Meta-analysis of miRNA expression profiles for prostate cancer recurrence following radical prostatectomy

Elnaz Pashaei1, Elham Pashaei1, Maryam Ahmady2, Mustafa Ozen3, Nizamettin Aydin1*

1 Department of Computer Engineering, Yildiz Technical University, Istanbul, Turkey, 2 Department of Computer Engineering and IT, Payame Noor University, Tehran, Iran, 3 Department of Pathology & Immunology Baylor College of Medicine, Houston, Texas, United States of America

* naydin@yildiz.edu.tr

Abstract

Background
Prostate cancer (PCa) is a leading reason of death in men and the most diagnosed malignancies in the western countries at the present time. After radical prostatectomy (RP), nearly 30% of men develop clinical recurrence with high serum prostate-specific antigen levels. An important challenge in PCa research is to identify effective predictors of tumor recurrence. The molecular alterations in microRNAs are associated with PCa initiation and progression. Several miRNA microarray studies have been conducted in recurrence PCa, but the results vary among different studies.

Methods
We conducted a meta-analysis of 6 available miRNA expression datasets to identify a panel of co-deregulated miRNA genes and overlapping biological processes. The meta-analysis was performed using the ‘MetaDE’ package, based on combined P-value approaches (adaptive weight and Fisher’s methods), in R version 3.3.1.

Results
Meta-analysis of six miRNA datasets revealed miR-125A, miR-199A-3P, miR-28-5P, miR-301B, miR-324-5P, miR-361-5P, miR-363*, miR-449A, miR-484, miR-498, miR-579, miR-637, miR-720, miR-874 and miR-98 are commonly upregulated miRNA genes, while miR-1, miR-133A, miR-133B, miR-137, miR-221, miR-340, miR-370, miR-449B, miR-492, miR-496, miR-541, miR-572, miR-583, miR-606, miR-624, miR-636, miR-639, miR-661, miR-760, miR-890, and miR-939 are commonly downregulated miRNA genes in recurrent PCa samples in comparison to non-recurrent PCa samples. The network-based analysis showed that some of these miRNAs have an established prognostic significance in other cancers and can be actively involved in tumor growth. Gene ontology enrichment revealed many target genes of co-deregulated miRNAs are involved in “regulation of epithelial cell proliferation” and “tissue morphogenesis”. Kyoto Encyclopedia of Genes and Genomes
(KEGG) analysis indicated that these miRNAs regulate cancer pathways. The PPI hub proteins analysis identified CTNNB1 as the most highly ranked hub protein. Besides, common pathway analysis showed that TCF3, MAX, MYC, CYP26A1, and SREBF1 significantly interact with those DE miRNA genes. The identified genes have been known as tumor suppressors and biomarkers which are closely related to several cancer types, such as colorectal cancer, breast cancer, PCa, gastric, and hepatocellular carcinomas. Additionally, it was shown that the combination of DE miRNAs can assist in the more specific detection of the PCa and prediction of biochemical recurrence (BCR).

**Conclusion**

We found that the identified miRNAs through meta-analysis are candidate predictive markers for recurrent PCa after radical prostatectomy.

**Introduction**

Prostate cancer (PCa) is the most diagnosed malignancy and the second most reason of cancer-related death for the men over the age of 50 in the western countries [1]. The prostate-specific antigen (PSA) is the most reliable biomarker for PCa, which is helpful for diagnosis, screening, and follow-up after surgery. For treatment of PCa, two treatment methods, radiation therapy or radical prostatectomy (RP) and hormone ablation therapy are used. Yet, these methods do not provide enhanced survival rates and nearly 30% of patients experience a biochemical recurrence with enhanced PSA levels after curative treatment of RP [2]. Moreover, metastatic and advanced tumors of PCa respond very poorly to chemotherapy [3]. All these facts emphasize the significance of developing early diagnostic biomarkers for PCa progression. Identifying effective predictors of tumor recurrence after the surgical operation to determine whether treatment is required or not is a main challenge in the PCa research. To predict biochemical recurrence (BCR) of PCa after RP and develop effective predictors of tumor recurrence, multiple studies have been conducted for gene expression profiling [4–6].

Recently, numerous studies have been published which show that the alterations in microRNAs are associated with PCa initiation and progression [7–9].

The miR-1, miR-133b, miR-519d, and miR-647 are new biomarkers with prognostic and diagnostic value for recurrence of PCa, which have been identified through miRNA expression profiling [10, 11]. The miR-449b, miR-21, miR-141 and miR-221 are also known as putative prognostic or predictive markers in PCa recurrence after RP [12–14].

Meta-analysis utilizes statistical methods to contrast and combines results from multiple studies in the hope of increasing the statistical power and reproducibility over individual studies and identifying patterns across studies [15]. A limited number of studies [1, 10–14, 16, 17] has been conducted on microRNA expression profiles to distinguish recurrent from non-recurrent prostate tumor tissues and to identify novel biomarkers for prediction of PCa progression. The average differential expression level (fold change) and some level of significance as measured by the t-test are common procedures for identifying the biomarkers. These miRNA microarray data sets provide a rich resource for genome-wide information on PCa progression and make an ideal chance to perform a meta-analysis study. We assumed that a meta-analysis of some miRNA expression datasets of PCa progression can give a potentially significant list of co-deregulated miRNAs in PCa progression, which is important to specify
pathways in which the miRNAs of interest and their target genes are involved. To increase the probability of revealing truly significant deregulated miRNA genes, which should have higher potentials to be utilized as consistent biomarkers for the disease, we analyzed miRNA expression profile in PCa progression considering 5 studies (6 datasets). This meta-analysis increases the significance of the results.

**Materials and methods**

**Literature analysis**

There are a limited number of reports in the literature studied miRNAs in PCa progression. We systematically queried for these studies from PubMed database.

The following Medical Subjective Heading (MeSH) and Embase tree were used: “recurrence” or “recurrence” and “prostatic neoplasms” or “prostate cancer” and “microrRNas” or “microRNA” and “gene expression” or “expression”. In addition, publicly available microRNA data sets were searched by “RISmed” package in R to ensure no relevant studies were missed. Through database searching, a total of 24 studies was identified. Of these, 19 studies were retained after rejecting repetition. According to the title and abstract, a total of 14 studies was excluded. Review, case report, animal experiment, no association with PCa, and experiment on DNA microarray were the reasons for excluding these articles. The full-text articles were evaluated for the remaining 5 studies, and all of them (6 datasets) were retained in the final meta-analysis. These miRNA data sets were obtained from the National Centers for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/).

**The microRNA datasets and individual data analysis**

In this study, a total of six microRNA datasets related to the recurrent PCa after RP (GSE55323 [10], GSE26245 and GSE26247 [11], GSE65061 [17], GSE62610 [12], and GSE46738 [14]) met the inclusion criteria and were selected for meta-analysis.

In the GSE55323, a total of 41 recurrent and 41 non-recurrent tumors after RP, which have been obtained from Baylor College of Medicine Prostate Cancer program, have been considered for performing miRNA profiling. Recurrence has been defined as a two consecutive serum PSAs greater than 0.2 ng/ml. To carry out microarray analysis, 20 samples from each group have been profiled using microRNA microarray chips.

In GSE26245 and GSE26247, total RNA from 71 formalin-fixed-paraffin-embedded (FFPE) specimens with known long-term outcome have been used for performing DASL expression profiling with a custom-designed panel of 522 PCa relevant genes. Recurrence has been defined as a two consecutive serum PSAs greater than 0.2 ng/ml. In the GSE26245, samples from 71 patients (29 with BCR and 42 without BCR) and in the GSE26247, samples from 82 patients (29 with BCR and 53 without BCR) have been used. In this study, the samples with unknown BCR have been removed.

For the GSE65061, total RNA has been extracted from tumor-enriched 1mm cores from 43 RP paraffin tissue blocks. Tissue isolated at the time of RP has been utilized for miRNA profiling. Thirty-six months has been considered as the cutoff, as it was near the median time to recurrence. From 43 patients, 19 were labeled as the samples with BCR (≤ 36 months) and 24 as the samples without BCR (> 36 months).

In the GSE62610, total RNA has been taken from tumor-enriched 1.5 mm cores in diameter from 36 formalin fixed paraffin embedded (FFPE) specimens. Then biochemical failure has been defined as two consecutive measurements of PSA > 0.2 ng/ml. From 36 patients 22 has been classified as the samples with BCR and 14 as the samples without BCR. In the GSE62610
most of microRNAs have null expression. After excluding miRNAs with no expression in any of the samples, 536 miRNAs have been kept for further analysis.

For GSE46738, total RNA has been taken from tumors from the 51 patients that underwent an RP by the same surgeon to treat localized PCa. In the GSE46738, the BCR status of samples is not mentioned explicitly. In the present study, according to the expression level of the miRNAs with greater statistical power, which has been reported in the third table of the study [14], tumors were divided into the positive BCR and the negative BCR by using clustering techniques. In GSE46738, from 51 samples, 34 were classified as the samples with BCR and 17 as the samples without BCR. Table 1 has provided detailed information of each dataset.

The microRNA microarray datasets were obtained from GEO NCBI. All GEO series matrix files (GSE), platform sets, and annotation files were downloaded and parsed using ‘GEOquery’ package of Bioconductor 3.2 in R version 3.2.2. To identify Differentially Expressed (DE) miRNAs in each individual dataset, moderated t-test was used.

**Microarray meta-analysis**

This meta-analysis was performed in accordance with the guidelines provided in [18]. First, each individual dataset was preprocessed using the log2 transformation and normalization. Then, any training and validation dataset (GSE26245 and GSE26247) was combined together. Next, gene matching was done for all microRNA probes. When multiple probe sets matched to an identical gene symbol, the probe that presented the greatest inter-quartile range (IQR) was selected to represent the target gene symbol. After matching all probes to a common microRNA gene symbol, differential expression analysis was performed with “MetaDE” package for each dataset independently using adjusted p-value < 0.05, based on the false discovery rate by the Benjamini–Hochberg procedure [19] and moderated t-test.

Data integrity was checked for all datasets, and the differential expression meta-analysis across recurrence and non-recurrence samples was carried out by p-values combination using Adaptive Weight (AW) and Fisher methods.

**Statistical analysis**

The meta-analysis was performed using the ‘MetaDE’ package in R [20]. The moderated t-statistic was utilized to identify DE miRNAs in each individual dataset. The Fisher and AW were
used to combine the p-values from moderated t-test for meta-analysis. Fisher’s method is a summation of–log (p-value) across studies. An adjusted p-value of < 0.05, based on the False Discovery Rate (FDR) using the Benjamini–Hochberg procedure was used to select DE microRNA genes.

Network analysis of common differentially expressed microRNA genes

Network-based analysis was performed using a MIROB web tool (http://mirob.interactome.ru/) which has been designed to support analysis of microRNA expression data. MIROB (microRNA OncoBase) scans the set of input miRNAs to build any cancer pathways, detects key targets and Transcription Factors (TF) of candidate miRNAs, identifies any possible correlation between key targets and TF of candidate miRNAs and other diseases, and makes pathogenesis network.

Functional gene set enrichment analysis of common differentially expressed microRNA genes

The TF and target genes of DE miRNAs were searched for the pathways in which they participate, using the EnrichR web tool [21]. Specifically, Gene Ontology (GO) analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and Reactome pathway analysis were performed. Moreover, in order to find metabolic pathways and biochemical reactions, pathway commons analysis was performed for candidate DE miRNAs using a pathway commons network visualizer (PCViz) web tool. To further investigate the function of the DE miRNAs, they were also mapped to the most significant KEGG pathway.

Diagnostic performance of common differentially expressed microRNA genes

Based on the hypothesis that miRNA classifiers may improve sensitivity and specificity over single markers, the diagnostic potential of 37 DE miRNAs classifier was trained and tested in each dataset by performing receiver operating characteristic (ROC) analysis using the normalized expression values of miRNA genes. Logistic regression classifier with leave-one-out cross validation (LOOCV) scheme was used for this analysis. To show the discriminatory power of 37 DE miRNAs for distinguishing recurrent PCa samples from non-recurrent samples, area under the ROC curve (AUC) was calculated using WEKA open source machine learning software. Furthermore, the best subset of DE microRNAs which improves the BCR prediction over original studies and 37 DE miRNAs set, was identified in each dataset by using geometric particle swarm optimization (PSO). The LOOCV AUC of logistic regression was utilized as the fitness function. Partial C4.5 decision tree (PART) was used to find the relation and rules between the DE microRNAs in each dataset for biological point of view.

Results

Identification of common differentially expressed microRNAs for prostate cancer recurrence by meta-analysis

To identify a common DE microRNAs for PCa recurrence, five miRNA studies (Table 1) were analyzed using “MetaDE” package in R. First, individual analysis was performed and the moderated t—test was used to calculate the p-values which frequently used in meta-analysis. Then, AW and Fisher’s method were utilized to combine the p-values and find miRNAs that were
differentially expressed between samples with recurrence and non-recurrence (+/− BCR) across all studies. From miRNA microarray meta-analysis, we identified a total of 37 DE miRNAs including 15 overexpressed and 22 under expressed microRNAs across at least two datasets under the significance threshold of adjusted p-value < 0.05. Fig 1 shows the number of DE microRNAs against FDR obtained from individual analysis as well as meta-analysis. It is clearly seen that the meta-analysis has detected more candidate markers. Fig 2 shows the heat map of those 37 microRNAs. A complete list of DE microRNAs has been provided in Table 2. The miR-449A, miR-484, and miR-579 were among the most significant overexpressed genes, while miR-449B, miR-1, miR-137, miR-370, miR-375 were the most under expressed genes across all miRNA datasets (See Table 2).

Identification of the TF and regulatory network for the differentially expressed microRNAs obtained from meta-analysis

MIROB tool was used to perform regulatory microRNA network analysis to identify regulators responsible for the observed patterns in miRNA meta-analysis studies. The interaction network was constructed between DE microRNAs, TF and target genes associated with the complete set of DE (Fig 3). Twenty four of DE miRNAs were found in the network. The details of those miRNA gene networks have been given in Table 3. Key targets, ontology information on target genes, TF and a descriptive analysis of expression of the DE miRNAs have been summarized in this table. In addition, it shows that DE miRNAs are highly associated with colorectal, PCa, breast, and gastric cancer.
Further enrichment analysis for identification of overrepresented biological pathways and gene ontology terms

We performed gene set enrichment analysis by EnrichR tool, using the complete list of key targets and TF of DE miRNAs. GO terms and biological pathways were significantly overrepresented in the gene list if they showed an adjusted p-value < 0.05. Results for gene ontology and enriched biological pathways (KEGG, Reactome) have been shown in Tables 4–6, respectively. DE microRNAs in meta-analysis results were associated with the enriched pathways with adjusted p-value < 0.05, including “MicroRNAs in cancer (hsa05206)”, “Pathways in cancer (hsa05200)”, “Proteoglycans in cancer (hsa05205)”, “PI3K-Akt signaling pathway (hsa04151)”, “Prostate cancer (hsa05215)” and “Signal Transduction (R-HSA-162582)”. The most important GO terms associated with key targets and TF of DE miRNA genes included “regulation of epithelial cell proliferation (GO: 0050678)”, “tissue morphogenesis (GO: 0048729)”, “regulation of cellular response to stress (GO: 0080135)”, and “positive regulation of cellular component movement (GO: 0051272)”.

To further investigate the function of DE miRNAs, we mapped them to the KEGG database. Eleven of them (miR-1, miR-125A, miR-133A, miR-133B, miR-137, miR-199A, miR-221, miR-28, miR-324, miR-363 and miR-449A) were found in the “miRNAs in cancer” pathway (KEGG-ID: hsa05206; Fig 4) with adjusted P-value of 7.55e-15 (Table 5). Moreover, common pathway analysis revealed that TCF3, MYC, MAX, CYP26A1, and SREBF1 significantly interact with DE miRNAs (Fig 5).
| miRNA   | Meta. Stat | Meta. P value | Meta. FDR |
|---------|------------|---------------|-----------|
| miR-1   | 25.6944    | 0.0039        | 0.0342    |
| miR-133A| 24.066     | 0.00245       | 0.04833   |
| miR-133B| 22.2085    | 0.0089        | 0.0294    |
| miR-137 | 30.7251    | 0.0005        | 0.019     |
| miR-221 | 19.26      | 0.00744       | 0.0477    |
| miR-340 | 20.7435    | 0.00044       | 0.04      |
| miR-370 | 26.1757    | 0.0033        | 0.0342    |
| miR-449B| 22.501     | 0.00038       | 0.042     |
| miR-489 | 19.9117    | 0.00744       | 0.0455    |
| miR-492 | 26.547     | 0.0012        | 0.025     |
| miR-496 | 26.6035    | 0.0003        | 0.008     |
| miR-541 | 21.215     | 0.0001        | 0.005     |
| miR-572 | 24.4192    | 0.00446       | 0.0416    |
| miR-583 | 27.205     | 0.00061       | 0.048     |
| miR-606 | 22.7104    | 0.00042       | 0.05      |
| miR-624 | 20.58      | 0.00039       | 0.038     |
| miR-636 | 95.9233    | <0.001        | <0.001    |
| miR-639 | 19.5331    | 0.0082        | 0.0455    |
| miR-661 | 23.87      | 0.00012       | 0.028     |
| miR-760 | 24.3088    | 0.0022        | 0.035     |
| miR-890 | 23.07      | 0.0014        | 0.013     |
| miR-939 | 16.61      | 0.0023        | 0.049     |
| miR-125A-5P | 22.9155   | 0.00028       | 0.038     |
| miR-199A-3P | 0.0016   | 0.00274       | 0.042     |
| miR-28-5P | 24.05      | 0.0024        | 0.04      |
| miR-301B | 20.0917    | 0.0066        | 0.0455    |
| miR-324-5P | 32.2291   | 0.00065       | 0.1625    |
| miR-361-5P | 0.00077   | 0.00122       | 0.038     |
| miR-363*| 22.176     | 0.0005        | 0.044     |
| miR-449A| 26.5308    | 0.0031        | 0.0342    |
| miR-484 | 25.4578    | 0.00034       | 0.0342    |
| miR-498 | 25.0151    | 0.0019        | 0.035     |
| miR-579 | 29.3       | 0.00025       | 0.1625    |
| miR-637 | 20.69      | 0.00055       | 0.0375    |
| miR-720 | 25.13      | 0.00125       | 0.0125    |
| miR-874 | 34.7751    | 0.000178      | 0.0208    |
| miR-98 | 23.903     | 0.0007        | 0.04625   |

The "moderated t-test" is used to perform individual analysis and calculate p-values. The corresponding p-values are adjusted, based on the false discovery rate using the Benjamini–Hochberg procedure used to select DE miRNAs across at least two datasets.

**”, denotes the mature miRNA sequence.

“NA”, represents "not available".

https://doi.org/10.1371/journal.pone.0179543.t002
Diagnostic performance

We assessed the diagnostic potential of the 37-miRNA signature identified by meta-analysis. ROC curve analysis gave AUCs from 0.55–0.84 for miRNAs set in each GEO dataset (See Fig 6). To investigate whether a miRNA signature may increase diagnostic accuracy over 37-miRNA signature, we employed a soft computing technique (PSO/ logistic regression) and trained and tested on miRNA expression profiles. The best subset of DE miRNAs was identified in each GEO dataset and shown in Table 7. Notably, the discriminating power of the identified signatures in each GEO dataset is higher than the case where 37-miRNA classifier was considered. For the best subset of DE miRNAs in each GEO dataset, the ROC curve analysis gave AUCs from 0.75–0.97 (See Fig 7). The highest diagnostic accuracy (97%) was given for GSE55323 with 11-miRNAs. Moreover, in order to correctly classify BCR+ vs. BCR- samples, simple rules were extracted using a decision tree classifier (Table 7). Among six GEO datasets, rules with high diagnostic potentials were extracted for GSE46738 and GSE26247.

Finally, a comparison between the expressions of co-deregulated microRNAs in BCR+ vs. BCR- was done by plotting boxplots (Fig 8). The boxplots were drawn for co-deregulated microRNAs that are involved in the PCa pathway.
Table 3. The details of 37 DE miRNAs that are involved in the interaction network, which has been drawn by MIROB.

| MicroRNAs | Transcription Factors | Target genes | Disease influence (expression) | pathogenesis of a disease |
|-----------|----------------------|--------------|-------------------------------|---------------------------|
| miR-1     | SNAI2                | FOXP1, HDAC4, PDLIM5, PIM1, CCND2, CXCL12, PNP, LASP1, SNAI2, PAX7, KLF4, MET, FN1, PTMA, TAGLN2, PAX3, GJA1, SOX6, ATP6V1B2, LARP4, CNN3, HSPD1, HSPA4, POQK, PGM2, SERP1, NETO2, Sxn1, CAND1, ADAR, KIF2A, G6PD, MEF2A, KCN2, PPP2R5A, HCN2, TFW1, HCN4, KCNE1, ANXA2, ETS1 | -                           | Metastasis, Angiogenesis, growth, Proliferation, Invasion, migration, Apoptosis, cell cycle arrest, differentiation, WNT signaling. |
| miR-125A  | NFATC1, TP53          | RHOA, FYN, CDKN1A, EDN1, BAK1, ARID3B, CD34, ERBB2, ERBB3, NTRK3, ELAVL1, TNFAIP3, PDPN, KLF13, CLEC5A, TRAF6, RAF1, ZBTB7A, VEGFA | Colorectal cancer (down)    | Proliferation, Invasion, migration, differentiation, cell cycle arrest, Angiogenesis, survival, Sorafenib resistance, myeloid differentiation |
| miR-133A  | -                    | CD47, LASP1, GSTP1, FSCN1, ARPC5, TAGLN2, CASP9, KCNH2, CACNA1C, HCN2, KCNQ1, EGFR, IGF1R, RFFL, SP1, ABC21, FOXC1, BCL2L1 | Prostate Cancer (down)      | Proliferation, Invasion, migration, Apoptosis, cell cycle arrest, colony formation, ERK pathway (MAPK pathway), Liver metastasis, Lung metastasis, tumor growth, Adriamycin (Adr) resistance, 5-fluorouracil resistance, cisplatin resistance |
| miR-133B  | TP63                 | BCL2L2, MCL1, FGFR1, FSCN1, MET, PITX3, IGF1R, CXCR4, UTRN, SP1, RHOA, MMP9, EGFR, TAGLN2, LASP1, SIRT1, PPP2R2D, FOXC1, PTBP1 | Colorectal cancer (up), Prostate Cancer (down), Gastric (down) | Proliferation, Invasion, migration, Apoptosis, cell cycle arrest, WNT signaling, tumor growth, cisplatin resistance, Cell growth |
| miR-137   | FOXD3, HMGA1         | CDK6, CDC42, SLC7A1, KDM1A, CSMD1, C10orf26, CACNA1C, TCF4, ESRRB, CTBP1, FMR1, MI1, GLI1R1, CSE1L, PTG2, MTF, PXN, PTBP1, NF1, EPHA7, AKT2, ZBTB7A, HEY2, KLF12, MYO1C, CUL4A, FOXO1, CDK6 | Colorectal cancer (down), Gastric (down) | Metastasis, Angiogenesis, growth, colony formation, Proliferation, Invasion, migration, Apoptosis, tumorgrowth, Cellgrowth, celledifferentiation, Stemness, cell viability, aerobic glycolysis, cell cycle |
| miR-199A1 | SP1, SNHG12, SNHG1, RELA | ST6GAL1, HSPAT5, A5F, ERN1, IKKBK, CACUL1, CAUV2, MTO, LIF, RELA, NFKB1, ATG7, CLTC, NLK, CDH1, SLC27A1, MAPK4, CD151, YAP1, OSCP1, HIF1A, VEGFA, IGF1R, IG2E, FL1, KDR, HGF, MMP2, E2F3, ACVR1B | -                           | Proliferation, Invasion, migration, Apoptosis, cell cycle arrest, Angiogenesis, colony formation, ERK pathway (MAPK pathway), Lung metastasis, tumor growth, cisplatin resistance, cell viability, Chemoresistance, survival, Sorafenib resistance, Autophagy, adhesion |
| miR-221   | RELA, JUN, ESR1, NCOR2, NCOR1, TP53 | CERS2, TRPS1, DICER1, KIT, NOS3, BBC3, MBD2, CDKN1B, GJA1, ICAM1, CDKN1B, DIRAS3, RAB1A, HECTD2, TICAM1, PTMRM, GMGT, FOXO3, RECK, MDM2, PTEN, SOCS1, CASKP3 | Breast cancer (up), Colorectal cancer (up), Gastric (up) | Proliferation, Invasion, migration, Apoptosis, cell cycle arrest, Metastasis, Cell growth, motility, cell cycle progression, Chemoresistance, doxorubicin resistance, Radioresistance, survival, Sorafenib resistance |
| miR-28    | STAT5B               | STAT5B, CDKN1A, CCND1, HOXB3, NME1, N4BP1, OTUB1, TEX261, MAPK1, E2F6, MPL, BAG1, MAD2L1, RAP1B, IL34, IGF1 | Colorectal cancer (down)    | Proliferation, Invasion, migration, Apoptosis, cell cycle arrest, Metastasis, ERK pathway (MAPK pathway), P38 signaling, AKT signalling, PI3K signaling |
| miR-301B  | -                    | FOXF2         | -                             | -                          |
| miR-324   | -                    | SMO, GLI1, WNT2B, ETS1, SP1 | -                             | Proliferation, Invasion, migration, cell cycle arrest, Metastasis, Radioresistance |
| miR-340   | RELA                 | RELA, MET, ROCK1, PTBP1, SOX2, MTF, RHOA, PLAT, DMD, JAK1, CCNG2 | Gastric (up)                | Proliferation, Invasion, migration, differentiation, cell cycle arrest, Metastasis, tumor growth, Cell growth, stemness, aerobic glycolysis, cell viability, cell cycle progression, Senescence, JAK/STAT signalling |

(Continued)
Table 3. (Continued)

| MicroRNAs | Transcription Factors | Target genes | Disease influence (expression) | Pathogenesis of a disease |
|-----------|-----------------------|--------------|--------------------------------|--------------------------|
| miR-361   | -                     | STAT6, VEGFA, TWIST1, WT1, SH2B1, CXCR6, SND1, PHB | -                            | Proliferation, Invasion, migration, Apoptosis, Metastasis, colony formation, tumor growth, Cell growth, stemness |
| miR-363   | -                     | CDKN1A, S1PR1, BCL2L11, CASP3, CD276, FBXW7, MCL1 | -                            | Proliferation, Apoptosis, cisplatin resistance, cell viability, Chemoresistance, survival |
| miR-370   | -                     | CPT1A, TGFBR2, FOXM1, FOXO1, ENG | Gastric (up) | colony formation, Proliferation, Apoptosis Chemoresistance, colony formation, cisplatin, resistance |
| miR-449A  | E2F1, EZH2, MYCN      | E2F3, CDC25A, MET, SIRT1, CDK6, BCL2, CCND1, CRHR1, LIF1, KLF4, NOTCH1, HDAC1, AR, IL6R, SOX4, CREB5, FOS, MYC | Prostate Cancer (down), Gastric (down) | Metastasis colony, formation, Proliferation, Invasion, migration, Apoptosis, motility, EMT, cell cycle arrest, cisplatin resistance, differentiation, Cell growth, cell viability, Radioresistance, Senescence, Antiapoptosis |
| miR-449B  | E2F1, AR              | CDK6 CDC25A, HDAC1, SOX4 | -                            | Proliferation, migration Apoptosis, Cell growth colony formation, cell viability |
| miR-489   | -                     | SMAD3, MMP7, PROX1 | -                            | Proliferation, Invasion, migration, Lung metastasis, Adriamycin (Adr) resistance, EMT |
| miR-492   | VDR, NCOA3            | BSG, SOX7 | -                            | Proliferation, Oxaliplatin, resistance |
| miR-498   | -                     | TERT, ERBB2 | -                            | Apoptosis, tumor growth, Cell growth |
| miR-661   | CEBPA                 | STARD10, PVRL1, MTA1, MCL1, MDM2, MDM4, PTEN | -                            | Proliferation, Invasion, migration, cell cycle arrest, Metastasis, tumor growth, motility, EMT |
| miR-760   | -                     | CSNK2A1, HIST1H3D, HIST1H2AD, PHLP2 | -                            | Proliferation, colony formation, Senescence |
| miR-874   | -                     | AQP3, PIN1, MAGEC2 | Gastric (down) | Proliferation, Invasion, Apoptosis, colony formation, Cell growth, mTOR signaling |
| miR-939   | -                     | APC2, NGFR | -                            | Proliferation, WNT signaling |
| miR-98    | EZH2                  | ACVR1B, MMP11, EZH2, SALL4, IGF2BP1, CTHR6 | Gastric (up) | Angiogenesis, growth, Proliferation, Invasion, migration, Apoptosis, EMT, cell cycle arrest, WNT signaling |

https://doi.org/10.1371/journal.pone.0179543.t003

Table 4. Top enriched gene ontology (GO) biological process identified by functional analysis of the target genes and TFs of the DE microRNAs in the meta-analysis.

| GO-ID       | Description                                           | Overlap * | Adjusted P-value |
|-------------|-------------------------------------------------------|-----------|------------------|
| GO:0050678  | regulation of epithelial cell proliferation           | 32/258    | 7.430E-18        |
| GO:0048729  | tissue morphogenesis                                 | 35/358    | 1.191E-16        |
| GO:0080135  | regulation of cellular response to stress            | 36/404    | 4.884E-16        |
| GO:0051272  | positive regulation of cellular component movement   | 31/296    | 1.209E-15        |
| GO:0070482  | response to oxygen levels                            | 29/259    | 2.118E-15        |
| GO:2001233  | regulation of apoptotic signaling pathway             | 33/356    | 2.466E-15        |
| GO:2000147  | positive regulation of cell motility                 | 30/287    | 2.699E-15        |
| GO:0040017  | positive regulation of locomotion                     | 30/304    | 1.184E-14        |

Gene sets functional analysis was performed using extended libraries of the EnrichR tool.

* Overlap: indicates the number of hits from the meta-analysis compared to each curated gene set library.

https://doi.org/10.1371/journal.pone.0179543.t004
Various miRNAs are DE in individuals with recurrent PCa, and identifying the most important miRNAs and pathways associated with the disease is very important. A meta-analysis of multiple miRNA datasets combines the generated p-values of individual studies, making the identification of DE microRNA genes more reliable.

In this study, we attempted to identify common miRNAs underlying recurrent PCa using meta-analysis of six publicly available microRNA datasets to focus deeply on identifying DE microRNA genes and risk factors shared between them.

By meta-analysis of six published miRNA expression datasets of recurrent PCa, we identified a common signature of a total of 37 DE microRNAs including 15 overexpressed and 22 underexpressed microRNA genes across at least two datasets under the significance threshold of adjusted p-value $< 0.05$ in recurrence compared to non-recurrence samples. The identified 37 microRNAs in this meta-analysis were discovered as DE microRNAs in at least one dataset in the prior individual analysis. Of the 37 DE miRNAs associated with BCR after RP (Table 2), all except miR-606 have been reported to be associated with cancer in general [22–35]. Fifteen miRNAs (miR-1, miR-133A, miR-133B, miR-449A, miR-137, miR-370, miR-221, miR-449B, miR-125A-5P, miR-199A-3P, miR-301B, miR-340, miR-361, miR-363, miR-98) have been

| Pathway ID   | Name                                        | Overlap | Adjusted P-value |
|--------------|---------------------------------------------|---------|------------------|
| hsa05206     | MicroRNAs in cancer                         | 56/297  | 3.476E-45        |
| hsa05200     | Pathways in cancer                          | 55/397  | 4.152E-37        |
| hsa05205     | Proteoglycans in cancer                     | 33/203  | 4.675E-24        |
| hsa04151     | PI3K-Akt signalling pathway                 | 38/341  | 7.293E-22        |
| hsa05215     | Prostate cancer                             | 23/89   | 1.259E-21        |
| hsa05212     | Pancreatic cancer                           | 20/66   | 2.252E-20        |
| hsa05218     | Melanoma                                     | 19/71   | 2.982E-18        |
| hsa05220     | Chronic myeloid leukemia                    | 19/73   | 4.676E-18        |
| hsa04520     | Adherens junction                           | 19/74   | 5.524E-18        |
| hsa04933     | AGE-RAGE signalling pathway in diabetic complications | 21/101 | 7.221E-18 |

Gene sets functional analysis was performed using extended libraries of the EnrichR tool. * Overlap: indicates the number of hits from the meta-analysis compared to each curated gene set library.

https://doi.org/10.1371/journal.pone.0179543.t005

Table 6. Top enriched reactome pathways identified by functional analysis of the target genes and TFs of the DE microRNAs in the meta-analysis.

| Pathway ID   | Name                        | Overlap | Adjusted P-value |
|--------------|-----------------------------|---------|------------------|
| R-HSA-162582 | Signal Transduction         | 100/2465| 4.220E-22        |
| R-HSA-1266738| Developmental Biology       | 46/786  | 2.574E-14        |
| R-HSA-1236394| Signalling by ERBB4         | 29/330  | 5.348E-13        |
| R-HSA-166520 | Signalling by NGF           | 33/450  | 8.395E-13        |
| R-HSA-180292 | GAB1 signalosome            | 19/125  | 1.065E-12        |
| R-HSA-198203 | PI3K/AKT activation         | 19/125  | 1.065E-12        |
| R-HSA-5654695| PI-3K cascade:FGFR2         | 18/122  | 4.702E-12        |
| R-HSA-1257604| PIP3 activates AKT signalling | 18/122  | 4.702E-12        |

Gene sets functional analysis was performed using extended libraries of the EnrichR tool. * Overlap: indicates the number of hits from the meta-analysis compared to each curated gene set library.

https://doi.org/10.1371/journal.pone.0179543.t006
Fig 4. The most significant enriched KEGG pathway for the DE microRNAs identified from meta-analysis. The microRNAs in the red box indicates co-deregulated microRNA genes in our list. The DE microRNAs identified from meta-analysis were mapped to "microRNAs in cancer" pathway (KEGG-ID: hsa03020) by using the KEGG mapper web tool.

https://doi.org/10.1371/journal.pone.0179543.g004
previously linked to PCa [36–46] and of those, miR-1, miR-133B, miR-449B, and miR-221 have been described as predictive markers in PCa recurrence after RP [10, 12, 13].

Among the overexpressed DE microRNAs, miR-449A and miR-579 had high combined P-values across all studies. Tumor-suppressive miR-449A targets HDAC1 and induces growth arrest in PCa [37]. It also causes Rb-dependent cell cycle arrest and senescence in PCa cells (Table 3) [47]. For a previously poorly characterized miRNA, namely miR-579, no PCa related functions have been reported. MiR-579-3p is only known as a master regulator of melanoma progression and drug resistance [48].

Among the under expressed DE microRNAs mir-496, miR-137, miR-1, and miR-370 had the highest combined P-values across all studies. MiR-496 is also a previously poorly characterized miRNA, which has no functions in PCa. Methylated DNA binding domain protein 2 (MBD2) is known as the only TF of miR-496, which coordinately silences gene expression through activation of the miR-496 promoter in breast cancer cell line [49].

Methylated mir-137 host gene is promising diagnostic and/or prognostic biomarker of PCa. The epigenetic silencing of miR137 is an important event in promoting androgen signaling during prostate carcinogenesis and progression. MiR-137 suppresses cell growth in several cancers such as ovarian, colorectal, and gastric [50].

MiR-1 is known as a biomarker of recurrence PCa, which is in agreement with the findings in present meta-analysis study. MiR-1 functions as a tumor suppressor which suppresses cancer cell proliferation, metastasis, angiogenesis, invasion, cell cycle arrest, WNT signaling and

![Fig 5. Common pathway analysis for DE microRNAs identified from meta-analysis. This analysis revealed that TCF3, MYC, MAX, CYP26A1 and SREBF1 are significantly interacting with candidate miRNA genes.](https://doi.org/10.1371/journal.pone.0179543.g005)
promotes apoptosis by ectopic expression. This miRNA is a potential prognostic biomarker of hepatocellular carcinoma (HCC) and colorectal cancer. The expression of miR-1 alters in several cancers such as lung, gastrointestinal, prostate, bladder, head and neck, and renal cancer [51].

MiR-370 plays an important role in the proliferation of human PCa cells by directly suppressing the tumor suppressor FOXO1 [39].

PPI Hub Proteins analysis of the TF and target genes of DE MicroRNAs was conducted for prioritization of the most important hub genes using the EnrichR web tool. CTNNB1 was the most important hub genes among TF and target genes of DE microRNAs across six microarray studies.

CTNNB1 (Catenin Beta 1) functions as a Key downstream component of the canonical WNT signaling pathway. WNTs and their downstream effectors have crucial roles in the regulation of various processes that are important for cancer progression, including tumor growth, tumor initiation, differentiation, cell senescence, cell death, differentiation and metastasis [52]. Nuclear accumulation and abnormal stabilization of CTNNB1 as a consequence of missense mutations occurs at a high frequency in a variety of epithelial cancers such as colorectal cancer, medulloblastoma, ovarian cancer, and pilomatrixoma. Upregulation of CTNNB1 is also associated with PCa [53].

To elucidate the role of DE microRNAs obtained from the meta-analysis, we performed pathway analysis and gene set enrichment analysis for TF and target genes of DE miRNAs.
using the EnrichR web tool. The most enriched pathway and Gene Ontology (GO) term among the TF and target genes of DE miRNAs were “MicroRNAs in cancer (hsa05206), "Pathways in cancer (hsa05200), Signal Transduction (R-HSA-162582), “regulation of epithelial cell proliferation (GO: 0050678)” and “tissue morphogenesis (GO: 0048729)”.

Common pathway analysis revealed that TCF3, MYC, MAX, CYP26A1 and SREBF1 were the most significant proteins associated with DE miRNA genes. Of note, these proteins were not identified as TF and target genes of DE microRNAs.

Previous studies have reported that the diminished activity of TCF3 plays a role in lymphoid malignancies, and up-regulation of it is involved in the development and progression of colorectal cancer. TCF3 is regulated by androgens and acts as a tumor promoter in PCa [54].

Overexpression, Mutations, translocation and rearrangement of MYC is related to several cancers such as breast, PCa, gastrointestinal, melanoma, and small cell lung cancer [55].

MAX is known as a tumor suppressor in renal oncocytomas and small cell lung cancer. The mutation of it has been identified in gastrointestinal stromal tumors [56]. High expression of CYP26A1 is associated with several cancers such as breast, head and neck, colorectal and ovarian. CYP26A1 is a methylation marker of PCs associated with ERG-positive cancers [57].

Sterol regulatory element-binding protein1 (SREBP1) is a key regulatory factor that controls lipid homeostasis. SREBP1 is a critical link between oncogenic signaling and tumor metabolism. The overexpression of SREBF1 is related to a variety of cancers such as PCa, breast, head and neck, colorectal, endometrial, glioblastoma, pancreatic, and ovarian [58].
To understand the association of the DE microRNAs list with the most significant target genes and transcription factors, we conducted a regulatory gene network analysis using the MIROB web tool. CDKN1A and LASP1 were amongst the most significant target genes associated with the DE microRNAs.

Cyclin-dependent kinase inhibitor 1 (CDKN1A) also is known as P21 is involved in p53/TP53 mediated inhibition of cellular proliferation in response to DNA damage and its overexpression results in cell cycle arrest and autophagy cell death. The expression of this gene is tightly controlled by the tumor suppressor protein p53 in a human brain tumor cell line [59]. The CDKN1A genotypes CT and TT are associated with an increased risk of advanced prostate carcinoma compared with the CC genotype [60]. Elevated p21 levels are associated with higher Gleason score, and increased PCa recurrence [61].

LIM and SH3 protein 1 (LASP1), a promoter of cell proliferation and migration, play a significant role in cancer development and progression. LASP-1 is involved in numerous biological and pathological processes. It plays an important role in the regulation of dynamic actin-based and cytoskeletal activities. LASP-1 is highly expressed in the central nervous system and contributes to the formation and progression of prostate cancer through a NF-KB pathway [62].

RELA, SNAI2, and TP53 were among the most significant transcription factors associated with the DE microRNAs.
REL A also known as NF-kappa-B is a ubiquitous transcription factor involved in many biological processes such as immunity, inflammation, cell growth, differentiation, tumorigenesis and apoptosis. Zinc finger protein (SNAI2) is known as a transcriptional repressor that modulates both activator-dependent and basal transcription. SNAI2 regulates cell proliferation and invasiveness of metastatic PCa cell lines. Cellular tumor antigen p53 (TP53) acts as a tumor suppressor in many tumor types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type.

Moreover, network analysis showed that ten of the 37 DE miRNAs (miR-125A, miR-133B, miR-137, miR-221, miR-28, miR-340, miR-370, miR-449A, miR-874, and miR-98) have an established prognostic significance in other cancers such as colorectal, gastric, and breast. This network also indicated that eight of 37 DE miRNAs (miR-133A, miR-133B, miR-137, miR-199A1, miR-340, miR-361, miR-498, and miR-661) can be actively involved in tumor growth.

In this study, we also built new miRNA diagnostic classifiers in each GEO datasets based on best subset of DE miRNAs in the meta-analysis. These classifiers predicted BCR after RP with very high accuracy. The highest diagnostic accuracy (97%) was given for GSE55323 with
11-miRNAs. The performance of our 11-miRNA diagnostic classifier (97%) exceeded that of a 2-miRNA classifier (miR-1+miR-133B; AUC: 71%) developed earlier by Karatas et al. [10]. One miRNA (miR-1) is shared between these classifiers, further supporting the validity of our findings.

Briefly, we used “MetaDE” package to perform a meta-analysis, which provides options for gene matching across studies, gene filtering before meta-analysis and functions for conducting several major meta-analysis methods such as Fisher and AW for differential expression analysis. Then performed the GO enrichment analysis, pathway analysis, network analysis, and ROC analysis.

In conclusion, this is the first report that provides biological insights on common microRNA expression signatures for recurrent PCa after RP. The candidate miRNAs are worthy to be validated in the wet lab.

Supporting information
S1 Fig. The PRISMA flow diagram.

Acknowledgments
The authors thank reverent reviewers for their precious comments that made considerable improvements in this work.

Author Contributions
Conceptualization: Elnaz Pashaei.
Data curation: Elnaz Pashaei.
Formal analysis: Elnaz Pashaei, Elham Pashaei, Maryam Ahmady.
Funding acquisition: Elnaz Pashaei.
Investigation: Elnaz Pashaei, Elham Pashaei, Maryam Ahmady.
Methodology: Elnaz Pashaei.
Project administration: Mustafa Ozen, Nizamettin Aydin.
Resources: Elnaz Pashaei.
Software: Elnaz Pashaei.
Supervision: Mustafa Ozen, Nizamettin Aydin.
Validation: Elnaz Pashaei.
Visualization: Elnaz Pashaei.
Writing – original draft: Elnaz Pashaei, Elham Pashaei, Maryam Ahmady.
Writing – review & editing: Elnaz Pashaei, Elham Pashaei, Nizamettin Aydin.

References
1. Fendler A, Jung M, Stephan C, Honey RJ, Stewart RJ, Pace KT, et al. miRNAs can predict prostate cancer biochemical relapse and are involved in tumor progression. Int J Oncol. 2011; 39(5):1183–92. https://doi.org/10.3892/ijo.2011.1128 PMID: 21769427
2. Barron N, Keenan J, Gammell P, Martinez VG, Freeman A, Masters JR, et al. Biochemical relapse following radical prostatectomy and miR-200a levels in prostate cancer. Prostate. 2012; 72: 1193–1199. https://doi.org/10.1002/pros.22469 PMID: 22161972

3. Bhatnagar N, Li X, Padi SK, Zhang Q, Tang MS, Guo B. Downregulation of miR-205 and miR-31 confers resistance to chemotherapy-induced apoptosis in prostate cancer cells. Cell Death Dis. 2010; 1: e105. https://doi.org/10.1038/cddis.2010.85 PMID: 21368878

4. Ross-Adams H, Lamb AD, Dunning MJ, Halim S, Lindberg J, Massie CM, et al. Integration of copy number and transcriptomics provides risk stratification in prostate cancer: A discovery and validation cohort study. EBioMedicine. 2015 Jul 29; 2 (9): 1133–44. https://doi.org/10.1016/j.ebiom.2015.07.017 PMID: 26501111

5. Sun Y, Goodison S. Optimizing molecular signatures for predicting prostate cancer recurrence. Prostate. 2009 Jul 1; 69 (10): 1119–27. https://doi.org/10.1002/pros.20961 PMID: 19343730

6. Mortensen MM, Hayer S, Lynnerup AS, Ørntoft TF, Sørensen KD, Borre M, et al. Expression profiling of prostate cancer tissue delineates genes associated with recurrence after prostatectomy. Scientific Reports. 2015; 5: 16018. https://doi.org/10.1038/srep16018 PMID: 26522007

7. Ozen M, Creighton CJ, Ozdemir M, Ittmann M. Widespread deregulation of microRNA expression in human prostate cancer. Oncogene. 2008; 27:1788–1793. https://doi.org/10.1038/sj.onc.1210809 PMID: 17891175

8. Schaefer A, Jung M, Mollenkopf HJ, Wagner I, Stephan C, Jentzkow F, et al. Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma. Int J Cancer. 2010; 126:1166–1176. https://doi.org/10.1002/ijc.24827 PMID: 19676045

9. Tong AW, Fulgham P, Jay C, Chen P, Khalil I, Liu S, et al. MicroRNA profile analysis of human prostate cancers. Cancer Gene Ther. 2008; 15:206–216. https://doi.org/10.1038/cgt.2008.77 PMID: 18949015

10. Karatas OF, Guzel E, Suer I, Ekici ID, Caskurlu T, Creighton CJ, et al. miR-1 and miR-133b are differentially expressed in patients with recurrent prostate cancer. PLoS One 2014; 9(6):e98675. PMID: 24967583. https://doi.org/10.1371/journal.pone.0098675

11. Long Q, Johnson BA, Osunkoya AO, Lai YH, Zhou W, Abramovitz M, et al. Protein-coding and microRNA biomarkers of recurrence of prostate cancer following radical prostatectomy. Am J Pathol. 2011; 179(1):46–54. https://doi.org/10.1016/j.ajpath.2011.03.008 PMID: 21703393

12. Mortensen MM, Hayer S, Ørntoft TF, Sørensen KD, Dyrysjø L, Borre M. High miR-449b expression in prostate cancer is associated with biochemical recurrence after radical prostatectomy. BMC Cancer. 2014 Nov 21; 14:859. https://doi.org/10.1186/1471-2407-14-859 PMID: 25416653

13. Zheng Q, Peskoe SB, Ribas J, Rafiqi F, Kudrolli T, Meeker AK, et al. Investigation of miR-21, miR-141, and miR-221 expression levels in prostate adenocarcinoma for associated risk of recurrence after radical prostatectomy. Prostate. 2014; 74:1655–1662 https://doi.org/10.1002/pros.22883 PMID: 25252191

14. Leite KR, Reis ST, Viana N, Morais DR, Moura CM, Silva IA, et al. Controlling RECK miR21 Promotes Tumor Cell Invasion and Is Related to Biochemical Recurrence of Prostate Cancer. Journal of Cancer. 2015 Jan 21; 6(3):292–301. https://doi.org/10.7150/jca.11038 PMID: 25663948

15. Hong F, Breitling R. A comparison of meta-analysis methods for detecting differentially expressed genes in microarray experiments. Bioinformatics. 2008; 24(3):374–82. https://doi.org/10.1093/bioinformatics/btn435 PMID: 18204063

16. Kristensen H, Thomsen AR, Haldrup C, Dyrysjø L, Hayer S, Borre M, et al. Novel diagnostic and prognostic classifiers for prostate cancer identified by genome-wide microRNA profiling. Oncotarget. 2016 May 24; 7(21):30760–71. https://doi.org/10.18632/oncotarget.8953 PMID: 27120795

17. Bell EH, Kirste S, Fleming JL, Stegmaier P, Drendel V, Mo X, et al. A novel miRNA-based predictive model for biochemical failure following post-prostatectomy salvage radiation therapy. PLoS One. 2015 Mar 11; 10(3):e0118745. https://doi.org/10.1371/journal.pone.0118745 eCollection 2015. PMID: 25760964

18. Ramasamy A, Mondry A, Holmes CC, Altman DG. Key Issues in Conducting a Meta-Analysis of Gene Expression Microarray Datasets PLoS Med. 2008 Sep 30; 5(9):e184. https://doi.org/10.1371/journal.pmed.0050184 Epub 2008 Sep 2. PMID: 18767902

19. Benjamin Y and Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. (Wiley: Journal of the Royal Statistical Society. Series B (Methodological), 1995).

20. Wang X, Kang DD, Shen K, Song C, Lu S, Chang LC, et al. An R package suite for microarray meta-analysis in quality control, differentially expressed gene analysis and pathway enrichment detection. Bioinformatics. 2012 Oct 1; 28(19): 2534–2536. Published online 2012 Aug 3 https://doi.org/10.1093/bioinformatics/bts485 PMID: 22637666

21. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Research. 2016 gkw377.
22. Xu J, Jiang N, Shi H, Zhao S, Yao S, Shen H. miR-28-5p promotes the development and progression of ovarian cancer through inhibition of NHBP1. Int J Oncol. 2017 Apr 27. https://doi.org/10.3892/ijo.2017.3977 PMID: 28498450

23. Kuo WT, Yu SY, Li SC, Lam HC, Chang HT, Chen WS. MicroRNA-324 in Human Cancer: miR-324-5p and miR-324-3p Have Distinct Biological Functions in Human Cancer. Anticancer Res. 2016 Oct; 36 (10):5189–5196. https://doi.org/10.21873/anticancer.11089 PMID: 27798879

24. Liu G, Wang H, Fu JD, Liu JY, Yan AG, Guan YY. A five-miRNA expression signature predicts survival in hepatocellular carcinoma. APMIS. 2017 Apr 21. https://doi.org/10.1111/apm.12697 PMID: 28430373

25. Peng L, Zhu H, Wang J, Sui H, Zhang H, Jin C, et al. MiR-492 is functionally involved in Oxaliplatin resistance in colon cancer cells LS174T via its regulating the expression of CD147. Mol Cell Biochem. 2015 Jul; 405(1–2):73–9. https://doi.org/10.1007/s11010-015-2397-z Epub 2015 Apr 11. PMID: 25862460

26. Lu YJ, Liu RY, Hu K, Wang Y. MiR-541-3p reverses cancer progression by directly targeting TGIF2 in non-small cell lung carcinoma. Tumour Biol. 2016 Sep; 37(9):12685–12695. Epub 2016 Jul 22. https://doi.org/10.1007/s13277-016-5241-5 PMID: 27448300

27. Wu AH, Huang YL, Zhang LZ, Tian G, Liao QZ, Chen SL. MiR-572 promoted cell proliferation of human ovarian cancer cells by suppressing PPP2R2C expression. Biomed Pharmacother. 2016 Feb; 77:92–7. https://doi.org/10.1016/j.biopha.2015.12.006 Epub 2015 Dec 28. PMID: 26796271

28. Yun S, Lee SU, Kim JM, Lee HJ, Song HY, Kim YK, et al. Integrated mRNA-microRNA profiling of human NK cell differentiation identifies MiR-583 as a negative regulator of IL2Rγ expression. PLoS One. 2014 Oct 14; 9(10):e108913. https://doi.org/10.1371/journal.pone.0108913 eCollection 2014. PMID: 25313504

29. Yang DS. Novel prediction of anticancer drug chemosensitivity in cancer cell lines: evidence of modulation by microRNA expressions. Conf Proc IEEE Eng Med Biol Soc. 2014; 2014:4780–6. https://doi.org/10.1109/EMBC.2014.694693 PMID: 25571061

30. Hsu CM, Lin PM, Wang YM, Chen ZJ, Lin SF, Yang MY. Circulating miRNA is a novel marker for head and neck squamous cell carcinoma. Tumour Biol. 2012 Dec; 33(6):1933–42. https://doi.org/10.1007/s13277-012-0454-8 Epub 2012 Jul 19. PMID: 22811001

31. Jang JY, LeeYS, Jeon YK, Lee K, Jang JJ, Kim CW. ANT2 suppression by shRNA restores miR-636 expression, thereby downregulating Ras and inhibiting tumorigenesis of hepatocellular carcinoma. Exp Mol Med. 2013 Jan; 45(5):63. https://doi.org/10.1038/emm.2013.1 PMID: 23530670

32. Lin Z, Sun L, Chen W, Liu B, Wang Y, Fan S, et al. miR-639 regulates transforming growth factor beta-induced epithelial-mesenchymal transition in human tongue cancer cells by targeting FOXC1. Cancer Sci. 2014 Oct; 105(10):1288–98. doi: 10.1111/cas.12499. Epub 2014 Sep 29. PMID: 25130698

33. Zhang X, Tang J, Zhi X, Xie K, Wang W, Li Z, et al. Correction: miR-874 functions as a tumor suppressor by inhibiting angiogenesis through STAT3/VEGF-A pathway in gastric cancer. Oncotarget. 2017 Apr 25; 8(17):29535. https://doi.org/10.18632/oncotarget.17402 PMID: 28468128

34. Hu SH, Wang CH, Huang YL, Zhang LZ, Liu JF, Xu CW, Li XL, et al. miR-760 mediates chemoresistance through inhibition of epithelial mesenchymal transition in breast cancer cells. Eur Rev Med Pharmacol Sci. 2016 Dec; 20(23):5002–5008. PMID: 27981531

35. Ying X, Li-ya Q, Feng Z, Yin W, Ji-hong L. MiR-939 promotes the proliferation of human ovarian cancer cells by repressing APC2 expression. Biomed Pharmacother. 2015 Apr; 71:64–9. https://doi.org/10.1016/j.biopha.2015.02.020 Epub 2015 Feb 26. PMID: 25960217

36. Tao J, Wu D, Xu B, Qian W, Li P, Lu Q, et al. microRNA-133 inhibits cell proliferation, migration and invasion in prostate cancer cells by targeting the epidermal growth factor receptor. Oncol Rep. 2012 Jun; 27(6):1967–75. https://doi.org/10.3892/or.2012.1711 Epub 2012 Mar 7. PMID: 22407299

37. Noonan EJ, Place RF, Pookot D, Basak S, Whitson JM, Hirata H, et al; miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. Oncogene 2009, 28:1714–1724. https://doi.org/10.1038/onc.2009.19 PMID: 19252544

38. Daniluainite K, Dubikaityte M, Gibas P, Bakavicius A, Lazutka JR, Ulys A, et al. Clinical significance of miRNA host gene promoter methylation in prostate cancer. Hum Mol Genet. 2017 Apr 7. https://doi.org/10.1093/hmg/ddx138 PMID: 28398479

39. Wu Z, Sun H, Zeng W, He J, Mao X. Upregulation of MicroRNA-370 induces proliferation in human prostate cancer cells by downregulating the transcription factor FOXC1. PLoS One. 2012; 7(9):e45825. https://doi.org/10.1371/journal.pone.0045825 PMID: 23029264

40. Fu Y, Cao F. MicroRNA-125a-5p regulates cell proliferation and migration through NAI1F1 in prostate carcinoma. Onco Targets Ther. 2015 Dec 17; 8:3827–35. https://doi.org/10.2147/OTT.S92314 eCollection 2015. PMID: 26719710

41. Qu F, Zheng J, Gan W, Lian H, He H, Li W, et al. MiR-199a-3p suppresses proliferation and invasion of prostate cancer cells by targeting Smad1. Oncotarget. 2017 Apr 18. https://doi.org/10.18632/oncotarget.17191 PMID: 28477026
42. Guo YJ, Liu JX, Guan YW. Hypoxia induced upregulation of miR-301a/b contributes to increased cell autophagy and viability of prostate cancer cells by targeting NDRG2. Eur Rev Med Pharmacol Sci. 2016; 20(1):101–8. PMID: 26813459

43. Huang K, Tang Y, He L, Dai Y. MicroRNA-340 inhibits prostate cancer cell proliferation and metastasis by targeting the MDM2-p53 pathway. Oncol Rep. 2016 Feb; 35(2):887–95. https://doi.org/10.3892/or.2015.4458 Epub 2015 Nov 26. PMID: 26718483

44. Liu D, Tao T, Xu B, Chen S, Liu C, Zhang L, et al. MiR-361-5p acts as a tumor suppressor in prostate cancer by targeting signal transducer and activator of transcription-6 (STAT6). Biochem Biophys Res Commun. 2014 Feb 28; 445(1):151–6. https://doi.org/10.1016/j.bbrc.2014.01.140 Epub 2014 Jan 31. PMID: 24491557

45. Chen Y, Lu X, Wu B, Su Y, Li J, Wang H. MicroRNA 363 mediated positive regulation of c-myc translation affect prostate cancer development and progress. Neoplasma. 2015; 62(2):191–8. https://doi.org/10.4149/neop_2015_024 PMID: 25591557

46. Ting HJ, Messing J, Yasmin-Karim S, Lee YF. Identification of microRNA-98 as a therapeutic target inhibiting prostate cancer growth and a biomarker induced by vitamin D. J Biol Chem. 2013 Jan 4; 288(1):1–9. https://doi.org/10.1074/jbc.M112.395947 Epub 2013 Feb 25. PMID: 23188821

47. Noonan EJ, Place RF, Basak S, Pookot D, Li LC: miR-449a causes Rb dependent cell cycle arrest and senescence in prostate cancer cells. Oncotarget 2010, 1:349–358. https://doi.org/10.18632/oncotarget.167 PMID: 20948969

48. Fattore L, Mancini R, Acunzo M, Romanò G, Laganà A, Pisanu ME, et al. miR-579-3p controls melanoma progression and resistance to target therapy. Proc Natl Acad Sci U S A. 2016 Aug 23; 113(34):E5005–13. https://doi.org/10.1073/pnas.1607753113 Epub 2016 Aug 8. PMID: 27503895

49. Alvarado S, Wyglinski J, Sederman M, Andrews SA, Szfy M. Methylated DNA binding domain protein 2 (MBD2) coordinately silences gene expression through activation of the microRNA hsa-mir-496 promoter in breast cancer cell line. PLoS One. 2013 Oct 29; 8(10):e74009. https://doi.org/10.1371/journal.pone.0074009 eCollection 2013. PMID: 24204564

50. Nilsson EM, Laursen KB, Whitchurch J, McWilliam A, Ødum N, Persson JL, et al. MiR137 is an androgen regulated repressor of an extended network of transcriptional co-regulators. Oncotarget. 2015 Nov 3; 6(34):35710–25. https://doi.org/10.18632/oncotarget.5958 PMID: 26461474

51. Han C, Yu Z, Duan Z, Kan Q. Role of MicroRNA-1 in Human Cancer and Its Therapeutic Potentials. BioMed Research International. 2014; 2014:428371. https://doi.org/10.1155/2014/428371 PMID: 24949449

52. Liu Y, Qin Z, Yang K, Liu R, Xu Y. Cripto-1 promotes epithelial-mesenchymal transition in prostate cancer via Wnt/β-catenin signaling. Oncol Rep. 2017 Mar; 37(3):1521–1528. https://doi.org/10.3892/or.2017.5378 Epub 2017 Jan 17. PMID: 28098905

53. Anastas JN, Moon RT. WNT signalling pathways as therapeutic targets in cancer. Nat Rev Cancer. 2013 Jan; 13(1):11–26. https://doi.org/10.1038/nrc3419 PMID: 23258168

54. Patel D, Chinaranagar S, Chaudhary J. Basic helix loop helix (bHLH) transcription factor 3 (TCF3, E2A) is regulated by androgens in prostate cancer cells. Am J Cancer Res. 2015 Oct 15; 5(11):3407–21. eCollection 2015. PMID: 26807321

55. Dang Chi V.. MYC on the Path to Cancer. Cell. 2012; 149(1): 22–35. https://doi.org/10.1016/j.cell.2012.03.003 PMID: 22464321

56. Romero OA, Torres-Diz M, Pros E, Savola S, Gomez A, Moran S, et al. MAX Inactivation in Small Cell Lung Cancer Disrupts MYC–SWI/SNF Programs and Is Synthetic Lethal with BRG1. Cancer Discov. 2014 Mar 14; 4(3):292–303. https://doi.org/10.1158/2159-8290.CD-13-0799 PMID: 24362264

57. Kron K, Liu L, Trudel D, Pethe V, Trachtenberg J, Fleshner N, et al. Correlation of ERG expression and DNA methylation biomarkers with adverse clinicopathologic features of prostate cancer. Clin Cancer Res. 2012 May 15; 18 (10):2896–904. https://doi.org/10.1158/1078-0432.CCR-11-2901 Epub 2012 Mar 27 PMID: 22452941

58. Guo D, Bell EH, Mischi P, Chakravarti A. Targeting SREBP-1-driven lipid metabolism to treat cancer. Curr Pharm Des. 2014; 20(15):2619–26. PMID: 23859617

59. Girinsky T, Koumenis C, Graeber TG, Peehl DM, Giaccia AJ. Attenuated response of p53 and p21 in primary cultures of human prostatic epithelial cells exposed to DNA-damaging agents. Cancer Res. 1995 Sep 1; 55(17):3726–31. PMID: 7543816

60. Kibel AS, Suarez BK, Belani J, Oh J, Webster R, Brophy-Ebbers M, et al. CDKN1A and CDKN1B polymorphisms and risk of advanced prostate carcinoma. Cancer Res. 2003 May 1; 63(9):2033–6. PMID: 12727815
61. Jain AK, Raina K, Agarwal R. Deletion of p21/Cdkn1a confers protective effect against prostate tumorigenesis in transgenic adenocarcinoma of the mouse prostate model. Cell Cycle. 2013 May 15; 12(10):1598–604. https://doi.org/10.4161/cc.24741 Epub 2013 Apr 25. PMID: 23624841

62. Sun W, Guo L, Shao G, Liu X, Guan Y, Su L, et al. Suppression of LASP-1 attenuates the carcinogenesis of prostatic cancer cell lines: Key role of the NF-κB pathway. Oncol Rep. 2017 Jan; 37(1):341–347. https://doi.org/10.3892/or.2016.5223 Epub 2016 Nov 7. PMID: 27840958