A Novel Analysis of the Differentiated Expression of Long Noncoding RNAs Profiles in Human Prostate Cancer

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Abstract

Background: Long noncoding RNAs (lncRNAs) have crucial roles in cancer biology. Increasing numbers of evidences have indicated that lncRNAs play an important role in the pathogenesis, invasion, and metastasis in almost all kinds of cancers. But, compared with the large amounts of patients, there is rare reports that showed the differential expression of lncRNAs in prostate cancer.

Methods: In this study, lncRNA expression profiles were screened in PCa, by using 5 pairs of clinical specimens in PCa and matched non-PCa tissues with lncRNA chip. To affirm further clinical value, we extended the samples consisting of another 5 tumor specimens and 7 para-cancerous/benign contrasts by qRT-PCR in top 10 up-regulated and down-regulated lncRNAs.

Results: A total of 817 lncRNAs were differentially expressed between PCa tumor and para-cancerous tissues (Fold Change ≥2.0, $p < 0.05$): 422 were upregulated, whereas 395 were downregulated in PCa tissues. Gene ontology and KEGG pathway analyses showed that many lncRNAs were implicated in carcinogenesis. Among differentially expressed lncRNAs, lnc-MYL2-4:1 (FC = 0.00141, $p = 0.01909$) and NR_125857 (FC = 59.27658, $p = 0.00128$) had the highest magnitude of change. The subsequent qPCR confirmed the expression of NR_125857 accorded with the clinical samples.

Conclusions: Our study detected a relatively novel complicated map of lncRNAs in PCa, which may have the potential to investigate for diagnosis, treatment and follow-up in PCa. Our study revealed the expression of NR_125857 in human PCa tissues was most up-regulated. Further study of these meaningful candidates are need to research deep mechanisms.

Backgroud

Prostate cancer (PCa) is one of the most common malignancy in males, for in the global, it causes estimated 1,276,106 cases and 358,989 deaths in 2018 [1]. In addition, PCa ranks the fifth leading cause of cancer death worldwide [2].With the development of economy and society, China is experiencing a transition stage from a developing country into a developed country, named as “westernized lifestyle-related cancer”, so we can effortlessly draw the conclusion that there is an ascendant tendency in the incidence rates of PCa [3]. Many conventional high-risk factors have been concerned with the period of tumorigenesis, invasion and metastasis of PCa, including genetic, environmental and life-style factors.

PCa is normally hormone-dependent at diagnosis indicating androgen receptor (AR) signaling is a distinctive feature in this disease. The AR is a ligand-activated transcription factor typically responsive to the androgen testosterone and dihydrotestosterone. Even though the majority of patients with PCa are treated successfully with surgery and/or radiotherapy, metastatic disease develops in a significant proportion of patients. Androgen deprivation therapy (ADT), urological surgery or chemical castration, is a standard treatment used in recurrent PCa [4]. However, eventually, patients will develop castration-resistant prostate cancer (CRPC) prompting additional therapies, many of which further block the androgen axis [5]. This stage of PCa, known as CRPC, is currently hopeless. Recent studies revealed that the frequency of AR-null CRPC is increasing, because of the application of more effective AR antagonists such as enzalutamide and abiraterone [6]. Since the effort of urologists for the patient in the end stage of this disease is limited, it is imperative for the scientists to progress effective biomarkers for very early detection and active target for clinical treatment.

Non-coding RNAs (ncRNAs) are a class of RNA molecules that lack protein-coding potential. Several important classes of ncRNA are transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), microRNAs (miRNAs), small nucleolar RNAs (snoRNA), small nuclear RNAs (snRNA), and long non-coding RNAs (lncRNAs) [7]. Accumulating genomic and transcriptomic sequencing results have revealed that only small proportion of the human genome is transcribed into protein-coding mRNAs, whereas the majority of the genome is transcribed into ncRNAs [8, 9]. Amongst the classes of ncRNAs, long noncoding RNAs (lncRNAs) are a class of transcripts longer than 200 nucleotides with limited protein coding potential [10]. Unlike microRNAs or proteins, lncRNAs function cannot be currently inferred from sequence or structure, with the diversity of long ncRNAs described to date precluding simple generalizations. The broad functional repertoire of long ncRNAs includes roles in high-order chromosomal dynamics, telomere biology and subcellular structural organization [11]. LncRNAs regulate local protein-coding gene expression at the level of chromatin remodeling, transcriptional control and post-transcriptional processing, which suggests that RNA has continued to evolve and expand alongside proteins and DNA and indicate they have multiple functions in a wide range of biological processes, such as proliferation, apoptosis, or cell migration [10, 12]. Unlike miRNAs, lncRNAs are able to fold into secondary and tertiary structures by which they carry out their

function [13]. Upward high-throughput transcriptomics studies propose long noncoding RNA (lncRNA) dysregulation in various diseases, including neuroblastoma, pancreatic ductal adenocarcinoma, lung cancer and other cancers through corresponding miRNAs [14–17]. Moreover, they are detected in circulating blood and/or urine [18–20]. LncRNAs are a novel class of potential biomarkers and therapeutic targets for the treatment of cancer [21].

Nevertheless, the function of most lncRNAs is still unknown. A growing amount of evidence has showed that lncRNAs play a vital role in the progression of PCa [22]. Especially, the expression levels and potential roles of lncRNAs in PCa are need to be further studied [23]. Through additional discovery of molecular mechanisms in lncRNAs to broaden the field of PCa, patients can profit from developing more effective healing innovations. Generally, the consequences of lncRNA chip vary from samples to samples. In this study, we considered the differential expression of lncRNAs in PCa tissues to find out several vital and potential biomarkers so that we could construct an independent bank of lncRNAs in PCa for research among the target cohorts in southeast coastal areas of China. Therefore, to provide research subjects in the further study, we firstly figured out the expression difference of lncRNAs in PCa through chip analysis, and then we carried out the validation of the distensible clinical samples.

Material And Method

Tissue samples

A group of five pairs of PCa and matched non-tumor normal tissues were collected from Huashan Hospital, Fudan University. To deep confirm, another cohort of prostate tissues were obtained from prostate needle biopsies in Huashan Hospital, Fudan University. Our study was permitted by the ethics committee of Huashan Hospital, Fudan University and written informed consent was obtained from all patients. All tissue was histologically identified by pathological section. If diagnosed as prostate adenocarcinoma, the Gleason score, PSA value, TNM stage and recurrence were according to the NCCN guideline. Otherwise, the tissues are recognized as normal contrast. A subset of patients had matched PCa tissues and normal tissues available in the qPCR. The initial screening step (Table 1) was conducted with microarray chip assay. Another cohort screening information, which was considered as the validation of the expanded clinical samples (Table 2), was listed with the qPCR.

Table 1
The main clinical information of patients with PCA included in our study.

NO	Gender	Age (Years)	Histological type	Initial total PSA	Gleason score	TNM stage
12	Man	64	Adenocarcinoma	9.39	3 + 4	T2cN0M0
34	Man	50	Adenocarcinoma	15.84	4 + 3	T3bN0M0
56	Man	62	Adenocarcinoma	14	4 + 3	T3bN0M1
78	Man	54	Adenocarcinoma	9.13	4 + 3	T2cN0M0
910	Man	62	Adenocarcinoma	54.66	5 + 4	T3bN1M1

Table 2
The basal clinical characteristics of extended samples by prostate biopsy for qPCR included in our study.

NO	Gender	Age (Years)	Histological type	Initial total PSA	Gleason score	Proportion of Cancer tissue
001	Man	62	Adenocarcinoma	10.44	3 + 4	80%
002	Man	51	Adenocarcinoma	8.08	4 + 5	70%
003	Man	53	Adenocarcinoma	9.11	4 + 4	30%
004	Man	54	Adenocarcinoma	8.48	3 + 3	5%
005	Man	55	Adenocarcinoma	11.84	4 + 3	60%
006	Man	63	Normal tissue	10.92	/	/
007	Man	78	Normal tissue	9.46	/	/
008	Man	50	Normal tissue	9.56	/	/
009	Man	63	Normal tissue	12.71	/	/
010	Man	72	Normal tissue	10.82	/	/
011	Man	77	Normal tissue	8.33	/	/
012	Man	58	Normal tissue	9.21	/	/

Table 3
The list of the primer sequence for the top 10 up-regulated and down-regulated lncRNAs.

The accession of lncRNA	The primer sequence
hGAPDH-Q-F	TCAAGGCTGAGAACGGGAAG
hGAPDH-Q-R	TCGCCCCACTTGATTTTGGA
NR_125857-F	CCCATCCTCATTTGGTGCTG
NR_125857-R	CAACAGACAACACGAGGCAG
NR_015342-F	GAAGCACCTCGCATTTGTGG
NR_015342-R	TTTCTCAAACCGCCTGATGC
NR_109832-F	TCCGTCTCCTGCATGTCCTTGG
NR_109832-R	ACCTTCACCCTCCAGCCACAG
ENST00000412654-F	AGCATGGTCCCCAATGTAGC
ENST00000412654-R	CCACCCATGAGGCGTAATCA
lnc-AC110080.1-5:1-F	CCATCTCCTGCAAGTCTAGTCA
lnc-AC110080.1-5:1-R	TGCCACTGAACCTATCTGGC
ENST00000415820-F	GAGGGTAGATGGAGCATCGC
ENST00000415820-R	TTCCAGTTCTTTGCTCCGCA
ENST00000558010-F	TGCCCGTAATCCCTTTGTCC
ENST00000558010-R	ATTTGGTGCCGTGTGCTAGA
ENST00000365110-F	TTGCACGTTGTTGGAGCTTG
ENST00000365110-R	AATTTGCCCCTCACGTAGCA
NONHSAT072254-F	CAGGGCCAGTCAGAGTCTTTC
NONHSAT072254-R	CCACTGAACCTGTCTGGGATG
NONHSAT072236-F	CAGGGCCAGTCAGAGTCTTTC
NONHSAT072236-R	CCACTGAACCTGTCTGGGATG
ENST00000424251-F	TGGCATGAGCAAACTTGGGC
ENST00000424251-R	CAGTGCCAATAACGGCCACA
lnc-TACC2-3:1-F	ACGCCTGCATCTTCACAAAG
lnc-TACC2-3:1-R	TCAAAGCTACACAATGCGGG
NR_125859-F	TTTGCCCATAAGTCTCCCTGG
NR_125859-R	TTCCAAGCCAGCGTTTTCAC
NONHSAT136589-F	TGCTGGCTGCTCTGAACTAAA
NONHSAT136589-R	TCCAGCTTTTAGGCACACACA
lnc-CHST2-2:3-F	TGCAGACAAGTGTGTATGAGT
lnc-CHST2-2:3-R	CTGTCTGCTAACAAAGGGTTCA
lnc-PDCD11-5:1-F	AATCCCATCAGGCGTAGGG

l the forward primer while -R presented the reverse primer.

The accession of lncRNA	The primer sequence
lnc-PDCD11-5:1-R	GCAGAAATCACACCCAGGTTC
lnc-PTEN-11:1-F	TGCCAGTCTCTAGGTCCCTG
lnc-PTEN-11:1-R	AGACGCCAGGCTCCCAA
lnc-MID1-4:1-F	CAGAGCAAGGCACCCACTAA
lnc-MID1-4:1-R	CCCACGACTGCTCCAAAGTA
lnc-C19orf73-1:1-F	ACTGCGACACAGCGGTA
lnc-C19orf73-1:1-R	GGAGCACGTTTATTCAGAGAAAT
lnc-MYL2-4:1-F	TATTGTTCCTGGGCTGCAGA
lnc-MYL2-4:1- R	GGAGAACACGTTGGAGTTGG
***-F presented the forward primer while -R presented the reverse primer.**	

Rna Extraction And Purification

Total RNA was extracted and purified using mirVana™ miRNA Isolation Kit (Cat#AM1561, Ambion, Austin, TX, US) following the manufacturer's instructions and checked for a RIN number to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US).

Rna Labeling

rRNA was amplified and labeled by Low Input Quick Amp WT Labeling Kit (Cat.# 5190 – 2943, Agilent technologies, Santa Clara, CA, US), following the manufacturer's instructions. Labeled cRNA were purified by RNeasy mini kit (Cat.# 74106, QIAGEN, GmBH, Germany).

Array Hybridization

Each slide was hybridized with 1.65 µg Cy3-labeled cRNA using Gene Expression Hybridization Kit (Cat.# 5188–5242, Agilent technologies, Santa Clara, CA, US) in Hybridization Oven (Cat.# G2545A, Agilent technologies, Santa Clara, CA, US), according to the manufacturer's instructions. After 17 hours hybridization, slides were washed in staining dishes (Cat.# 121, Thermo Shandon, Waltham, MA, US) with Gene Expression Wash Buffer Kit (Cat.# 5188–5327, Agilent technologies, Santa Clara, CA, US), followed the manufacturer's instructions. Differentially expressed lncRNAs were analyzed with independent samples t-test. LncRNAs with $\geq$ 2.0 fold-changes (FC) and $p < 0.05 < / > cript >$ were selected as lncRNAs with significant differential expression.

Data Acquisition

Slides were scanned by Agilent Microarray Scanner (Cat#G2565CA, Agilent technologies, Santa Clara, CA, US) with default settings, Dye channel: Green, Scan resolution = 3 µm, PMT 100%, 20bit. Data were extracted with Feature Extraction software 10.7 (Agilent technologies, Santa Clara, CA, US). Raw data were normalized by Quantile algorithm, Limma packages in R.

Bioinformatics Analysis

LncRNA targets correlated with mRNAs were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses using GO (http://www.geneongoloty.org/) and KOBAS software (KEGG Orthology-Based Annotation System, https://www.kegg.jp/). The differentially expressed lncRNAs-targeted miRNAs were sought and predicted by miRanda

software (http://miranda.org.uk/) coupled with statistical analysis. The lncRNAs expression profile for microarray chip assay, besides data and bioinformatics analysis were carried out by Shanghai Biotechnology Corporation (Shanghai, China).

Qpcr Analysis

Total RNA from another normal tissues (9 samples) and PCa tissues (7 samples) sustained by pathology after perineal prostate biopsy guided by ultrasound was prepared by Trizol Isolation Reagent (Invitrogen). Dimethylcarbinol, ethanol and trichloromethane were of analytic grade. DNase I, SYBR Green Realtime PCR Master Mix Plus and the ReverTra Ace qPCR RT Kit are from Toyobo Co. Japan. The reverse transcription kit steps are strictly followed to transcribe to cDNA. cDNA was used as template, and hGAPDH as internal parameter. The primer concentration was set as 0.4 μmol/L. Three parallel samples were set for each sample, tested as 15 μl system used for amplification. For qPCR solution, THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan) was utilized. qPCR was performed on the LightCycler 96 (Roche, Indianapolis, IN, USA) following the instruction. The reaction conditions of qPCR were: pre-denaturation at 95 °C for 3 min, denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s, extension at 72 °C for 20 s and totally 40 cycles. Assay numbers got involved in the top 10 up-regulated and down-regulated expression of lncRNAs and GAPDH, respectively. The sequences of primer are listed in the Table 1. Differentiated gene expression was calculated by the comparative Ct method.

The Co-expression Network Of Lncrna-mirna-mrna

To supplementary achieve perceptions of the lncRNAs' biological functions in the complex biological processes and cellular regulation, the lncRNA-miRNA-mRNA co-expression network was constructed to investigate the potential interaction between miRNAs, mRNAs and lncRNAs. As shown in the Fig. 5, the co-expression network of lncRNA-miRNA-mRNA included 20 nodes of miRNAs and 84 connections consisting of various lncRNAs and mRNAs. Among the 17 networks, one of the most known co-expression networks was miR-17-5p because it had been proven that miR-17-5p repressed metallopeptidase inhibitor 3 expression in PCa while in this study we found the network of miR-17-5p also got involved in the gene EIF3H, HELLS and DNAL1, which was regulated by the same lncRNA URS000048C392 (also named ENST00000555037.1) [24]. With one edge networks like URS00008B6496(ENST00000547292.1), URS00000B8AF9(ENST00000482003.1), URS0000EEB1F2(ENST00000436764) and URS00007CEE5E(lnc-DHX38-3:6), it should be simple to confirm their roles in PCa by further experiment. Some complicated networks like URS00008C2FEF(ENST00000591956), URS00008BBA94(ENST00000452731) and URS00009BE037(ENST00000492250) were associated with two diverse miRNA signal pathways, which indicated their might have different influence on PCa. URS00005D043E(ENST00000464382), URS000046AFA0(ENST00000534169) and URS0000EF6BD5(ENST00000435802) were connected to the same miR-375,and URS0000DB7AD5(ENST00000580175) and URS000032BFFB(ENST00000558749) were affected by miR-582-5p in the meanwhile. Although with several edges in mRNAs, the rest of lncRNA and miRNAs had the relationship of one-one correspondence. As demonstrated, those lncRNAs, miRNAs and mRNAs were vastly linked as the key hub of the co-expression network, which implied their vitally potential impact on lncRNAs in the progress of regulating particular target genes in PCa.

Survival Curve Analysis

We used the GEPIA (http://gepia.cancer-pku.cn/) as tool to calculate the survival curve of the top 10 upregulated and downregulated lncRNAs original gene.

Statistical analysis

All data are shown as mean ± standard deviation (SD). Statistical significance was determined using Student's t-test by SPSS 13.0 and Graphpad Prism 5. $p < 0.05 < / > cript >$ was considered statistically significant.

Result

lncRNA expression profiles in PCa

The microarray screening identified 68,424 lncRNAs in PCa, non-PCa or both tissues. As illustrated in Fig. 1, totally, 817 lncRNAs were differentially expressed between PCa tumor and paracancerous tissues ($FC \geq 2.0 and p < 0.05 < / > cript >$) (Table S2): among which 422 were upregulated, and the remaining 395 were downregulated in PCa tissues. The magnitude of FC was the highest for NR_125857 for upregulated lncRNAs ($FC = 59.27658, p = 0.00128$) while it was the lowest for lnc-MYL2-4:1 in downregulated lncRNAs ($FC = 0.00141, p = 0.01909$). Hierarchical clustering (Fig. 2D), volcano plot (Fig. 3), and scatter plots (Fig. 3) shown that the different expression profiles of lncRNAs between PCa and non-PCa tissues were diverse. The top each twenty up- and down-regulated lncRNAs were listed in Table 4.

Table 4
Top each twenty up- and down-regulated lncRNAs and corresponding gene information of lncRNAs

Accession	*P*-values	Fold change	Regulation	Chromosome	Strand	Gene Symbol	Associated gene description
NR_125857	0.0012796	59.276583	up	chr6	+	EVADR	-
NR_015342	0.003856	28.092424	up	chr9	+	PCA3	prune homolog 2 (Drosophila)
NR_109832	0.0087905	21.726513	up	chr22	+	PCAT14	-
ENST00000412654	0.0026089	19.731117	up	chr9	+	PCA3	prune homolog 2 (Drosophila)
lnc-AC110080.1-5:1	0.0214716	17.480829	up	chr2	-	—	-
ENST00000415820	0.0175281	16.656541	up	chr21	-	AP001610.9	-
ENST00000558010	0.0150573	15.600412	up	chr15	-	RP11-279F6.2	-
ENST00000365110	0.0289324	13.988218	up	chr11	+	SNORA62	-
NONHSAT072254	0.0075766	12.035524	up	chr2	-	—	-
NONHSAT072236	0.0072588	11.671845	up	chr2	-	—	-
ENST00000439575	0.0439698	11.653131	up	chr10	+	RP11-118K6.2	-
lnc-AC110080.1-1:1	0.0177589	11.267734	up	chr2	-	—	-
lnc-AC233264.5-3:1	0.0367401	10.224691	up	chr2	+	—	-
ENST00000616913	0.0034881	9.6291064	up	chr9	+	PCA3_1	prune homolog 2 (Drosophila)
NONHSAT072252	0.013674	9.0948737	up	chr2	-	—	-
lnc-MX1-1:1	0.0217773	8.6735934	up	chr21	+	—	-
lnc-LRCH4-3:1	0.0161447	8.2237523	up	chr7	-	—	ArfGAP with FG repeats 2
lnc-AC110080.1-15:1	0.0140691	8.1295177	up	chr2	-	—	-
ENST00000621752	0.0168726	7.9573305	up	chr10	-	RP11-122K13.15	-
lnc-TOMM70A-1:1	0.0397496	0.2638873	down	chr3	-	—	filamin A interacting protein 1-like
ENST00000623595	0.0256375	0.2592142	down	chr9	+	RP11-392A14.8	-
lnc-ABHD10-1:1	0.0328931	0.2574471	down	chr3	+	—	pleckstrin homology-like domain, family B, member 2
lnc-GADD45B-1:2	0.0217582	0.2560995	down	chr19	+	—	guanine nucleotide binding protein (G protein), gamma 7
lnc-C9orf43-5:1	0.0179094	0.2529903	down	chr9	+	—	regulator of G-protein signaling 3

Accession	*P*-values	Fold change	Regulation	Chromosome	Strand	Gene Symbol	Associated gene description
lnc-C2CD4A-8:3	0.0070292	0.2501496	down	chr15	+	—	RAR-related orphan receptor A
lnc-NDRG1-5:1	0.0451373	0.250131	down	chr8	-	—	ST3 beta-galactoside alpha-2,3-sialyltransferase 1
lnc-HFM1-3:3	0.0435927	0.2433965	down	chr1	-	—	transforming growth factor, beta receptor III
ENST00000617916	0.0078552	0.2319187	down	chr19	-	LLNLR-268E12.1	-
NR_125886	0.0124719	0.2278954	down	chr4	+	LOC101927636	-
ENST00000623273	0.0117887	0.2264097	down	chr5	-	CTB-174D11.3	slit homolog 3 (Drosophila)
ENST00000424251	0.0143991	0.2204195	down	chrX	+	RP1-146A15.1	interleukin 1 receptor accessory protein-like 1
lnc-TACC2-3:1	0.0146426	0.2146128	down	chr10	+	—	transforming, acidic coiled-coil containing protein 2
NR_125859	0.0322068	0.2054874	down	chr6	+	LOC101928540	filamin A interacting protein 1
NONHSAT136589	0.0022274	0.1790319	down	chrX	-	—	dystrophin
lnc-CHST2-2:3	0.023774	0.1771025	down	chr3	+	—	-
lnc-PDCD11-5:1	0.000183	0.1635704	down	chr10	+	—	neuralized E3 ubiquitin protein ligase 1
lnc-PTEN-11:1	0.0032078	0.1472261	down	chr10	+	—	-
lnc-MID1-4:1	0.00866	0.1373993	down	chrX	-	—	Rho GTPase activating protein 6
lnc-C19orf73-1:1	0.034911	0.030913	down	chr19	-	—	histidine rich calcium binding protein
lnc-MYL2-4:1	0.0190929	0.0014053	down	chr12	-	—	myosin, light chain 2, regulatory, cardiac, slow

The Results Of Bioinformatics Analysis

Top each twenty up- and down-regulated lncRNAs and corresponding gene information of lncRNAs were shown in Table 4. Moreover, each Top 30 enrichments about GO and KEGG analyses suggested that these differentially expression lncRNAs were relevant to several vital physiological processes, such as cardiac muscle hypertrophy, muscle hypertrophy, neural precursor cell proliferation, establishment or maintenance of cell polarity, cardiac muscle tissue development, striated muscle cell development, postsynaptic membrane. Intriguingly, most of them are associated with the muscle

tissue development, including cardiac muscle and striated muscle, which may hint the reorganization of the excellular matrix on behalf of the smooth muscle surrounding the PCa. Moreover, the upregulation of the neuron formation shows the nerve paracrine factor involving in the tumorigenesis. Except for the famous pathways, such as TGF-β, Wnt, MARK and mTOR that have been proven to be closely correlated to proliferation, invasion and metastasis in PCa, astonishingly, the pathway of aldosterone-regulated sodium reabsorption, dilated cardiomyopathy, hypertrophic cardiomyopathy, pathogenic Escherichia coli infection and vascular smooth muscle contraction also implies the revegetation of smooth muscle may interfere with the microenvironment of PCa. Additionally, the pathogenic Escherichia coli infection may link to the common urinary disease, prostatitis, which also causes the tissue recovery (Fig. 4).

The Results Of Qpcr

The outcome of qPCR showed significant statistic differences in NR_125857, NR_015342, NR_109832, ENST00000412654, lnc-AC110080.1-5:1, ENST00000415820, ENST00000558010 ($p < 0.05 < / > cript >$) in PCa tissues (n = 5) compared to normal prostate tissues (n = 7) while there was no statistic difference of all the top ten downregulated lncRNA expression (Fig. 6A, B). These results indicated that the expression of NR_125857, NR_015342, NR_109832, ENST00000412654, lnc-AC110080.1-5:1, ENST00000415820, ENST00000558010 were up-regulated in PCa and it revealed whether the downregulation of ENST00000424251, lnc-TACC2-3:1, NR_125859, NONHSAT136589, lnc-CHST2-2:3,lnc-PDCD11-5:1,lnc-PTEN-11:1, lnc-MID1-4:1, lnc-C19orf73-1:1, lnc-MYL2-4:1 was still doubted. Compared with the original outcomes of RNA-seq array, the relative expression of qPCR in the additional samples showed consistency in figure (Fig. 6C).

The Analysis Of Survival Curve

As the top seven upregulated lncRNAs in our study revealed the coherence of bioinformatics analysis and qPCR analysis, we further analyzed their survival curves of original gene in PCa by the tool of GEPIA (http://gepia.cancer-pku.cn/) (Fig. 7). Higher expression of prostate-specific DD3(PCa3) in patients of PCa showed lower survival rate after about 80 months while the higher expression of PCa associated transcript-14 (PCAT14) demonstrated higher survival rate since approximately 60 months. The high expression of AP001610.9 led to a dramatic decline of survival rate after 110 months despite the phenomenon that it revealed moderately higher survival rate from the 80th to 110th month. Moreover, differentiated expression of RP11-279F6.2 showed a subtle difference that the high expression would result in lower survival rate in the duration of 80th and 105th month. Nevertheless, there was no recorded data of NR_125857, which was the most upregulated lncRNA in our study.

Discussion

Traditionally, by means of measuring the assay of PSA protein in the blood, folk with high risks in PCa screen for that illness. However, in the United States, the US Preventive Services Task Force (USPSTF) reviewed the evidence on the benefits and harms of PSA-based screening for PCa and subsequent treatment of screen-detected PCa [25]. For men aged 55 to 69 years, the decision to undergo periodic PSA-based screening for PCa should be various from person to person and should take the balance of the possible benefits and troubles of screening PSA with their clinician [26]. Through screening PSA, for some men, it just gives the patients a limited potential profit of the reductions in risk of dying of PCa and metastatic disease in the end stage [27]. Many men will suffer potential harms of screening, including false-positive results that entail extra testing and even, invasive prostate biopsy, to separate them from the real patients; overdiagnosis and overtreatment; and it may arouse a lot of treatment complications, such as incontinence and erectile dysfunction, which wastes unnecessary time, influence the normal life and lowers the quality of life [28–30]. Clinicians should not screen men who do not express a preference for screening (C recommendation) [31–33]. The USPSTF recommends against PSA-based screening for PCa in men 70 years and older (D recommendation) [25, 34, 35]. As there is progressively amounts of argument and distrust about the specificity and sensitivity of PSA, it is essential to develop more dependable biomarkers for early screening of PCa. Recent developments in the detection of lncRNAs have acknowledged lineage- and cancer-specific biomarkers that may be applicable in the clinical utilization of PCa. Herein, we will combine our analysis of RNA-seq datasets, from 5 patient samples, including PCa and adjacent benign prostate tissue with the other investigation to exploit and corroborate differentially expressed lncRNA connected with PCa. After we concluded the lncRNA candidates, which had

ges, we proved the consistency of RNA-seq results and qRT-PCR outcomes by extending

the clinical samples that consisted of another 5 tumor specimens and 7 para-cancerous/benign contrasts through prostate biopsy. The top each twenty up-regulated and down-regulated lncRNAs was listed in Table 4.

We noticed that NR_125857, related to the gene EVADR, ranked the first line of upregulation in our database. EVADR is the written abbreviation of Endogenous retroViral-associated ADenocarcinoma RNA (EVADR), by analyzing RNA-seq data derived from colorectal tumors and matched normal control tissues [36]. This lncRNA demonstrated nominal to low expression in normal tissue, but is significantly upregulated in cancer, particularly in colon, rectal, lung, stomach and pancreas adenocarcinomas. It was reported the EVADR lncRNA determined the promoter activity of the MER48 long terminal repeat (LTR) in vitro, mapped the genome-wide MER48 LTR expression [36, 37]. Regardless of a biological function, the specificity of EVADR activation in adenocarcinomas coupled with the poorer survival probability that tracks with elevated EVADR expression suggested that further characterization of EVADR as a candidate adenocarcinoma biomarker is warranted [36].Nevertheless, the original article did not mention any details about the EVADR in PCa. In our study, it was totally clear that the expression of NR_125857 is up-regulated in PCa by RNA-seq and qPCR. Since it was described as the highest upregulated lncRNA in our research, it seemed to be promising in the PCa research, for, without any doubt, PCa is also a kind of adenocarcinoma. The mechanism mediated by the high expression of NR_125857 in PCa requires further cavernous research and investigation.

In the top five of the upregulation in lncRNA, NR_015342 and ENST00000412654 are associated with the PCa3, accounting for a large proportion. PCa3 was located on chromosome 9q21-22 [38]. PCa3, as one of the oldest identified lncRNAs, is an accepted diagnostic urinary biomarker for PCa [39]. Because PCa3 is over-expressed in 95% of PCa, with up to 100-fold up-regulation compared to adjacent non-neoplastic cells [40, 41]. Highly overexpression of PCa3 in PCa tissue was found to be a potential non-invasively prediction of prostate biopsy which might be a promising biomarker in clinical diagnosis [42]. However, PCa3 assays also have limited utility in detecting men with higher grade diseases due to low PCa3 levels [43]. For instance, a patient was observed to be negative for PCa as assessed by urinary PCa3, but was later diagnosed to have very high-grade disease (Gleason Score 9) and high Decipher metastasis risk [44]. Thus, it warns us that single usage of PCa3 as a stand-alone marker for PCa may deliver false negative outcomes for patients with higher grade tumor.

Ranking at the third up-regulation of genes, NR_109832 suggests the gene PCAT14 also play an important role in PCa tumorigenesis. PCAT-14 is commonly up-regulated in primary tumors. PCAT14 is an AR-regulated transcript while PCAT14 is highly expressed in low grade disease and loss of PCAT14 predicts for disease aggressiveness and recurrence, and its overexpression suppresses invasion of PCa cells [45]. PCAT14 lower expression is significantly prognostic for multiple clinical endpoints supporting its significance for predicting metastatic disease that could be used to improve patient management [46].

The sixth up-regulated gene symbol is related with AP001610.9, and ENST00000415820 may links to LOC111099027, LOC105372809, TMPRSS2 and MX1. TMPRSS2, also named as PP9284 or PRSS10, is transmembrane serine protease 2, which is a member of the membrane-anchored serine proteases family [47]. It has been figured out that TMPRSS2 mediates a proteolytic cascade regulated by androgen signaling, which promotes the progression, invasion, and metastasis of PCa cells by activating the matriptase and disordering the extracellular matrix [48–50]. TMPRSS2 mainly affects degradation of extracellular matrix nidogen-1 and laminin β1 [48]. Therefore, it indicates an innovative approach for targeting these two proteases in treatment development, and the intimate connection between tumor cells and extracellular matrix in the PCa. Moreover, MX1 has many aliases, such as IFI-78K, IFI78, MX, MxA and lncMX1-215. It belongs to the class of dynamin-like large guanosine triphosphatases (GTPases) acknowledged to be involved in intracellular vesicle trafficking and organelle homeostasis, which chiefly participates in the cellular antiviral response against a wide range of RNA viruses, including influenza viruses and members of the bunyavirus family [51]. It is an interferon stimulated antiviral protein that is required for a complete antiviral response [52]. Preceding study found that down-regulation of MxA in LNCaP cells by dihydrotestosterone suggests that MxA appears to be meaningfully associated with cell cycle and further cancer development while the loss of MxA expression leads to increased metastasis and decreased sensitivity to Docetaxel, which shows that MxA expression could regulate the outcome of chemotherapy [53, 54].

Although the ENST00000365110 ranked the eighth contender, its interrelated gene SNORA62 has the full name as small nucleolar RNA, H/ACA box 62. snoRNAs are one of the most ancient and numerous families of non-protein-coding RNAs. Eukaryotic snoRNAs are conserved from archaeal sRNAs in both function and structure [55]. Therefore, the main function of snoRNAs - to guide site-specific rRNA modification - is the same in archaea and all eukaryotic lineages [56]. Owing to the presence of a conserved H box (5'-

ox snoRNAs form a double hairpin structure [55]. The box H/ACA snoRNAs direct the

site-specific pseudouridylation of pre-rRNAs [57].Recent findings have proven that deregulation of the pseudouridinylation process is connected with the progression of PCa [58]. Another research demonstrates SNORA62 are encoded from the host gene RPSA or Laminin receptor (LAMR) while it is observed that the mutations in the LAMR/RPSA gene may be related to congenital asplenia, the inborn absence of spleen [59, 60].

The lowest down-regulation lncRNA is the anonymous lnc-MYL2-4:1. In our study, it suggests this lncRNA is interrelated to myosins, which are a large and diverse family of molecular motors important for cell migration and motility [61]. In PCa, Myo1b, Myo6, Myo9b, Myo10, and Myo18a were expressed at higher levels in high metastatic potential cells, and especially Myo1b and Myo10 were expressed at higher levels in metastatic tumors [62–64]. Changes in expression of several myosin isoforms may contribute to metastasis in PCa [62]. Though the outcome of qPCR in this study was no significant different in PCa tissues and normal tissues, the exact interaction between our candidate lncRNA and myosin is still needed to research.

The second down-regulation lncRNA lnc-C19orf73-1:1 is related to histidine rich calcium binding protein (HRC). The HRC is a novel regulator of sarcoplasmic reticulum (SR) Ca2+-uptake, storage and release, so the HRC plays a pivotal role in Ca2+-homeostasis.2 Calcium (Ca2+) is an essential intracellular signaling molecule involved in the regulation of cancer progression, including cell proliferation, apoptosis, invasion and migration [65, 66]. It has been proved that HRC promotes growth of hepatocellular carcinoma in vitro and in vivo [67]. Furthermore, HRC also plays a significant role in myocyte differentiation and in anti-apoptotic cardioprotection against ischemia/reperfusion induced cardiac injury [68].

Lnc-MID1-4:1, located on the chromosome X, is associated with Rho GTPase activating protein 6. Rho GTPases have been figured out to be critical signal transducers, which mediate growth factor-induced changes to the actin cytoskeleton and activating the phagocyte NADPH oxidase [69]. As a result, they get involved in abundant cellular processes. For example, cell migration, cell survival, transcriptional regulation and vesicle transferring [70]. The deleted in liver cancer 1 (DLC-1) gene encodes a GTPase activating protein that acts as a negative regulator of the Rho family of small GTPases, and DLC-1 is assumed as a bona fide tumor suppressor gene in different types of human cancer [71]. It hints that the down-regulation of Lnc-MID1-4:1 may influence on the particular cellular functions in PCa.

lnc-PDCD11-5:1 is connected to neutralized E3 ubiquitin protein ligase 1. The E3 ubiquitin ligase NEDD4 negatively regulates HER3/ErbB3 level and signaling [72]. Many preceding studies reveals NEDD4 has been acknowledged to play a critical role in the regulation of a number of membrane receptors, endocytic machinery components and the tumor suppressor PTEN [73]. The loss of PTEN expression was associated with worse survival and shorter time on abiraterone treatment [74]. Ubiquitin Ligases are also involved in the regulation of Wnt, TGF-β, and Notch Signaling Pathways [75].

There is scarcely any information about the rest of up-regulation lncRNAs, such as ENST00000558010, ENST00000365110, NONHSAT072254, NONHSAT072236, lnc-PTEN-11:1, and ENST00000439575. More experiments are needed to prove their impression and function.

In our analysis, there are ten qualified samples, so our study still has boundedness in the number of samples. Yet the feature of our study was that our patients are typical Mongoloid men and our results exhibited very specificity in east Asia area. Moreover, to highlight the coherence of our outcomes and practical issues and value, we further extended the clinical samples for qPCR and drew the survival curves of meaningful genes of lncRNAs after the confirmation of qPCR. The top seven upregulation lncRNAs, like NR_125857, NR_015342, NR_109832, ENST00000412654, lnc-AC110080.1-5:1, ENST00000415820 and ENST00000558010 are promising research candidates for extra investigation. The present study of lncRNAs in PCa tissues is a proof-of-principle that lncRNAs have a possible character in PCa formation and progression. As demonstrated in the tables, there are so many lncRNAs has the relationship with PCa, the information, even, the name of their majority is blank. Lots of verification test are need to be completed. Our current study on the potential link between lncRNAs and PCa presents a novel analysis for further investigations into the biomarker and target genes of such lncRNAs, leading to clinical research for the disease.

The treatment paradigm of PCa has progressed rapidly in the last decade due to wider availability and choice of therapy. Thus, we have the reason to believe in, with the deep investigation, the potential mechanism of lncRNA will be disclosed stepwise, which provides new breakthroughs in the early diagnosis, prognosis, and therapy targets of PCa.

Conclusion

Our study detected a relatively novel complicated map of lncRNAs in PCa, which may have the potent to figure out a completely new and useful biomarkers or molecule for diagnosis, treatment and follow-up in PCa. As a candidate, we found that NR_125857 expression in human PCa tissues was up-regulated. Further researches with numerous sample sizes of PCa tissues are needed to evaluate the relationships among the expression of NR_125857, clinicopathological features, and prognosis of PCa patients. In addition, more comprehensive *in vivo* study is essential.

Declarations

Declarations

Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

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Authors' contributions

Shengyang Ge and Yuanyuan Mi equally dedicated to this article.

Contributions

FZ, YZ, GWX and SCY conceived and designed the experiments. YYM and CYS performed the experiments. YYM, SYG, YJG and QFH analyzed the data. YZ and FZ contributed analysis tools. SYG and YYM wrote the paper. CYS, YZ and GWX revised the manuscript. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Huashan Hospital, Fudan University. All enrolled patients signed written informed consent prior to sample collection.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

List of abbreviations

PCa: Prostate Cancer

AR: Androgen Receptor

CRPC: Castration-Resistant Prostate Cancer

ncRNAs: Non-Coding RNAs

tRNAs: Transfer RNAs

rRNAs: Ribosomal RNAs

miRNAs: microRNAs

snoRNAs: Small Nucleolar RNAs

sRNAs: Small Nuclear RNAs

lncRNAs : Long Non-Coding RNAs

GO: Gene Ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

PCA3 : Prostate-Specific Dd3

PCAT14: Prostate Cancer Associated Transcript-14

USPSTF: Us Preventive Services Task Force

EVADR: Endogenous retroViral-Associated ADenocarcinoma RNA

LTR : Long Terminal Repeat

GTPases : Guanosine Triphosphatases

TMPRSS2 : Transmembrane Serine Protease 2

SNORA62: Small Nucleolar RNA, H/Aca Box 62

LAMR: Laminin Receptor

HRC: Histidine Rich Calcium Binding Protein

DLC-1: Deleted in Liver Cancer 1

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Figures

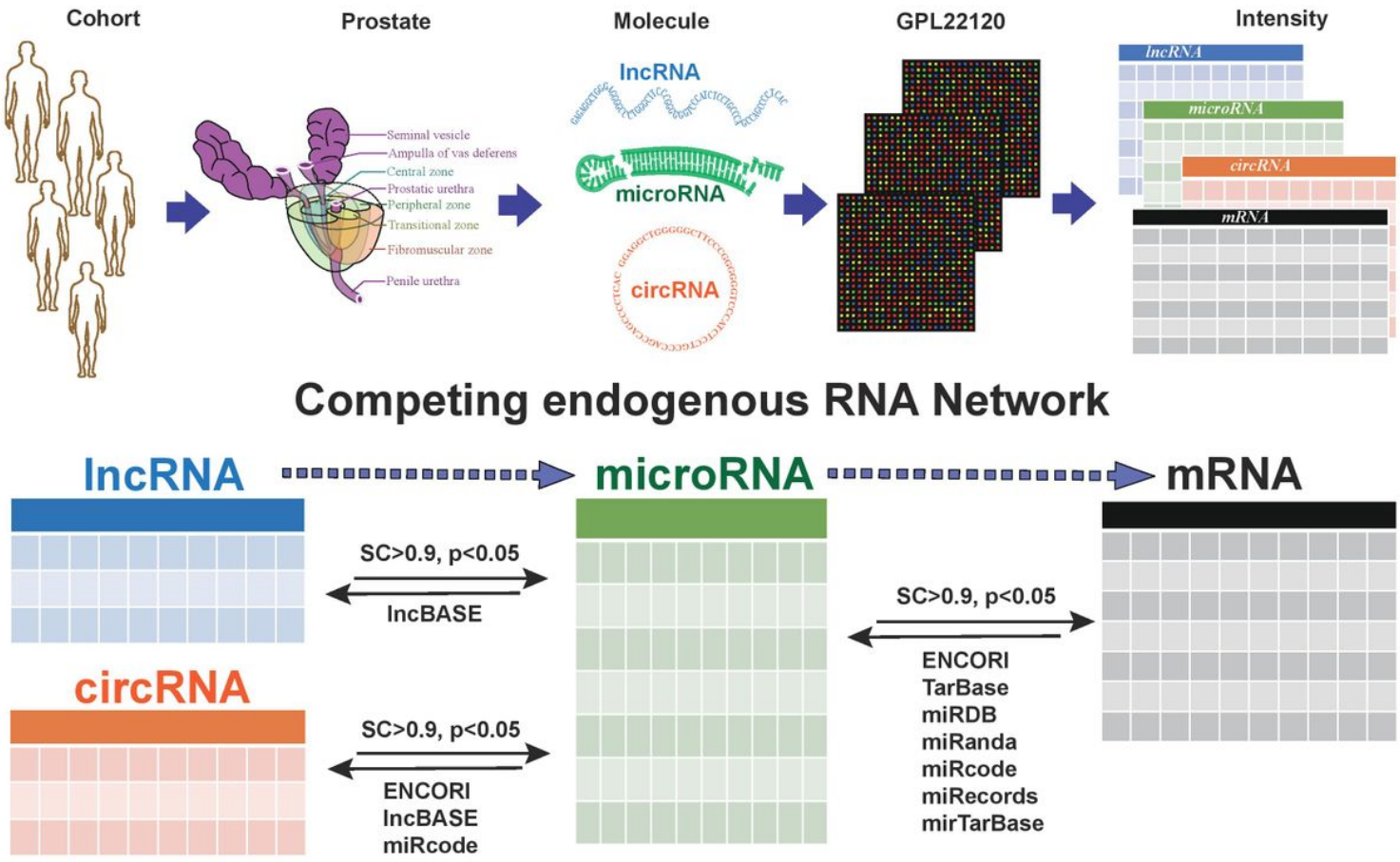

Figure 1

The diagram of data processing. In this study, lncRNA expression profiles were screened in PCa, by using five pairs of clinical specimens in PCa and matched non-PCa tissues with lncRNA chip GPL22120. The abundance of each lncRNA against each miRNA was calculated using Spearman correlation, and then filtered by comparison with the theoretical databases. The theoretical databases included ENCORI, lncBase, miRcode for the relations of lncRNA-miRNA and miRcode, ENCORI, TarBase, miRTarBase, miRDB, miRanda, miRecords for the relations of miRNA-mRNA.

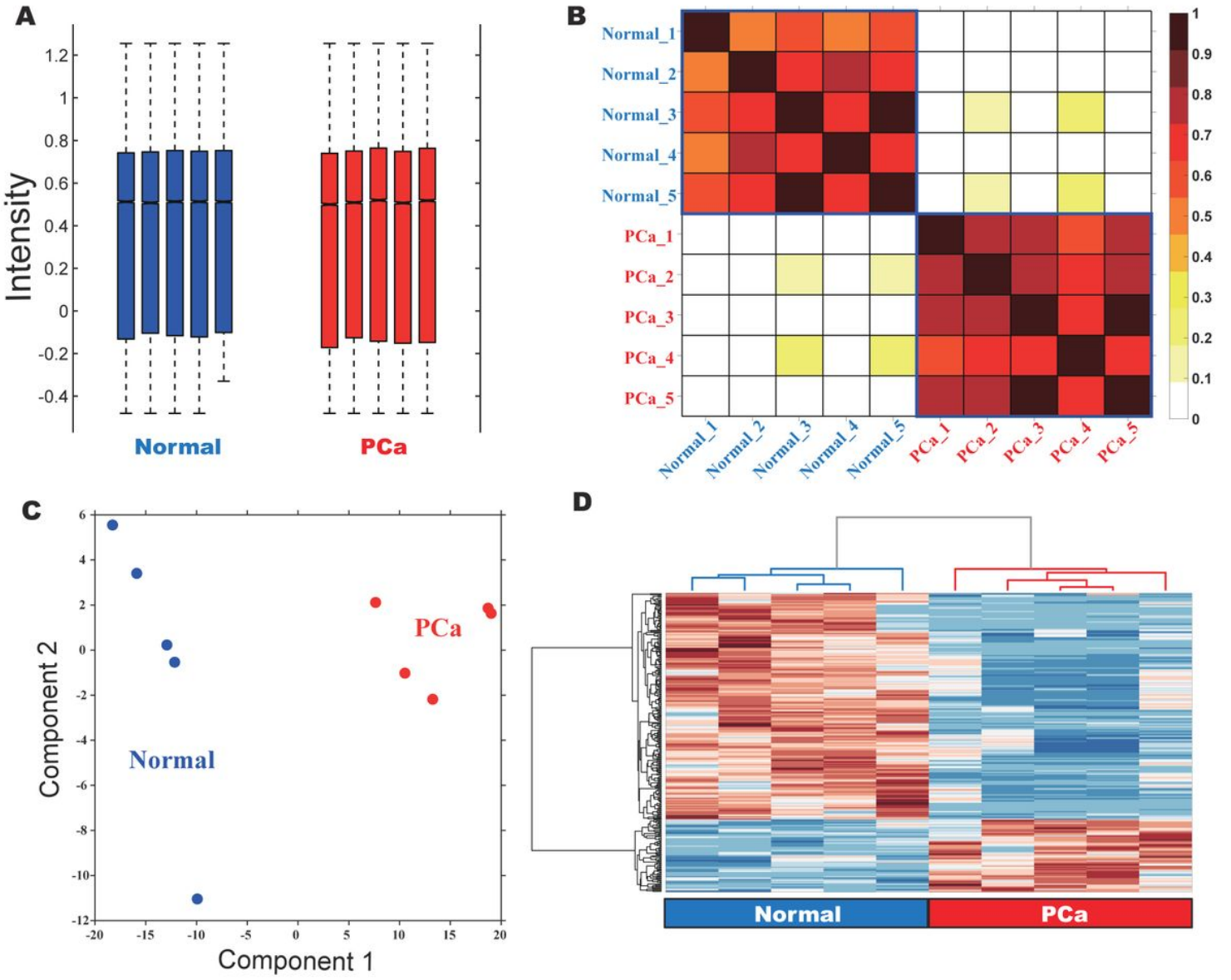

Figure 2

Global view of all lncRNAs expression in PCa tissues compared to paired non-PCa tissues. (A) Boxplot. The medians between samples were roughly flat, and the ranges of expressions were similar. (B) Sample correlation matrix. The correlation coefficient within the groups was significantly higher than that between the groups, which indicated the larger differences between PCa tissues and paired non-PCa tissues. (C) Principal component analysis. The difference between normal and tumor was large in the first principal component, but slightly in the second principal component, which showed there was a big difference between the samples. (D) Hierarchy Clustering Analysis. Repeated samples are clustered together, indicating the repeatability of samples and the differences between samples. The black dotted line divides lncRNAs into two categories: above the line, it presented the part of high expression in non-PCa tissues and low expression in PCa, and below the line, it presented the part of high expression in PCa and low expression in non-PCa tissues. Overall, through a variety of global analysis, we concluded that our tissue samples used in our study presented good reproducibility and the large differences between groups.

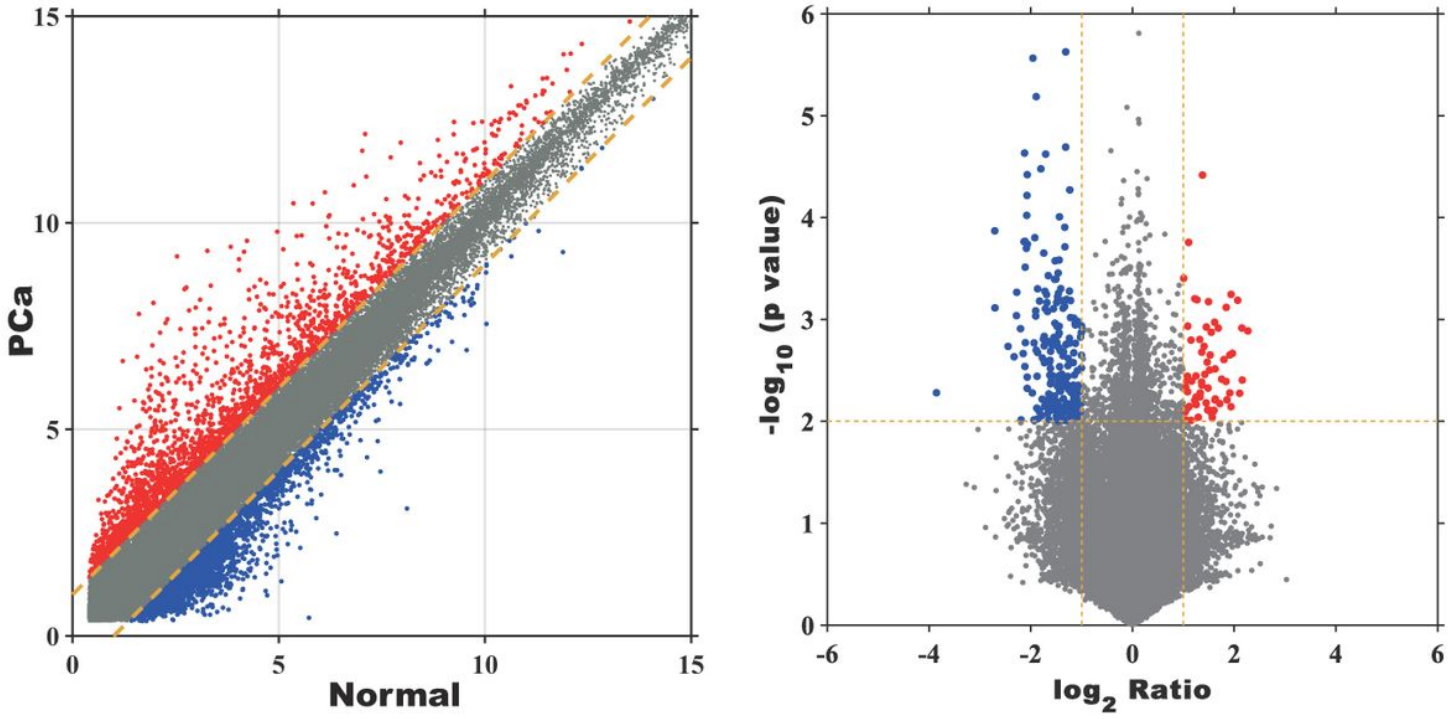

Figure 3

Selection of differentially expressed lncRNAs. The scatter plots and volcano plots exhibited the differentially expressed lncRNAs in PCa tissues compared to paired non-PCa tissues. The left figure presented the scatter plots while the right figure presented the volcano plots.

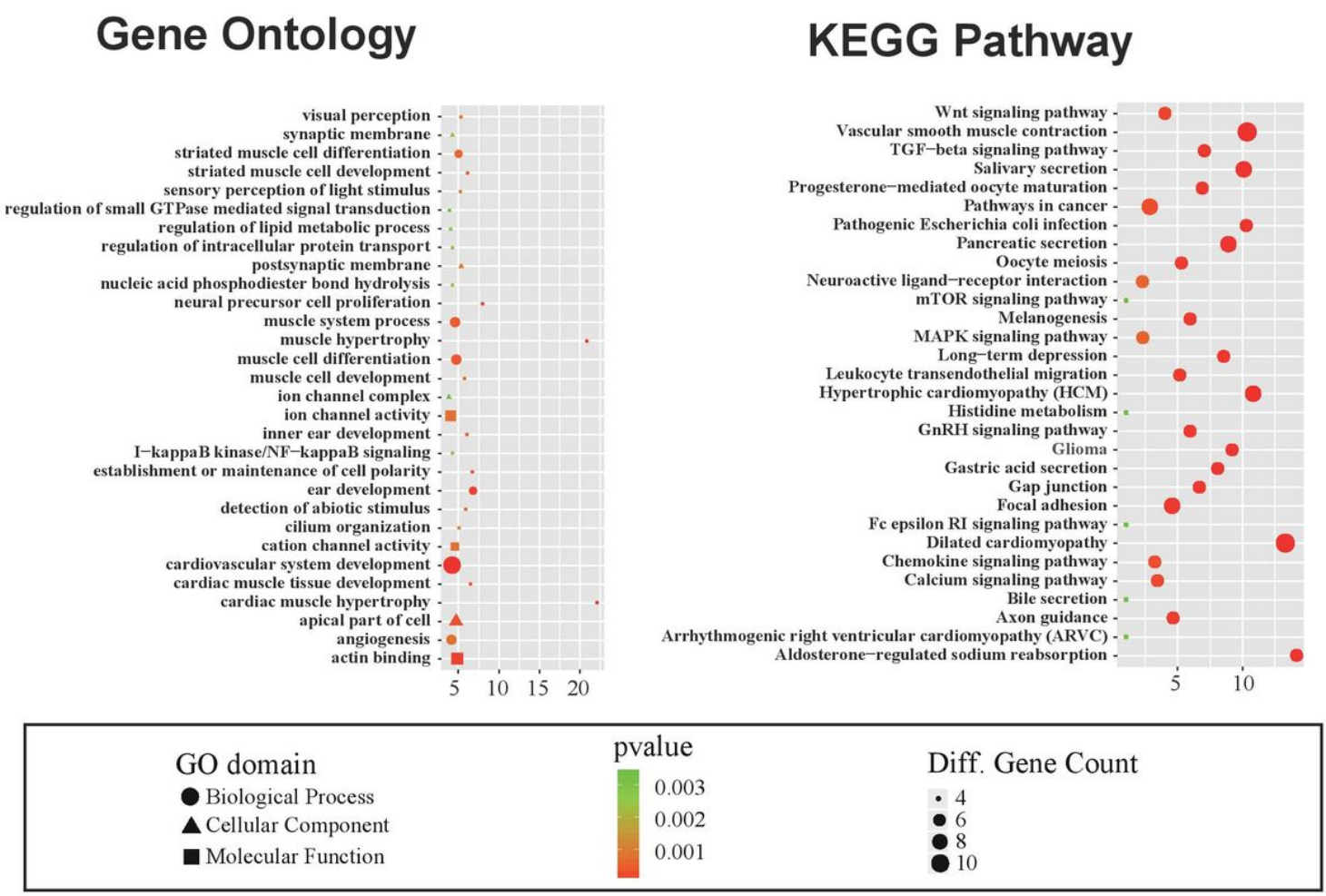

Figure 4

Results of Gene Ontology and KEGG pathway analysis. (A) Top 30 classes of GO enrichment terms. (B) Top 30 classes of KEGG pathway enrichment terms.

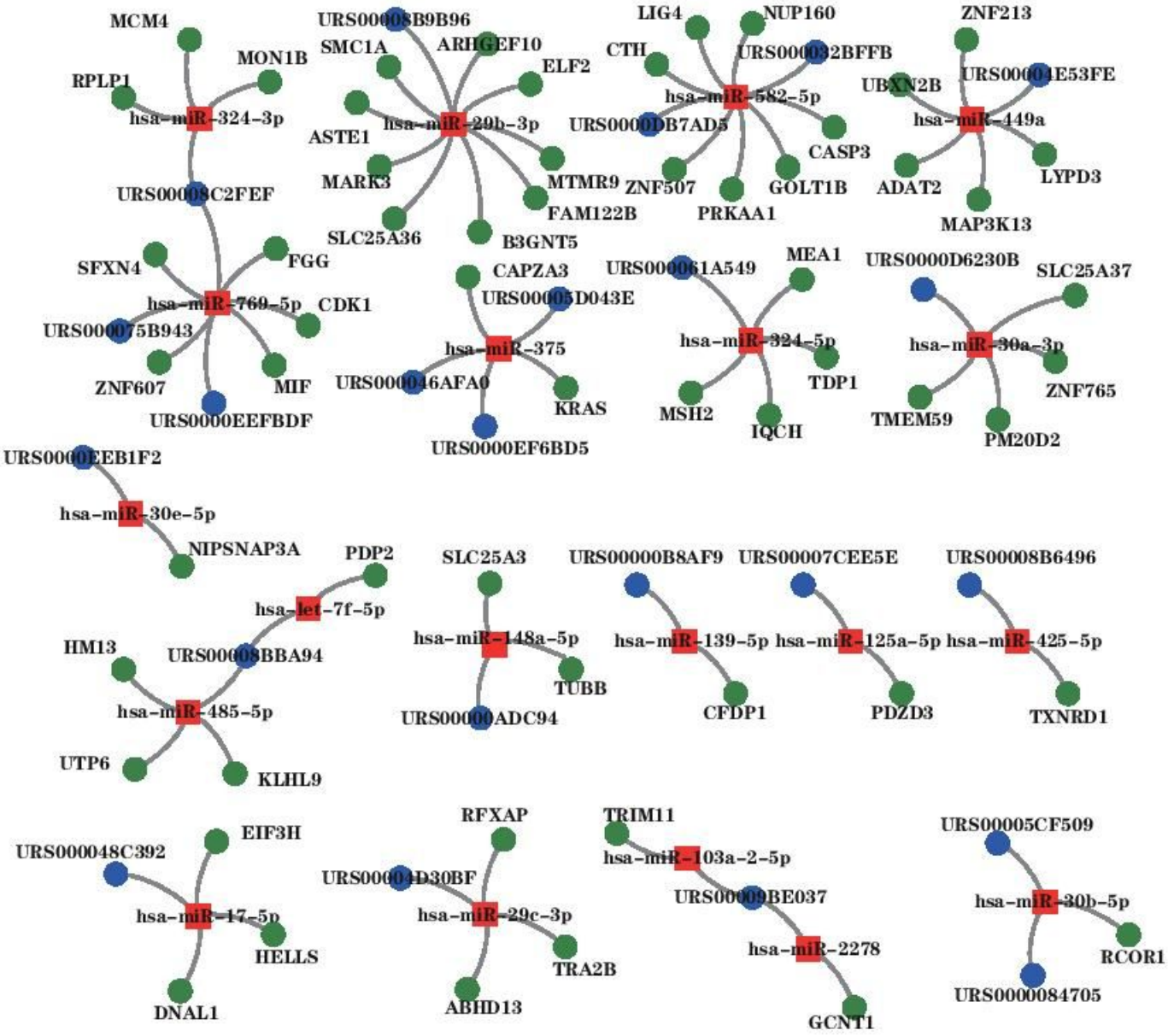

Figure 5

The co-expression network of lncRNA-miRNA-mRNA. In this figure, the red squares represented the miRNAs, the blue circle represented the lncRNAs and the green circles represented the mRNAs.

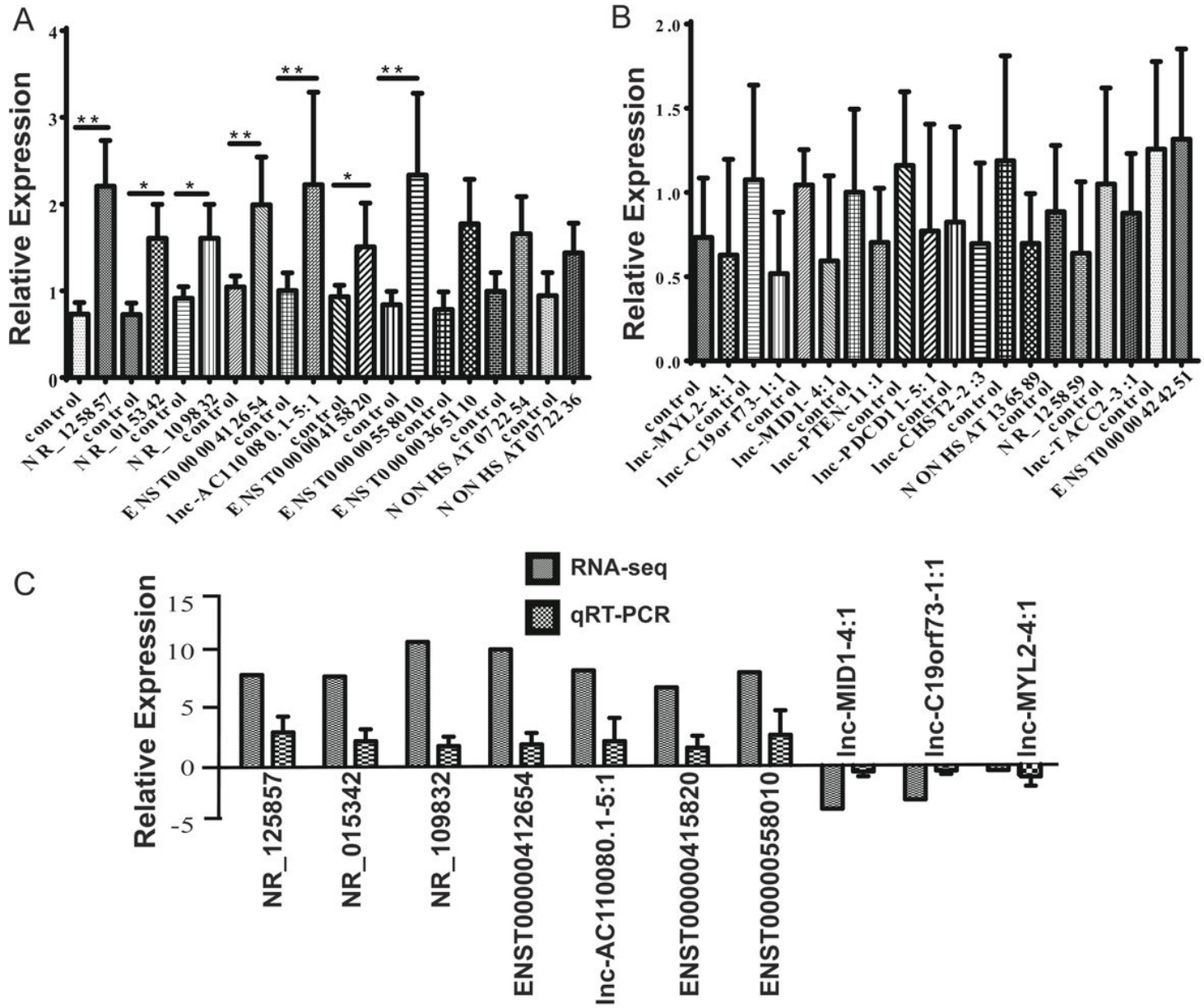

Figure 6

The outcomes of subsequent verification tests by qPCR. (A)The relative expression of top ten upregulated lncRNAs in qPCR. There were significant statistic differences in NR_125857, NR_015342, NR_109832, ENST00000412654, lnc-AC110080.1-5:1, ENST00000415820, ENST00000558010 ($p < 0.05$). (B) The relative expression of top ten downregulated lncRNAs in qPCR. There was no significant statistic difference between them ($p>0.05$), which may be due to the small sample size. (C) Confirmation of the expression patterns of lncRNAs by comparing the results of qRT-PCR and original array outcomes of RNA-seq. Top 7 up-regulated lncRNAs and top 3 down-regulated lncRNAs.

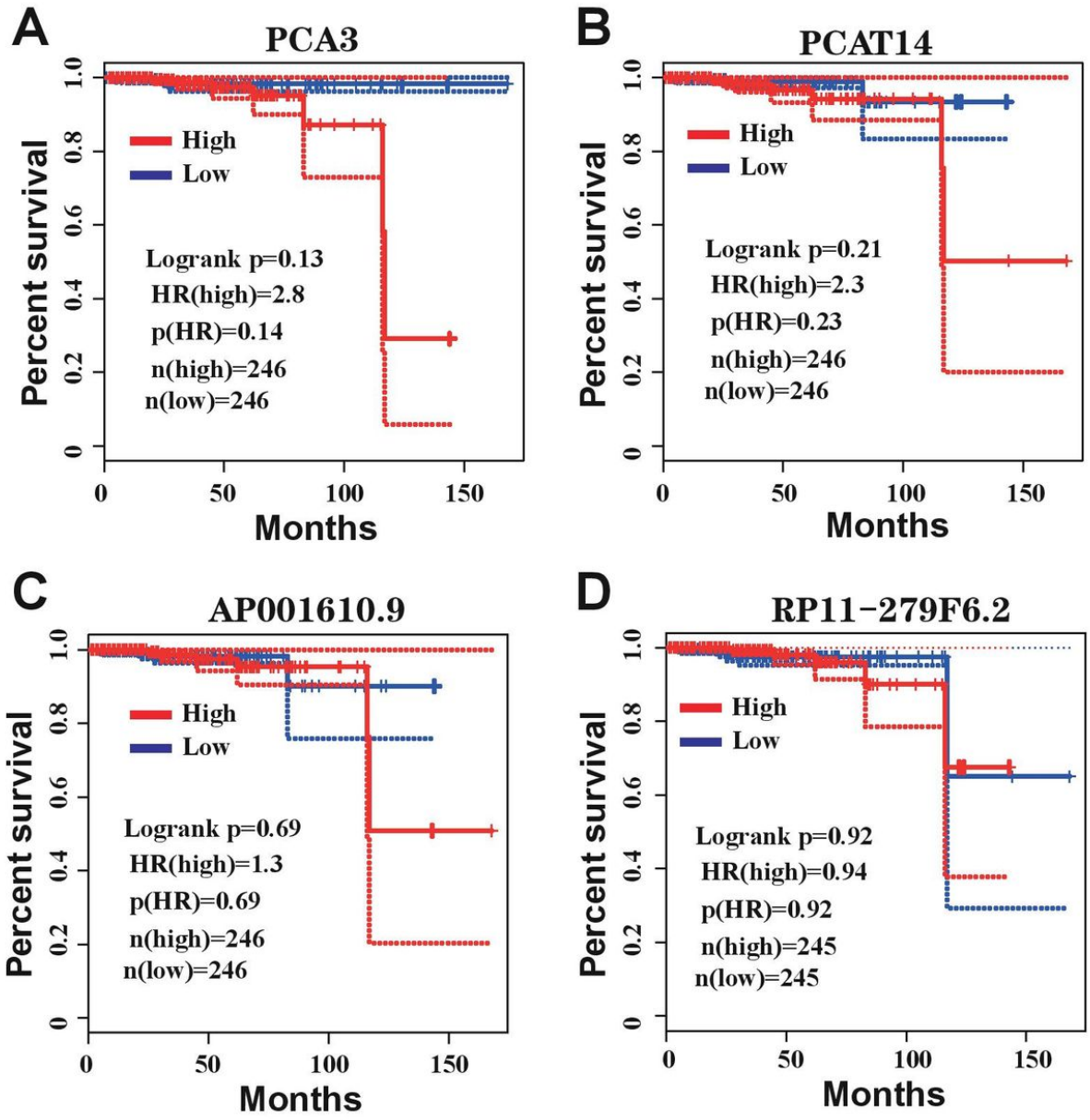

Figure 7

The results of survival curve analysis by GEPIA. (A) The survival curve analysis of PCA3. (B) The survival curve analysis of PCAT4. (C) The survival curve analysis of RP11-279F6.2. (D) The survival curve analysis of AP001610.9

Supplementary Files

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