwaves Improved Glucose Tolerance in Mouse Under High-Fat Diet

**Wonkyoung Cho[1], Young Mi Park[1*]**

[1] Department of Molecular Medicine, Ewha Womans University, Medical School, Korea

*parkym@gmail.com*

Obesity is a serious medical condition in which body fat mass is increased. It is related to metabolic diseases such as type II diabetes, cardiovascular diseases, and cancer. Shockwaves are a sequence of mechanical pulses characterized by high peak pressure (100 MPa), fast rise (<10 ns), and short lifecycle (10 μs). Low-dose energy shockwave therapy has been proven to be beneficial for several medical conditions including orthopedic diseases. Recent studies revealed that the therapeutic effects of shockwave treatment are derived from differentiation of mesenchymal stem cells, and releases of angiogenetic factors. Also, our group revealed that shockwaves suppress adipocyte differentiation via decrease in PPAR gamma recently. The goal of this study is to evaluate the effect of shockwaves on high-fat induced obesity.

We treated wild-type (WT, C57BL/6) male mice with or without shockwaves to high-fat diet (HFD) for 16 weeks. Mice body weight was measured every week during HFD. After low-dose shockwaves treatment, western blots and quantitative real-time PCR (qRT-PCR) were performed with adipose tissues (epididymal fat). glucose tolerance test (GTT) was done before mice necropsy.

We found that shockwaves improved glucose tolerance under HFD in mouse compared shockwaves-untreated group. There was no significant body weight difference between shockwaves treated and untreated group. Also, shockwaves increased GLUT4 expression in adipose tissue both protein level and RNA level.

Therefore, we concluded that shockwaves have an effect in glucose tolerance in mouse under HFD.

PO-847

## Bacterial Type III Effector Protein HopQ Inhibits Melanoma Motility Through Autophagic Degradation of Vimentin

**Seung-Ho Park[1], Sung-Jin Yoon[1], Young-Jun Park[1*]**

[1] Environmental Diseases Research Center, KRIBB, Korea

*PYJ71@kribb.re.kr*

Malignant melanoma is a fatal disease that rapidly spreads to the whole body. Treatments have limited use due to drug resistance and various side effects. Pseudomonas syringae pv. tomato (Pto) is an opportunistic bacterial pathogen capable of severe infection in plants. Pto injects the effector protein HopQ into the plant cytosol via a type III secretion system and suppresses the host defense mechanisms. The present study aimed to examine the suitability of HopQ as a possible drug against melanoma metastasis.

The effects of HopQ on B16F10 cell motility were examined using wound-healing assay. The interaction between HopQ and 14-3-3 or vimentin was examined by co-immunoprecipitation (co-IP) and immunofluorescence. The HopQ-overexpressing B16F10 cells injected into the C57BL/6 mice through the tail vein to determine the effects of HopQ on melanoma metastasis in vivo.

In melanoma cells, over-expressed HopQ is phosphorylated and bound to 14-3-3 through its N-terminal domain, and this binding increased the interaction between HopQ and vimentin. The binding of HopQ to vimentin allowed for degradation of vimentin via p62-dependent selective autophagy. Decreased expression of vimentin by HopQ inhibited melanoma motility and in vivo metastasis.

Pseudomonas syringae effector HopQ directly degraded vimentin in melanoma, and could be an inhibitor of melanoma metastasis.

PO-848

# DGCR8 Is Indispensable for Myeloid Cell Differentiation in the Bone Marrow Microenvironment

**Jayoung Kim[1], Soo-Yeon Woo[1], Hee-Sun Choi[1], Dongjun Lee[1*]**

[1] School of Medicine, Pusan National University, Korea

*lee.dongjun@pusan.ac.kr*

MicroRNAs are small regulatory RNAs that modulate protein expression and have been shown to play key regulatory roles in all aspects of biology including cell proliferation, differentiation, development, apoptosis, metabolism, cancer, and hematopoiesis.

The generation of normal microRNAs requires enzymes including DGCR8 and DICER. DGCR8, a double-stranded RNA binding protein, participates in the miRNAs biogenesis pathway by interacting with the DROSHA. DGRCR8 has also been implicated in stem cell homeostasis. Dicer deletion in bone marrow microenvironments causes myeloid dysplasia and acute myeloid leukemia in the mice.

Here, we report that Dgcr8 deletion in the BM microenvironment (Dgrcr8Δ/Δ) is indispensable for maintaining mature hematopoietic lineage cells in the blood and showed increased myeloid cells and decreased B cells in the BM. Also, HSCs did not preserve from the Dgrcr8Δ/Δ setting under transplantation stress. Further, under transplantation condition, Dgrcr8Δ/Δ recipient mice displayed myeloid expansion in the BM.

Collectively, our findings suggest that DGRCR8 is important in myeloid cell differentiation to function in the context of the hematopoietic compartment.

PO-849

# The Oxysterols 27-Hydroxycholesterol Influences Pools of Hematopoietic Stem and Progenitor Cells

**Hee Seon Choi[1], Soo-Yeon Woo[1], Jayoung Kim[1], Dongjun Lee[1*]**

[1] The Department of Convergence Medicine, Pusan National University, Korea

*ldj319@gmail.com*

Oxysterols are oxygenated derivatives of cholesterol and, compared to cholesterol, contain an additional hydroxy, epoxide or ketone group in the sterol nucleus, and/or a hydroxyl group in the side chain. 27-hydroxycholesterol (27HC) is a side-chain oxysterol oxygenated at the 27 the carbon atom of cholesterol. This oxysterol is produced via oxidation by sterol 27-hydroxylase (CYP27A1) and metabolized via 7α-hydroxylation for bile acid synthesis in the liver. The previous study demonstrated that treatment with the alternative ERα ligand 27HC induced ERα-dependent HSC mobilization. Also, Cyp27a1-deficient mice had significantly reduced 27HC levels and HSC mobilization.

27-Hydroxycholetsterol is metabolites of cholesterol that activates monocytes and macrophages and inflammation and immune reaction. To know what this oxysterol function is in the bone marrow, 27HC treated into bone marrow cell. Cells were treated with cholesterol and 27-hydroxycholesterol dissolved in ethanol. The transfected cells were harvested by centrifuge and western blot analysis. The bone marrow cells were analyzed by flow cytometry.

27HC treated into bone marrow cell. The number of hematopoietic stem and progenitor cells was decreased after 27HC treatment. This observation indicates that a decrease of hematopoietic stem and progenitor cells is related to ROS level.

we report that 27HC exhibit impaired hematopoietic stem and progenitor cell (HSPC) numbers, but did not influence mature cell output in vitro. Furthermore, 27HC has increased ROS levels from HSPCs. Thus, 27HC is indispensable for regulating pools of hematopoietic stem and progenitor cells.

PO-850

# Resistance Exercise Confers Cardioprotection and Improves Mitochondrial Function in Diabetic Rats

**Jubert Marquez[1,2], Tae Hee Ko[3], Hyoung Kyu Kim[2,3], Jin Han[2,3*]**

[1] Department of Biology, University of the Philippines-Manila, Philippines
[2] Department of Health Sciences and Technology, College of Medicine, Inje University, Busan, Korea
[3] National Research Laboratory for Mitochondrial Signaling, Cardiovascular and Metabolic Disease Center, Inje University, Busan, Korea
[4] Department of Internal Medicine, College of Medicine, Sanggye Paik Hospital, Inje University, Seoul, Korea

*phyhanj@gmail.com*

Metabolic dysregulation and mitochondrial dysfunction are hallmarks of diabetic cardiomyopathy (DC). Resistance exercise (RE) has recently been observed to provide beneficial effects to subjects with cardiovascular diseases. However, the exact mechanism how RE confers cardioprotection in DC and regulates mitochondrial dysfunction are still unclear. Therefore, this study investigated wheth-

er RE attenuates DC by improving mitochondrial function using diabetic rats.

Fourteen Otsuka Long-Evans Tokushima Fatty rats were assigned to sedentary control (SC, n = 7) and RE (n = 7) groups at 28 weeks of age. Long-Evans Tokushima Otsuka rats were used as the non-diabetic control. The RE rats were trained 20 repetitions of climbing a ladder 5 days per week. Glucose and lipid profiles were taken after the exercise protocol, and various mitochondrial function parameters were investigated. Molecular mechanism was studies using western blotting.

RE rats exhibited higher glucose uptake and lower lipid profiles, indicating changes in energy metabolism. RE rats significantly increased the ejection fraction and fractional shortening compared with the SC rats. Isolated mitochondria in RE rats showed increase in mitochondrial numbers, which were accompanied by higher expression of mitochondrial biogenesis proteins such as proliferator-activated receptor-γ coactivator-1α and TFAM. Moreover, RE rats reduced proton leakage and reactive oxygen species production, with higher membrane potential. These results were accompanied by higher superoxide dismutase 2 and lower uncoupling protein 2 (UCP2) and UCP3 levels in RE rats.

Overall these data indicate the effectiveness of RE in ameliorating DC by improving mitochondrial function, which may contribute to the maintenance of diabetic cardiac contractility.

PO-851

# Thioredoxin-Interacting Protein Promotes Phagosomal Acidification Upon Exposure to Escherichia Coli Through Inflammasome Mediated Caspase-1 Activation in Macrophages

**Sung-Jin Yoon[1], Seung-Ho Park[1], Young-Jun Park[1*]**

[1] Environmental Disease Research Center, Korea Research Institute of Bioscience & Biotechnology(KRIBB), Korea

*pyj71@kribb.re.kr*

In host defense, it is crucial to maintain the acidity of the macrophage phagosome for effective bacterial clearance. However, the mechanisms governing phagosomal acidification upon exposure to gram-negative bacteria have not been fully elucidated. In this study, we demonstrate that in macrophages exposed to Escherichia coli, the thioredoxin-interacting protein (TXNIP)-associated inflammasome plays a role in pH modulation through the activated caspase-1-mediated inhibition of NADPH oxidase.

Mouse peritoneal macrophage cells were plated in 24-well plates at 5x105 cells per well and incubated with GFP-expressing E. coli .Cells were analyzed immediately using a FACSCanto II flow cytometer (BD) and the data were processed using the FACSDiva software (BD). For the treatment of inhibitors, cells were incubated with 10 μM Wortmannin (Selleckchem) or 20 nM bafilomycin A (Selleckchem) for 30 minutes before the addition of bacteria. For the phagosomal maturation assay using pHrodo™ Red E. coli Bioparticles, cells were plated in 48-well plates at 2x105 cells per well and incubated with

pHrodo™ Red E. coli Bioparticles at 20 μg per well at the indicated periods.

While there was no difference in early phase bacterial engulfment between Txnip knockout (KO) macrophages and wild-type (WT) macrophages, Txnip KO macrophages were less efficient at destroying intracellular bacteria in the late phase and their phagosomes failed to undergo appropriate acidification. These phenomena were associated with reactive oxygen species production and were reversed by treatment with an NADPH oxidase inhibitor or a caspase inhibitor

In line with these results, Txnip KO mice were more susceptible to both intraperitoneally administered Escherichia coli and sepsis induced by cecum ligation and puncture than WT mice. Taken together, this study suggests that the TXNIP-associated-inflammasome-caspase-1 axis regulates NADPH oxidase to modulate the pH of the phagosome, controlling bacterial clearance by macrophages.

PO-852

# Mitochondrial Biogenesis in Cardiac Cell Line Increases Upon Stimulation via Cyclic Stretch

**Hyoung Kyu Kim[1,2*], Nammi Park[1], Jubert Marquez[1], Amy Kim[1], Jin Han[1]**

[1] Cardiovascular and Metabolic Disease Center, Department of Health Sciences and Technology, Inje University, College of Medicine, Korea
[2] Department of Integrated Biomedical Science, Inje University, College of Medicine, Korea

*estrus74@gmail.com*

To assess the effect of mimetic cyclic stretch on mitochondria in a cardiac cell line, as mitochondria play an essential role in maintaining heart function by producing biological energy molecules.

Fabrication of polydimethylsiloxane (PMDS) substrates, culture and seeding of HL-1 cells, application of uniaxial strain, cell viability assay, real-time PCR, immunoblot analysis, fluorescence associated mitochondrial function analysis, intracellular ATP level, statistics

Cyclic stretch induced mitochondria-related gene and protein alteration as the expression of mitochondria biogenesis-related genes and mitochondria oxidative phosphorylation-related genes and respective protein levels were increased in the cyclic stretch stimulated cell lines as opposed to the non-stimulated controls. Consequently, cyclic stretch increased mitochondrial mass and ATP production in treated cells without mitochondrial stress. Our results suggest that cyclic stretch transcriptionally enhanced mitochondria biogenesis and oxidative phosphorylation without detrimental effects in the cultured cardiac cell line as stretch did not affect cell viability.

Cyclic stretch (10% elongation, 0.5 Hz, 4 h/day) increased mitochondrial biogenesis in the HL-1 cardiac cell line under cultured environment at the transcriptional level, inducing changes to a property closer to true cardiac cells. This system may prove especially useful for studying mitochondria biology in cardiac-like conditions.

PO-853

## Protective and Preservative Effect of NecroX-5 During Hypoxia/Reoxygenation Injury Through Mitochondrial Oxidative Phosphorylation and PGC1α Expression

**Thi Thu Vu[3], Maria Victoria Faith Garcia[1,2], Hyoung Kyu Kim[2,3], Jubert Marquez[2], Jin Han[2,3*]**

[1] Biology Department, De La Salle University Manila, Philippines
[2] Department of Health Science and Technology, Inje University, Korea
[3] College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Korea

*phyhanj@gmail.com*

To verify the role of NecroX-5 in protecting mitochondrial oxidative phosphorylation capacity during hypoxia-reoxygenation (HR)

Necrox-5 treatment (10 μM) and non-treatment were employed on isolated rat hearts during hypoxia/reoxygenation treatment using an ex vivo Langendorff system. Proteomic analysis was performed using liquid chromatography-mass spectrometry (LC-MS) and non-labeling peptide count protein quantification. Real-time PCR, western blot, citrate synthases and mitochondrial complex activity assays were then performed to assess heart function.

Treatment with NecroX-5 during hypoxia significantly preserved electron transport chain proteins involved in oxidative phosphorylation and metabolic functions. NecroX-5 also improved mitochondrial complex I, II, and V function. Additionally, markedly higher peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC1α) expression levels were observed in NecroX-5-treated rat hearts.

These novel results provide convincing evidence for the role of NecroX-5 in protecting mitochondrial oxidative phosphorylation capacity and in preserving PGC1α during cardiac HR injuries.

PO-854

## NecroX-5 Protects Hypoxia/Reoxygenation-Treated Rat Hearts via Modulation of the TNFα/Dcn/TGFβ1/Smad2 Pathway

**Trong Kha Pham[1*], Thu Vu Thi[1], Hyoung Kyu Kim[2], Jin Han[2]**

[1] Department of Physiology, Vietnam National University, Hanoi, Vietnam
[2] National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Inje University, Korea

*phamtrongkha@hus.edu.vn*

Ischemia-reperfusion injury is a major contributor to acute myocardial infarction associated with coronary artery disease. NecroX-5 compounds have

been shown to protect the liver and heart from this condition. However, the exact mechanism is still unknown. The aim of this study was to further define the role and mechanism of action of NecroX-5 in regulating inflammation and fibrosis responses in a model of hypoxia/reoxygenation.

We utilized hypoxia/reoxygenation -treated rat hearts and lipopolysaccharide (LPS)-treated H9C2 culture cells in the presence or absence of NecroX-5 (10 μmol/L) treatment as experimental models. TNFα/Dcn/TGFβ1/Smad2 – mediated signaling was assessed in whole hearts, isolated cardiomyocytes and H9C2 cells.

NecroX-5 signifi cantly increased Dcn expression levels in hypoxia/reoxygenation-treated hearts. In contrast, expression of TGFβ1 and Smad2 phosphorylation was strongly attenuated in NecroX-5-treated hearts. In addition, signifi cantly increased production of TNFα, TGFβ1, and pSmad2, and markedly decreased Dcn expression levels, were observed in LPS-stimulated H9C2 cells. Interestingly, NecroX-5 supplementation effectively attenuated the increased expression levels of TNFα, TGF1β, and pSmad2, as well as the decreased expression of Dcn.

The data highlight the potential anti-inflammatory and anti-fibrotic effects of NecroX-5 in acute cardiac injury, and these effects may ultimately prevent further pathological remodeling of heart and heart failure.

PO-856

# Reversal of Lung Emphysema by Endothelial Directed Attenuation of LRG-1 Signaling

**Alexandra Racanelli[1], Shu Hisata[1], Brisa Palikuqi[1], Balvir Kunar[1], Aiyuan Zhou[1], Pouneh Rabbany[1], Keith McConn[1], David Redmond[1], Ryan Schreiner[1], Bi-sen Ding[1], Joseph Scaundura[1], Fernando Martinez[1], Suzanne Cloonan[1], Shahin Rafii[1], Augustine Choi[1*]**

[1] Internal Medicine, Weill Cornell Medicine, USA
[2] Internal Medicine, Mount Sinai Medical Center, USA

*amc2056@med.cornell.edu*

Chronic obstructive pulmonary disease (COPD) is a disorder marked by airway occlusion and airspace enlargement (emphysema) leading to progressive airflow obstruction and clinical symptoms. Microvasculature abnormalities are features of COPD lungs and correlate with disease severity. Lung-specific endothelium coordinate propagation and behavior of adjacent parenchymal cells through the release of angiocrine factors. We hypothesized that maladaptive ECs are critical to the pathogenesis of COPD and that targeting lung EC biology has great therapeutic potential.

We performed in silico analyses on sequencing datasets from the Lung Genomics Research Consortium (LGRC) in COPD samples. We used an elastase murine model of COPD/emphysema to study ECs in the disease state. We isolated lung ECs from treated mice and performed RNA sequencing. Supervised gene ontology analyses identified key pathways. We utilized a mouse line in which Lrg1 (Lrg1iΔEC/iΔEC) was deleted in adult mouse ECs following injection with tamoxifen and exposed this line to our elastase model.

Analyses of the LGRC data sets identified loss of endothelial marks with increased emphysema. LRG1, a known glycoprotein that binds to the TGF-beta accessory receptor, was upregulated and correlated with severity of emphysema. In our elastase model, angiogenesis pathways were up-regulated in ECs from elastase treated mice. Additionally, LRG1 was also up-regulated. Furthermore, Lrg1iΔEC/iΔEC mice were protected from elastase-induced lung injury compared to controls.

We identified that in COPD samples there is a loss of key EC markers, suggesting that a maladaptive vascular niche helps drive disease. LRG1 was found to be elevated in both COPD and elastase-treated tissue and loss of EC Lrg1 perturbs the development of emphysema in the elastase model. Hence, LRG1 is a critical factor involved in promoting the development of the maladaptive vascular niche observed in the disease state of emphysema.

PO-857

# The Role of IGF-1 Signaling in Injury-Induced Cardiac Fibrosis

**Sangmi Ock[1], Woo Jin Ham[1], Chae Won Kang[1], Wang Soo Lee[1], Jaetaek Kim[1*]**

[1] Department of Internal Medicine, College of Medicine, Chung-Ang University, Korea

*jtkim@cau.ac.kr*

In the heart, IGF-1 regulates cardiomyocyte hypertrophy, proliferation, metabolism, and protection from cell death. After injury, fibroblasts transdifferentiate into contractile myofibroblasts for tissue remodeling. However, the role of IGF-1 signaling in cardiac fibrosis has not been directly evaluated in vivo.

To examine the mechanisms linking IGF-1 signaling to cardiac fibrosis, we generated fibroblast-restricted IGF-1R deficient mice.

Mice deficient fibroblast-specific IGF-1R exhibited enhanced cardiac interstitial fibrosis and significantly more severe cardiac dysfunction following 7-day angiotensin II/phenylephrine (Ang II/PE) infusion compared to wild-type mice. Ang II/PE increased TGF-β signaling, as shown by the increased phosphorylation of SMAD2, an activating SMAD, while Ang II/PE–induced SMAD2 activation was markedly accelerated in fibroblast-specific IGF-1R KO mice. In vitro studies with adult primary rat cardiac fibroblasts revealed that Ang II induced the expression of α-SMA, which was completely blocked by co-incubation with IGF-1.

These results demonstrate IGF-1 negatively regulates cardiac fibrosis by attenuating Ang II induced transdifferentiation of fibroblasts to myofibroblasts and may represent a novel therapeutic approach against fibrotic cardiac remodeling.

PO-858

# Sphingosine Kinase 2 Inhibition Mitigates Psoriasis-Like Skin Symptom

**Joo-Won Park**[1*]

[1] Department of Biochemistry/College of Medicine, Ewha Womans University, Korea

*joowon.park@ewha.ac.kr*

Sphingosine-1-phosphate (S1P) functions as an important bioactive signalling molecule in cell processes, including cell proliferation and apoptosis. We examined the potency of sphingosine kinase inhibition using an imiquimod-induced psoriasis mouse model.

To induce psoriasis-like skin inflammation, mice were treated once daily with a topical dose of 83 mg of imiquimod cream applied to the shaved back region for six consecutive days.

Topical sphingosine kinase 1/2 inhibition, which blocks S1P generation, alleviated imiquimod-induced skin lesions and reduced the serum interleukin 17-A levels induced by application of imiquimod. These treatments also normalized skin mRNA levels of genes associated with inflammation and keratinocyte differentiation. Inhibition of sphingosine kinase 2, but not sphingosine kinase 1, diminished levels of suppressor of cytokine signalling 1 and blocked T helper type 17 differentiation of naïve CD4+ T cells; imiquimod-induced psoriasis-like skin symptoms were also ameliorated.

These results indicate the distinct effects of sphingosine kinase 1 and sphingosine kinase 2 inhibition on T helper type 17 generation and suggest molecules that inhibit S1P formation, including ceramidase and sphingosine kinase 2 inhibitors, as novel therapeutic candidates for psoriasis.

# The 21st International Vascular Biology Meeting

in conjunction with the 9th International Congress on Lipid and Atherosclerosis (ICoLA) &
the 5th Annual Meeting of the Korean Society for Vascular Biology and Medicine (KVBM)

# Author Index

September 9 (Wed) ~ October 10 (Sat), 2020 I Virtual Meeting (On demand)
September 9 (Wed) ~ 12 (Sat), 2020 I IVBM 2020 LIVE for Korean

KSoLA
The Korean Society of Lipid and Atherosclerosis

KVBM
Korean Society for Vascular Biology and Medicine

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# AUTHOR INDEX

# SPONSORS

## Platinum Sponsor

MSD
JW Pharmaceutical
YUHAN
DAEWOONG www.daewoong.co.kr

## Gold Sponsor

AstraZeneca
Hanmi Hanmi Pharm. Co., Ltd.
AMGEN
Takeda
Chong Kun Dang Pharm. Seoul, Korea
Pfizer 한국화이자업존(주)
SERVIER 한국세르비에(주)
Boehringer Ingelheim
Lilly
SANOFI

## Basic Sponsor

IVIM TECHNOLOGY
NOVARTIS
THE CURACLE 주식회사 큐라클
ThermoFisher SCIENTIFIC

## Additional Sponsor

KIIT Kangwon Institute of Inclusive Technology
ibs Institute for Basic Science
National Creative Initiatives Center for Immune and Vascular Cell Network, Ewha Womans University, National Research Foundation of Korea
인하대학교의과대학 저산소표적질환연구센터 HDRC Inha University School of Medicine Hypoxia-related Disease Research Center
KRIBB Korea Research Institute of Bioscience & Biotechnology
한림제약주식회사
BAYER Bayer
건강노화한의과학연구센터 HEALTHY AGING KOREAN MEDICAL RESEARCH CENTER
MERCK
iNtRON BIOTECHNOLOGY
KOREA BIOMICS (주)코리아바이오믹스
CUREBIO CARE & CURE
AbelBio Research & Sales
Jung Jin Int'l Intellectual Property Law Firm
BMS
DREAMCELL
GCTRC Gene & Cell Therapy Research Center for Vessel-associated Diseases
스마트에이징융복합연구센터 Smart-aging Convergence Research Center
BK21 Brain Korea21
DAWINBIO TECH

IVBM 2020

The 21st International Vascular Biology Meeting

September 9-12, 2020 ∥ Conrad Hotel, Seoul, Korea


## Secretariat of IVBM 2020

**A** 1002 Botanic Park Tower, Magok-joongang-ro 161-17, Ganseo-gu, Seoul, Korea

**T** +82-2-2135-3617, 3618, 3621

Scientific Program: +82-2-2135-3618 Registration: +82-2-6263-2054 Sponsorship & Exhibition: +82-2-576-9945

**E** office@ivbm2020.org **W** www.ivbm2020.org