How to Activate Vascular Endothelial Growth Factor-C

Sawan Kumar Jha[1], Khushbu Rauniyar[1], Terhi Karpanen[4], Veli-Matti Leppänen[1,2], Pascal Brouillard[5],
Miikka Vikkula[5,6], Kari Alitalo[1,2,3], Michael Jeltsch[1,2]

[1]Translational Cancer Biology Research Program, [2]Wihuri Research Institute, Biomedicum Helsinki, University of Helsinki, Finland
[3]Helsinki University Central Hospital, Helsinki, Finland, [4]Institute for Clinical Medicine, University of Oslo, Oslo, Norway
[5]de Duve Institute, University of Louvain, Brussels, Belgium, [6]Walloon Excellence in Life Sciences and Biotechnology (WELBIO), University of Louvain, Brussels, Belgium

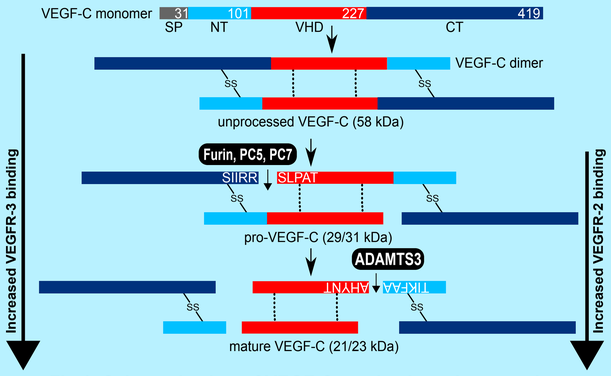

Fig 1: Schematic view of VEGF-C synthesis and processing

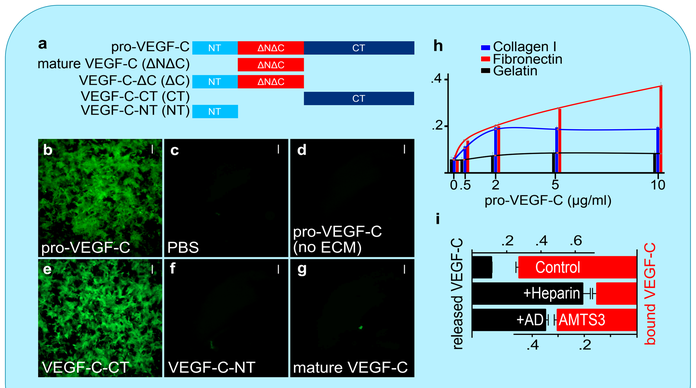

Fig 2: pro-VEGF-C binds to extracellular matrix via its C-terminal domain

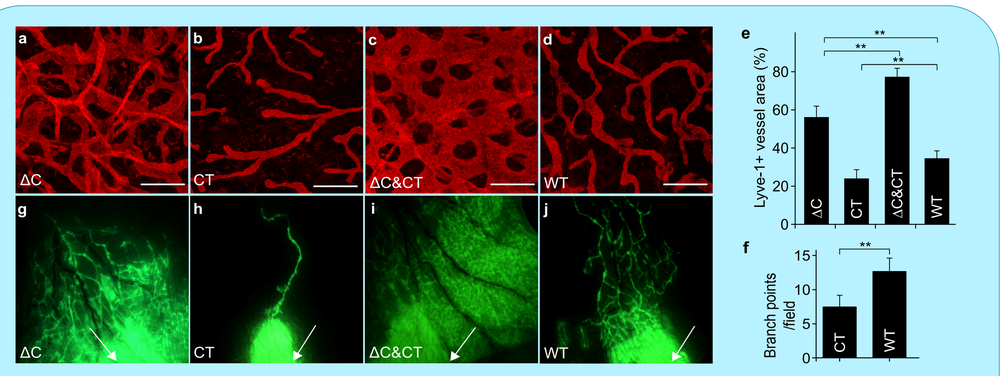

Fig 3: The C-terminus of VEGF-C enhances the lymphangiogenic response to VEGF-Clacking the C-terminus, but it represses lymphangiogenesis on its own

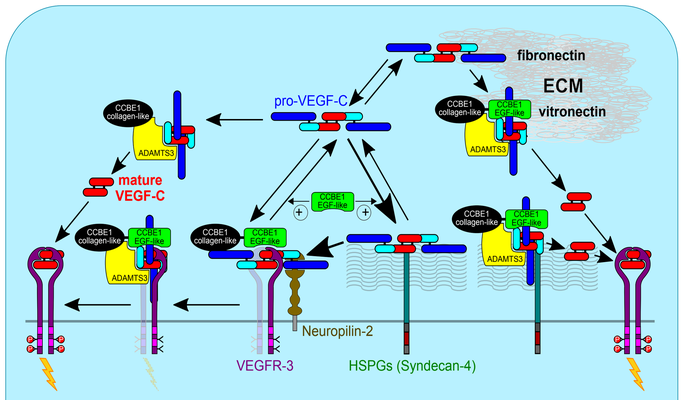

Fig 8: Schematic view of VEGF-C activation based on current experimental evidence

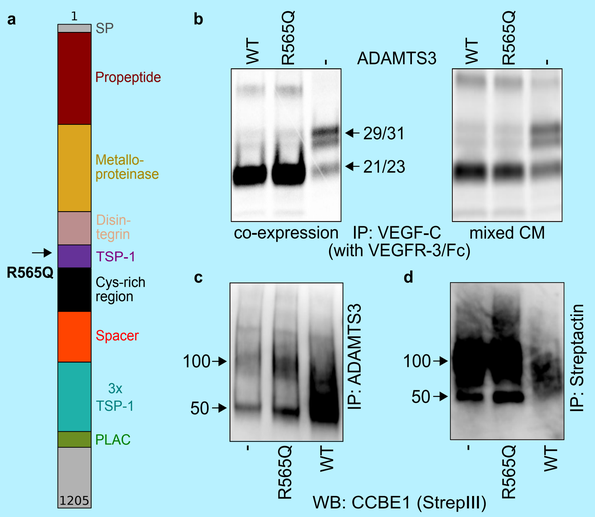

Fig 7: The R565Q variant in ADAMTS3 interferes with the interaction of ADAMTS3 with CCBE1 and increases the amount of free CCBE1

michael@jeltsch.org
http://jeltsch.org
http://lab.jeltsch.org

K. Albin Johanssons Stiftelse
Cancer Society of Finland
German Society of Lymphology
JANE AND AATOS ERKKO FOUNDATION
ACADEMY OF FINLAND

The collagen- and calcium-binding EGF domains 1 (CCBE1) protein has been shown to be necessary for the activation of vascular endothelial growth factor-C (VEGF-C) and thus for lymphangiogenesis. In previous studies the N- and C-terminal domains of CCBE1 enhanced lymphangiogenesis independently from each other. The VEGF-C activation-enhancing function was contained in CCBE1's C-terminal domain, while it remained unclear how its N-terminal domain contributed to increased VEGFR-3 signaling. Because ADAMTS3-deleted mice showed a complete lack of lymphangiogenesis, it also remained unknown whether other proteases besides ADAMTS3 could activate VEGF-C in a physiologic context.

Here, we show that efficient activation of VEGF-C requires both the C-terminal domain of VEGF-C and the N-terminal domain of CCBE1. The N-terminal domain of CCBE1 increased VEGFR-3 signaling by colocalizing pro-VEGF-C with its activating protease and the lymphatic endothelial cell surface. In assays where ADAMTS3 amounts are limited, proteolytic activation of pro-VEGF-C required colocalization, which was mediated by the N-terminal domain of CCBE1. Interestingly, abnormal localization of CCBE1 was also associated with a variant of ADAMTS3, which we found in a lymphedema patient. We identified alternative proteases which are capable of activating VEGF-C. Among these, KLK3, aka prostate specific antigen (PSA), specifically and efficiently activated VEGF-C. KLK3-mediated VEGF-C cleavage was, similar to ADAMTS3, enhanced by CCBE1. Such alternative activation of VEGF-C was observed in seminal plasma, and exposure of sperm cells to mature VEGF-C resulted in a significant increase in sperm mobility.

These results show that CCBE1 promotes VEGFR-3 signaling and lymphangiogenesis by two different mechanisms, which are carried out independently by the two domains of CCBE1: by enhancing the cleavage activity of VEGF-C-activating proteases and by mediating the colocalization of substate and protease. These new insights should be valuable in developing new strategies to therapeutically target VEGF-C/VEGFR-3-induced signaling. In addition, the cleavage of VEGF-C by the major seminal protease KLK3, the significant amounts of VEGF-C in seminal plasma and the increased sperm motility in response to mature VEGF-C argue for a role of VEGF-C in reproductive biology.

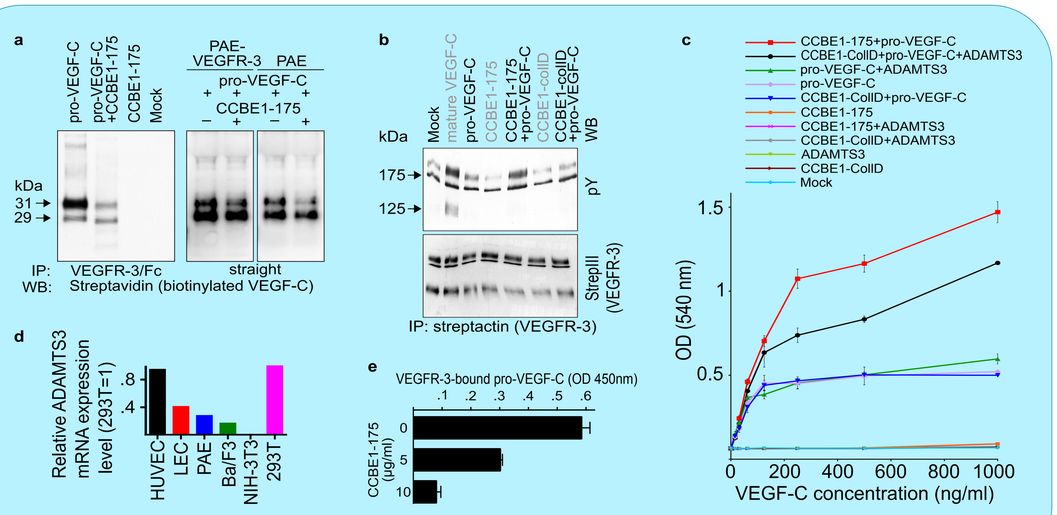

Fig 6: The N-terminal domain of CCBE1 affects the distribution and activity of pro-VEGF-C in cell-based assays

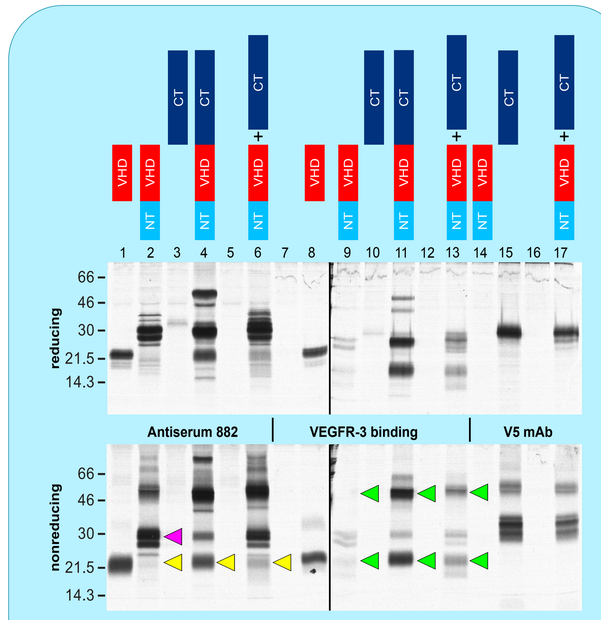

Fig 4: The VEGF-C C-terminus rescues activation and receptor binding of VEGF-C- ΔC *in vitro*

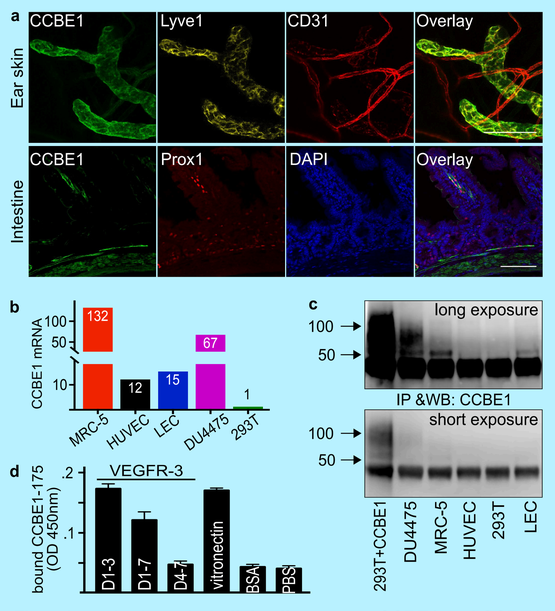

Fig 5: Lymphatic endothelial cells express CCBE1 and CCBE1 localizes to lymphatic endothelial cell surfaces