Original Article

Screening key miRNAs and genes in prostate cancer by microarray analysis

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Background: Prostate cancer (PCa) is the second most frequent cancer and the fifth leading cause of cancer-related death in men while the mechanisms remain unclear.

Methods: Differentially expressed mRNAs (DEmRNAs) and miRNAs (DEmiRNAs) between PCa and non-tumor controls were identified by using microarray analysis. Functional annotation of DEmRNAs, construction of protein-protein interaction (PPI) network and prediction of upstream transcription factors and downstream target DEmRNAs of DEmiRNAs were conducted to further research functions of key DEmRNAs and DEmiRNAs. Validation of selected DEmRNAs and survival analysis were conducted by using The Cancer Genome Atlas (TCGA).

Results: Total of 91 DEmRNAs and 62 DEmiRNAs were obtained. Thrombospondin-4 precursor (THBS4) was the most significantly up-regulated DEmRNA whose product was predicted to interact with the hub protein of the PCa-specific PPI network, collagen type I alpha 1 chain (COL1A1). Both ATP binding cassette subfamily C member 4 (ABCC4) and endothelin receptor type B (EDNRB) have great prognostic value for PCa. Thrombospondin type 1 domain containing 4 (THSD4) was a down-regulated DEmRNA regulated by several cancer-related miRNAs including has-miR-107, hsa-miR-3175 and hsa-miR-484. Two miRNAs (hsa-miR-428 and hsa-miR-4284) involve in PCa by regulating BMP5-BAMBI interaction and TGF-beta signaling pathway. The expression of selected DEmRNAs between PCa and non-tumor controls in TCGA was consistent with that in our microarray analysis, generally.

Conclusions: Key DEmRNAs and DEmiRNAs between PCa and non-tumor controls were identified in this study which provided clues for exploring the molecular mechanism and developing potential biomarkers and therapeutic target sites for PCa.

Keywords: Prostate cancer (PCa); microarray analysis; differentially expressed genes; miRNA

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Introduction

Prostate cancer (PCa) is the second most frequent cancer and the fifth leading cause of cancer-related death in men (1). It is estimated that there will be 1.7 million new cases annually worldwide by 2030 (2). However, the mechanisms underlying the development and progression of PCa remain to be fully elucidated. Hence, there is an urgent needed for exploring the molecular pathogenesis and developing novel biomarkers and therapeutic strategies for PCa.

PCa carcinogenesis have been reported to be associated with multiple alterations in oncogenes and tumor suppressor genes (3-5). MicroRNAs (miRNAs) are short non-coding RNAs (~22 nucleotides) that regulate up to 30% of human genes post-transcriptionally which represses translation or degrades messenger RNA (mRNA) and play a crucial role in multiple cellular processes such as proliferation, differentiation, apoptosis, migration, and invasion (6,7). Furthermore, accumulated evidence indicated that miRNA play important roles in the processes of PCa (8-10).

In this present study, differentially expressed mRNAs (DEmRNAs) and miRNAs (DEmiRNAs) were identified between PCa and non-tumor tissues by microarray analysis. Functional annotation of DEmRNAs, construction of protein-protein interaction (PPI) network and prediction of upstream transcription factors and downstream target DEmRNAs of DEmiRNAs were conducted to further research functions of key DEmRNAs and DEmiRNAs. Our study may provide new clues for exploring molecular mechanism of PCa and developing PCa-associated diagnostic and therapeutic approaches.

Methods

Patients

Seven patients with PCa and two patients with benign prostatic hyperplasia (BPH) were recruited in this study from The 2nd Hospital of Tianjin medical university between June 2010 and June 2011.The inclusion criteria were as follows: (I) patients with PCa (T2N0M0) were diagnosed based on pathological examination and MRI results; (II) age and sex matched patients with BPH were diagnosed via biopsy. *Table 1* displayed the detailed information of all these participants.

This study has been approved by the ethics institute of The 2nd Hospital of Tianjin medical university (KY2014K016). All these participants signed the informed consent. This research complied with the principles of the Declaration of Helsinki (as revised in 2013).

Preparation of RNA

Pca tumor tissue samples (PCa_1-PCa_7) were obtained from the seven patients with PCa (PCa1-PCa7). Five adjacent non-tumor tissue samples (Con_1-Con_5) obtained from five patients with PCa (PCa1-PCa5) and two prostate tissue samples (Con_6-Con_7) obtained from two patients with BPH (BPH1-BPH2) were serve as non-tumor controls. According to the manufacturer's instructions, total RNA from each sample was isolated and purified by using Trizol (Invitrogen, Gaithersburg, MD, USA) and NucleoSpin® RNA clean-up (MACHEREY-NAGEL, Germany), respectively. The concentration and purity of the total RNA were assayed with a Nanodrop spectrophotometer (Thermo Fisher Scientifc Inc., Waltham, MA, USA). The integrity of the total RNA was confirmed using electrophoresis in agarose gel containing formaldehyde. By using MirVana® miRNA isolation Kit (Ambion, AM1560), miRNA was isolated from each sample.

Gene array profiling

By using Jingxin cRNA linear amplification and labeling kit (CapitalBio) (11), RNA from each sample was labeled with Cy3-dCTP or Cy5-Dctp. Then, labeled products were purified with the PCR NucleoSpin Extract II kit (Machery-Nagel Inc.) and hybridized with the 35k human Genome Array (CapitalBio Inc., Beijing, China). After hybridization, the gene arrays were washed and scanned with CapitalBio LuxScan™10K-A scanners. LuxScan 3.0, BoaoAnalyzer6_step1.pl and BoaoAnalyzer6_step2.pl software products (CapitalBio Inc.) were used to extract and analyze the data. The fluorescence intensity was normalized prior to analysis.

MiRNA array profiling

By using the FlashTagTM Biotin RNA Labeling Kit (Genisphere, LLC, Hatfeld, PA, USA), miRNA was biotin labeled according to the manufacturer's instructions. Then, biotin labeled miRNAs was hybridized to Affymetrix® GeneChip® miRNA Arrays (Affymetrix, Santa Clara, CA, USA) with the Eukaryotic Hybridization Control Kit (Affymetrix, P/N 900454) in the Hybridization Oven 640 (Affymetrix). Hybridized miRNA arrays were washed and stained using the Hybridization, Wash and Stain Kit (Affymetrix) on the Fluidics Station 450 (Affymetrix) and

Table 1 Patient characteristic

Index	PCa1	PCa2	PCa3	PCa4	PCa5	PCa6	PCa7	BPH_1	BPH_2
Gender	Male	Male	Male	Male	Male	Male	Male	Male	Male
Age (years)	70	62	66	68	72	68	61	60	64
Pathological type	PRAD	PRAD and DAP	PRAD	PRAD	PRAD	PRAD	PRAD	BPH	BPH
Gleason score	6	6	7	5	7	7	8	NA	NA
TNM stage	T2N0M0	T2N0M0	T2N0M0	T2N0M0	T2N0M0	T3aN0M0	T3bN0M0	NA	NA

PCa, prostate cancer; PRAD, prostate adenocarcinoma; DAP, ductal adenocarcinoma of the prostate.

scanned on a GeneChip® Scanner 3000 (Affymetrix) using Command Console™ 1. Data summarization and quality control were performed by using Affymetrix miRNA QC tool. With the Robust Multichip Average (RMA), background correction and normalization were conducted.

Identification of DEmRNAs and DEmiRNAs

The gene array data and the miRNA array data obtained were normalized by locally weighted scatterplot smoothing (LOWESS) method (12). Then, the *t*-test method in the LIMMA package of R software (13) was used to identify the DEmRNAs and DEmiRNAs between PCa and non-tumor controls. |log2Foldchange| >1 and P value <0.05 were the thresholds for the DEmRNAs and P value <0.05 was the threshold for DEmiRNAs. Hierarchical clustering analysis of DEmiRNAs and DEmRNAs were conducted by using R package "pheatmap".

PPI network

To further research the biological functions of DEmRNAs between PCa and non-tumor controls, the PPI network was constructed by using STRING (http://string-db.org/cgi/about.pl?sessionId=DfAjFN3R1SaY&footer_active_subpage=content) and the Cytoscape software. Nodes and edges were used to represent the proteins and interactions between two proteins, respectively. Proteins with a degree of ≥5 were defined as hub proteins of this PPI network.

Functional annotation

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of DEmRNAs between PCa and non-tumor controls were conducted by using online-based software DAVID (https://david.ncifcrf.gov/). Statistical significance was defined as P value <0.05.

Prediction of potential transcription factors of DE-miRNAs

FunRich software, is a tool mainly used for functional enrichment and interaction network analysis of genes and proteins (14). By using FunRich software, the upstream transcription factors of DEmiRNAs were predicted.

DEmiRNA-DEmRNA interaction analysis

Four bioinformatic algorithms (miRDB, TarPmiR, miRTarBase and Targetscan) with miRwalk 3.0 (http://miRwalk.umm.uni-heidelberg.de/) were used to predict the downstream target genes. Then, DEmiRNA-DEmRNA pairs predicted by these four bioinformatic algorithms in which DEmRNA was negatively correlated with DEmiRNAs in this study were retained for our following research. Based on these above DEmiRNA-DEmRNA pairs, the DEmiRNA-DEmRNA interaction network was constructed by the Cytoscape software.

Validation for the expression of DEmRNAs

The Cancer Genome Atlas (TCGA; https://tcga-data.nci.nih.gov/tcga/) is a public-funded project which consists of multi-dimensional data of for multiple cancers at DNA, RNA, and protein levels. The mRNA profile of 492 prostate adenocarcinoma (PRAD) cases and 52 adjacent non-tumor controls was stored in TCGA. Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) is a web-based tool to deliver fast and customizable functionalities based on TCGA and GTEx data (15). By

using GEPIA, expression of selected DEmRNAs obtained in this study were validated by TCGA.

Survival analysis

To evaluate the prognostic value of all these obtained DEmRNAs in PCa, survival analysis was performed for all these DEmRNAs by using GEPIA.

Results

DEmRNAs and DEmiRNAs between PCa and non-tumor controls

Total of 91 DEmRNAs (51 up-regulated and 40 down-regulated DEmRNAs) and 62 DEmiRNAs (39 up-regulated and 23 down-regulated DEmiRNAs) between PCa and non-tumor controls were obtained. Top 20 significantly DEmRNAs and DEmiRNAs were displayed in *Tables 2* and *3*, respectively. Hierarchical cluster analysis of DEmRNAs and DEmiRNAs were shown in *Figure 1A* and *B*, respectively.

Functional annotation

The most significantly enriched GO terms in PCa were extracellular space (P=5.71E-05), extracellular exosome (P=3.44E-04), cell adhesion (P=4.65E-04), extracellular matrix (P=1.11E-03) and membrane raft (P=1.33E-03). The significantly enriched GO terms including "biological process", "molecular function", and "cellular component" were displayed in *Figure 2A*. TGF-beta signaling pathway (P=5.62E-03) was the only pathway that enriched for DEmRNAs between PCa and non-tumor controls. Four DEmRNAs including inhibin subunit beta A (INHBA) and BMP and activin membrane bound inhibitor (BAMBI), inhibitor of DNA binding 4, HLH protein (ID4) and bone morphogenetic protein 5 (BMP5) were enriched in this pathway (*Figure 2B*).

Prediction of upstream transcription factors of DEmiRNAs

After analysis by using FunRich software, upstream transcription factors of DEmiRNAs were predicted. The top 10 transcription factors for DEmiRNAs (*Figure 3A*) were early growth response 1 (EGR1), POU class 2 homeobox 1 (POU2F1), NK6 homeobox 1 (NKX6-1), Sp1 transcription factor (SP1), NOBOX oogenesis homeobox (NOBOX), AT-rich interaction domain 3A (ARID3A), YY1 transcription factor (YY1), homeobox A9 (HOXA9), myogenic factor 5 (MYF5), POU class 4 homeobox 3 (POU4F3).

DEmiRNAs-DEmRNAs interaction network

A total of 54 DEmiRNA-DEmRNA pairs consisted of 24 DEmiRNAs (12 up-regulated miRNAs and 2 down-regulated mRNAs) and 14 DEmRNAs (5 up-regulated mRNAs and 19 down-regulated mRNAs) were obtained. The DEmiRNA-DEmRNA interaction network was displayed in *Figure 3B*, hsa-miR-484, hsa-miR-1231 and hsa-miR-941 were three hub miRNAs.

PPI network

The PPI network of DEmRNAs were consisted of 36 nodes and 30 edges (*Figure 4*). Two hub proteins including collagen type I alpha 1 chain (COL1A1, degree =7) and Thy-1 cell surface antigen (THY1, degree =5) were identified based on this PPI network (*Figure 4*).

Validation of selected DEmRNAs in TCGA

Based on our microarray analysis, three up-regulated DEmRNAs including thrombospondin-4 precursor (THBS4), PDZ and LIM domain protein 5 (PDLIM5) and ATP binding cassette subfamily C member 4 (ABCC4) and five down-regulated DEmRNAs including bone morphogenetic protein 5 precursor (BMP5), FXYD domain-containing ion transport regulator 1 (FXYD1), inhibitor of DNA binding 4 (ID4) , thrombospondin type 1 domain containing 4 (THSD4) and endothelin receptor type B (EDNRB) were selected to perform the expression validation by TCGA. The different expression levels of these eight DEmRNAs between PRAD and adjacent non-tumor controls in TCGA were analyzed and depicted through box-plots (*Figure 5*) which were consistent with our microarray analysis, generally.

Survival analysis

Up-regulation of ABCC4 is significantly associated with poor survival of patients with PCa (P=0.045, *Figure 6A*). Decreased EDNRB predicts poor survival in PCa (P=0.027, *Figure 6B*). Both these two genes have great prognostic value for PCa.

Discussion

Table 2 Top 20 up-regulated and down-regulated DEmRNAs between PCa and non-tumor controls

Gene ID	Gene symbol	Log2FC	P value
ENSG00000113296	*THBS4*	3.420962	0.030016
ENSG00000198542	*ITGBL1*	3.116457	0.036118
ENSG00000169507	*NP_775783.1*	3.067172	0.049361
ENSG00000144355	*DLX1*	2.998112	0.044163
ENSG00000105664	*COMP*	2.532081	0.047278
ENSG00000106819	*ASPN*	2.481949	0.025225
ENSG00000132821	*C20orf102*	2.271759	0.006654
ENSG00000153982	*GDPD1*	2.24658	0.025275
ENSG00000109625	*CPZ*	2.028859	0.031082
ENSG00000147676	*MAL2*	1.979245	0.047306
ENSG00000170369	*CST2*	1.893333	0.027599
ENSG00000164687	*FABP5*	1.891178	0.044616
ENSG00000163110	*PDLIM5*	1.807621	0.02019
ENSG00000154917	*RAB6B*	1.771981	0.029096
ENSG00000187210	*GCNT1*	1.756058	0.009711
ENSG00000177707	*PVRL3*	1.654416	0.020207
ENSG00000105707	*HPN*	1.653384	0.00697
ENSG00000183010	*PYCR1*	1.647304	0.011148
ENSG00000140479	*PCSK6*	1.612633	0.02487
ENSG00000122641	*INHBA*	1.59196	0.025026
ENSG00000074590	*NUAK1*	–3.677414	0.033604
ENSG00000112175	*BMP5*	–2.569483	0.031053
ENSG00000143196	*DPT*	–2.213348	0.018534
ENSG00000172005	*MAL*	–2.184924	0.034631
ENSG00000101938	*CHRDL1*	–2.024137	0.044497
ENSG00000186847	*KRT14*	–1.955376	0.048046
ENSG00000049319	*SRD5A2*	–1.933853	0.041699
ENSG00000096141	*LY6G6D*	–1.920051	0.048276
ENSG00000110244	*APOA4*	–1.89642	0.046703
ENSG00000204789	*ZNF204*	–1.877634	0.016781
ENSG00000154188	*ANGPT1*	–1.7652	0.036458
ENSG00000126258	*FXYD1*	–1.747271	0.03719
ENSG00000162641	*C1orf62*	–1.721416	0.008241
ENSG00000151778	*C13orf21*	–1.703242	0.041983
ENSG00000165646	*SLC18A2*	–1.686371	0.047647
ENSG00000176049	*JAKMIP2*	–1.471425	0.025972
ENSG00000137273	*FOXF2*	–1.463853	0.035233
ENSG00000161055	*SCGB3A1*	–1.463547	0.00583
ENSG00000173376	*C4orf31*	–1.446799	0.046379
ENSG00000172201	*ID4*	–1.420409	0.020618

PCa, prostate cancer; DEmRNAs, differentially expressed mRNAs; Log2FC, log2Fold change.

PCa is the most lethal urogenital system malignancy in men. Therefore, investigating the molecular mechanisms involved in the progression of PCa is crucial and may reveal novel biomarkers and therapeutic strategies. In this study, DEmRNAs and DEmiRNAs between PCa and non-tumor controls were identified by microarray analysis and their potential roles were further explored by bioinformatics analysis.

THBS4 was the most significantly up-regulated gene between PCa and non-tumor controls in this study. As a member of the extracellular calcium-binding protein family (16), THBS4 was reported to involve with various cancers including colorectal, gastric and prostate cancers (17-19). Furthermore, increased THBS4 has been demonstrated to play a significant role in the proliferation and migration of PCa by regulating the matrix metalloproteinases-9 (MMP-9) and p38 Mitogen-activated protein kinase (MAPK) signaling pathway (4). In this present study, interaction between THBS4 and a potential regulator in metastasis of PCa, COL1A1 (5,8) was found which provided clues for exploring the precise role of THBS4 in PCa.

Two DEmRNAs including ABCC4 and EDNRB with great prognostic value for PCa were identified in this study which might serve as potential biomarkers. Highly methylated EDNRB (20,21) and dysregulated ABCC4 (22,23) have been reported to be regulators of PCa. In addition, EDNRB could be regulated by both hsa-miR-107 and hsa-miR-762. Hsa-miR-107 was a potential regulator of multiple cancers (24-26). Aberrant expressed hsa-miR-107 has been found in meningioma (25) which promoted the proliferation and inhibited apoptosis of colon cancer cells (26). Therefore, hsa-miR-107/hsa-miR-762-EDNRB interactions might play a key role in the processes of PCa.

According to the KEGG enrichment analysis, TGF-

Table 3 Top 20 up-regulated and down-regulated DEmiRNAs between PCa and non-tumor controls

miRNA	log2FC	P value
hsa-miR-183	1.572899	0.047641
hsa-miR-182	1.553273	0.03888
hsa-miR-4284	1.318899	0.04523
hsa-miR-663b	1.283888	0.016842
hsa-miR-375	1.229287	0.021521
hsa-miR-941	1.083061	0.018677
hsa-miR-3180-3p	0.926267	0.043474
hsa-miR-93	0.893455	0.015524
hsa-miR-106b	0.885544	0.047635
hsa-miR-1231	0.866421	0.034641
hsa-miR-217	0.823899	0.04281
hsa-miR-1292	0.742308	0.020546
hsa-miR-25	0.733483	0.015988
hsa-miR-484	0.692888	0.027458
hsa-miR-1307	0.687092	0.023083
hsa-miR-339	0.653019	0.03867
hsa-miR-200c	0.620878	0.013658
hsa-miR-3175	0.610865	0.023216
hsa-miR-23a	0.601889	0.022816
hsa-miR-1972	0.550624	0.016358
hsa-miR-376c	−1.10784	0.031947
hsa-miR-1	−1.04235	0.046145
hsa-miR-30e	−0.82655	0.014027
hsa-miR-221	−0.81939	0.006079
hsa-miR-503	−0.77411	0.025611
hsa-miR-99a	−0.75539	0.006023
hsa-miR-338	−0.74427	0.042402
hsa-miR-15a	−0.73052	0.041203
hsa-miR-29a	−0.7237	0.007211
hsa-miR-455	−0.7106	0.01735
hsa-miR-455	−0.70809	0.033923
hsa-miR-221	−0.70405	0.044713
hsa-miR-27b	−0.63631	0.005391
hsa-miR-422a	−0.63095	0.021855
hsa-miR-379	−0.60175	0.04474
hsa-miR-376a	−0.59835	0.033791
hsa-miR-494	−0.59444	0.011013
hsa-let-7g	−0.5942	0.043972
hsa-miR-337	−0.58311	0.039703
hsa-miR-224	−0.52878	0.034879

PCa, prostate cancer; DEmiRNAs, differentially expressed miRNAs; Log2FC, log2 fold change.

beta signaling pathway is the sole pathway that enriched for DEmRNAs of PCa. Previous studies have confirmed that TGF-beta signaling pathway is involved in cancer invasion, metastasis and angiogenesis (27). Furthermore, TGF-beta might involve with the regulation of collagen (28), fbronectin (29), laminin (30) and MMP-9 (31) to affect migratory and invasive capacity. BMP5 and BAMBI were two DEmRNAs that enriched in TGF-beta signaling pathway. Aberrant transcriptional expression levels of several bone morphogenetic proteins (BMPs) such as BMP 2–7 have been observed in PCa compared to normal prostate tissues which highlighted the importance of the BMP family in PCa (32). Based on our PPI network, BMP5, a member of BMP family was found to interact with BAMBI, and BAMBI could make an inhibition on BMP as well as TGF-beta signaling pathway (33). Moreover, BMP5 was a shared downstream target of two cancer-related miRNAs, hsa-miR-4284 (34) and hsa-miR-484 (35,36). We speculated that these two miRNAs play potential roles in PCa by regulating BMP5-BAMBI interaction and TGF-beta signaling pathway.

Based on DEmiRNA-DEmRNA interaction network, THSD4 is a down-regulated DEmRNA regulated by a maximum of DEmiRNAs including hsa-miR-107, hsa-miR-1972, hsa-miR-3175, hsa-miR-3196, hsa-miR-484, hsa-miR-663b, hsa-miR-762 and hsa-miR-941. Although no previous study reports the association between THSD4 and PCa, THSD4 has been indicated to involve in tumorigenesis and development of various cancers including breast cancer, glioblastoma and esophageal carcinoma (37-39). Moreover, has-miR-107, hsa-miR-3175 and hsa-miR-484 have been proved to be potential mediators of PCa (26,40,41) which suggested that THSD4 might be potential regulators of PCa with regulation by these PCa-related miRNAs.

Conclusions

In conclusion, key DEmRNAs and DEmiRNAs between PCa and non-tumor controls were identified in this study. Both ABCC4 and EDNRB have great prognostic value for PCa which were potential prognostic biomarkers. THSD4 serves as a potential regulator of PCa regulated by several cancer-related miRNAs including has-miR-107, hsa-miR-3175 and hsa-miR-484. Hsa-miR-428 and hsa-miR-4284 might play potential roles in PCa by regulating BMP5-BAMBI interaction and TGF-beta signaling pathway. All these findings provided clues for exploring the

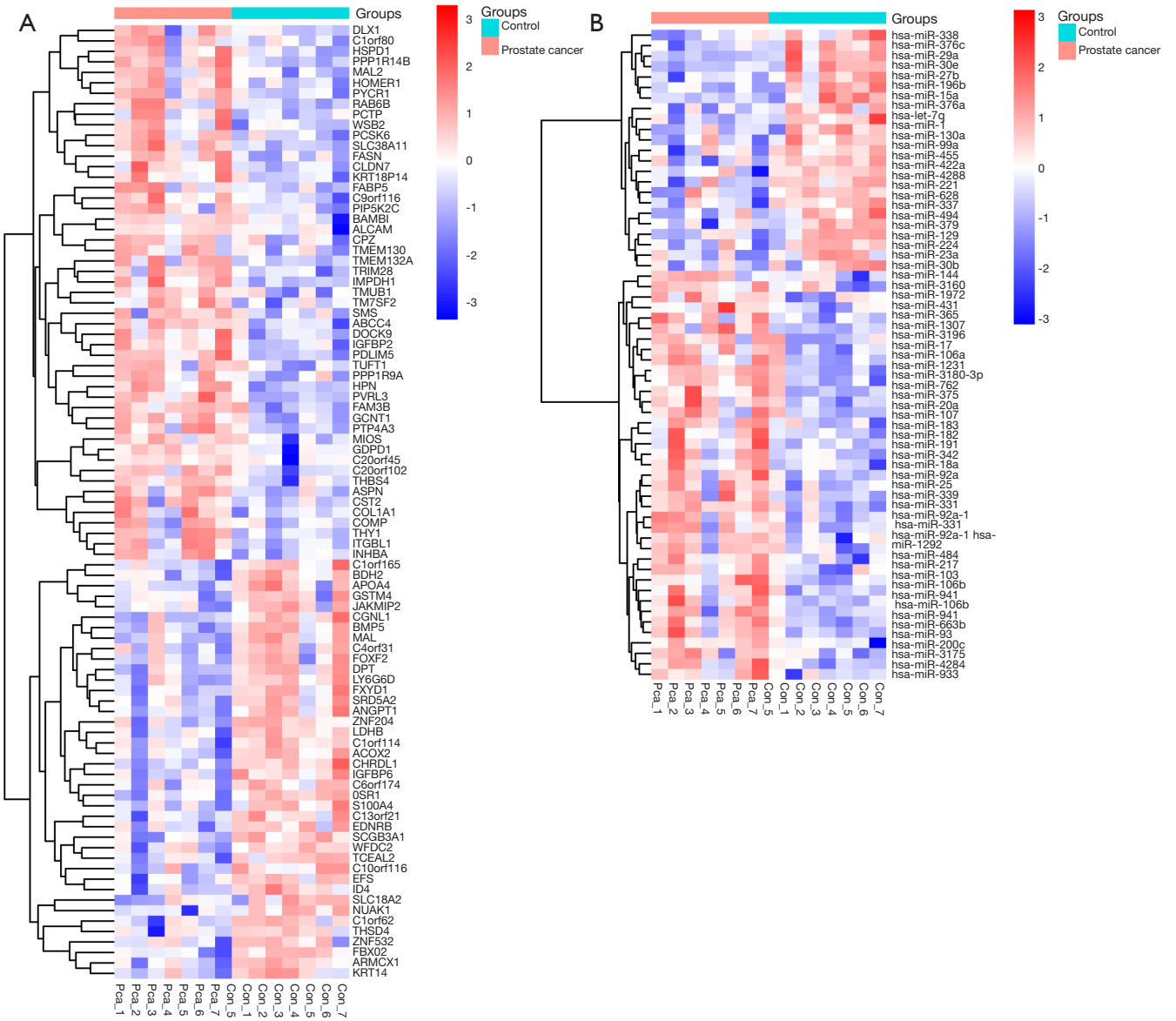

Figure 1 Hierarchical clustering analysis of DEmRNAs and DEmiRNAs between PCa and non-tumor controls. (A) DEmRNAs; (B) DEmiRNAs. Row and column represent DEmRNAs/DEmiRNAs and samples, respectively. The color scale indicated the log2-transformed expression levels of DEmRNAs and DEmiRNAs. Red and blue color indicate up- and down-regulation, respectively. DEmRNAs, differentially expressed mRNAs; DEmiRNAs, differentially expressed miRNAs; PCa, prostate cancer.

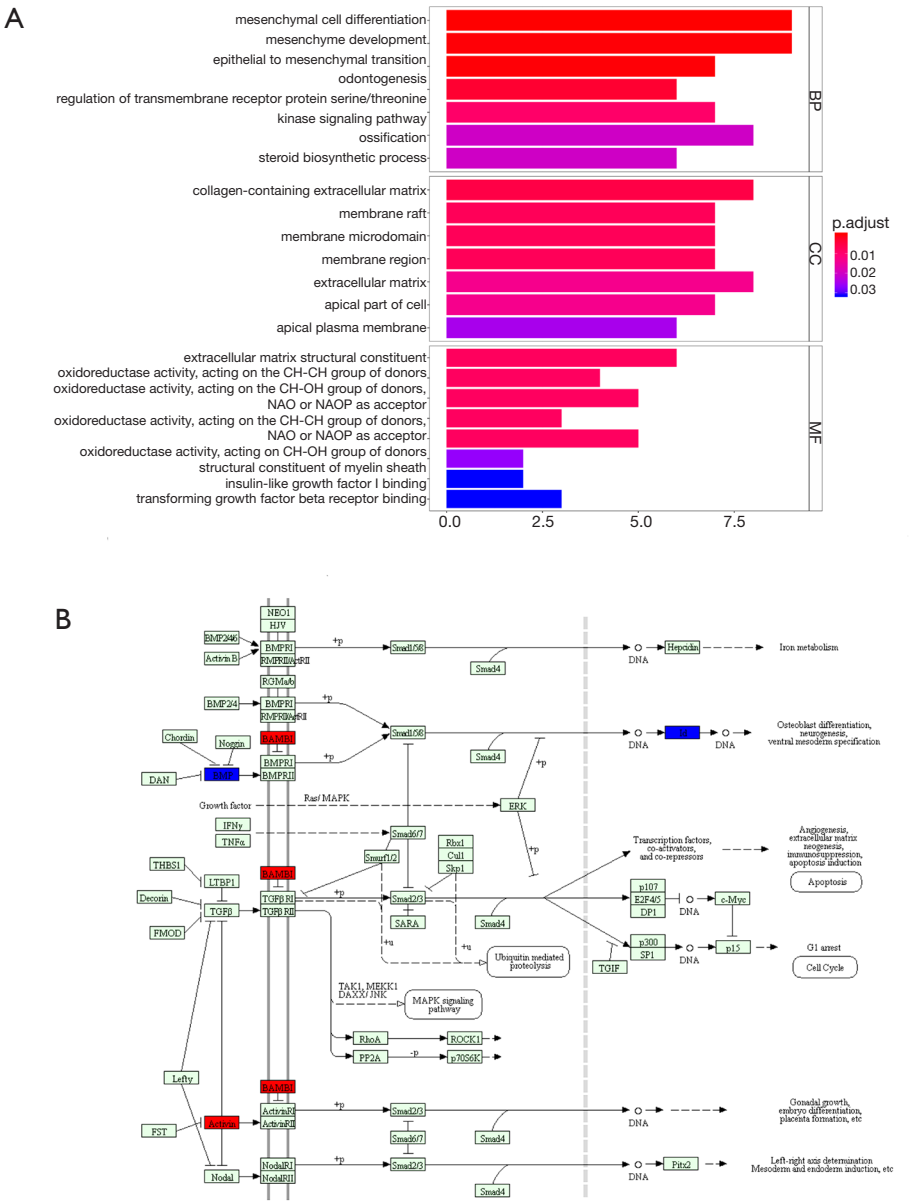

Figure 2 Functional annotation of DEmRNAs. (A) Significantly enriched GO terms including "biological process (BP)", "molecular function (MF)", and "cellular component (CC)" for DEmRNAs between PCa and non-tumor controls. The Y-axis shows GO terms and the X-axis represents counts of DEmRNAs enriched in GO terms. The color scale represents adjust P value. (B) TGF-beta signaling pathway. The red and blue rectangles represented the particles which regulated by the up- and down-regulated DEmRNAs between PCa and non-tumor controls, respectively. DEmRNAs, differentially expressed mRNAs; GO, Gene ontology; PCa, prostate cancer.

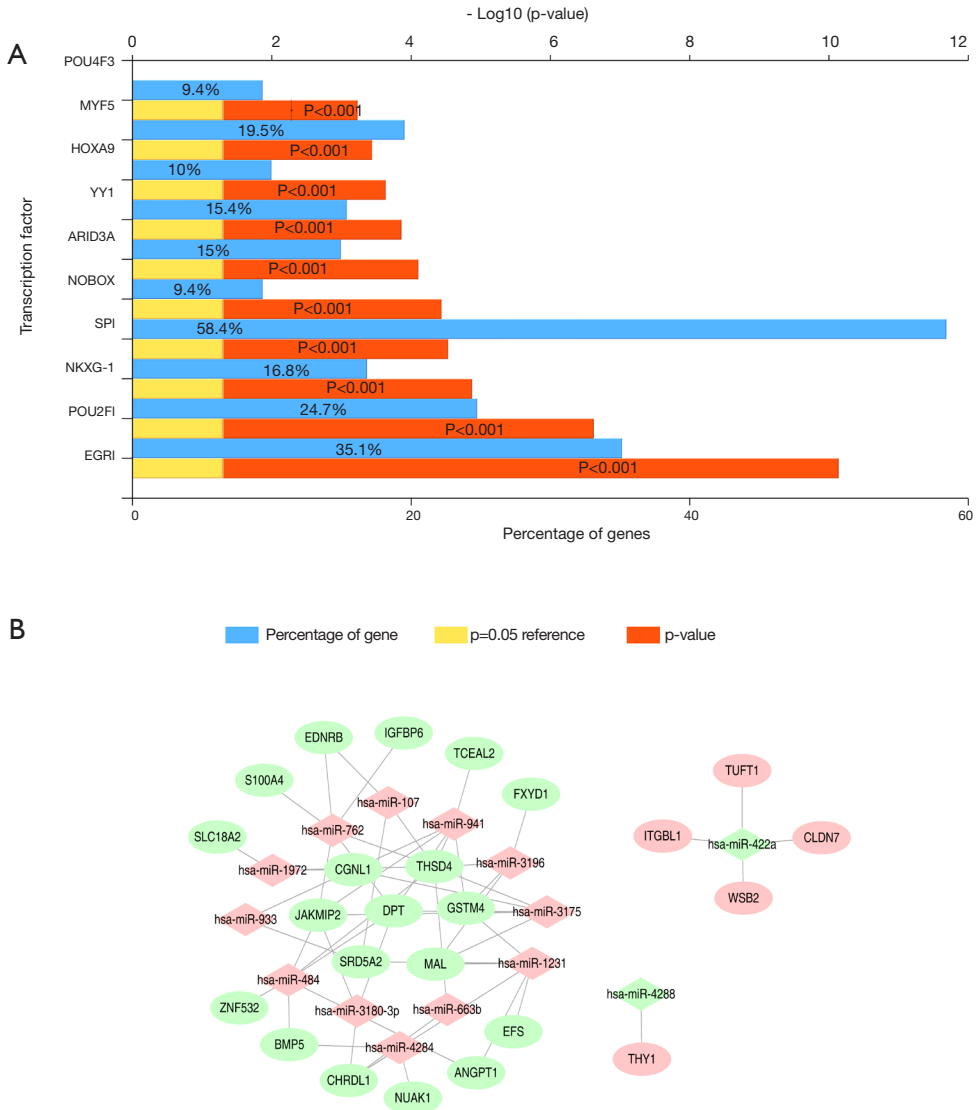

Figure 3 DEmiRNA-DEmRNA interaction network. (A) Top 10 upstream transcription factors of DEmiRNAs. The X-axis shows percentage of genes and the Y-axis represents transcription factors enriched for DEmiRNAs. (B) DEmiRNA-DEmRNA interaction network. The ellipses and rhombuses represent DEmRNAs and DEmiRNAs between PCa and non-tumor control, respectively. Red and green color indicates up- and down-regulation, respectively. DEmRNAs, differentially expressed mRNAs; DEmiRNAs, differentially expressed miRNAs; PCa, prostate cancer.

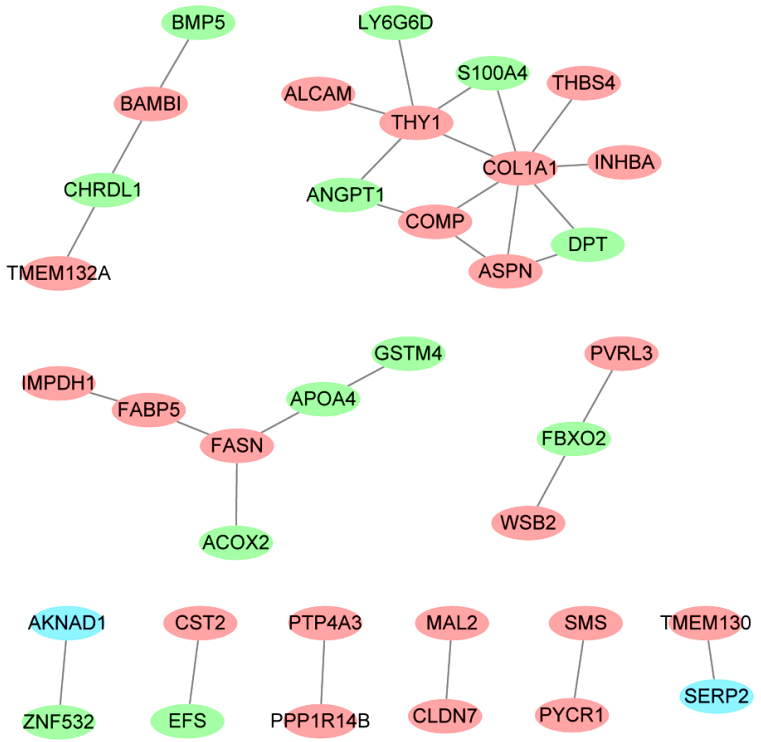

Figure 4 PPI network. Red and green ellipses represent proteins encoded by up- and down-regulated DEmRNAs, respectively. Edges indicate integrations between proteins. Blue ellipses represent other proteins. PPI, protein-protein interaction; DEmRNAs, differentially expressed mRNAs.

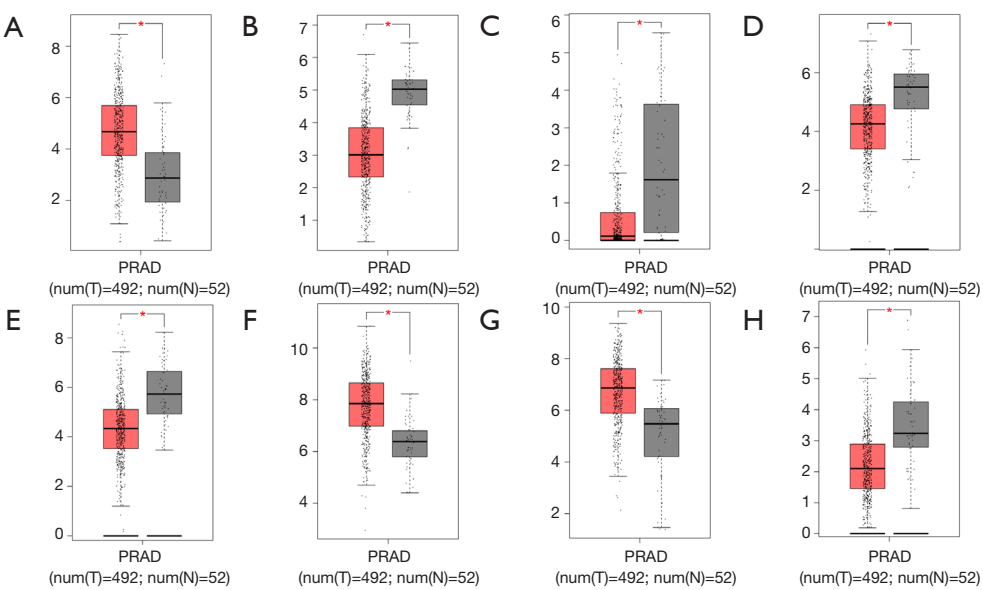

Figure 5 Validation of the expression levels of selected DEmRNAs in PCa based on TCGA database. (A) THBS4; (B) ID4; (C) BMP5; (D) THSD4; (E) FXYD1; (F) PDLIM5; (G) ABCC4; (H) EDNRB. The x-axis shows PRAD group (T) and adjacent non-tumor group (N) and y-axis shows the relative expression levels of DEmRNAs. * indicates P value <0.05. DEmRNAs, differentially expressed mRNAs; PCa, prostate cancer; TCGA, The Cancer Genome Atlas.

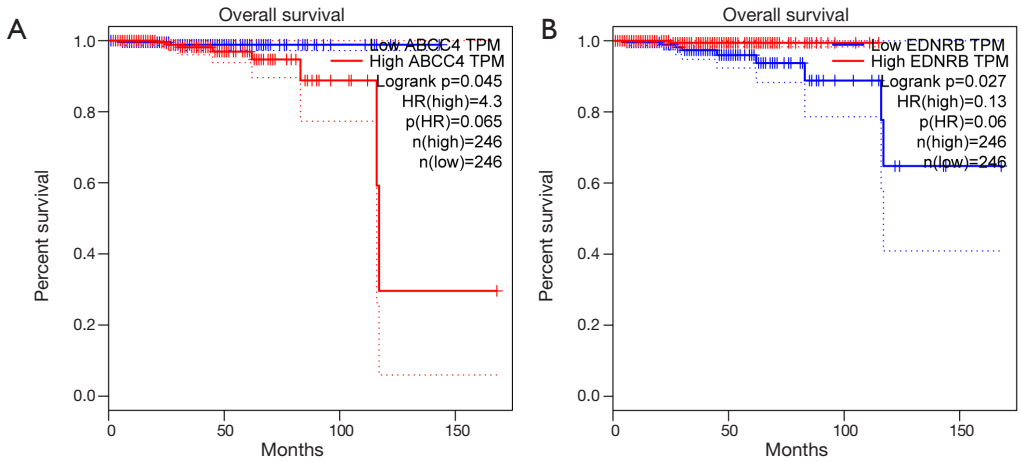

Figure 6 Survival analysis of ABCC4 and EDNRB in PCa. The x-axis shows times (months) and y-axis shows the survival rate. (A) ABCC4; (B) EDNRB. PCa, prostate cancer.

underlying mechanism of PCa and further experiment was needed to confirm our conclusion.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.12.30). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study has been approved by the ethics institute of The Second Hospital of Tianjin medical university (KY2014K016). All these participants signed the informed consent. This research complied with the principles of the Declaration of Helsinki (as revised in 2013).

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