

## Prostate Cancer

# Systematic Identification of MicroRNAs That Impact on Proliferation of Prostate Cancer Cells and Display Changed Expression in Tumor Tissue

Anna Aakula<sup>a,b,c,\*</sup>, Pekka Kohonen<sup>b,d</sup>, Suvi-Katri Leivonen<sup>b,e</sup>, Rami Mäkelä<sup>b,f</sup>,  
Petteri Hintsanen<sup>a</sup>, John Patrick Mpindi<sup>a,b</sup>, Elena Martens-Uzunova<sup>g</sup>, Tero Aittokallio<sup>a</sup>,  
Guido Jenster<sup>g</sup>, Merja Perälä<sup>b,h</sup>, Olli Kallioniemi<sup>a,b</sup>, Päivi Östling<sup>a,b</sup>

<sup>a</sup>Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland; <sup>b</sup>VTT Technical Research Centre of Finland, Medical Biotechnology, Turku, Finland<sup>1</sup>; <sup>c</sup>Turku Centre for Biotechnology, University of Turku, Turku, Finland<sup>1</sup>; <sup>d</sup>Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden; <sup>e</sup>Genome-Scale Biology Research Program, University of Helsinki, Helsinki, Finland; <sup>f</sup>Misvik Biology Corporation, Turku, Finland; <sup>g</sup>Department of Urology, Erasmus MC, Rotterdam, The Netherlands; <sup>h</sup>Natural Resources Institute Finland (Luke), Helsinki, Finland

## Article info

## Article history:

Accepted September 14, 2015

## Associate Editor:

James Catto

## Keywords:

Functional high-throughput  
screening  
MicroRNA  
Prostate cancer  
Reverse-phase protein array

## Abstract

**Background:** Systematic approaches to functionally identify key players in microRNA (miRNA)-target networks regulating prostate cancer (PCa) proliferation are still missing.

**Objective:** To comprehensively map miRNA regulation of genes relevant for PCa proliferation through phenotypic screening and tumor expression data.

**Design, setting, and participants:** Gain-of-function screening with 1129 miRNA molecules was performed in five PCa cell lines, measuring proliferation, viability, and apoptosis. These results were integrated with changes in miRNA expression from two cohorts of human PCa (188 tumors in total). For resulting miRNAs, the predicted targets were collected and analyzed for patterns with gene set enrichment analysis, and for their association with biochemical recurrence free survival.

**Outcome measurements and statistical analysis:** Rank product statistical analysis was used to evaluate miRNA effects in phenotypic screening and for expression differences in the prostate tumor cohorts. Expression data were analyzed using the significance analysis of microarrays (SAM) method and the patient material was subjected to Kaplan-Meier statistics.

**Results and limitations:** Functional screening identified 25 miRNAs increasing and 48 miRNAs decreasing cell viability. Data integration resulted in 14 miRNAs, with aberrant expression and effect on proliferation. These miRNAs are predicted to regulate >3700 genes, of which 28 were found up-regulated and 127 down-regulated in PCa compared with benign tissue. Seven genes, *FLNC*, *MSRB3*, *PARVA*, *PCDH7*, *PRNP*, *RAB34*, and *SORBS1*, showed an inverse association to their predicted miRNA, and were identified to significantly correlate with biochemical recurrence free survival in PCa patients.

**Conclusions:** A systematic in vitro screening approach combined with in vivo expression and gene set enrichment analysis provide unbiased means for revealing novel miRNA-target links, possibly driving the oncogenic processes in PCa.

**Patient summary:** This study identified novel regulatory molecules, which impact on PCa proliferation and are aberrantly expressed in clinical tumors. Thus, our study reveals regulatory nodes with potential for therapy.

© 2015 European Association of Urology. Published by Elsevier B.V. All rights reserved.

<sup>1</sup> Previous affiliation.

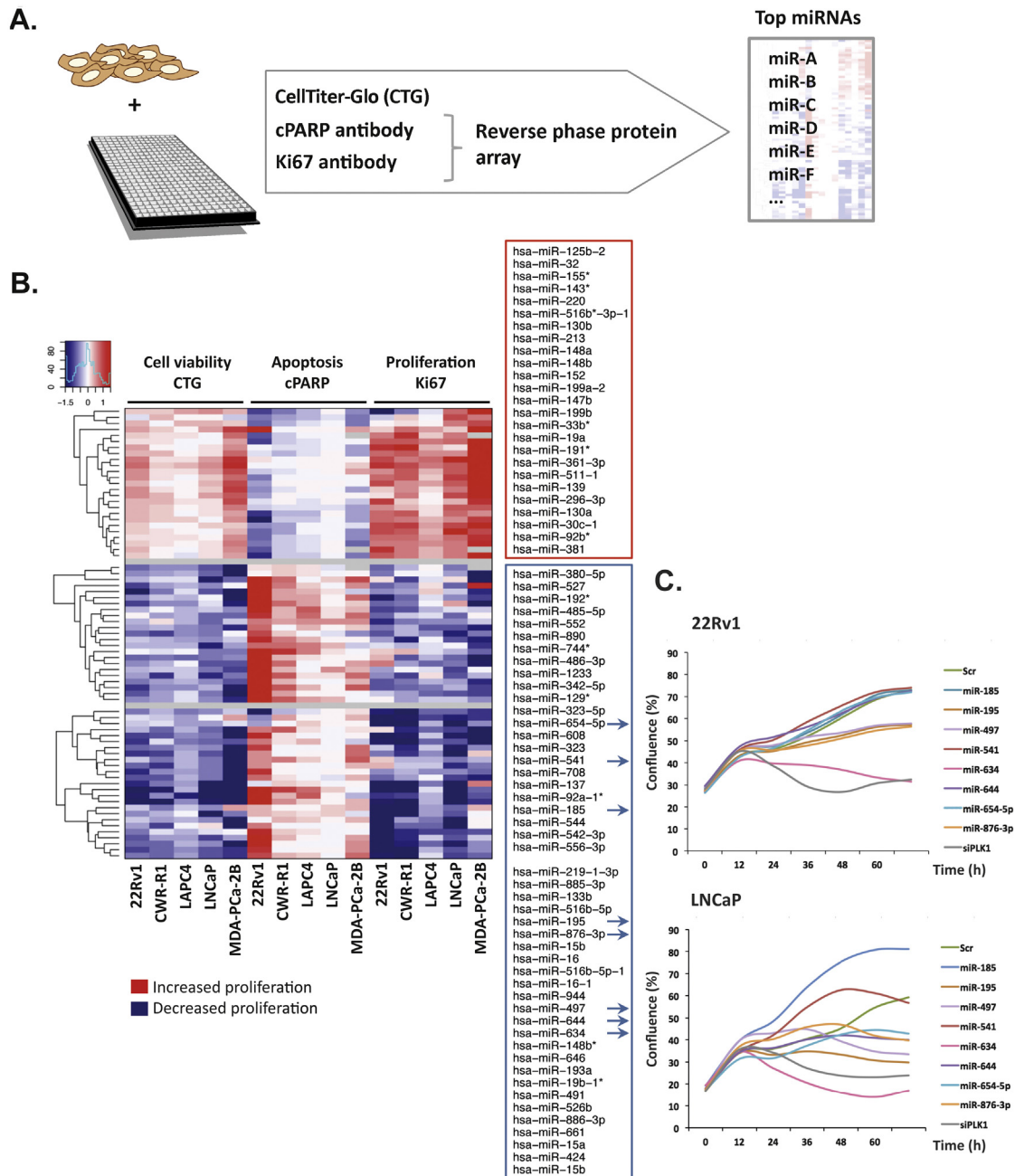
\* Corresponding author. Institute for Molecular Medicine Finland (FIMM), Nordic EMBL Partnership for Molecular Medicine, Biomedicum Helsinki 2U (D301b), P.O. Box 20 (Tukholmankatu 8), FI-00014 Helsinki, Finland. Tel. +358 400828256.  
E-mail address: [anna.aakula@fimm.fi](mailto:anna.aakula@fimm.fi) (A. Aakula).

1. Introduction

There is an urgent need to widen our understanding of prostate cancer (PCa) proliferation and signaling mechanisms beyond androgen regulation. The cost of PCa

treatment is a significant burden to our society and despite the recent advent of many new drugs, the metastatic progression of PCa remains a clinical challenge [1–4].

MicroRNAs (miRNAs) have the ability to target many genes, also within the same pathway, making them central



**Fig. 1 – Functional microRNA (miRNA) screen for identification of effect on prostate cancer (PCa) cell proliferation.** (A) PCa cell lines were reverse-transfected with 1129 precursor miRNA molecules in 384-well plates for 72 h. CellTiter-glo (CTG) luminescence measure for adenosine triphosphate-content of cells was measured directly from plates, whereas for reverse-phase protein array analyses cells in parallel plates were lysed and printed onto nitrocellulose covered glass slides. Ki67 and cPARP protein levels were measured with specific antibodies and general protein content of lysates with Sypro Ruby stain. Hits were analyzed as previously described by ranking (false discovery rate q-value < 0.01) [6]. (B) Heat map displaying all top ranking miRNAs having parallel effects on CTG and Ki67 and opposing effects on cPARP. Top box denotes 25 miRNAs increasing cell growth and bottom box 48 miRNAs decreasing cell growth. (C) Eight of the miRNAs decreasing proliferation (marked with blue arrows in B) were validated by following the effect on growth in Incucyte ZOOM live content imager, where confluence was imaged every hour. Cells were transiently transfected using human miRNA precursors and a pre-miR negative control (Scr), as well as with siPLK1 as positive control. The cells were grown on 24-well plates. Growth curves are representative growth curves, of the average of three independent experiments.

regulatory nodes. In PCa, altered miRNA regulation has been shown to contribute to progression and endocrine resistance [5]. We have previously identified 13 miRNAs targeting the androgen receptor (AR) [6] and shown that miR-183, regulates the diagnostic biomarker prostate-specific antigen (PSA) [7]. Aberrant miRNA expression and specific miRNA signatures have been identified by many groups, demonstrating the involvement of miRNAs in the pathophysiology of PCa [8–11]. High-throughput approaches to assess miRNA function have, however, remained less common.

Here, we combined systematic functional screening with in vivo expression to identify key players of PCa proliferation that could be clinically valuable. Our study identified 73 miRNAs with an effect on the growth of PCa cells and characterized the potential miRNA-target network with aberrant expression in tumor samples. MiR-19a, miR-32, miR-124a, miR-130b, miR-148a, and miR-583 were identified as potential regulators of *FLNC*, *MSRB3*, *PARVA*, *PCDH7*, *PRNP*, *RAB34*, and *SORBS1*. The aberrant expression of these genes was significantly associated with biochemical recurrence free survival of 140 PCa patients.

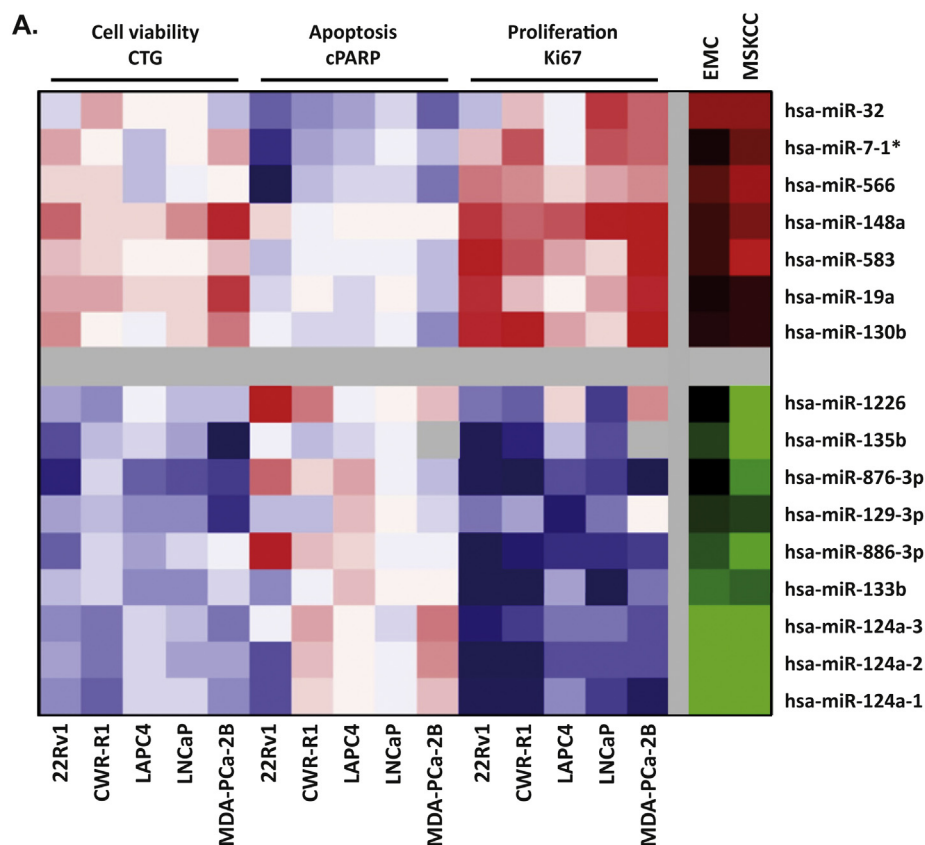
## 2. Materials and methods

### 2.1. Cell culture

Cells were cultured according to the provider's recommendations and as previously described, for less than 4 mo prior to experiments [6].

### 2.2. Functional screening and data analysis

Functional screening using well-based viability and reverse-phase protein array readouts were performed as previously described [6,12]. Briefly, the cells were reverse-transfected with 20 nM human pre-miR miRNA Precursor library v2 (Ambion Inc., Austin, TX, USA, 319 molecules) and miRIDIAN microRNA Mimic library v10.1, (Dharmacon, CO, USA, 810 molecules). Viability was measured using CellTiter-Glo (CTG) (Promega Corp, Madison, WI, USA). Ki67 and cPARP protein levels were detected with primary antibodies (no. M7240, Dako, Glostrup, Denmark and no. ab32064, E51 Abcam, Cambridge, UK, respectively). Raw data were normalized and analyzed as previously described, false discovery rate (FDR) q-value cut-off <0.01 [6]. Eight miRNAs were validated using Incucyte ZOOM live cell imaging upon miRNA overexpression, as previously described in [23].



**Fig. 2 – Integration of microRNA (miRNA) effect on proliferation with prostate tumor miRNA expression.** To integrate miRNAs affecting proliferation with miRNAs having changed expression in tumor cells versus normal cells, we combined miRNA expression with our functional read-outs (CTG, cPARP, and Ki67). On both expression data sets, Erasmus University Medical Center (EMC) [14] and Memorial Sloan Kettering Cancer Center (MSKCC) [15], we performed significance analysis of microarrays, after which the fold changes were ranked [false discovery rate (FDR) q-value < 0.05]. Also, the screening results were ranked (FDR q-value < 0.1, maximum permutation-based *p*-value < 0.0124), after which the ranks from each data type were combined (FDR q-value < 0.05). Red and blue colors (left panel) denote increased and decreased proliferation, respectively. The right most panel displays expression values. Red and green denotes increased and decreased expression in primary prostate tumors compared with benign tissue, respectively (EMC; PCa *n* = 50 and normal *n* = 11, and MSKCC; PCa *n* = 99 and normal *n* = 28).

### 2.3. Integration of miRNA functional screening with tumor miRNA expression analyses

First, the screening results were ranked by rank product  $q$ -value  $<0.1$ , using 100 rounds of gene-row/sample-column permutations [13]. In this analysis, unidirectional statistical testing was carried out after the signs for cPARP measurements were reversed ( $x' = -1 \cdot x$ ). This approach was used to find miRNAs having parallel effects on CTG and Ki67, with opposing effects on cPARP in all five PCa cell lines.

The miRNA expression data were obtained from: (1) Erasmus University Medical Center (EMC; primary PCa  $n = 50$ , normal  $n = 11$ ) [14]; and (2) Memorial Sloan Kettering Cancer Center (MSKCC; primary PCa  $n = 99$ , normal  $n = 28$ ) [15]. These have previously been published in the ArrayExpress database with accession number E-TABM-794 and in the Gene Expression Omnibus with accession number GSE21032/GSE21036, respectively. The expression values underwent quantile normalization (using the R/Bioconductor limma package) and subsequently significance analysis of microarrays to define miRNAs with similar expression differences between tumor and normal in both data sets. Permutation based significance analysis, using sample-level permutations, was carried out within blocks defined by each study, FDR cut-off  $<0.05$ .

For integration, functional screening data was combined with the miRNA expression data using 100 permutation replicates and with an FDR  $q$ -value cut-off  $<0.05$ . In combining the results to produce a single list of differentially expressed and functionally active miRNAs, each individual cut-off was applied together (ie, each miRNA was required to pass three cut-offs to be selected for further characterization).

### 2.4. Expression analyses of predicted miRNA targets in prostate tumor material

A list, of  $>3700$  predicted miRNA target genes, was collected using TargetScan 5.2 for the 14 miRNAs from the integration analysis. The predicted target genes were ranked according to their differential expression ratio in cancer versus normal ( $n = 127$ ) [15]. Next, gene set enrichment analysis (GSEA) (MSigDB, C2, C5, and C6 gene sets, v4.02, Broad Institute, Cambridge, MO, USA) was performed on aberrantly expressed target genes to identify the most significantly enriched and depleted mRNA signatures, at a FDR  $q$ -value cut-off  $<0.01$ . The GSEA identified genes were further analyzed using Gene Ontology database (released June 06, 2015).

### 2.5. Kaplan-Meier analyses to identify the clinically relevant miRNA targets

To assess the clinically relevant miRNA-target data, biochemical relapse free survival data on 140 samples from MSKCC was downloaded from cBioPortal and survival analysis was performed with R statistical software [15]. For each target/miRNA the data was stratified into two groups based on the 50th quartile. Levels of statistical significance were set at log-rank  $p < 0.05$ .

## 3. Results

### 3.1. Gain-of-function screening identifies miRNAs impacting on PCa cell proliferation

To systematically analyze the effect of miRNA overexpression on the viability and proliferation, functional screening using a total of 1129 miRNA mimics in five PCa cell lines (22Rv1, CWR-1R, LAPC4, LNCaP, MDA-PCa-2b) was performed (Fig. 1A). Our analysis focused on finding miRNAs, which inhibited cell viability (CTG), and proliferation (Ki67),

and induced apoptosis (cPARP) in all cell lines. The results showed 25 miRNAs which increased, and 48 miRNAs which decreased cell growth (Fig. 1B). The rank of each miRNA are shown in Supplementary Table 1. From this, it is evident that miR-634 was the most efficient in decreasing proliferation and miR-148a/b in increasing proliferation. Eight growth decreasing miRNAs were validated, by measuring the confluence of the cells every hour post-miRNA overexpression in 22Rv1 and LNCaP cells. MiR-634 was most effective, but also miR-195, miR-497, and miR-876-3p reduced growth in both cell lines (Fig. 1C). Furthermore, miR-644 and miR-654-5p affected growth of LNCaP cells.

### 3.2. Integration of miRNAs influencing proliferation with miRNA expression in PCa tumor tissue

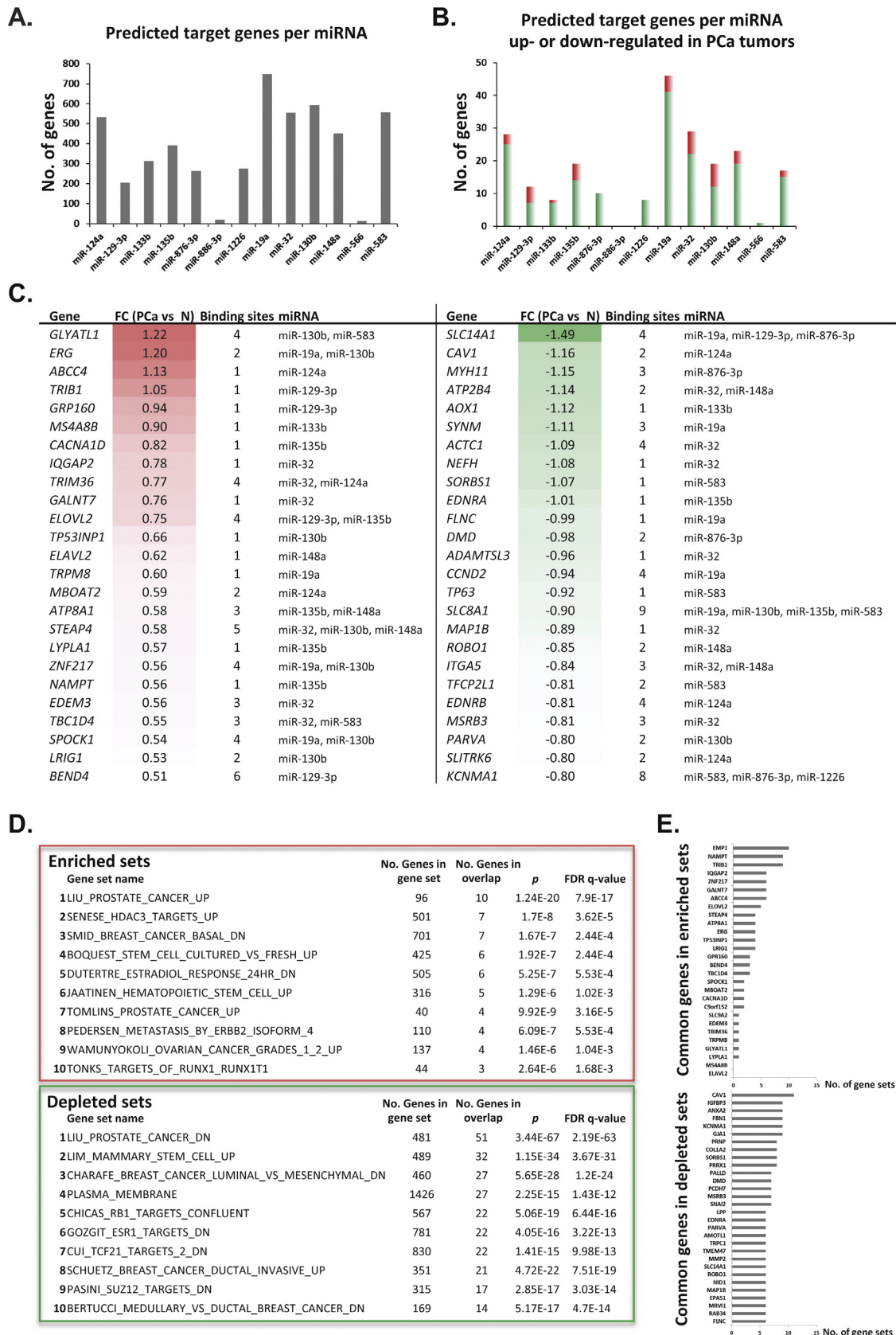
To identify the potentially clinically relevant miRNAs from our functional screen, these results were integrated with miRNA expression data from PCa tumors. Our hypothesis was that miRNAs increasing cell proliferation would be up-regulated in PCa tumors, whereas miRNAs decreasing proliferation would be down-regulated in tumor tissue. Tumor miRNA expression data were obtained from two publicly available datasets, EMC [14] and MSKCC [15], from which primary PCa tumors were compared with benign prostate tissue. A summary of the clinical characteristics for the study cohorts is available in Supplementary Table 2. Integration of functional screening and miRNA expression was done based on rank (Supplementary Table 3). The integration resulted in 14 miRNAs (unique sequences), miR-7, miR-19a, miR-32, miR-124a, miR-129-3p, miR-130b, miR-133b, miR-135b, miR-148a, miR-556, miR-583, miR-876-3p, miR-886, and miR-1226 that influence proliferation of PCa cells and display the expected change in expression in tumors (Fig. 2).

### 3.3. Expression analyses to find relevant miRNA targets

To study how and through which pathways these 14 miRNAs regulate proliferation we analyzed their potential target gene expression in cell lines and tumor samples. First, to identify putative targets we used the target prediction algorithm TargetScan and listed the  $>3700$  predicted target genes (Supplementary Table 4). Figure 3A shows the overall number of predicted gene targets per miRNA. Next, we calculated the mRNA fold change of these targets in primary PCa tumors compared with benign tissue (right most panel, Supplementary Table 4) [15]. Figure 3B shows the number of the potential target genes with a changed expression per miRNA where, the red or green color indicates up-regulation or down-regulation, respectively. Figure 3C lists the top changers for the 14 miRNAs.

Expression fold change analysis was furthermore carried out in cell lines, (Supplementary Table 5 and Supplementary Figure 1). The overlap of putative target genes with a changed expression in cell lines and tumors is limited. Only 15 of the genes were the same and thus, we continued our studies with the genes showing an altered expression in PCa patient samples.





**Fig. 3 – Expression analyses to find relevant microRNA (miRNA) target genes.** To identify the relevance of the miRNAs influencing proliferation in cell lines and with a changed expression in prostate cancer (PCA) samples, we searched for their potential target genes. TargetScan was utilized to predict potential target genes for the top 14 miRNAs identified by integration. (A) The number of predicted target genes per miRNA is displayed in the graph. (B) The number of predicted target genes that also are differentially expressed (up in red, and down in green) in PCA patient samples (Memorial Sloan Kettering Cancer Center) [15]. (C) The top genes with a changed expression [15]. FC (PCA vs N) denotes the expression change, where green

In order to elucidate the function of the aberrantly expressed genes and to focus on the most prominent expression patterns, we performed GSEA. We identified >100 negatively enriched gene sets, and 20 positively enriched genes sets, and the top 10 of these gene sets are shown in Figure 3D. The most commonly identified genes (58) in the gene sets are presented in Figure 3E. Gene Ontology analysis identified 19 genes linked to localization regulation, 29 genes localized to the cell periphery, and 12 genes to cytoskeletal binding.

### 3.4. The impact of the top recognized genes on biochemical relapse free survival

Next, to explore the clinical value of the identified miRNA-gene pairs, we performed Kaplan-Meier analyses of the top genes that were shared between the gene sets identified by GSEA (Supplementary Table 6). To give a comprehensive view of the connections with putative clinical value, arising from our integrated analysis and the expression of their predicted targets, we visualized them in a network (Fig. 4A). Triangles display miRNAs with changed expression, whereas red or green squares denote increased or decreased target mRNA expression, respectively. MiR-7-1, miR-566, miR-886-3p, and miR-1226 are not included in the network because they were predicted to target only a few mRNAs with a changed expression.

A highly significant ( $p < 0.01$ ) link to biochemical relapse free survival was observed for nine genes: filamin C gamma (*FLNC*), methionine sulfoxide reductase B3 (*MSRB3*), parvin alpha (*PARVA*), protocadherin 7 (*PCDH7*), prion protein (*PRNP*), RAB34 member RAS oncogene family (*RAB34*), and sorbin SH3 domain containing 1 (*SORBS1*), solute carrier family 14, member 1 (*SLC14A1*), and snail family zinc finger 2 (*SNAI2*). Seven of these genes are negatively associated with their putative miRNA regulator (Fig. 4B). Furthermore, we analyzed the 14 miRNAs for their effect on the time to PSA increase or biochemical relapse. MiR-130b was the only miRNA with a significant association with survival ( $p = 0.004$ ) (Fig. 4C). Thus, miR-130b and its putative target *PARVA* may form a pair that regulates key biological processes that associate with aggressive disease as evident from biochemical relapse free survival in PCa patients.

## 4. Discussion

Here we performed miRNA high-throughput overexpression screening in PCa cell lines and miRNA expression analyses in clinical PCa tumors. We studied the relationship of miRNAs to aberrantly expressed predicted target genes, as well as analyzed their association with biochemical relapse free survival of patients.

We identified altogether 73 miRNAs that either enhanced or inhibited growth in all the five PCa cell lines

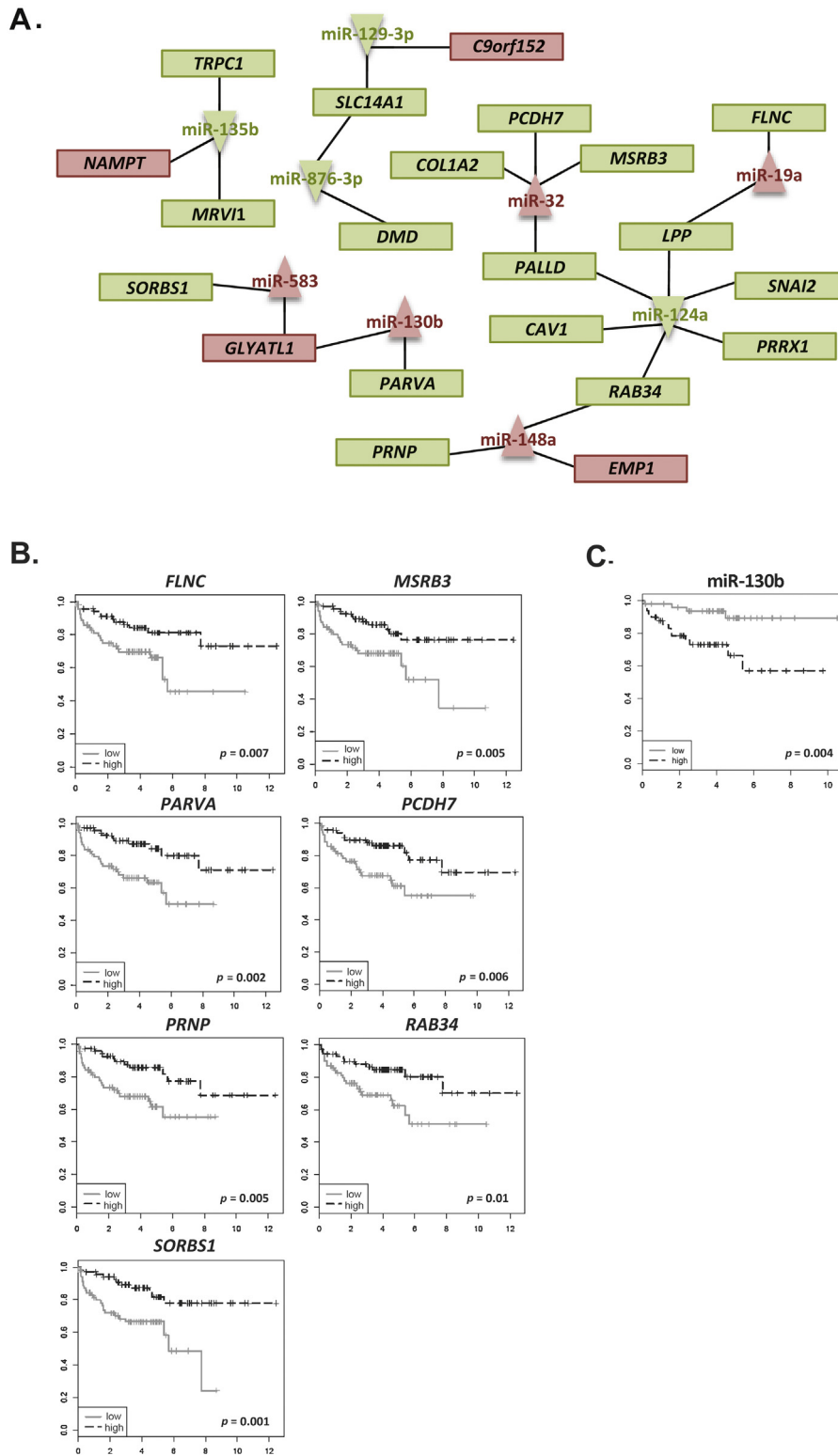
screened. Seven miRNAs, miR-7, miR-19a, miR-32, 130b, miR-148a, miR-566, and miR-583 increased the growth in cell lines and were up-regulated in PCa samples, whereas seven miRNAs miR-124a, miR-129, miR-133b, miR-135b, miR-876, miR-886, and miR-1226 decreased the growth in our functional screens and had a lower expression in tumor than in benign tissue.

To the best of our knowledge, only a few of these miRNAs have been more extensively studied in PCa. MiR-19a, part of the miR-17-92 cluster, has been shown to be directly regulated by AR binding, enhance viability of dihydrotestosterone-treated LNCaP cells, and be elevated in high-risk PCa compared with healthy samples [16,17]. These results nicely corroborate with our observation. Similarly, miR-32 is AR-regulated and enhances growth of LNCaP cells when overexpressed [18]. MiR-148a is an androgen-responsive miRNA attenuating paclitaxel resistance in hormone refractory PCa [19,20]. MiR-124 has recently been identified to reduce migration in PCa cells [21]. Equally, miR-133b expression has been identified low in DU145 and PC3 cells, where ectopic expression inhibited proliferation [22]. MiR-135b affects the growth of AR+ PCa cells and ER $\alpha$ + breast cancer (BCa) cells when overexpressed, and directly regulates AR and ER $\alpha$  in PCa and BCa cells respectively, as well as HIF1AN in PCa and BCa [6,23].

The putative target gene list for the 14 miRNAs contained >3700 genes. The mere presence of miRNA-binding sites is insufficient for predicting target regulation, and therefore we analyzed the expression of the identified genes in cell lines and prostate tumors. The limited overlap between differently expressed target genes in cell lines and tumors, compared with the control, emphasizes the importance of expression data interpretation, also highlighted by Domcke et al [24]. By GSEA, we aimed to further focus the list to the most relevant pattern of expression and tissue identity was confirmed by these analyses. Network visualization was used to display the links between the predicted targets enriched through GSEA and viability-influencing miRNAs. These genes were analyzed for their effect on the time to PSA increase, and patients were more prone to biochemical recurrence when seven genes, (*FLNC*, *MSRB3*, *PARVA*, *PCDH7*, *PRNP*, *RAB34*, *SORBS1*) also negatively associated with their miRNA, were down-regulated. Of these, *MSRB3* and *PCDH7* for example, have been identified to inhibit proliferation, and are thought to function in cell-cell recognition and adhesion, respectively [25,26]. *FLNC* expression has been shown to be decreased in PCa compared with normal cells [27]. Underexpression of *SORBS1*, involved in actin stress fiber and focal adhesion formation, has been identified in another PCa cohort, further implicating a role in PCa development and progression [28].

Our analyses identified a novel interesting miRNA-target pair; miR-130b and *PARVA*. MiR-130b promotes proliferation in the studied cell lines. Moreover, its expression is elevated in tumors, whereas its predicted target *PARVA* is

indicates decreased expression whereas red indicates increased expression. The number of predicted miRNA binding sites and the miRNAs that are predicted to regulate the genes according to TargetScan are also indicated. (D) The predicted miRNA regulated genes were subjected to gene set enrichment analysis (*MSigDB*, C2, C5, and C6 gene sets, v4.02, Broad Institute) that identified overlapping genes in gene sets at a false discovery rate q-value cut-off < 0.01. The top 10 gene sets with overlapping genes for up-regulated and down-regulated genes are displayed. (E) The up-regulated and down-regulated predicted miRNA target genes most frequently identified in these gene sets. FC = fold change; FDR = false discovery rate; miRNA = microRNA; N = normal; PCa = prostate cancer.



**Fig. 4 – Network visualization and Kaplan-Meier analyses of microRNAs (miRNAs) and their core target genes. (A) The genes most frequently identified in the gene sets by gene set enrichment analysis with link to prostate-specific antigen-relapse, and the miRNAs that are predicted to regulate them, are visualized in the network. Genes in red boxes are up-regulated in prostate cancer (PCa) samples, and genes in green boxes are down-regulated in PCa compared with benign tissue. The expression of the miRNAs in PCa samples is denoted by red (up-regulated) and green (down-regulated) triangles. (B) Genes with a statistically significant ( $p \leq 0.01$ ) link to biochemical relapse free survival in PCa patients, when subjected to Kaplan-Meier analyses. (C) The link of miR-130b to biochemical relapse free survival in PCa patients, when subjected to Kaplan-Meier analyses ( $p = 0.004$ ). For each gene/miRNA the data was stratified into two groups based on the 50th quartile.**

down-regulated in clinical PCa tissue. PARVA is a focal adhesion protein, and loss of it enhances lamellipodia formation, which is a critical driving force of motility. The MAPK ERK mediated phosphorylation of PARVA also plays a critical role in the regulation of cell spreading and migration [29]. In our analyses, both miR-130b and PARVA significantly correlate with the biochemical recurrence free survival. Taken together, our study identifies miRNA-mRNA links critical for cell adhesion and motility. To the best of our knowledge, none of the miRNA-mRNA relationships in the network have been validated previously.

This study further highlights the complexity of miRNA regulation. This study has not accounted for all potential interactions and further studies will be needed to validate the identified interactions and elucidate the role of them. However, we believe that this systems biological approach yields important data for further investigations. The miRNAs offer ample prospects as future predictors of prognosis and treatment. Furthermore, a miRNA approach offer means for more effective functional restoration of abnormal pathways at multiple points at the same time as compared to more conventional one-point targeting.

## 5. Conclusions

A comprehensive approach of functional miRNA screening and expression analyses identifies miR-19a, miR-32, miR-124a, miR-130b, miR-148a, miR-583 as potential regulators of *FLNC*, *MSRB3*, *PARVA*, *PCDH7*, *PRNP*, *RAB34*, and *SORBS1*, which correlate with PSA-relapse in PCa patients.

**Author contributions:** Anna Aakula had full access to all the data in the study and takes responsibility for data integrity and accuracy of data analysis.

**Study concept and design:** Aakula, Östling.

**Acquisition of data:** Aakula, Leivonen, Mäkelä, Martenz-Uzunova.

**Analysis and interpretation of data:** Aakula, Kohonen, Leivonen, Mäkelä, Mpindi, Hintsanen, Östling.

**Drafting of the manuscript:** Aakula, Östling.

**Critical revision of the manuscript for important intellectual content:** Aakula, Kohonen, Leivonen, Mäkelä, Hintsanen, Mpindi, Martenz-Uzunova, Aittokallio, Jenster, Perälä, Kallioniemi, Östling.

**Statistical analysis:** Kohonen, Mpindi.

**Obtaining funding:** Kallioniemi.

**Administrative, technical, or material support:** Perälä.

**Supervision:** Kallioniemi, Östling.

**Other:** None.

**Financial disclosures:** Anna Aakula certifies that all conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript (eg, employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

**Funding/Support and role of the sponsor:** The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement No. 201438.25, Academy of Finland Centre of Excellence in "Translational Genome-Scale Biology", Cancer Society of Finland, Sigrid Juselius Foundation, Swedish Research Council for Medicine and personal grants from Academy of Finland to S-KL, TA, and PÖ.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.eururo.2015.09.019>.

## References

- [1] BCC Research Market report. Prevention and treatment of prostate cancer: technologies and global markets (PHM113A). Massachusetts, 2013. <http://www.bccresearch.com>.
- [2] Loeb S, Bjurlin MA, Nicholson J, et al. Overdiagnosis and overtreatment of prostate cancer. *Eur Urol* 2014;65:1046–55.
- [3] Mateo J, Smith A, Ong M, de Bono JS. Novel drugs targeting the androgen receptor pathway in prostate cancer. *Cancer Metastasis Rev* 2014;33:567–79.
- [4] Ahmed A, Ali S, Sarkar FH. Advances in androgen receptor targeted therapy for prostate cancer. *J Cell Physiol* 2014;229:271–6.
- [5] Ottman R, Nguyen C, Lorch R, Chakrabarti R. MicroRNA expressions associated with progression of prostate cancer cells to antiandrogen therapy resistance. *Mol Cancer* 2014;13:1.
- [6] Östling P, Leivonen SK, Aakula A, et al. Systematic analysis of microRNAs targeting the androgen receptor in prostate cancer cells. *Cancer Res* 2011;71:1956–67.
- [7] Larne O, Östling P, Haflidadóttir BS, et al. miR-183 in prostate cancer cells positively regulates synthesis and serum levels of prostate-specific antigen. *Eur Urol* 2015;68:581–8.
- [8] Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T. MicroRNA expression profiling in prostate cancer. *Cancer Res* 2007;67:6130–5.
- [9] Volinia S, Calin GA, Liu CG, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc Natl Acad Sci U S A* 2006;103:2257–61.
- [10] Hart M, Nolte E, Wach S, et al. Comparative microRNA profiling of prostate carcinomas with increasing tumor stage by deep sequencing. *Mol Cancer Res* 2014;12:250–63.
- [11] Yang Q, Zheng Y, Zhu D. Diagnostic performance of microRNAs expression in prostate cancer. *Tumour Biol* 2014;35:10529–38.
- [12] Leivonen SK, Mäkelä R, Östling P, et al. Protein lysate microarray analysis to identify microRNAs regulating estrogen receptor signaling in breast cancer cell lines. *Oncogene* 2009;28:3926–36.
- [13] Hong F, Breitling R, McEntee CW, Wittner BS, Nemhauser JL, Chory J. RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. *Bioinformatics* 2006;22:2825–7.
- [14] Martens-Uzunova ES, Jalava SE, Dits NF, et al. Diagnostic and prognostic signatures from the small non-coding RNA transcriptome in prostate cancer. *Oncogene* 2012;31:978–91.
- [15] Taylor BS, Schultz N, Hieronymus H, et al. Integrative genomic profiling of human prostate cancer. *Cancer Cell* 2010;18:11–22.
- [16] Mo W, Zhang J, Li X, et al. Identification of novel AR-targeted microRNAs mediating androgen signalling through critical pathways to regulate cell viability in prostate cancer. *PLoS One* 2013;8:e56592.
- [17] Moltzahn F, Olshen AB, Baehner L, et al. Microfluidic-based multiplex qRT-PCR identifies diagnostic and prognostic microRNA signatures in the sera of prostate cancer patients. *Cancer Res* 2011;71:550–60.
- [18] Jalava SE, Urbanucci A, Latonen L, et al. Androgen-regulated miR-32 targets BTG2 and is overexpressed in castration-resistant prostate cancer. *Oncogene* 2012;31:4460–71.
- [19] Murata T, Takayama K, Katayama S, et al. miR-148a is an androgen-responsive microRNA that promotes LNCaP prostate cell growth by repressing its target CAND1 expression. *Prostate Cancer Prostatic Dis* 2010;13:356–61.



- [20] Fujita Y, Kojima K, Ohhashi R, et al. MiR-148a attenuates paclitaxel resistance of hormone-refractory, drug-resistant prostate cancer PC3 cells by regulating MSK1 expression. *J Biol Chem* 2010;285:19076–84.
- [21] Kang S, Zhao Y, Hu K, et al. miR-124 exhibits antiproliferative and antiaggressive effects on prostate cancer cells through PACE4 pathway. *Prostate* 2014;74:1095–106.
- [22] Tao J, Wu D, Xu B, et al. microRNA-133 inhibits cell proliferation, migration and invasion in prostate cancer cells by targeting the epidermal growth factor receptor. *Oncol Rep* 2012;27:1967–75.
- [23] Aakula A, Leivonen SK, Hintsanen P, et al. MicroRNA-135b regulates ER $\alpha$ , AR and HIF1AN and affects breast and prostate cancer cell growth. *Mol Oncol* 2015;9:1287–300.
- [24] Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat Commun* 2013;4:2126.
- [25] Lee E, Kwak GH, Kamble K, Kim HY. Methionine sulfoxide reductase B3 deficiency inhibits cell growth through the activation of p53-p21 and p27 pathways. *Arch Biochem Biophys* 2014;547:1–5.
- [26] Yoshida K, Yoshitomo-Nakagawa K, Seki N, Sasaki M, Sugano S. Cloning, expression analysis, and chromosomal localization of BH-protocadherin (PCDH7), a novel member of the cadherin superfamily. *Genomics* 1998;49:458–61.
- [27] Amaro A, Esposito AI, Gallina A, et al. Validation of proposed prostate cancer biomarkers with gene expression data: a long road to travel. *Cancer Metastasis Rev* 2014;33:657–71.
- [28] Vanaja DK, Ballman KV, Morlan BW, et al. PDLIM4 repression by hypermethylation as a potential biomarker for prostate cancer. *Clin Cancer Res* 2006;12:1128–36.
- [29] Clarke DM, Brown MC, LaLonde DP, Turner CE. Phosphorylation of actopaxin regulates cell spreading and migration. *J Cell Biol* 2004;166:901–12.

[www.seem16.org](http://www.seem16.org)

**SEEM16**

**EAU 12th South Eastern European Meeting**

23-24 September 2016, Antalya, Turkey

**Call for Abstracts**

Deadline 15 June 2016

EAU Events are accredited by the EBU in compliance with the UEMS/EACCME regulations

**EAU** European Association of Urology