

RESEARCH ARTICLE

Construction and analysis of mRNA, miRNA, lncRNA, and TF regulatory networks reveal the key genes associated with prostate cancer

Yun Ye¹*, Su-Liang Li¹*, Sheng-Yu Wang²

1 Department of Clinical Laboratory, The First Affiliated Hospital of Xi'an Medical University, Xi'an, Shaanxi, China, **2** Department of Respiration, The First Affiliated Hospital of Xi'an Medical University, Xi'an, Shaanxi, China

* These authors contributed equally to this work.

* yeyun236@163.com



Abstract

Purpose

Prostate cancer (PCa) causes a common male urinary system malignant tumour, and the molecular mechanisms of PCa are related to the abnormal regulation of various signalling pathways. An increasing number of studies have suggested that mRNAs, miRNAs, lncRNAs, and TFs could play important roles in various biological processes that are associated with cancer pathogenesis. This study aims to reveal functional genes and investigate the underlying molecular mechanisms of PCa with bioinformatics.

Methods

Original gene expression profiles were obtained from the GSE64318 and GSE46602 datasets in the Gene Expression Omnibus (GEO). We conducted differential screens of the expression of genes (DEGs) between two groups using the online tool GEO2R based on the R software limma package. Interactions between differentially expressed miRNAs, mRNAs and lncRNAs were predicted and merged with the target genes. Co-expression of miRNAs, lncRNAs and mRNAs was selected to construct mRNA-miRNA-lncRNA interaction networks. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed for the DEGs. Protein-protein interaction (PPI) networks were constructed, and transcription factors were annotated. Expression of hub genes in the TCGA datasets was verified to improve the reliability of our analysis.

Results

The results demonstrate that 60 miRNAs, 1578 mRNAs and 61 lncRNAs were differentially expressed in PCa. The mRNA-miRNA-lncRNA networks were composed of 5 miRNA nodes, 13 lncRNA nodes, and 45 mRNA nodes. The DEGs were mainly enriched in the nuclei and cytoplasm and were involved in the regulation of transcription, related to sequence-specific DNA binding, and participated in the regulation of the PI3K-Akt signalling

OPEN ACCESS

Citation: Ye Y, Li S-L, Wang S-Y (2018) Construction and analysis of mRNA, miRNA, lncRNA, and TF regulatory networks reveal the key genes associated with prostate cancer. PLoS ONE 13(8): e0198055. <https://doi.org/10.1371/journal.pone.0198055>

Editor: Geraldo A. Passos, University of São Paulo, BRAZIL

Received: May 11, 2018

Accepted: August 2, 2018

Published: August 23, 2018

Copyright: © 2018 Ye et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

pathway. These pathways are related to cancer and focal adhesion signalling pathways. Furthermore, we found that 5 miRNAs, 6 lncRNAs, 6 mRNAs and 2 TFs play important regulatory roles in the interaction network. The expression levels of EGFR, VEGFA, PIK3R1, DLG4, TGFBR1 and KIT were significantly different between PCa and normal prostate tissue.

Conclusion

Based on the current study, large-scale effects of interrelated mRNAs, miRNAs, lncRNAs, and TFs established a new prostate cancer network. In addition, we conducted functional module analysis within the network. In conclusion, this study provides new insight for exploration of the molecular mechanisms of PCa and valuable clues for further research into the process of tumourigenesis and its development in PCa.

Introduction

Prostate cancer (PCa) involves a common male urinary system malignant tumour that has the highest incidence among European and American populations [1,2]. This disease not only seriously affects the quality of life of patients but is also associated with financial burdens for society and the family [3,4]. PCa is closely regulated by various cytokines, related genes and intercellular signalling networks in the course of its development. However, the specific molecular mechanisms remain unclear. Therefore, it is important to study the molecular mechanisms of prostate cancer and to prevent the disease and develop effective treatments.

miRNAs are a class of non-coding small RNAs that can be combined with the 3'-UTR of target mRNA to regulate gene expression, which leads to abnormal expression of target genes. A large number of studies have reported abnormal expression of miRNAs in prostate cancer involving multiple miRNAs, such as miR-16, 221, 375, 1290 and 141 [5–7]. Abnormal miRNA expression has been confirmed to be closely related with long non-coding RNAs (lncRNAs) and transcription factors (TFs). lncRNAs regulate miRNA expression by binding and sequestering target miRNAs and participating in mRNA expression regulation [8,9]. lncRNAs are emerging as novel diagnostic markers of PCa, and Chang et al. found that HOTAIR may participate in PCa progression [10]. Tian et al. revealed that RNCR3 functions as a tumour-promoting lncRNA in prostate cancer [11]. Li et al. found long noncoding RNA BDNF-AS is associated with clinical outcomes and plays a functional role in human prostate cancer [12]. As important factors in gene transcription and post-transcriptional regulation, TFs are also involved in controlling signaling pathways with miRNAs [13,14]. A previous study found that CREB1 and FoxA1 associated with advanced prostate cancer, and CREB1/FoxA1 target gene panels can predict prostate cancer recurrence [15]. However, further research is needed to determine the regulatory mechanisms of miRNAs, lncRNAs, TFs and mRNAs in PCa.

Microarray analysis can quickly identify all genes that are expressed at the same time-point [16]. Future research could benefit from integration and analysis of these data [17]. In this work, we identified differentially expressed genes (DEGs) in prostate cancer from the GSE64318 [18] and GSE46602 [19] datasets. We performed Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis for significant DEGs. Furthermore, we analysed mRNA-miRNA-lncRNA and protein-protein interaction (PPI) networks to reveal interactions and identified some factors that may be associated with PCa regulatory

mechanisms. Finally, we analysed hub genes based on the PPI network and TCGA datasets. This study will contribute to understanding the molecular mechanism of prostate cancer, providing valuable clues for further research.

Material and methods

Raw data

The datasets used in the present study were downloaded from the National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>) [20]. The experimental articles that were used to compare the RNAs in prostate cancer tissue and noncancerous prostate tissue from patients were included. The original gene expression profiles were obtained from the GSE64318 [18] and GSE46602 datasets [19] (S1 and S2 Tables). The GSE64318 dataset includes microRNA expression profiles of prostate biopsy samples from 54 prostate biopsy specimens (27 tumours and 27 normal tissues). The platform used for these data is the GPL8227 Agilent-019118 Human miRNA Microarray 2.0 G4470B (miRNA ID version). The GSE46602 dataset includes genome expression profiling of tumour tissue specimens from 36 patients with prostate cancer and normal prostate biopsies from 14 patients. The platform used to analyse these data was the GPL570 Affymetrix Human Genome U133 Plus 2.0 Array.

Identification of differentially expressed genes

GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>) is an interactive web tool based on the R language limma package [21] that can be used to compare two or more groups of samples to identify differential expression in a GEO series. In the present study, GEO2R was used to filter differentially expressed mRNAs and miRNAs between normal and tumour samples separately in each of the datasets. The false discovery rate (FDR) is a method of conceptualizing the rate of type I errors in null hypothesis testing when conducting multiple comparisons. GEO2R calculates the FDR automatically. The multiple t test was used to detect statistically significant genes at the same time with FDR correction. Fold change (FC) >2 and P-value <0.05 were set as the cut-off criteria. Probes without a corresponding gene symbol were then filtered.

Prediction of mRNA-miRNA-lncRNA interactions

Interactions between differentially expressed miRNAs and differentially expressed mRNAs were predicted using miRWalk 3.0 (<http://mirwalk.umm.uni-heidelberg.de/>), which integrated the prediction results of both TargetScan [22] and miRDB [23], and a score ≥ 0.95 was considered as the cutoff criterion for the prediction analysis in miRWalk. Only the target mRNAs included in all of these databases were selected for the further analysis. The interaction between miRNA and lncRNA was predicted by using DIANA-LncBase v2.0 [24] and the score ≥ 0.4 was considered as cutoff criterion for the prediction analysis in the experimental module of LncBase. After the predicted targets were intersected with DEGs, miRNAs, lncRNAs and mRNAs were selected for further analysis. Cytoscape software (version 3.40) was used to visualize the regulatory network.

Gene function analysis

The DAVID database (<http://david.abcc.ncifcrf.gov/>) was used to conduct gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis on significant DEGs and target genes of miRNAs with differential expression. The species was limited to *Homo sapiens*, and the “adjusted P-value (from the Benjamini–Hochberg method), 0.05” was

considered statistically significant [25]. The GO terms included these three criteria: molecular function (MF), cellular component (CC), and biological process (BP).

Protein-protein interaction (PPI) network construction and analysis

The protein-protein interaction network was constructed using the STRING online database. PPI pairs with a combined score ≥ 0.4 were used to construct the PPI network. Transcription factors were annotated using a TF checkpoint [26]. Then, the regulatory relationship between genes was visualized using Cytoscape software (version 3.4.0) and analysed through a topological property of the computing network including the degree distribution of the network using the CentiScaPe app [27]. Furthermore, the gene with a degree > 5 was defined as a hub gene in the regulatory network according to a previous study [28].

TCGA dataset analysis

TCGA is a platform for researchers to download and assess free public datasets (<https://cancergenome.nih.gov/>) [29]. In the present study, we verified the expression of hub genes in TCGA datasets to improve the reliability of our analysis using an online tool, UALCAN (<http://ualcan.path.uab.edu/>) [30]. UALCAN used TCGA level 3 RNA-seq and clinical data from 31 cancer types. As described by Li and Dewey [31], the “scaled_estimate” was multiplied by 10^6 to obtain transcripts per million (TPM) expression value using an in-house PERL (Practical Extraction and Report Language) program. As TPM has been suggested to be more comparable across samples than fragments per kilobase of transcript per million mapped reads (FPKM) and reads per kilobase of transcript per million mapped reads (RPKM) [32], TPM was used as the measure of expression here. Fifty-two normal samples and 497 primary tumour samples of prostate adenocarcinoma (PRAD) in TCGA datasets were included in the present study. Box and whisker plots were used to show hub gene expression levels between normal samples and primary tumour samples in PRAD.

Results

Identification of differentially expressed mRNAs, miRNAs and lncRNAs

The results show that 60 miRNAs, 1578 mRNAs and 128 lncRNAs were differentially expressed in prostate cancer (S3, S4 and S5 Tables). Analysis of the GSE64318 dataset led to the identification of 60 miRNAs in PCa tumour tissues compared with normal prostate tissues, including 35 up-regulated miRNAs and 25 down-regulated miRNAs. Analysis of the GSE46602 dataset led to the identification of 1578 mRNAs, including 583 up-regulated and 995 down-regulated mRNAs, and 128 lncRNAs, including 49 up-regulated and 79 down-regulated lncRNAs. We selected all significantly up-regulated and down-regulated mRNAs, miRNAs and lncRNAs to plot their expression on heat-maps and volcano plots (Fig 1). The top 10 significantly up-regulated and down-regulated miRNAs, mRNAs and lncRNAs are shown in Tables 1, 2 and 3, respectively.

mRNA-miRNA-lncRNA network analysis

The miRNA-mRNA regulatory network was constructed. Interaction analysis shows that 33 differentially expressed miRNAs targeted 143 mRNAs, up-regulating or down-regulating them (Fig 2). In detail, 25 over-expressed miRNAs down-regulate 104 mRNAs, while 8 under-expressed miRNAs up-regulate 15 mRNAs. Subsequently, we reconstructed the lncRNA-miRNA-mRNA network after selecting the pre-treated data with P -values < 0.05 and fold change > 2 (Fig 3). In the network, there are 5 miRNA nodes, 13 lncRNA nodes, and 45

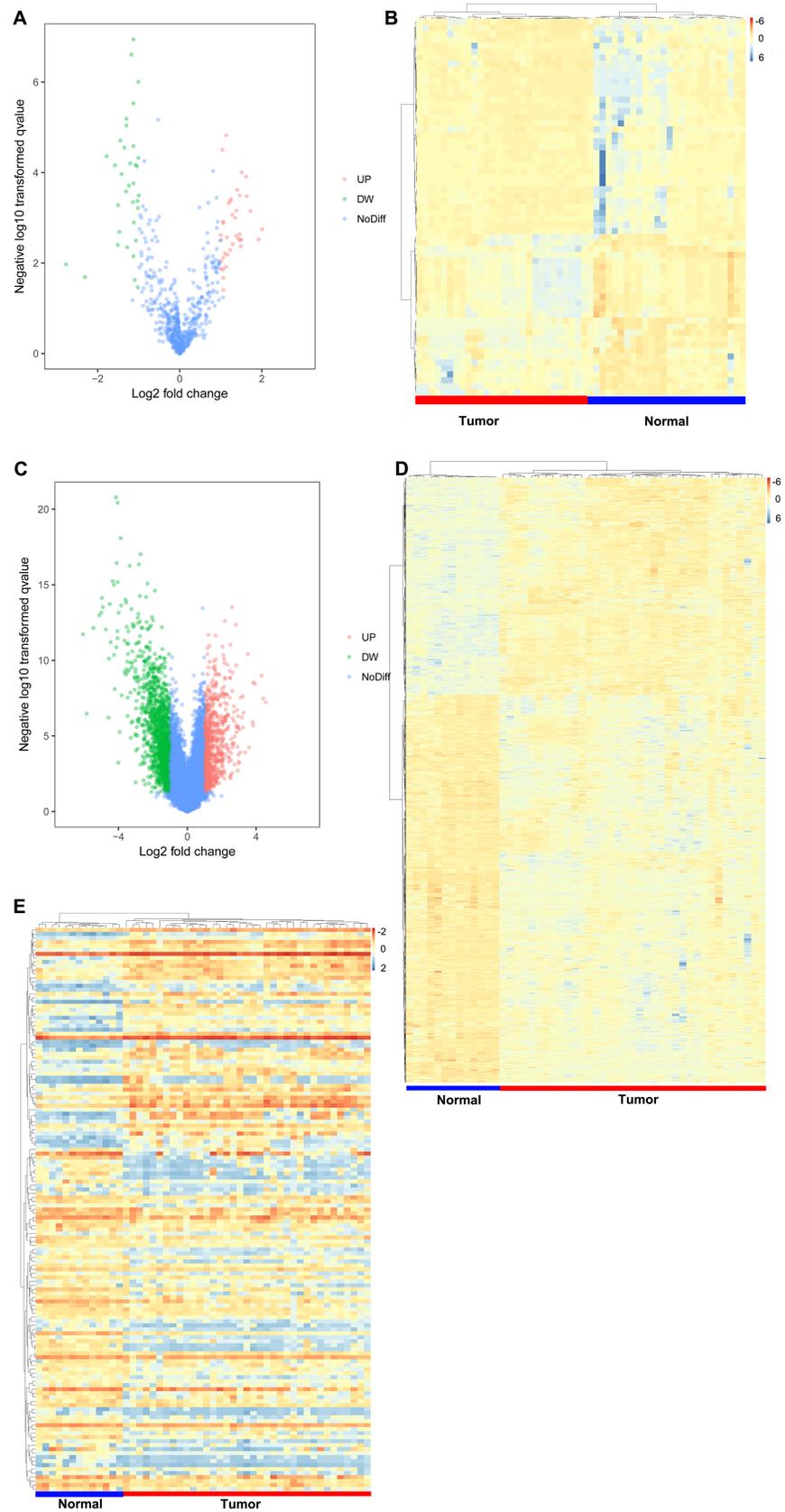


Fig 1. Differentially expressed genes in prostate cancer (PCa) and normal prostate tissue. (A. Volcano plot of differentially expressed miRNAs. B. Heatmap of differentially expressed miRNAs. C. Volcano plot of differentially expressed mRNAs. D. Heatmap of differentially expressed mRNAs. E. Heatmap of differentially expressed lncRNAs).

<https://doi.org/10.1371/journal.pone.0198055.g001>

mRNA nodes. In detail, 9 under-expressed lncRNAs up-regulate 19 miRNAs, while 5 over-expressed miRNAs down-regulate 44 mRNAs.

Function enrichment analysis of DEGs

GO enrichment analyses for DEGs based on the mRNA-miRNA-lncRNA network were performed. The top 10 most significant GO terms of each group were shown. On the MF level, the DEGs were mainly enriched in sequence-specific DNA binding. On the CC level, the DEGs were mainly enriched in the nucleus, cytoplasm and plasma membrane. On the BP level, the DEGs were mainly enriched in positive regulation of transcription from the RNA polymerase II promoter, cell proliferation and cell migration. The top 20 most significant KEGG pathway terms are shown. The genes were mainly enriched in the PI3K-Akt signalling pathway, pathways in cancer and the focal adhesion signalling pathway (Fig 4, S1 File).

Protein-protein interaction network (PPI) analysis

Using the STRING online database and Cytoscape software, a total of 128 of the 1578 DEGs were mapped into the PPI network complex. In this network, with a degree >5, 32 nodes were chosen as hub nodes, including 2 TFs, 14 miRNAs, and 16 mRNAs; the results are presented in Table 4, Fig 5 and S2 File. Moreover, we found that 6 mRNAs (i.e., EGFR, VEGFA, PIK3R1, DLG4, TGFBR1 and KIT) exhibited higher degrees (>10) and miRNA-mRNA pairs. These findings suggest that these nodes may play important roles in the development of PCa.

Table 1. Top 10 significantly up-regulated and down-regulated miRNAs in the GSE64318 dataset.

miRNA_ID	Down/Up	logFC	adj.P.Val	P.Value
hsa-miR-671-5p	Down	-1.58	0.003129	6.91E-05
hsa-miR-923	Down	-1.5	0.01131	5.23E-04
hsa-miR-483-5p	Down	-1.47	0.027441	2.04E-03
hsa-miR-92b	Down	-1.42	0.004019	1.08E-04
hsa-miR-150	Down	-1.35	0.002095	2.81E-05
hsa-miR-498	Down	-1.31	0.007999	2.63E-04
hsa-miR-933	Down	-1.3	0.000927	6.46E-06
hsa-miR-765	Down	-1.3	0.001072	9.14E-06
hsa-miR-563	Down	-1.24	0.006355	1.94E-04
hsa-miR-665	Down	-1.21	0.003129	6.32E-05
hsa-miR-221	Up	1.46	0.009099	3.21E-04
hsa-miR-454	Up	1.46	0.029644	2.31E-03
hsa-miR-26b	Up	1.47	0.034161	3.16E-03
hsa-let-7a	Up	1.5	0.034161	3.02E-03
hsa-miR-203	Up	1.51	0.003856	9.86E-05
hsa-miR-148a	Up	1.62	0.004387	1.23E-04
hsa-miR-183	Up	1.63	0.009145	3.34E-04
hsa-miR-200a	Up	1.72	0.01323	7.09E-04
hsa-miR-1	Up	1.92	0.034161	3.02E-03
hsa-let-7f	Up	2.01	0.024917	1.79E-03

<https://doi.org/10.1371/journal.pone.0198055.t001>

Table 2. Top 10 significantly up-regulated and down-regulated mRNAs in the GSE46602 dataset.

Gene Symbol	Down/Up	logFC	adj.P.Val	P.Value
CD177	Down	-6.06431	2.03E-09	1.89E-12
NEFH	Down	-5.84333	3.13E-05	3.34E-07
SLC14A1	Down	-5.45679	8.83E-10	7.15E-13
KRT5	Down	-5.10424	2.09E-10	1.03E-13
KRT15	Down	-4.99578	1.56E-10	7.00E-14
TRIM29	Down	-4.96486	2.68E-11	7.36E-15
TP63	Down	-4.90695	7.76E-11	2.98E-14
JUP	Down	-4.73608	1.03E-09	9.03E-13
NEFH	Down	-4.57684	5.07E-05	6.18E-07
DST	Down	-4.55381	4.33E-08	7.60E-11
CXCL14	Up	3.356898	2.96E-03	1.22E-04
SIM2	Up	3.518509	2.60E-08	3.80E-11
F5	Up	3.592734	1.63E-03	5.63E-05
LUZP2	Up	3.789194	8.41E-05	1.21E-06
PCA3	Up	3.822882	1.28E-04	2.08E-06
AMACR	Up	3.830373	7.49E-07	2.88E-09
GLYATL1	Up	3.928912	8.57E-07	3.45E-09
AMACR	Up	4.307398	3.25E-07	1.02E-09
DLX1	Up	4.410439	5.09E-06	3.35E-08
PCA3	Up	4.558841	7.89E-06	5.87E-08

<https://doi.org/10.1371/journal.pone.0198055.t002>

Table 3. Top 10 significantly up-regulated and down-regulated lncRNAs in the GSE46602 dataset.

Gene Symbol	Location	adj.P.Val	P.Value	logFC	Down/Up
LINC00844	chr10:58999518–59001617	5.83E-09	6.18E-12	-4.45143	Down
MIR205HG	chr1:209428820–209432552	4.37E-13	5.59E-17	-3.34821	Down
RP11-44F21.5	chr4:75081702–75084717	2.14E-07	6.06E-10	-3.01543	Down
RP11-680G24.5	chr16:15018106–15020488	1.49E-05	1.28E-07	-2.57293	Down
RP11-203B7.2	chr4:146052604–146056762	2.96E-07	9.10E-10	-2.25844	Down
MAGI2-AS3	chr7:79452957–79471208	1.29E-07	3.08E-10	-1.94617	Down
LSAMP-AS1	chr3:116360024–116370085	4.05E-06	2.48E-08	-1.94409	Down
LINC00937	chr12:8356963–8390752	1.63E-04	2.83E-06	-1.93046	Down
LINC00883	chr3:107240692–107326964	3.22E-04	6.79E-06	-1.86947	Down
PGM5-AS1	chr9:68355164–68357866	4.00E-02	4.20E-03	-1.86179	Down
RP5-1092A3.4	chr1:28239509–28241453	1.16E-01	2.21E-02	1.132616	Up
LINC01023	chr5:108727825–108728261	9.06E-04	2.61E-05	1.13526	Up
LINC01128	chr1:827591–859446	4.49E-02	5.00E-03	1.199525	Up
BAIAP2-AS1	chr17:81029133–81034719	1.48E-03	4.96E-05	1.201015	Up
RP11-48B3.4	chr8:80541300–80543104	1.15E-02	7.49E-04	1.284279	Up
RP11-339B21.15	chr9:128305161–128305515	6.32E-05	8.40E-07	1.310892	Up
GABPB1-AS1	chr15:50354174–50358312	8.11E-04	2.26E-05	1.367447	Up
RP11-391M1.4	chr3:40466466–40468587	7.89E-06	5.87E-08	1.489649	Up
RP11-295G20.2	chr1:231522517–231528556	7.32E-04	1.96E-05	1.673082	Up
LINC00992	chr5:117415512–117579744	1.90E-04	3.44E-06	2.288701	Up

<https://doi.org/10.1371/journal.pone.0198055.t003>

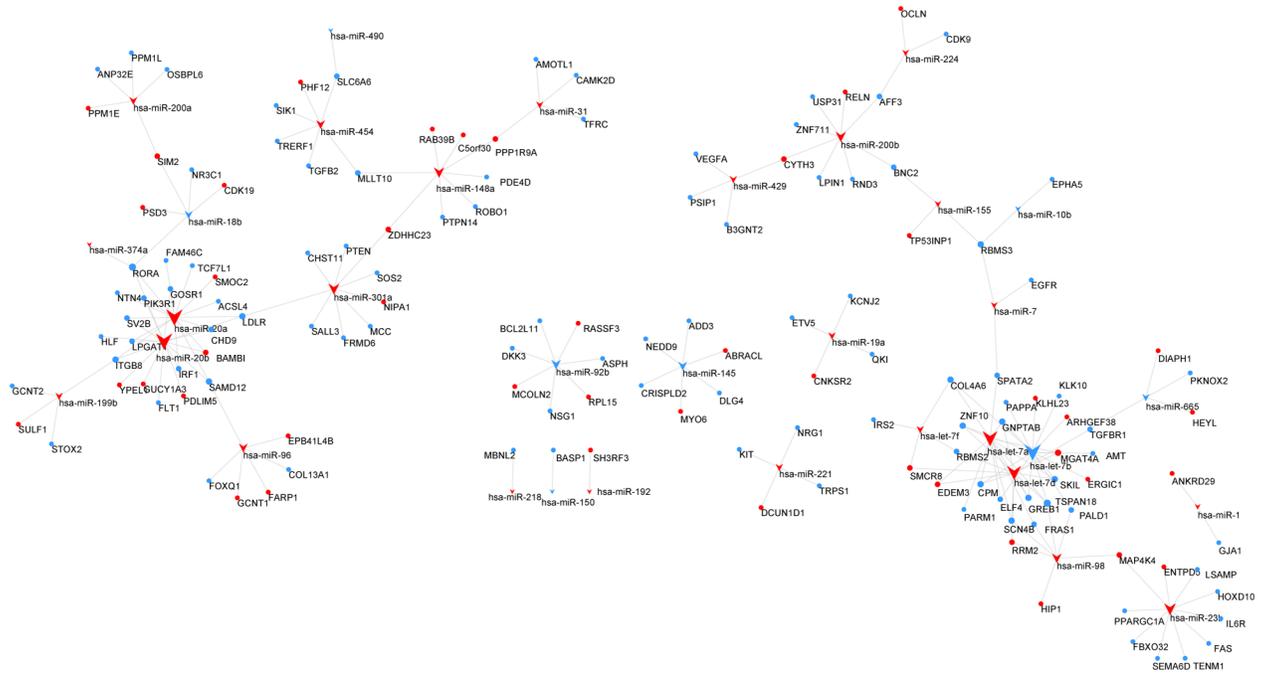


Fig 2. miRNA–mRNA regulatory network. Every node represents one gene, and each edge represents the interaction between genes. miRNAs are indicated with a V-shape, and mRNAs are indicated with circles. The colour red represents high expression, and blue represents low expression. There were 33 miRNAs, 143 mRNAs and 179 edges in the network.

<https://doi.org/10.1371/journal.pone.0198055.g002>

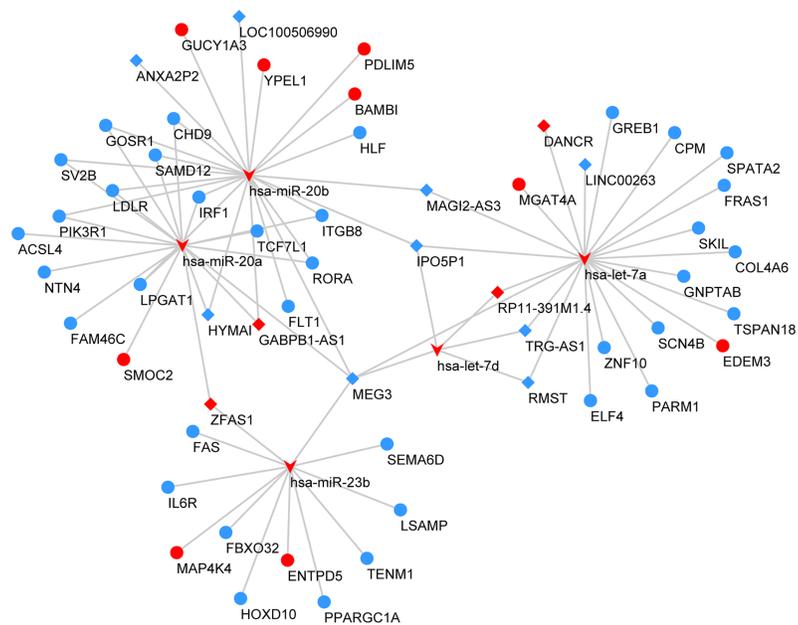


Fig 3. mRNA–miRNA–lncRNA regulatory network. Every node represents one gene, and each edge represents the interaction between genes. mRNAs, miRNAs and lncRNAs are indicated with circles, V-shapes and diamond shapes, respectively. The colour red represents high expression, and blue represents low expression. There were 13 lncRNAs, 5 miRNAs, 45 mRNAs and 81 edges in the network.

<https://doi.org/10.1371/journal.pone.0198055.g003>

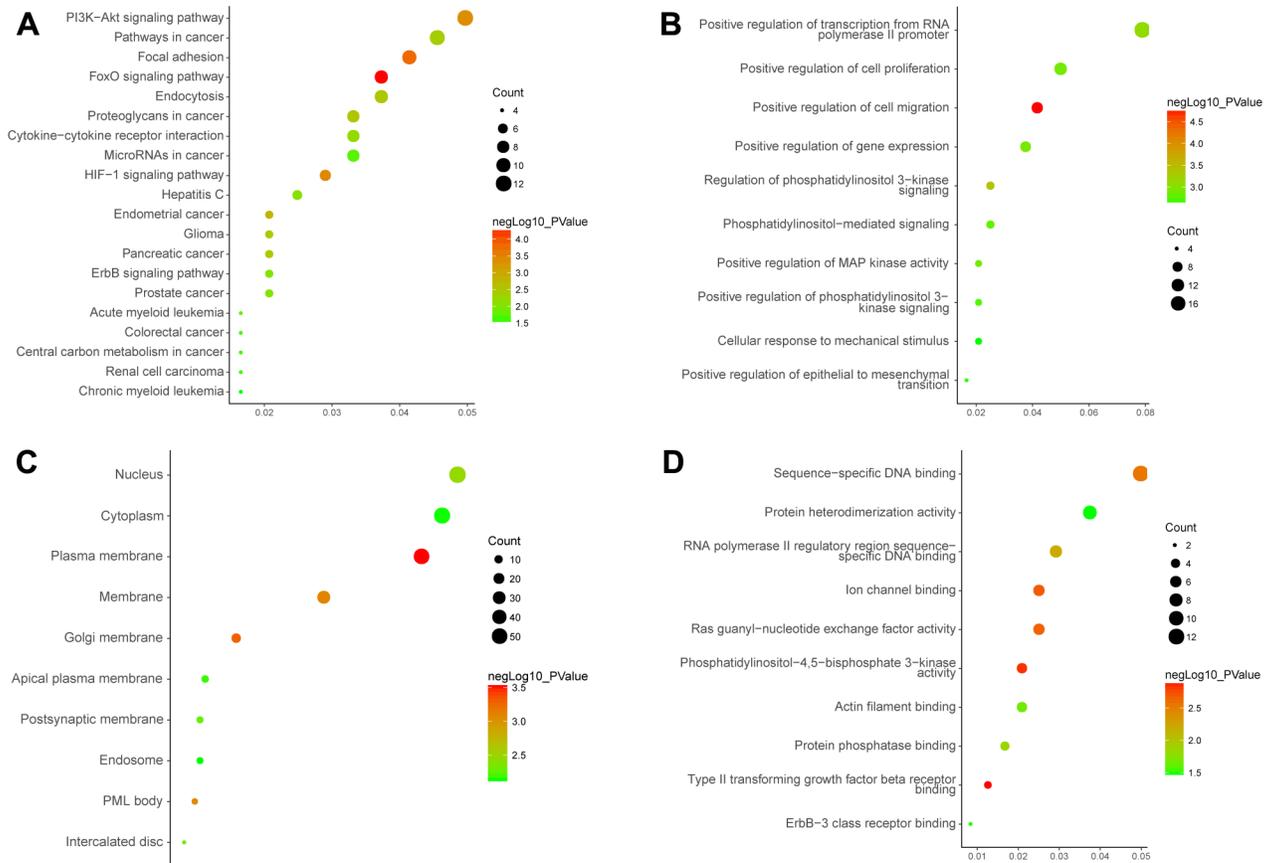


Fig 4. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genome (KEGG) enrichment analysis. (A. The top 20 most significant KEGG pathway terms. B. The top 10 most significant changes in the GO biological process. C. The top 10 most significant changes in the GO cellular component. D. The top 10 most significant changes in the GO molecular function.)

<https://doi.org/10.1371/journal.pone.0198055.g004>

TCGA dataset analysis

TCGA dataset analyses were performed to demonstrate that aberrant expression of the hub genes, including EGFR, VEGFA, PIK3R1, DLG4, TGFBR1 and KIT, were significantly different between PCa and normal prostate tissues (Fig 6).

Discussion

Prostate cancer (PCa) has become a public health issue of great concern worldwide [33]. However, the molecular mechanisms involved in PCa progression are still unclear. Therefore, it is crucial to study the mechanism and identify molecular targets for diagnosis and treatment. A number of studies have found that the development of PCa is related to a variety of signalling pathways, and mRNAs, miRNAs, lncRNAs and TFs play important regulatory roles. In this study, we performed a comprehensive bioinformatics analysis and retrieved mRNAs, miRNAs, lncRNAs and TFs in the interaction network, revealing key genes associated with prostate cancer.

miRNA expression in tumour tissues often differs from that of normal tissue, and this differential expression affects the occurrence, development and prognosis of tumours [34,35]. Studies have confirmed that miRNA expression profiles can be used as biomarkers for the early detection, classification and prognosis of tumours [36,37]. lncRNAs act as miRNA

Table 4. Topology parameters of hub genes (degree >5) in the miRNA-mRNA-TF regulatory network.

Gene	Closeness	Betweenness	Degree
EGFR	0.00212766	4046.701286	22
VEGFA	0.002159827	4331.82023	20
hsa-miR-20b	0.001855288	2752.2272	16
hsa-miR-20a	0.001821494	2973.867274	16
hsa-let-7b	0.001481481	2436.558748	16
hsa-let-7a	0.001582278	1383.644882	14
hsa-let-7d	0.00174216	2553.049636	13
PIK3R1	0.002057613	2045.442867	12
DLG4	0.001869159	3167.065846	12
TGFBR1	0.001976285	3691.770395	11
KIT	0.001904762	1517.550434	11
hsa-miR-23b	0.001587302	1375.820161	10
PTEN	0.001923077	1345.754047	10
hsa-miR-301a	0.001610306	1995.023424	9
LDLR	0.001960784	1888.001784	9
FLT1	0.001930502	1179.255452	9
hsa-miR-200b	0.001445087	1353.57487	8
hsa-miR-148a	0.00140647	1235.527073	8
PPARGC1A	0.001801802	1571.190602	8
BCL2L11	0.001834862	1606.829419	8
NR3C1*	0.00177305	733.3472529	7
NRG1*	0.001872659	1359.250724	7
hsa-miR-98	0.001618123	802.5761652	7
hsa-miR-92b	0.001506024	1655.33632	7
GJA1	0.001745201	1149.048086	7
hsa-miR-96	0.001335113	1416.966025	6
hsa-miR-454	0.001420455	1854.243032	6
hsa-miR-145	0.001461988	1257.608267	6
TFRC	0.001821494	445.8511305	6
ITGB8	0.001808318	2026.300029	6
EPHA5	0.001689189	845.4402231	6
CAMK2D	0.001620746	1045.62274	6

* transcription factors (TFs).

<https://doi.org/10.1371/journal.pone.0198055.t004>

sponges and can regulate miRNA abundance and compete with mRNA for miRNA binding [38]. By constructing an mRNA-miRNA-lncRNA network, we found that the aberrant expression of lncRNAs led to abnormal expression of 5 miRNAs (i.e., has-miR-20a, has-miR-20b, has-miR-23b, has-let-7a and has-let-7d) in PCa and thus regulated the expression of target mRNAs. A previous study demonstrated that 5 miRNAs regulate the development of prostate cancer by targeting specific mRNAs and play an important role in the regulation of prostate cancer [39–41]. The involvement of key lncRNAs including HYMAI, MEG3, IPO5P1, MAG12-AS3, RMST and TRG-AS1 are important. Among them, MEG3 is an important tumour suppressor gene that inhibits cell proliferation and induces apoptosis in PCa [42]. The discovery of these lncRNAs suggests potential diagnostic and therapeutic targets for PCa.

Next, we conducted a functional enrichment analysis of the DEGs based on the mRNA-miRNA-lncRNA network. We found that the DEGs were mainly enriched in the nucleus and

cytoplasm, were involved in transcriptional regulation, were related to sequence-specific DNA binding and participated in regulation of the PI3K-Akt signalling pathway, which is related to cancer, and the focal adhesion signalling pathway. To further analyse the key genes related to PCa, we constructed a PPI network. More significantly, we found that the transcription factor NR3C1 was a hub gene that participates in regulation of the expression of multiple miRNAs (has-miR-20a, has-miR-20b, has-miR-23b). Puhf M et al. assessed NR3C1 expression and the functional significance in tissues from PCa and found that it is a key factor for the development of PCa [43]. Moreover, we found that its regulatory function is similar to MEG3 and its down-regulation leads to increased expression of miRNAs (has-miR-20a, has-miR-20b, and has-miR-23b). Based on these results, we speculate that some regulatory relationship may exist between NR3C1 and MEG3.

Finally, through PPI and TCGA dataset analyses, we found that 6 mRNAs (i.e., EGFR, VEGFA, PIK3R1, DLG4, TGFBR1 and KIT) had higher degrees and miRNA-mRNA pairs. This expression was lower and significantly different between PCa and normal prostate tissues. EGFR belongs to a family of cell membrane receptor tyrosine kinases and is a key factor in tumour cell growth and invasion [44,45]. Previous studies have demonstrated that abnormal expression of EGFR and its downstream signalling contributes to disease progression in PCa [46]. EGFR downregulation is associated with enhanced signalling [47], which can lead to the development of cancer [48]. VEGFA is a mitogen with high endothelial cell specificity that plays a major regulatory role in the development of PCa [49]. A previous study found that PIK3R1, TGFBR1 and KIT might have clinical utility in distinguishing PCa [50–52]. EGFR can regulate VEGF expression by influencing MAPK and PI3K signalling pathways [53]. TGFBR1, DLG4 and KIT are also related to the regulation of MAPK and PI3K signalling pathways [54]. Our results further confirmed the potential biological relevance of 6 mRNAs in PCa. We also found that 6 mRNAs (EGFR, VEGFA, PIK3R1, DLG4, TGFBR1 and KIT) were associated with abnormal expression of 5 miRNAs (has-miR-20a, has-miR-20b, has-miR-23b, has-let-7a and has-let-7d) in PCa. In conclusion, the hub genes that we identified may play crucial roles in PCa.

Conclusion

We constructed and analysed mRNAs, miRNAs, lncRNAs, and TF interaction networks to reveal the key genes associated with prostate cancer. We found that 5 miRNAs (has-miR-20a, has-miR-20b, has-miR-23b, has-let-7a and has-let-7d), 6 lncRNAs (HYMAI, MEG3, IPO5P1, MAG12-AS3, RMST and TRG-AS1), 6 mRNAs (EGFR, VEGFA, PIK3R1, DLG4, TGFBR1 and KIT) and 2 TFs (NR3C1, NRG1) play important regulatory roles in the interaction network. The expression levels of EGFR, VEGFA, PIK3R1, DLG4, TGFBR1 and KIT were significantly different between PCa and normal tissues. Further research is needed to specify the molecular mechanism of these hub genes in PCa.

Supporting information

S1 Table. Clinical and histopathological variables of the study cohort in GSE64318.
(DOCX)

S2 Table. Clinical and histopathological variables of the study cohort in GSE46602.
(DOCX)

S3 Table. Differentially expressed miRNAs in the GSE64318 dataset.
(DOC)

S4 Table. Differentially expressed mRNAs in the GSE46602 dataset.
(DOC)

S5 Table. Differentially expressed lncRNAs in the GSE46602 dataset.
(DOC)

S1 File. GO and KEGG analysis dataset.
(XLSX)

S2 File. PPI analysis dataset.
(XLSX)

Acknowledgments

The authors would like to thank the study subjects and research personnel for their involvement in the study. The authors would also like to thank Professor Xiaoke Hao and Professor Yueyun Ma for their excellent theoretical assistance.

Author Contributions

Conceptualization: Yun Ye.

Data curation: Sheng-Yu Wang.

Investigation: Su-Liang Li.

Methodology: Su-Liang Li.

Resources: Sheng-Yu Wang.

Software: Su-Liang Li.

Writing – original draft: Yun Ye.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010; 127(12):2893–2917. <https://doi.org/10.1002/ijc.25516> PMID: 21351269
2. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin*. 2017; 67(1):7–30. <https://doi.org/10.3322/caac.21387> PMID: 28055103
3. Larson SR, Zhang X, Dumpit R, Coleman I, Lakely B, Roudier M, et al. Characterization of osteoblastic and osteolytic proteins in prostate cancer bone metastases. *Prostate*. 2013; 73(9): 932–940. <https://doi.org/10.1002/pros.22639> PMID: 23334979
4. Rajpar S, Fizazi K. Bone targeted therapies in metastatic castration-resistant prostate cancer. *Cancer J*. 2013; 19(1):66–70. <https://doi.org/10.1097/PPO.0b013e31827f123e> PMID: 23337759
5. Schaefer A, Jung M, Mollenkopf HJ, Wagner I, Stephan C, Jentzmik F, et al. Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma. *Int J Cancer*. 2010; 126(5):1166–1176. <https://doi.org/10.1002/ijc.24827> PMID: 19676045
6. Nguyen HC, Xie W, Yang M, Hsieh CL, Drouin S, Lee GS, et al. Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer. *Prostate*. 2013; 73(4):346–354. <https://doi.org/10.1002/pros.22572> PMID: 22887127
7. Huang X, Yuan T, Liang M, Du M, Xia S, Dittmar R, et al. Exosomal miR-1290 and miR-375 as prognostic markers in castration-resistant prostate cancer. *Eur Urol*. 2015; 67(1):33–41. <https://doi.org/10.1016/j.eururo.2014.07.035> PMID: 25129854
8. Guttman M, Rinn JL. Modular regulatory principles of large non-coding RNAs. *Nature*. 2012; 482(7385):339–346. <https://doi.org/10.1038/nature10887> PMID: 22337053

9. Chen L, Yao H, Wang K, Liu X. Long Non-Coding RNA MALAT1 Regulates ZEB1 Expression by Sponging miR-143-3p and Promotes Hepatocellular Carcinoma Progression. *J Cell Biochem.* 2017; 118(12):4836–4843. <https://doi.org/10.1002/jcb.26158> PMID: 28543721
10. Chang YT, Lin TP, Tang JT, Campbell M, Luo YL, Lu SY, et al. HOTAIR is a REST-regulated lncRNA that promotes neuroendocrine differentiation in castration resistant prostate cancer. *Cancer Lett.* 2018; pii: S0304-3835 (18)30435-X. <https://doi.org/10.1016/j.canlet.2018.06.029> PMID: 29944905
11. Tian C, Deng Y, Jin Y, Shi S, Bi H. Long non-coding RNA RNCR3 promotes prostate cancer progression through targeting miR-185-5p. *Am J Transl Res.* 2018; 10(5):1562–1570 PMID: 29887969
12. Li W, Dou Z, We S, Zhu Z, Pan D, Jia Z, et al. Long noncoding RNA BDNF-AS is associated with clinical outcomes and has functional role in human prostate cancer. *Biomed Pharmacother.* 2018; 102:1105–1110. <https://doi.org/10.1016/j.biopha.2018.03.118> PMID: 29710528
13. Afshar AS, Xu J, Goutsias J. Integrative Identification of Deregulated miRNA/TF-Mediated Gene Regulatory Loops and Networks in Prostate Cancer. *PLoS One.* 2014; 9(6): e100806. <https://doi.org/10.1371/journal.pone.0100806> PMID: 24968068
14. Xu H, He JH, Xiao ZD, Zhang QQ, Chen YQ, Zhou H, et al. Liver-enriched transcription factors regulate microRNA-122 that targets CUTL1 during liver development. *Hepatology.* 2010; 52(4):1431–1442. <https://doi.org/10.1002/hep.23818> PMID: 20842632
15. Sunkel B, Wu D, Chen Z, Wang CM, Liu X, Ye Z, et al. Integrative analysis identifies targetable CREB1/FoxA1 transcriptional co-regulation as a predictor of prostate cancer recurrence. *Nucleic Acids Res.* 2016; 44(9):4105–4122. <https://doi.org/10.1093/nar/gkv1528> PMID: 26743006
16. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science.* 2013; 339(6127): 1546–1558. <https://doi.org/10.1126/science.1235122> PMID: 23539594
17. Guo Y, Bao Y, Ma M, Yang W. Identification of Key Candidate Genes and Pathways in Colorectal Cancer by Integrated Bioinformatical Analysis. *Int J Mol Sci.* 2017; 18(4). pii: E722. <https://doi.org/10.3390/ijms18040722> PMID: 28350360
18. Wang BD, Ceniccola K, Yang Q, Andrawis R, Patel V, Ji Y, et al. Identification and Functional Validation of Reciprocal microRNA-mRNA Pairings in African American Prostate Cancer Disparities. *Clin Cancer Res.* 2015; 21(21):4970–4984. <https://doi.org/10.1158/1078-0432.CCR-14-1566> PMID: 26089375
19. Mortensen MM, Høyer S, Lynnerup AS, Ørntoft TF, Sørensen KD, Borre M2, et al. Expression profiling of prostate cancer tissue delineates genes associated with recurrence after prostatectomy. *Sci Rep.* 2015; 5:16018. <https://doi.org/10.1038/srep16018> PMID: 26522007
20. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 2002; 30(1): 207–210. PMID: 11752295
21. Diboun I, Wernisch L, Orengo CA, Koltzenburg M. Microarray analysis after RNA amplification can detect pronounced differences in gene expression using limma. *BMC Genomics.* 2006; 7:252. <https://doi.org/10.1186/1471-2164-7-252> PMID: 17029630
22. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell.* 2005; 120 (1): 15–20. <https://doi.org/10.1016/j.cell.2004.12.035> PMID: 15652477
23. Wong N, Wang X. miRDB: an online resource for microRNA target prediction and functional annotations. *Nucleic Acids Res.* 2015; 43(Database issue):D146–152. <https://doi.org/10.1093/nar/gku1104> PMID: 25378301
24. Paraskevopoulou MD, Vlachos IS, Karagkouni D, Georgakilas G, Kanellos I, Vergoulis T, et al. DIANA-LncBase v2: indexing microRNA targets on non-coding transcripts. *Nucleic Acids Res.* 2016; 44(D1): D231–238. <https://doi.org/10.1093/nar/gkv1270> PMID: 26612864
25. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet.* 2000; 25(1): 25–29. <https://doi.org/10.1038/75556> PMID: 10802651
26. Chawla K, Tripathi S, Thommesen L, Lægreid A, Kuiper M. TFcheckpoint: a curated compendium of specific DNA-binding RNA polymerase II transcription factors. *Bioinformatics.* 2013; 29(19):2519–2520. <https://doi.org/10.1093/bioinformatics/btt432> PMID: 23933972
27. Scardoni G, Petterlini M, Laudanna C. Analyzing biological network parameters with CentiScaPe. *Bioinformatics.* 2009; 25(21):2857–2859. <https://doi.org/10.1093/bioinformatics/btp517> PMID: 19729372
28. Han JD, Bertin N, Hao T, Goldberg DS, Berriz GF, Zhang LV, et al. Evidence for dynamically organized modularity in the yeast protein-protein interaction network. *Nature.* 2004; 430(6995):88–93. <https://doi.org/10.1038/nature02555> PMID: 15190252

29. Tomczak K, Czerwinska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemp Oncol (Pozn)*. 2015; 19(1A): A68–77. <https://doi.org/10.5114/wo.2014.47136> PMID: 25691825
30. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B VSK, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia*. 2017; 19(8):649–658. <https://doi.org/10.1016/j.neo.2017.05.002> PMID: 28732212
31. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics*. 2011; 12:323. <https://doi.org/10.1186/1471-2105-12-323> PMID: 21816040
32. Li B, Ruotti V, Stewart RM, Thomson JA, Dewey CN. RNA-Seq gene expression estimation with read mapping uncertainty. *Bioinformatics*. 2010; 26(4):493–500. <https://doi.org/10.1093/bioinformatics/btp692> PMID: 20022975
33. Mano R, Eastham J, Yossepowitch O. The very-high-risk prostate cancer: a contemporary update. *Prostate Cancer Prostatic Dis*. 2016; 19(4): 340–348. <https://doi.org/10.1038/pcan.2016.40> PMID: 27618950
34. Song C, Chen H, Wang T, Zhang W, Ru G, Lang J. Expression profile analysis of micro RNAs in prostate cancer by next-generation sequencing. *Prostate*. 2015; 75(5): 500–516. <https://doi.org/10.1002/pros.22936> PMID: 25597612
35. Avril S. [micro RNA expression in breast development and breast cancer]. *Pathologie*. 2013; 34 Suppl 2: 195–200. <https://doi.org/10.1007/s00292-013-1878-7> PMID: 24196612
36. Ferracin M, Veronese A, Negrini M. Micromarkers: miRNAs in cancer diagnosis and prognosis. *Expert Rev Mol Diagn*. 2010; 10(3):297–308. <https://doi.org/10.1586/erm.10.11> PMID: 20370587
37. Søskilde R, Vincent M, Møller AK, Hansen A, Høiby PE, Blondal T, et al. Efficient identification of miRNAs for classification of tumor origin. *J Mol Diagn*. 2014; 16(1):106–115. <https://doi.org/10.1016/j.jmoldx.2013.10.001> PMID: 24211363
38. Wang JB, Liu FH, Chen JH, Ge HT, Mu LY, Bao HB, et al. Identifying survival-associated modules from the dysregulated triplet network in glioblastoma multiforme. *J Cancer Res Clin Oncol*. 2017; 143(4):661–671. <https://doi.org/10.1007/s00432-016-2332-z> PMID: 28168356
39. Liu DF, Wu JT, Wang JM, Liu QZ, Gao ZL, Liu YX. MicroRNA expression profile analysis reveals diagnostic biomarker for human prostate cancer. *Asian Pac J Cancer Prev*. 2012; 13(7):3313–3317. PMID: 22994753
40. Cai S, Chen R, Li X, Cai Y, Ye Z, Li S, et al. Downregulation of microRNA-23a suppresses prostate cancer metastasis by targeting the PAK6-LIMK1 signaling pathway. *Oncotarget*. 2015; 6(6):3904–3917. <https://doi.org/10.18632/oncotarget.2880> PMID: 25714010
41. Wagner S, Ngezahayo A, Murua Escobar H, Nolte I. Role of miRNA let-7 and its major targets in prostate cancer. *Biomed Res Int*. 2014; 2014:376326. <https://doi.org/10.1155/2014/376326> PMID: 25276782
42. Luo G, Wang M, Wu X, Tao D, Xiao X, Wang L, et al. Long Non-Coding RNA MEG3 Inhibits Cell Proliferation and Induces Apoptosis in Prostate Cancer. *Cell Physiol Biochem*. 2015; 37(6):2209–2220. <https://doi.org/10.1159/000438577> PMID: 26610246
43. Pühr M, Hofer J, Eigentler A, Ploner C, Handle F, Schaefer G, et al. The glucocorticoid receptor is a key player for prostate cancer cell survival and a target for improved antiandrogen therapy. *Clin Cancer Res*. 2018; 24(4):927–938. <https://doi.org/10.1158/1078-0432.CCR-17-0989> PMID: 29158269
44. Lim SO, Li CW, Xia W, Lee HH, Chang SS, Shen J, et al. EGFR Signaling Enhances Aerobic Glycolysis in Triple-Negative Breast Cancer Cells to Promote Tumor Growth and Immune Escape. *Cancer Res*. 2016; 76(5):1284–1296. <https://doi.org/10.1158/0008-5472.CAN-15-2478> PMID: 26759242
45. Daizumoto K, Yoshimaru T, Matsushita Y, Fukawa T, Uehara H, Ono M, et al. A DDX31/mutant-p53/EGFR axis promotes multistep progression of muscle invasive bladder cancer. *Cancer Res*. 2018; 78(9):2233–2247. <https://doi.org/10.1158/0008-5472.CAN-17-2528> PMID: 29440146
46. Di Lorenzo G, Tortora G, D'Armiento FP, De Rosa G, Staibano S, Autorino R, et al. Expression of epidermal growth factor receptor correlates with disease relapse and progression to androgen-independence in human prostate cancer. *Clin Cancer Res*. 2002; 8(11): 3438–3444. PMID: 12429632
47. Sorkin A, von Zastrow M. Endocytosis and signalling: intertwining molecular networks. *Nat Rev Mol Cell Biol*. 2009; 10(9):609–622. <https://doi.org/10.1038/nrm2748> PMID: 19696798
48. Roepstorff K, Grøvdal L, Grandal M, Lerdrup M, van Deurs B. Endocytic downregulation of ErbB receptors: mechanisms and relevance in cancer. *Histochem Cell Biol*. 2008; 129(5): 563–578. <https://doi.org/10.1007/s00418-008-0401-3> PMID: 18288481

49. Lee C, Whang YM, Campbell P, Mulcrone PL, Elefteriou F, Cho SW, et al. Dual targeting c-met and VEGFR2 in osteoblasts suppresses growth and osteolysis of prostate cancer bone metastasis. *Cancer Lett.* 2018; 414:205–213. <https://doi.org/10.1016/j.canlet.2017.11.016> PMID: 29174801
50. Hellwinkel OJ, Rogmann JP, Asong LE, Luebke AM, Eichelberg C, Ahyai S, et al. A comprehensive analysis of transcript signatures of the phosphatidylinositol-3 kinase/protein kinase B signal-transduction pathway in prostate cancer. *BJU Int.* 2008; 101(11):1454–1460. <https://doi.org/10.1111/j.1464-410X.2008.07540.x> PMID: 18336616
51. Wen J, Li R, Wen X, Chou G, Lu J, Wang X, et al. Dysregulation of cell cycle related genes and micro-RNAs distinguish the low- from high-risk of prostate cancer. *Diagn Pathol.* 2014; 9:156. <https://doi.org/10.1186/s13000-014-0156-1> PMID: 25257132
52. Fonseca-Alves CE, Kobayashi PE, Palmieri C, Laufer-Amorim R. Investigation of c-KIT and Ki67 expression in normal, preneoplastic and neoplastic canine prostate. *BMC Vet Res.* 2017; 13(1):380. <https://doi.org/10.1186/s12917-017-1304-0> PMID: 29207991
53. Song ZY, Wang F, Cui SX, Qu XJ. Knockdown of CXCR4 Inhibits CXCL12-Induced Angiogenesis in HUVECs through Downregulation of the MAPK/ERK and PI3K/AKT and the Wnt/ β -Catenin Pathways. *Cancer Invest.* 2018; 36(1):10–18. <https://doi.org/10.1080/07357907.2017.1422512> PMID: 29381400
54. Hamidi A, Song J, Thakur N1, Itoh S, Marcusson A, Bergh A, et al. TGF- β promotes PI3K-AKT signaling and prostate cancer cell migration through the TRAF6-mediated ubiquitylation of p85 α . *Sci Signal.* 2017; 10(486): pii: eaal4186. <https://doi.org/10.1126/scisignal.aal4186> PMID: 28676490