Comprehensive analysis of differentially expressed profiles and reconstruction of a competing endogenous RNA network in papillary renal cell carcinoma

QING LUO[1], MENG CUI[1], QINFU DENG[2] and JINBO LIU[1]

Departments of [1]Laboratory Medicine and [2]Urology, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan 646000, P.R. China

Received August 22, 2018; Accepted March 27, 2019

DOI: 10.3892/mmr.2019.10138

Abstract. Long noncoding RNAs (lncRNAs) function as competing endogenous RNAs (ceRNAs). ceRNA networks may serve important roles in various tumors, as demonstrated by an increasing number of studies; however, papillary renal cell carcinoma (PRCC)-associated ceRNA networks mediated by lncRNAs remain unknown. Increased knowledge of ceRNA networks in PRCC may aid the identification of novel targets and biomarkers in the treatment of PRCC. In the present study, a comprehensive investigation of mRNA, lncRNA, and microRNA (miRNA) expression in PRCC was conducted using sequencing data from The Cancer Genome Atlas. Differential expression (DE) profiles of mRNAs, lncRNAs and miRNAs were evaluated, with 1,970 mRNAs, 1,201 lncRNAs and 96 miRNAs identified as genes with significantly different expression between PRCC and control paracancerous tissues. Based on the identified DEmRNAs, a protein-protein interaction network was generated using the STRING database. Furthermore, a ceRNA network for PRCC was determined using a targeted assay combined with the DE of miRNAs, mRNAs and lncRNAs, enabling the identification of important lncRNA-miRNA and miRNA-mRNA pairs. Analysis of the ceRNA network led to the extraction of a subnetwork and the identification of lncRNA maternally expressed 3 (MEG3), lncRNA PWRN1, miRNA (miR)-508, miR-21 and miR519 as important genes. Reverse transcription-quantitative polymerase chain reaction analysis was conducted to validate the results of the bioinformatics analyses; it was revealed that lncRNA MEG3 expression levels were downregulated in PRCC tumor tissues compared with adjacent non-tumor tissues. In addition, survival analysis was conducted to investigate the association between identified genes and the prognosis of patients with PRCC, indicating the potential involvement of 13 mRNAs, 15 lncRNAs and six miRNAs. In conclusion, the present study may improve understanding of the regulatory mechanisms of ceRNA networks in PRCC and provide novel insight for future studies of prognostic biomarkers and potential therapeutic targets.

Correspondence to: Professor Jinbo Liu, Department of Laboratory Medicine, The Affiliated Hospital of Southwest Medical University, 25 Taiping Street, Luzhou, Sichuan 646000, P.R. China
E-mail: liujb7203@163.com

Key words: papillary renal cell carcinoma, competing endogenous RNA network, The Cancer Genome Atlas, prognosis

Introduction

Papillary renal cell carcinoma (PRCC) is a complex malignant neoplasm and the second most frequent renal cell carcinoma (RCC) following renal clear cell carcinoma (CCRC), comprising ~18.5% of the total cases of RCC (1). In 1997, Delahunt and Elbe (2) described PRCC as comprising two subtypes, type 1 and type 2; however, Chevarie-Davis *et al* (3) reported that the frequency of 'overlapping' PRCC, which possessed a certain overlapping features, was ~47%. Furthermore, the survival of patients with PRCC varies, particularly in cases of sporadic PRCC. Ha *et al* (4) reported that the subclassification of PRCC did not affect the prognosis of patients with PRCC, whereas Tsimafeyeu *et al* (5) demonstrated that the expression of fibroblast growth factor receptor 2 was a prognostic factor in the survival of patients with metastatic PRCC. At present, there is no effective treatment for patients with advanced PRCC (6).

A number of genes have been previously identified to be frequently mutated in PRCC, including MET, SETD2, NF2, KDM6A, SMARCB1, FAT1, BAP1, PBRM1, STAG2, NFE2L2 and TP53 (7,8). At present, the research conducted regarding PRCC-associated biomarkers is insufficient to meet clinical requirements for the diagnosis and prognosis of patients, and there remains a lack of knowledge regarding the use of PRCC-associated noncoding RNAs as biomarkers and their internal interactions. A previous study reported that the classification of different molecular subtypes may aid the prognosis of PRCC (9). The requirement for improved understanding of the molecular mechanisms underlying the development and progression of PRCC is increasing. A recent study analyzed the expression of long noncoding RNAs (lncRNAs), which may serve as useful biomarkers for tumor staging, in PRCC using The Cancer Genome Atlas (TCGA) (10). At present,

there has been limited analysis of PRCC-associated competing endogenous RNA (ceRNA) networks involving lncRNAs in an entire genome.

The ceRNA hypothesis involves a complex post-transcriptional regulatory network in which lncRNAs, mRNAs and other RNAs act as natural microRNA (miRNA) sponges to suppress miRNA function by sharing one or more miRNA response elements (11). At present, increasing evidence indicates that regulatory networks serve important roles in the occurrence, development and regulation of tumors, including breast, ovarian, kidney, colon and liver cancers (12-17).

To investigate the regulatory mechanisms of ceRNA networks in PRCC, and aid improvements in the diagnosis and prognosis of PRCC, a comprehensive analysis of the genomic and epigenomic landscape of PRCC was conducted to identify statistically significant genetic alterations in tumors in the present study. Following a series of analyses, an lncRNA-miRNA-mRNA ceRNA network was constructed and important genes were identified, which may aid the identification of the functions of noncoding RNAs in PRCC, and the associations between miRNA, lncRNA and mRNA. Reverse transcription-quantitative PCR (RT-qPCR) analysis revealed that the expression of a central lncRNA in the ceRNA network, lncRNA maternally expressed 3 (MEG3), was downregulated in PRCC tumor tissues compared with adjacent non-tumor tissues. Furthermore, Kaplan-Meier survival analyses identified 13 mRNAs, 15 lncRNAs and six miRNAs that significantly predicted the prognosis of patients with PRCC. The results of the present study provide a novel approach for the investigation of molecular mechanisms and prognostic biomarkers in PRCC.

Materials and methods

Data source. mRNA and miRNA expression profiles and clinical characteristics of patients with PRCC were obtained from TCGA using the Data Transfer Tool (cancergenome.nih.gov/), using the search terms 'Kidney', 'Kidney Renal Papillary Cell Carcinoma' and 'Transcriptome Profiling', resulting in 289 PRCC sample tissues and 32 non-tumor tissues. mRNA and miRNA sequence (seq) data are available open access, and the present research met the requirements of TCGA publishing guidelines. Following the acquisition of mRNA-seq and miRNA-seq data, lncRNA expression data were obtained by relocating probes in mRNA expression profiles to lncRNAs based on annotations from the GENCODE project (version 28; gencodegenes.org/) (18,19).

Identification and analysis of PRCC-associated mRNAs, lncRNAs and miRNAs. Differential expression analysis of mRNAs, lncRNAs and miRNAs was conducted using data from tumor and adjacent non-tumor tissues from patients with PRCC using edgeR, a Bioconductor package (version 3.6) in R software (version 3.5.0) (20), with thresholds of |log2-fold change| ≥2.0 and adjusted $P<0.05$; the false discovery rate (FDR) was adjusted using the Benjamini-Hochberg method (21).

Functional annotation of differentially expressed mRNAs (DEmRNAs) and construction of the protein-protein interaction (PPI) network. To determine the biological significance of DEmRNAs, Gene Ontology (GO) terms were assigned using the GO database (geneontology.org/) (22) via the Database for Annotation Visualization and Integrated Discovery (DAVID; version 6.8; david.ncifcrf.gov/) (23,24). Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) (25-27) pathway enrichment analysis of DEmRNAs was performed using KOBAS 3.0 (http://kobas.cbi.pku.edu.cm/index.php) (28).

To further understand the protein-protein interactions between DEmRNAs, a PPI network was generated using the Search Tool for the Retrieval of Interacting Genes (version 10.5; string-db.org/) database (29,30). PPI networks were visualized using Cytoscape version 3.6.1 software (31). Furthermore, the CytoHubba (Version 0.1) plug-in for Cytoscape (32) was used to identify hub genes by ranking the participation degree in PPI networks.

Construction of the ceRNA network. DElncRNAs were compared using the miRcode database (version 11; mircode.org/) (33), then miRNAs in selected pairs were compared with previously identified DEmiRNAs to obtain the final integrated lncRNA-miRNA pairs. Prediction of target genes for selected lncRNA-miRNA pairs of miRNAs was performed using three databases, miRTarBase (version 7.0) (34), miRanda (version 3.3a) (35), and TargetScan (version 7.1) (36). Candidate target mRNAs were included in the three databases and intersected with previously identified DEmRNAs. Then, the lncRNA-miRNA-mRNA ceRNA network was reconstructed by assembling DElncRNA-DEmiRNA-DEmRNA associations visualized using Cytoscape (version 3.6.1). The sub-network of the ceRNA network was extracted using the Cytoscape plug-in MCODE (version 1.5.1) (37).

RNA extraction and RT-qPCR validation. lncRNA MEG3 was selected from the ceRNA network for expression analysis in 12 PRCC tumor tissues and adjacent non-tumor tissues. A total of 12 pairs of paraffin-embedded tissue samples from PRCC patients were collected at the Affiliated Hospital of Southwest Medical University (Luzhou, China) between January 2017 and February 2018. The patients ranged in age from 39 to 80 years, with a median age of 60.5 years, including 8 males and 4 females. All patients were confirmed with primary PRCC by pathological examination of surgical specimens and were not subject to any preoperative radiotherapy or chemotherapy, other malignant disease, or acute injury. The study was approved by the Ethical Review Committee of the Affiliated Hospital of Southwest Medical University. Written informed consent was obtained from all patients prior to sample collection. Total RNA was extracted from PRCC tumor and adjacent normal tissues using a paraffin-embedded tissue RNA extraction kit (Bioteke Corporation, Beijing, China). RT was performed using a ReverTra Ace qPCR RT Master Mix with gDNA Remover (Toyobo Life Science, Tokyo, Japan), according to the manufacturer's protocols. Expression level of the lncRNA was detected by qPCR using SYBR Green Realtime PCR Master Mix (Toyobo Life Science) in an Applied Biosystems™ 7500 Fast Real-Time PCR System (Applied Biosystems; Thermo

Table I. Top 10 upregulated and downregulated DEmRNAs, DElncRNAs and DEmiRNAs.

A, Upregulated

DEmRNA			DElncRNA			DEmiRNA		
Name	log2FC	FDR P-value	Name	log2FC	FDR P-value	Name	log2FC	FDR P-value
DDB2	2.1	$9.44x10^{-53}$	AL365181.2	5.8	$1.53x10^{-40}$	hsa-mir-21	2.95	$8.32x10^{-53}$
BBC3	2.59	$1.22x10^{-52}$	AC019069.1	3.37	$6.25x10^{-38}$	hsa-mir-561	3.08	$1.08x10^{-17}$
HK2	3.56	$5.82x10^{-44}$	AL365181.3	4.61	$1.46x10^{-35}$	hsa-mir-592	3.03	$2.78x10^{-17}$
LRRC20	2.07	$1.25x10^{-43}$	AC083862.2	2.15	$1.05x10^{-31}$	hsa-mir-1254-1	2.62	$2.02x10^{-14}$
GPRIN1	3.34	$1.62x10^{-40}$	AC005041.3	2.37	$1.52x10^{-31}$	hsa-mir-3934	2.01	$9.04x10^{-14}$
TMSB10	2.63	$1.97x10^{-37}$	AC092535.4	4.43	$4.79x10^{-29}$	hsa-mir-4768	2.14	$2.40x10^{-13}$
TNFSF9	4.06	$3.18x10^{-37}$	LACTB2-AS1	2.49	$1.73x10^{-28}$	hsa-mir-1293	3.34	$4.41x10^{-12}$
HAMP	5.16	$2.97x10^{-35}$	PAQR9-AS1	5.21	$2.75x10^{-27}$	hsa-mir-584	2.29	$9.54x10^{-12}$
APOC1	6.5	$3.13x10^{-34}$	GAS6-AS1	3.8	$4.41x10^{-26}$	hsa-mir-7156	4.01	$3.23x10^{-11}$
TREM2	4.7	$4.32x10^{-34}$	AL590666.2	3.64	$8.35x10^{-25}$	hsa-mir-4777	2.53	$4.57x10^{-11}$

B, Downregulated

DEmRNA			DElncRNA			DEmiRNA		
Name	log2FC	FDR P-value	Name	log2FC	FDR P-value	Name	log2FC	FDR P-value
MFSD4A	-4.72	$1.48x10^{-274}$	AC068631.1	-5.58	$1.62x10^{-177}$	hsa-mir-184	-5.3	$4.38x10^{-83}$
UMOD	-12.3	$4.41x10^{-252}$	AC002401.1	-6.42	$1.11x10^{-173}$	hsa-mir-216b	-5.48	$2.32x10^{-67}$
CALB1	-8.29	$1.71x10^{-214}$	AC079310.1	-8.36	$9.26x10^{-153}$	hsa-mir-126	-2.15	$3.64x10^{-48}$
EGF	-6.21	$4.85x10^{-206}$	AC008264.2	-3.96	$8.45x10^{-146}$	hsa-mir-33a	-2.57	$1.37x10^{-45}$
GP2	-8.23	$4.61x10^{-199}$	AC019080.1	-3.69	$1.11x10^{-138}$	hsa-mir-129-2	-3.3	$1.45x10^{-41}$
GGACT	-3.57	$4.85x10^{-191}$	AC106772.2	-6.95	$5.04x10^{-127}$	hsa-mir-129-1	-3.2	$2.57x10^{-39}$
DDN	-7.01	$4.88x10^{-187}$	AC027309.1	-6.33	$1.66x10^{-114}$	hsa-mir-145	-2.49	$4.73x10^{-36}$
CRHBP	-6.08	$5.81x10^{-186}$	AC010442.1	-3.21	$6.42x10^{-112}$	hsa-mir-323a	-2.72	$1.45x10^{-31}$
PTGER1	-5.4	$1.20x10^{-156}$	AC099684.2	-4.27	$9.72x10^{-111}$	hsa-mir-206	-3.16	$1.70x10^{-30}$
SLC26A4	-5.18	$3.04x10^{-149}$	AC105384.1	-5.39	$7.77x10^{-110}$	hsa-mir-489	-3.19	$3.16x10^{-27}$

DE, differentially expressed; lncRNA, long noncoding RNA; miRNA, microRNA; log2FC, log2 fold change; FDR, false discovery rate.

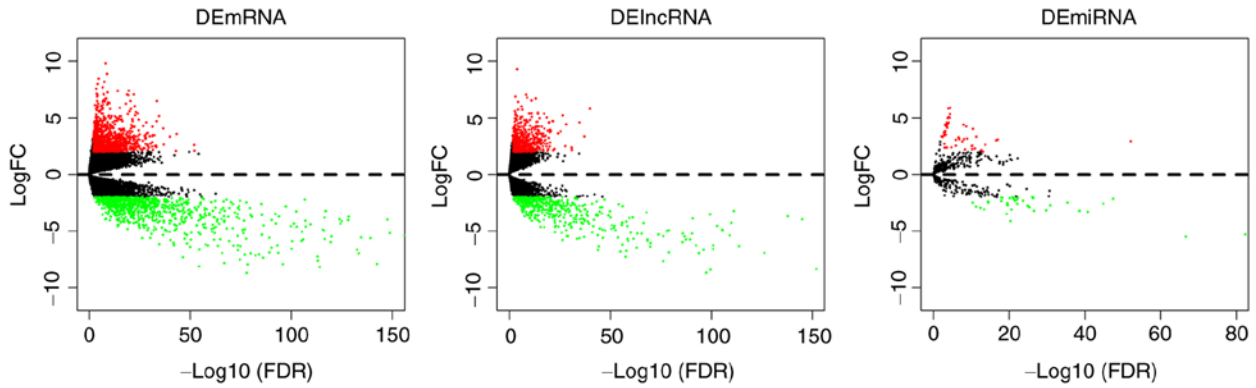

Figure 1. Volcano plots of DEmRNAs, DElncRNAs and DEmiRNAs in papillary renal cell carcinoma. Red points indicate upregulated genes; green points indicate downregulated genes. DE, differentially expressed; lncRNA, long noncoding RNA; miRNA, microRNA; FC, fold change; FDR, false discovery rate.

Fisher Scientific, Inc., Waltham, MA, USA). β-actin served as an internal control. The following primer sequences were used for qPCR: lncRNA MEG3, forward 5'-GCCTGCTGC CCATCTACAC-3', reverse 5'-CCTCTTCATCCTTTGCCA

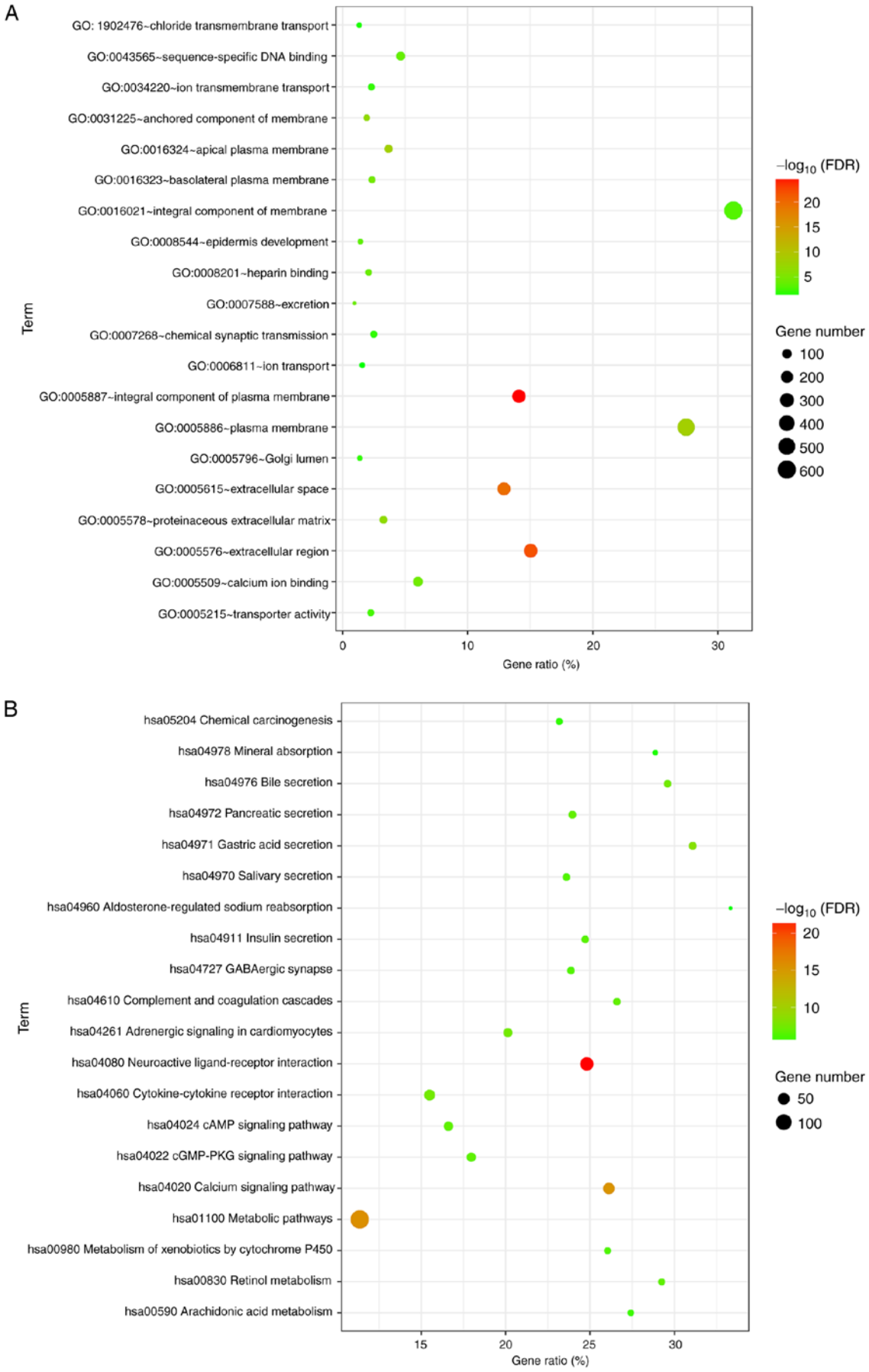

Figure 2. Enriched pathways associated with PRCC. (A) Top 20 GO terms for DEmRNAs associated with PRCC. (B) Top 20 Kyoto Encyclopedia of Genes and Genomes pathways enriched by DEmRNAs associated with PRCC. The color and size of points indicate the significance of the association and the number of genes, respectively. PRCC, papillary renal cell carcinoma; DE, differentially expressed; GO, Gene Ontology; FDR, false discovery rate; GABA, γ-aminobutyric acid; cAMP, cyclic AMP; cGMP, cyclic GMP; PKG, protein kinase G.

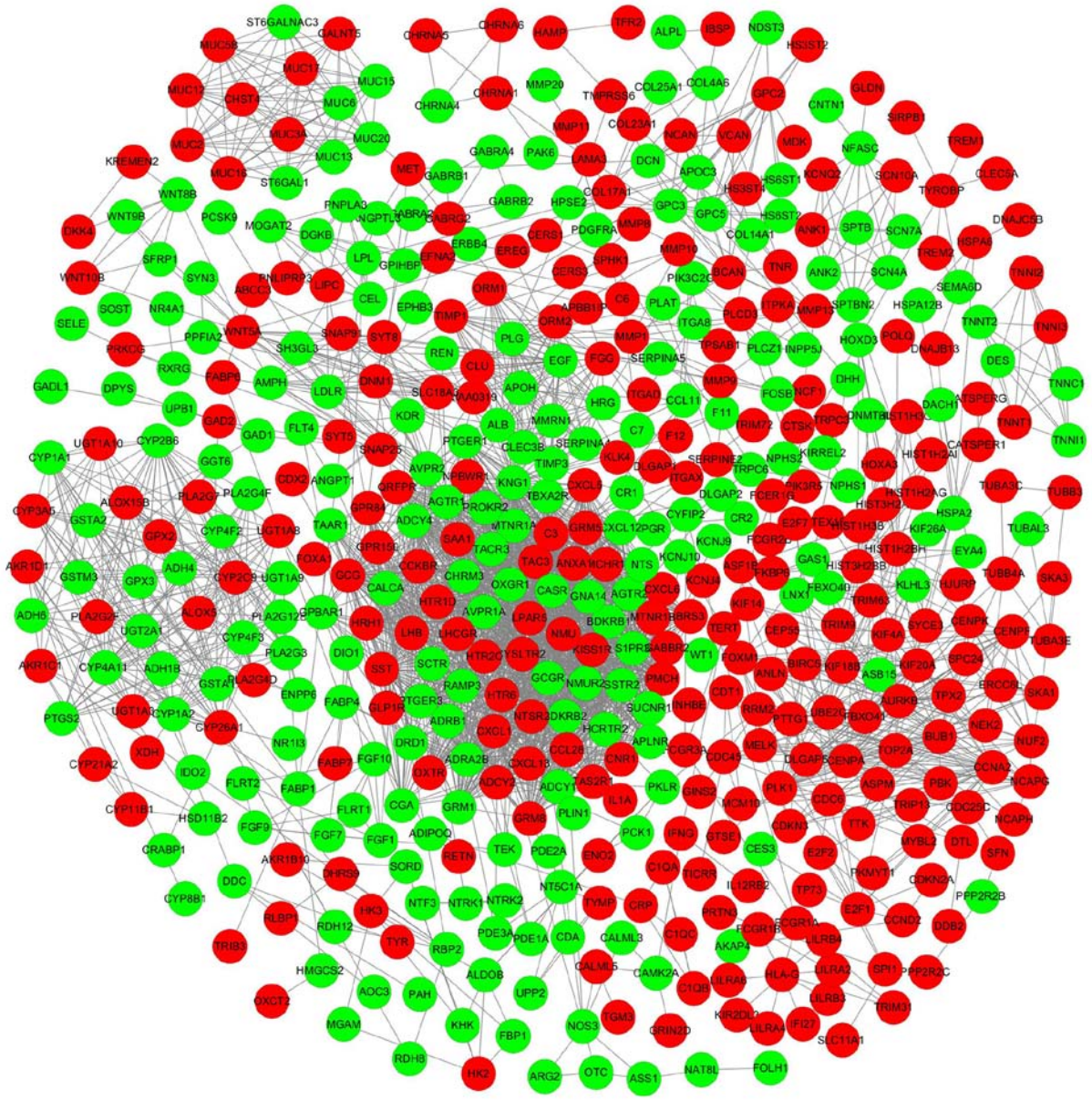

Figure 3. Protein-protein interaction network of papillary renal cell carcinoma-associated DEmRNAs constructed using the Search Tool for the Retrieval of Interacting Genes. Circles indicate protein-coding genes; green indicates downregulated DEmRNAs; red indicates upregulated DEmRNAs. DE, differentially expressed.

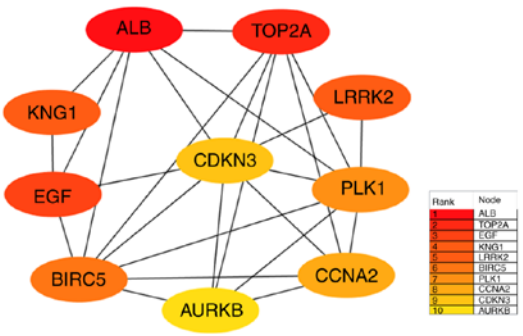

Figure 4. Top 10 hub DEmRNAs extracted from the protein-protein interaction network. Circles indicate protein-coding genes; lines between DEmRNAs indicate direct interactions. DE, differentially expressed; ALB, albumin; TOP2A, DNA topoisomerase II α; KNG1, kininogen 1; LRRK2, leucine-rich repeat kinase 2; CDKN3, cyclin dependent kinase inhibitor 3; EGF, epidermal growth factor; PLK1, polo like kinase 1; BIRC5, baculoviral IAP repeat containing 5; CCNA2, cyclin A2; AURKB, aurora kinase B.

TC-3'; and β-actin, forward 5'-TCCTCTCCCAAGTCCACACA-3' and reverse 5'-GCACGAAGGCTCATCATTCA-3'. qPCR was conducted as follows: 95˚C for 30 sec; 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec; and a dissociation cycle of 95˚C for 15 sec, 60˚C for 60 sec, 95˚C for 15 sec and 60˚C for 15 sec. Each reaction was performed in triplicate, and the relative expression levels (fold change) were calculated using the $2^{-\Delta\Delta Cq}$ method (38). A paired t-test was performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA) to analyze differences in the expression of lncRNA MEG3 between PRCC tumor tissues and adjacent nontumor tissues (n=12/group). $P<0.05$ was considered to indicate a statistically significant difference.

Survival analysis. To identify prognostic DERNAs for patients with PRCC from TCGA, clinical data were obtained and mapped Kaplan-Meier curves for various DElncRNAs,

Table II. Key DEmRNAs, DElncRNAs and DEmiRNAs comprising the papillary renal cell carcinoma-associated competing endogenous RNA network.

RNA type	Name
DEmRNA	SFRP1, NTF3, GPC5, RAPGEF4, TIMP3, E2F2, LDLR, DACH1, ELE, PRLR, SLC43A1, POLQ, ERG, RRM2, NR4A2, DDC, AHNAK2, IL11, CREB5, HAS2, E2F1, SALL3, TBXA2R, HOXC13, OXGR1, SLC22A6
DElncRNA	AC021066.1, TMEM72-AS1, ZRANB2-AS1, AC084262.1, TTTY14, KCNC4-AS1, LINC00330, UCA1, C15orf56, SFTA1P, LINC00494, MEG3, AP002478.1, GRM7-AS3, AC012379.1, LINC00269, COL18A1-AS1, AC079341.1, GPC5-AS1, LY86-AS1, LINC00518, PCGEM1, LINC00299, AL359815.1, LINC00221, LINC00310, GLIS3-AS1, LINC00473, TCL6, LINC00355, DLEU7-AS1, F10-AS1, BX255923.1, MIR4500HG, CNTN4-AS1, LINC00327, LINC00173, LRRC3-AS1, AC012640.1, ZFY-AS1, AC009061.1, SACS-AS1, SOX2-OT, LINC00460, AC061975.6, LINC00443, HOTTIP, LINC00462, AC092811.1, ERVMER61-1, ATP1B3-AS1, GAS6-AS1, AC025278.1, DNM3OS, CRNDE, MYCNOS, AP000525.1, MIR205HG, GDNF-AS1, PWRN1, RERG-AS1, AL590369.1, VCAN-AS1, GRM5-AS1, PVT1
DEmiRNA	hsa-mir-214, hsa-mir-31, hsa-mir-519d, hsa-mir-184, hsa-mir-211, hsa-mir-217, hsa-mir-508, hsa-mir-206, hsa-mir-216b, hsa-mir-506, hsa-mir-216a, hsa-mir-489, hsa-mir-145, hsa-mir-507, hsa-mir-21

DE, differentially expressed; lncRNA, long noncoding RNA; miRNA, microRNA.

DEmiRNAs and DEmRNAs were calculated. Patients with PRCC were divided into high expression and low expression groups according to the median value of gene expression. Significant differences in survival between groups were determined using log-rank tests; $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of DEmRNAs, DElncRNAs and DEmiRNAs in PRCC. Transcriptome sequencing data for mRNAs, lncRNAs and miRNAs were analyzed separately using 289 PRCC tumor samples and 32 paracancerous tissue samples. A total of 1,970 DEmRNAs, 1,201 DElncRNAs and 96 DEmiRNAs were identified to have significantly different expression (|log2-fold change| ≥2.0 and adjusted $P<0.05$) in tumor tissues compared with the adjacent tissue. Volcano plots were generated for the identified DEmRNAs, DElncRNAs and DEmiRNAs (Fig. 1). The top 10 upregulated and downregulated DEmRNAs, DElncRNAs and DEmiRNAs are listed in Table I.

Analysis of DEmRNAs. Of the 1,970 identified DEmRNAs, 1,092 mRNAs were upregulated and 878 were downregulated. To understand the biological significance of these DEmRNAs, GO and pathway enrichment analyses were conducted. GO analysis provides three categories of information: 'Biological process', 'cellular component' and 'molecular function'. Identified DEmRNAs were primarily enriched in 'metabolic pathways', 'neuroactive ligand-receptor interaction', 'calcium signaling pathways', 'pathways in cancer' and 'cytokine-cytokine receptor interactions'. GO and KEGG pathway analysis results are presented in Fig. 2.

A PPI network was constructed to investigate the interactions between the identified DEmRNAs. The PPI network was visualized using Cytoscape (Fig. 3). In addition, the top 10 hub DEmRNAs were identified using the CytoHubba plug-in according to degree levels: Albumin (ALB), DNA topoisomerase II α (TOP2A), epidermal growth factor (EGF), kininogen 1 (KNG1), leucine rich repeat kinase 2 (LRRK2), baculoviral IAP repeat containing 5 (BIRC5), polo like kinase 1 (PLK1), cyclin A2 (CCNA2), cyclin dependent kinase inhibitor 3 (CDKN3), and aurora kinase B (AURKB); the interaction network is presented in Fig. 4.

Construction and analysis of the lncRNA-miRNA-mRNA ceRNA network. To investigate how lncRNAs and miRNAs cooperate to regulate mRNA expression in PRCC, miRNA-mRNA and lncRNA-miRNA regulatory associations were used to construct an lncRNA-miRNA-mRNA ceRNA network. The differential expression profile consisted of 26 DEmRNA nodes, 65 DElncRNA nodes, 15 DEmiRNA nodes (Table II) and 287 edges. This reconstructed ceRNA network was visualized using Cytoscape (Fig. 5). From the networks, it was determined that DElncRNA MEG3 exhibited potential interactions with 14 DEmiRNAs (hsa-miR-507, hsa-miR-145, hsa-miR-519d, hsa-miR-184, hsa-miR-206, hsa-miR-211, hsa-miR-21, hsa-miR-214, hsa-miR-216a, hsa-miR-216b, hsa-miR-217, hsa-miR-508, hsa-miR-31 and hsa-miR-506). Therefore, it was hypothesized that lncRNA MEG3 may have an important role in regulating the ceRNA network in PRCC. Furthermore, miR-519d interacted with 18 DElncRNAs [testis-specific transcript Y-linked 14 (TTTY14), AP002478.1, long intergenic non-protein coding RNA (LINC)00221, T cell leukemia/lymphoma 6 (TCL6), LINC00173, AC009061.1, AC061975.6, AC025278.1, MEG3, LINC00269, glutamate metabotropic receptor 7-antisense RNA 3 (GRM7-AS3), LINC00462, zinc finger RANBP2-type containing 2-antisense RNA 1 (ZRANB2-AS1), LINC00330, HOXA distal transcript antisense RNA (HOTTIP), versican-antisense RNA 1 (VCAN-AS1), Pvt1 oncogene (PVT1) and glutamate metabotropic receptor 5-antisense RNA 1 (GRM5-AS1)] and possessed a targeted regulatory association with eight DEmRNAs [Rap guanine nucleotide exchange factor 4 (RAPGEF4), E2F transcription factor 2 (E2F2), hyaluronan

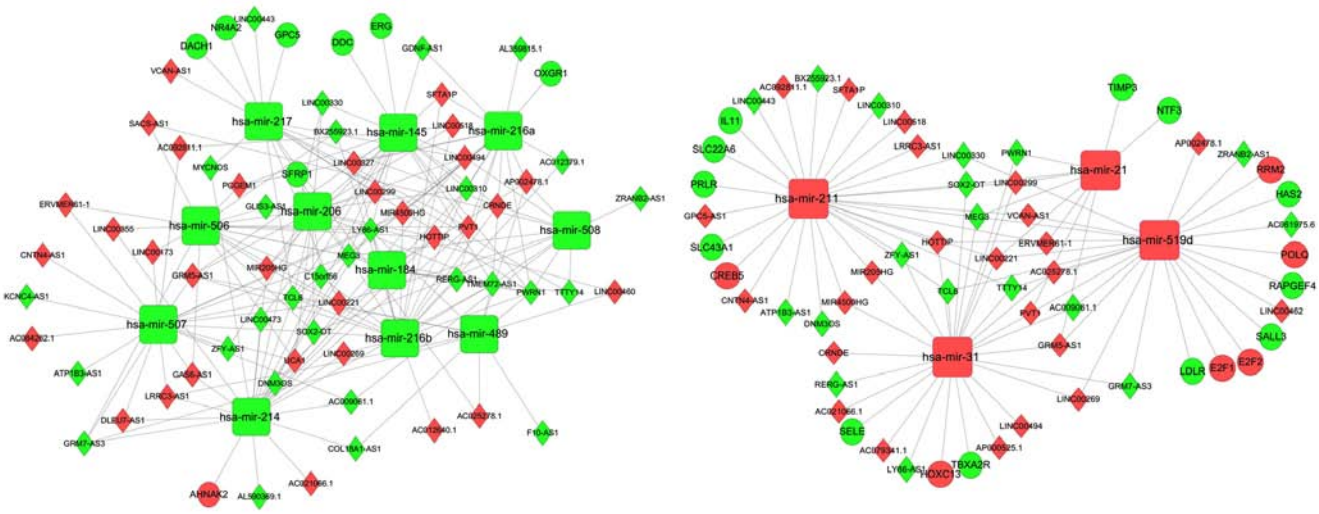

Figure 5. Reconstruction of lncRNA-miRNA-mRNA network. Diamonds indicate lncRNAs; circles indicate mRNA; rounded rectangles indicate miRNAs; edges indicate interactions. Red indicates upregulation; green indicates downregulation. lncRNA, long noncoding RNA; miRNA/mir, microRNA.

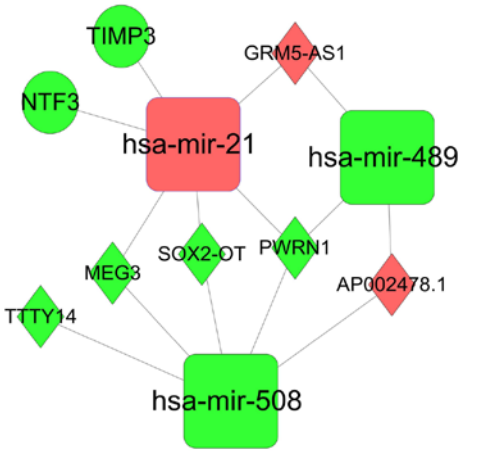

Figure 6. Subnetwork of the lncRNA-miRNA-mRNA ceRNA network. Diamonds indicate lncRNAs; circles indicate mRNA; round rectangles indicate miRNAs; edges indicate interactions. Red indicates upregulation; green indicates downregulation. lncRNA, long noncoding RNA; miRNA/mir, microRNA; ceRNA, competing endogenous RNA; TIMP3, tissue inhibitor of metalloproteinase 3; GRM5-AS1, glutamate metabotropic receptor 5-antisense RNA 1; NTF, neurotrophin 3; TTTY14, testis-specific transcript Y-linked 14; MEG3, maternally expressed 3; SOX2-OT, SOX2 overlapping transcript; PWRN1, Prader-Willi region non-protein coding RNA 1.

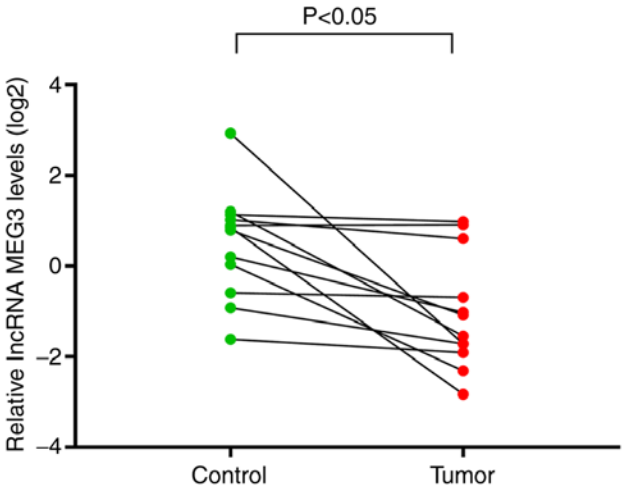

Figure 7. Expression of lncRNA MEG3 in PRCC tissues. Relative expression of lncRNA MEG3 (normalized to β-actin) in PRCC tumor and adjacent non-tumor control tissue samples. PRCC, papillary renal cell carcinoma; lncRNA MEG3, long noncoding RNA maternally expressed 3.

synthase 2 (HAS2), Sal-like protein 3 (SALL3), ribonucleotide reductase regulatory subunit M2 (RRM2), low density lipoprotein (LDL), DNA polymerase θ (POLQ) and E2F transcription factor 1 (E2F1)]. The ceRNA subnetwork was extracted using the plug-in MCODE for Cytoscape (Fig. 6). This subnetwork consists of hub genes, including lncRNA MEG3, lncRNA Prader-Willi region non-protein coding RNA 1 (PWRN1), hsa-miR-508 and hsa-miR-21, plus certain first neighbors, including neurotrophin 3 (NTF3), tissue inhibitor of metalloproteinase 3 (TIMP3), GRM5-AS1, AP002478.1, TTTY14 and hsa-miR-489.

RT-qPCR validation. Of the 15 DEmiRNAs that formed the ceRNA network, lncRNA MEG3 exhibited potential interactions with 14 DEmiRNAs, more than any other DElnRNA, suggesting that this lncRNA may be most likely to serve an important role in PRCC. To validate the bioinformatics results, lncRNA MEG3 was selected for expression analysis. LncRNA MEG3 was revealed to be significantly downregulated in PRCC tumor tissue compared with adjacent non-tumor tissues (Fig. 7), consistent with the aforementioned bioinformatics analysis.

Survival analysis using lncRNAs, miRNAs and mRNAs. In the ceRNA network, 13 DEmRNAs were analyzed to determine associations between expression levels and patient survival, including E2F1, E2F2, ETS transcription factor, glypican 5 (GPC5), HAS2, homeobox C13 (HOXC13), interleukin 11 (IL11), LDL receptor (LDLR), POLQ, RAPGEF4, RRM2, selectin E (SELE) and secreted frizzled related protein 1 (SFRP1), all of which were upregulated in patients with PRCC; expression levels of all these genes were associated with overall survival (P<0.05; Fig. 8A). Similarly,

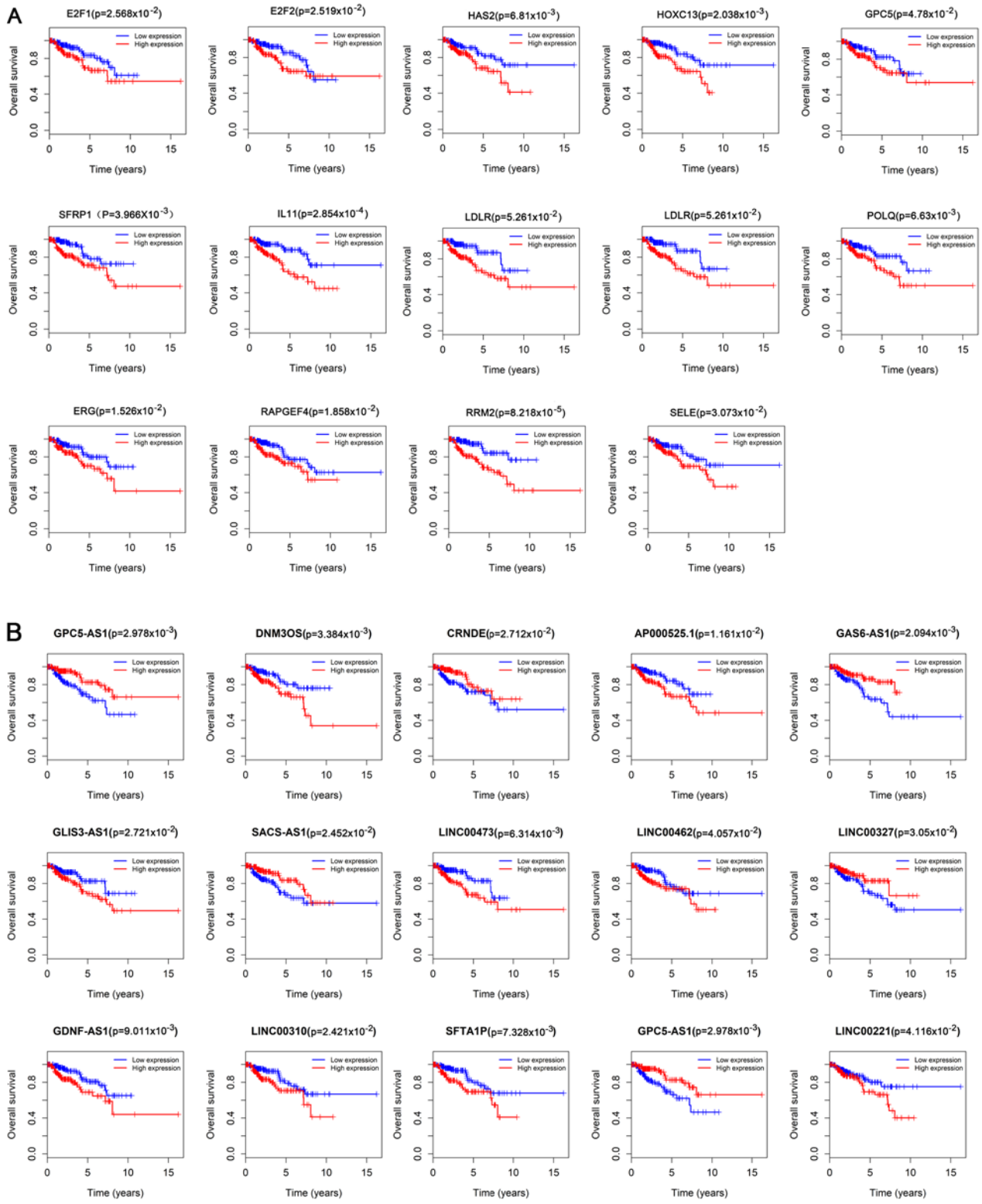

Figure 8. Kaplan-Meier survival analysis for papillary renal cell carcinoma-associated RNAs. According to the median value of gene expression, 288 patients with PRCC from The Cancer Genome Atlas were divided into high expression and low expression groups, whose survival data were complete. Survival curves for (A) mRNAs and (B) long noncoding RNAs.

the expression of five DElncRNAs [colorectal neoplasia differentially expressed (CRNDE), GAS6 antisense RNA 1 (GAS6-AS1), GPC5-antisense RNA 1 (GPC5-AS1), LINC00327 and SACS antisense RNA 1 (sacsin-AS1)] were significantly positively associated with patient survival, whereas 10 [AP000525.1, glial cell-derived neurotrophic factor-antisense RNA 1 (GDNF-AS1), GLIS family zinc finger 3-antisense RNA 1 (GLIS3-AS1), LINC00221, LINC00310, LINC00462, LINC00473, LRR containing 3-antisense RNA 1 (LRRC3-AS1), surfactant associated 1, pseudogene

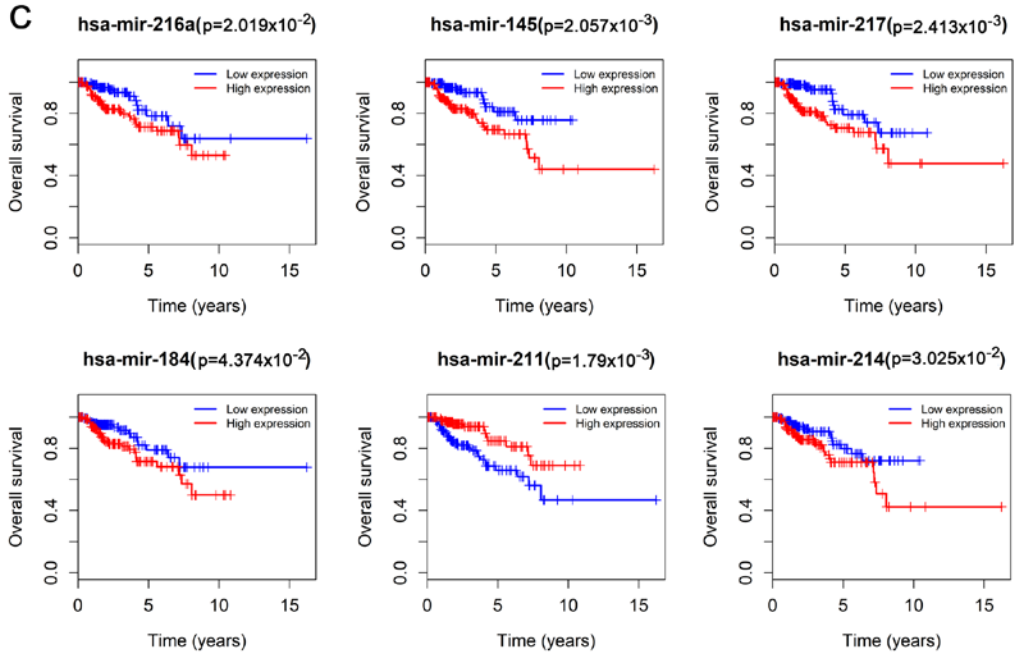

Figure 8. Continued. Kaplan-Meier survival analysis for papillary renal cell carcinoma-associated RNAs. According to the median value of gene expression, 288 patients with PRCC from The Cancer Genome Atlas were divided into high expression and low expression groups, whose survival data were complete. Survival curves for (C) microRNAs.

(SFTA1P) and DNM3 opposite strand (DNM3OS)] were negatively associated (P<0.05; Fig. 8B). One (hsa-miR-211) and five DEmiRNAs (hsa-miR-145, hsa-miR-184, hsa-mir-214, hsa-miR-216a, hsa-miR-217) were significantly positively and negatively associated with overall survival in PRCC, respectively (P<0.05; Fig. 8C).

Discussion

PRCC accounts for ~18.5% of total cases of RCC (1), and it is generally considered to exhibit an improved prognosis compared with CRCC (3). Therefore, there is a notably reduced level of research into PRCC. Prior to the TCGA report into PRCC (8), no large-scale study systematically investigated the pathogenesis of this disease or aimed to identify prognosis-associated biomarkers. Using TCGA, an in-depth analysis was conducted involving a comprehensive genomics approach to characterize the pathology of 161 cases of PRCC in subtypes 1 and 2 (8); however, due to the heterogeneity of PRCC, the pathogenesis, development and prognosis remain unclear, particularly concerning important ceRNA network-associated mechanisms. PRCC is considered to be highly heterogeneous; however, it was previously reported that ~50% of cases exhibit a certain degree of overlap between type I and type II (3). Therefore, in the present study, sample data for PRCC in TCGA were analyzed to identify factors frequently associated with the pathogenesis, development and prognosis of PRCC.

A large number of samples were obtained from the TCGA database, and gene pathways and hub genes associated with PRCC were identified to determine the mechanisms underlying PRCC incidence. A previous study involving TCGA data mining observed that type 2 tumors were characterized by CDKN2A silencing (8). In the present study, it was reported that CDKN3 was one of 10 hub genes in the ceRNA network. GO and KEGG pathway analyses are frequently used to determine the biological functions of DE coding genes. It was revealed via GO analysis of DEmRNAs that there was significant enrichment of 170 GO 'biological processes' (P<0.01), including 'excretion', 'epidermis development', 'integral components of plasma membrane', 'extracellular regions', 'calcium ion binding' and 'heparin binding'. The biological functions of the aforementioned DE genes are consistent with the formation and function of renal cells. It has been established that calcium ions affect almost every aspect of cellular life (39), and that variations in cytosolic calcium concentrations induce important cellular events (40). Intracellular calcium overload can initiate mitochondrial-dependent apoptosis (40), which may be a strategy for inhibiting the proliferation of cancer cells (41,42). Identified DEmRNAs were also significantly enriched in calcium signaling pathways, as determined by KEGG pathway analysis. Raynal *et al* (43) reported that targeting calcium signaling can reverse the epigenetic silencing of tumor suppressor genes. Additionally, renal cell carcinoma is closely associated with abnormal alterations in metabolic pathways involved in oxygen sensing, energy sensing and nutrient sensing cascades (44-46). A previous study demonstrated that metabolic pathways were altered in metastatic RCC, with downregulation of citric acid cycle genes and upregulation of the pentose phosphate pathway (47). Furthermore, identified DEmRNAs were also enriched in 'pathways in cancer', providing a theoretical basis for further research. Therefore, investigation of these signaling pathways may have notable implications for the identification of biological processes and molecular functions involved in tumorigenesis, progression and metastasis.

The roles of noncoding RNA have been identified following advancements in genetic research, including their central role in the diagnosis, treatment and prognosis of various tumors (48-50). Therefore, a lncRNA-miRNA-mRNA ceRNA network was reconstructed to investigate the roles of noncoding RNAs associated with PRCC via a combination of differential gene expression profile and target analyses. From the ceRNA network, it was hypothesized that lncRNA MEG3 and miR-519d may serve important roles in the regulation of ceRNA networks associated with PRCC. It has been reported that lncRNA MEG3 acts as a lncRNA tumor suppressor in numerous tumors (51-56) via interactions with the tumor suppressor p53 and the regulation of the expression of p53 target genes (57); however, the role of lncRNA MEG3 in PRCC has not yet been investigated.

It was also revealed that miR-519d may occupy an important position in the constructed ceRNA network. Downregulation of miR-519d was reported in studies investigating the molecular mechanisms underlying various tumors, including gastric, ovarian and colorectal cancers (58-60). Additionally, the extraction of a subnetwork identified potentially important RNAs, including lncRNA MEG3, lncRNA PWRN1, hsa-miR-508 and hsa-miR-21. lncRNA PWRN1 has been reported to target miR-425-5p and suppress the development of gastric cancer via p53 signaling (61). miR-508 suppressed the epithelial-mesenchymal transition, migration and invasion of ovarian cancer cells via the mitogen-activated protein kinase 1/ERK signaling pathway (62). Similarly, miR-21 has been reported be involved in numerous molecular mechanisms underlying tumorigenesis (63-65), including ceRNA network regulatory mechanisms (66).

To validate the results of the bioinformatics analyses, lncRNA MEG, a core lncRNA in the ceRNA network, was selected for expression analysis in PRCC tumor tissues and adjacent tissues. RT-qPCR analysis revealed that lncRNA MEG3 was downregulated in tumor tissues compared with adjacent non-tumor tissues. It was recently reported that lncRNA MEG3 expression was decreased in CCRC tissues and cells, affecting the apoptosis, proliferation, migration and invasion of CCRC cells by regulating miR-7/RAS like family 11 member B (67).

Survival analysis revealed that the expression of 13 out of 26 DEmRNAs, 15 out of 65 DElncRNAs and 6 out of 15 DEmiRNAs were significantly associated with survival, indicating that these RNAs may be potential biomarkers for the prognosis of patients with PRCC ($P<0.05$). It was observed that the expression of RRM2 was the most significantly associated with survival out of all the RNAs. Wang *et al* (68) reported that low expression of RRM2 was associated with increased time to progression and overall survival in patients with non-small cell lung cancer. Similarly, Zhang *et al* (69) revealed that reduced expression of GPC5 was an independent prognostic marker for the overall survival of patients with prostate cancer. GPC5 protein expression exhibited an association with tumorigenesis and tumor progression in prostate cancer, suggesting a potential application as a novel biomarker for the prediction of diagnosis and prognosis of prostate cancer (70). Furthermore, decreased expression of lncRNA GAS6-AS1 predicted poor prognosis in patients with non-small cell lung cancer (71). These prognosis-associated genes may be potential targets for future clinical treatments, and were identified to be significantly associated with the prognosis of PRCC in the present study.

In conclusion, an lncRNA-miRNA-mRNA ceRNA network was constructed via differential expression and target analyses, demonstrating that lncRNA MEG3 and miR-519d may serve important roles in PRCC. The expression of lncRNA MEG3 was observed to be downregulated in PRCC tumor tissues compared with adjacent non-tumor tissues. The present findings improve understanding of ceRNA network regulatory mechanisms associated with PRCC and may aid future studies into the molecular mechanisms underlying PRCC and the identification of prognostic biomarkers.

Acknowledgements

Not applicable.

Funding

The present study received financial supported by the Sichuan Science and Technology Program (grant nos. 2018TJPT0011, 2017TJPT0003, 2017HH0105 and 17KJFWSF0059).

Availability of data and materials

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

Authors' contributions

QL and JL conceived and designed the study. MC and QL collected the data. QL and MC analyzed the database. QD and QL performed the experiments. QL prepared the diagrams and drafted the manuscript. JL and QD reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

For the use of human tissue samples, the study was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University (Luzhou, China) and all patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Humphrey PA, Moch H, Cubilla AL, Ulbright TM and Reuter VE: The 2016 WHO classification of tumours of the urinary system and male genital organs-part B: Prostate and bladder tumours. Eur Urol 70: 106-119, 2016.
2. Delahunt B and Eble JN: Papillary renal cell carcinoma: A clinicopathologic and immunohistochemical study of 105 tumors. Mod Pathol 10: 537-544, 1997.

3. Chevarie-Davis M, Riazalhosseini Y, Arseneault M, Aprikian A, Kassouf W, Tanguay S, Latour M and Brimo F: The morphologic and immunohistochemical spectrum of papillary renal cell carcinoma: Study including 132 cases with pure type 1 and type 2 morphology as well as tumors with overlapping features. Am J Surg Pathol 38: 887-894, 2014.
4. Ha YS, Chung JW, Choi SH, Lee JN, Kim HT, Kim TH, Chung SK, Byun SS, Hwang EC, Kang SH, *et al*: Clinical significance of subclassification of papillary renal cell carcinoma: Comparison of clinicopathologic parameters and oncologic outcomes between papillary histologic subtypes 1 and 2 using the Korean renal cell carcinoma database. Clin Genitourin Cancer 15: e181-e186, 2017.
5. Tsimafeyeu I, Khasanova A, Stepanova E, Gordiev M, Khochenkov D, Naumova A, Varlamov I, Snegovoy A and Demidov L: FGFR2 overexpression predicts survival outcome in patients with metastatic papillary renal cell carcinoma. Clin Transl Oncol 19: 265-268, 2017.
6. Motzer RJ, Bacik J, Mariani T, Russo P, Mazumdar M and Reuter V: Treatment outcome and survival associated with metastatic renal cell carcinoma of non-clear-cell histology. J Clin Oncol 20: 2376-2381, 2002.
7. Durinck S, Stawiski EW, Pavía-Jiménez A, Modrusan Z, Kapur P, Jaiswal BS, Zhang N, Toffessi-Tcheuyap V, Nguyen TT, Pahuja KB, *et al*: Spectrum of diverse genomic alterations define non-clear cell renal carcinoma subtypes. Nat Genet 47: 13-21, 2015.
8. Cancer Genome Atlas Research Network; Linehan WM, Spellman PT, Ricketts CJ, Creighton CJ, Fei SS, Davis C, Wheeler DA, Murray BA, Schmidt L, *et al*: Comprehensive molecular characterization of papillary renal-cell carcinoma. N Engl J Med 374: 135-145, 2016.
9. Yang XJ, Tan MH, Kim HL, Ditlev JA, Betten MW, Png CE, Kort EJ, Futami K, Furge KA, Takahashi M, *et al*: A molecular classification of papillary renal cell carcinoma. Cancer Res 65: 5628-5637, 2005.
10. Lan H, Zeng J, Chen G and Huang H: Survival prediction of kidney renal papillary cell carcinoma by comprehensive LncRNA characterization. Oncotarget 8: 110811-110829, 2017.
11. Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP: A ceRNA hypothesis: The rosetta stone of a hidden RNA language? Cell 146: 353-358, 2011.
12. Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ and Pandolfi PP: A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature 465: 1033-1038, 2010.
13. Hou P, Zhao Y, Li Z, Yao R, Ma M, Gao Y, Zhao L, Zhang Y, Huang B and Lu J: LincRNA-ROR induces epithelial-to-mesenchymal transition and contributes to breast cancer tumorigenesis and metastasis. Cell Death Dis 5: e1287, 2014.
14. Chen P, Fang X, Xia B, Zhao Y, Li Q and Wu X: Long noncoding RNA LINC00152 promotes cell proliferation through competitively binding endogenous miR-125b with MCL-1 by regulating mitochondrial apoptosis pathways in ovarian cancer. Cancer Med 7: 4530-4541, 2018.
15. Liu K, Yao H, Wen Y, Zhao H, Zhou N, Lei S and Xiong L: Functional role of a long non-coding RNA LIFR-AS1/miR-29a/TNFAIP3 axis in colorectal cancer resistance to pohotodynamic therapy. Biochim Biophys Acta Mol Basis Dis 1864: 2871-2880, 2018.
16. Qu Y, Xiao H, Xiao W, Xiong Z, Hu W, Gao Y, Ru Z, Wang C, Bao L, Wang K, *et al*: Upregulation of MIAT regulates LOXL2 expression by competitively binding MiR-29c in clear cell renal cell carcinoma. Cell Physiol Biochem 48: 1075-1087, 2018.
17. Bai N, Peng E, Qiu X, Lyu N, Zhang Z, Tao Y, Li X and Wang Z: circFBLIM1 act as a ceRNA to promote hepatocellular cancer progression by sponging miR-346. J Exp Clin Cancer Res 37: 172, 2018.
18. Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, *et al*: The GENCODE v7 catalog of human long noncoding RNAs: Analysis of their gene structure, evolution, and expression. Genome Res 22: 1775-1789, 2012.
19. Frankish A, Diekhans M, Ferreira AM, Johnson R, Jungreis I, Loveland J, Mudge JM, Sisu C, Wright J, Armstrong J, *et al*: GENCODE reference annotation for the human and mouse genomes. Nucleic Acids Res 47: D766-D773, 2019.
20. Robinson MD, McCarthy DJ and Smyth GK: edgeR: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139-140, 2010.
21. Benjamini Y and Hochberg Y: Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Statisti Soc Series B: Methodol 57: 289-300, 1995.
22. Gene Ontology Consortium: The gene ontology (GO) project in 2006. Nucleic Acids Res 34 (Database Issue): D322-D326, 2006.
23. Huang da W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44-57, 2009.
24. Huang da W, Sherman BT and Lempicki RA: Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37: 1-13, 2009.
25. Kanehisa M, Sato Y, Furumichi M, Morishima K and Tanabe M: New approach for understanding genome variations in KEGG. Nucleic Acids Res 47 (D1): D590-D595, 2019.
26. Kanehisa M, Furumichi M, Tanabe M, Sato Y and Morishima K: KEGG: New perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 45(D1): D353-D361, 2017.
27. Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S, Tokimatsu T and Yamanishi Y: KEGG for linking genomes to life and the environment. Nucleic Acids Res 36 (Database Issue): D480-D484, 2008.
28. Xie C, Mao X, Huang J, Ding Y, Wu J, Dong S, Kong L, Gao G, Li CY and Wei L: KOBAS 2.0: A web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res 39: W316-W322, 2011.
29. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, *et al*: The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 45 (D1): D362-D368, 2017.
30. Jensen LJ, Kuhn M, Stark M, Chaffron S, Creevey C, Muller J, Doerks T, Julien P, Roth A, Simonovic M, *et al*: STRING 8-a global view on proteins and their functional interactions in 630 organisms. Nucleic Acids Res 37 (Database Issue): D412-D416, 2009.
31. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504, 2003.
32. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT and Lin CY: CytoHubba: Identifying hub objects and sub-networks from complex interactome. BMC Syst Biol 8 (Suppl 4): S11, 2014.
33. Jeggari A, Marks DS and Larsson E: miRcode: A map of putative microRNA target sites in the long non-coding transcriptome. Bioinformatics 28: 2062-2063, 2012.
34. Hsu SD, Tseng YT, Shrestha S, Lin YL, Khaleel A, Chou CH, Chu CF, Huang HY, Lin CM, Ho SY, *et al*: miRTarBase update 2014: An information resource for experimentally validated miRNA-target interactions. Nucleic Acids Res 42 (Database Issue): D78-D85, 2014.
35. John B, Enright AJ, Aravin A, Tuschl T, Sander C and Marks DS: Human MicroRNA targets. PLoS Biol 2: e363, 2004.
36. Agarwal V, Bell GW, Nam JW and Bartel DP: Predicting effective microRNA target sites in mammalian mRNAs. Elife: 4, 2015 (doi: 10.7554/eLife.05005).
37. Bader GD and Hogue CW: An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics 4: 2, 2003.
38. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
39. Boehning D, Patterson RL, Sedaghat L, Glebova NO, Kurosaki T and Snyder SH: Cytochrome c binds to inositol (1,4,5) trisphosphate receptors, amplifying calcium-dependent apoptosis. Nat Cell Biol 5: 1051-1061, 2003.
40. Pinton P, Giorgi C, Siviero R, Zecchini E and Rizzuto R: Calcium and apoptosis: ER-mitochondria Ca2+ transfer in the control of apoptosis. Oncogene 27: 6407-6418, 2008.
41. Kim KY, Cho HJ, Yu SN, Kim SH, Yu HS, Park YM, Mirkheshti N, Kim SY, Song CS, Chatterjee B and Ahn SC: Interplay of reactive oxygen species, intracellular Ca2+ and mitochondrial homeostasis in the apoptosis of prostate cancer cells by deoxypodophyllotoxin. J Cell Biochem 114: 1124-1134, 2013.
42. Xue J, Li R, Zhao X, Ma C, Lv X, Liu L and Liu P: Morusin induces paraptosis-like cell death through mitochondrial calcium overload and dysfunction in epithelial ovarian cancer. Chem Biol Interact 283: 59-74, 2018.

43. Raynal NJ, Lee JT, Wang Y, Beaudry A, Madireddi P, Garriga J, Malouf GG, Dumont S, Dettman EJ, Gharibyan V, *et al*: Targeting calcium signaling induces epigenetic reactivation of tumor suppressor genes in cancer. Cancer Res 76: 1494-1505, 2016.
44. Massari F, Ciccarese C, Santoni M, Brunelli M, Piva F, Modena A, Bimbatti D, Fantinel E, Santini D, Cheng L, *et al*: Metabolic alterations in renal cell carcinoma. Cancer Treat Rev 41: 767-776, 2015.
45. Wettersten HI, Aboud OA, Lara PN Jr and Weiss RH: Metabolic reprogramming in clear cell renal cell carcinoma. Nat Rev Nephrol 13: 410-419, 2017.
46. Lucarelli G, Galleggiante V, Rutigliano M, Sanguedolce F, Cagiano S, Bufo P, Lastilla G, Maiorano E, Ribatti D, Giglio A, *et al*: Metabolomic profile of glycolysis and the pentose phosphate pathway identifies the central role of glucose-6-phosphate dehydrogenase in clear cell-renal cell carcinoma. Oncotarget 6: 13371-13386, 2015.
47. White NM, Newsted DW, Masui O, Romaschin AD, Siu KW and Yousef GM: Identification and validation of dysregulated metabolic pathways in metastatic renal cell carcinoma. Tumour Biol 35: 1833-1846, 2014.
48. Renganathan A and Felley-Bosco E: Long noncoding RNAs in cancer and therapeutic potential. Adv Exp Med Biol 1008: 199-222, 2017.
49. Bhan A, Soleimani M and Mandal SS: Long noncoding RNA and cancer: A new paradigm. Cancer Res 77: 3965-3981, 2017.
50. Huarte M: The emerging role of lncRNAs in cancer. Nat Med 21: 1253-1261, 2015.
51. Dan J, Wang J, Wang Y, Zhu M, Yang X, Peng Z, Jiang H and Chen L: LncRNA-MEG3 inhibits proliferation and metastasis by regulating miRNA-21 in gastric cancer. Biomed Pharmacother 99: 931-938, 2018.
52. Feng SQ, Zhang XY, Fan HT, Sun QJ and Zhang M: Up-regulation of LncRNA MEG3 inhibits cell migration and invasion and enhances cisplatin chemosensitivity in bladder cancer cells. Neoplasma 65: 925-932, 2018.
53. Li Z, Yang L, Liu X, Nie Z and Luo J: Long noncoding RNA MEG3 inhibits proliferation of chronic myeloid leukemia cells by sponging microRNA21. Biomed Pharmacother 104: 181-192, 2018.
54. Long J and Pi X: lncRNA-MEG3 suppresses the proliferation and invasion of melanoma by regulating CYLD expression mediated by sponging miR-499-5p. Biomed Res Int 2018: 2086564, 2018.
55. Sun KX, Wu DD, Chen S, Zhao Y and Zong ZH: LncRNA MEG3 inhibit endometrial carcinoma tumorigenesis and progression through PI3K pathway. Apoptosis 22: 1543-1552, 2017.
56. Zhang SZ, Cai L and Li B: MEG3 long non-coding RNA prevents cell growth and metastasis of osteosarcoma. Bratisl Lek Listy 118: 632-636, 2017.
57. Wei GH and Wang X: lncRNA MEG3 inhibit proliferation and metastasis of gastric cancer via p53 signaling pathway. Eur Rev Med Pharmacol Sci 21: 3850-3856, 2017.
58. Pang Y, Mao H, Shen L, Zhao Z, Liu R and Liu P: MiR-519d represses ovarian cancer cell proliferation and enhances cisplatin-mediated cytotoxicity in vitro by targeting XIAP. Onco Targets Ther 7: 587-597, 2014.
59. Ye X and Lv H: MicroRNA-519d-3p inhibits cell proliferation and migration by targeting TROAP in colorectal cancer. Biomed Pharmacother 105: 879-886, 2018.
60. Yue H, Tang B, Zhao Y, Niu Y, Yin P, Yang W, Zhang Z and Yu P: MIR-519d suppresses the gastric cancer epithelial-mesenchymal transition via Twist1 and inhibits Wnt/β-catenin signaling pathway. Am J Transl Res 9: 3654-3664, 2017.
61. Chen Z, Ju H, Yu S, Zhao T, Jing X, Li P, Jia J, Li N, Tan B and Li Y: Prader-Willi region non-protein coding RNA 1 suppressed gastric cancer growth as a competing endogenous RNA of miR-425-5p. Clin Sci (Lond) 132: 1003-1019, 2018.
62. Hong L, Wang Y, Chen W and Yang S: MicroRNA-508 suppresses epithelial-mesenchymal transition, migration, and invasion of ovarian cancer cells through the MAPK1/ERK signaling pathway. J Cell Biochem 119: 7431-7440, 2018.
63. Zhou B, Wang D, Sun G, Mei F, Cui Y and Xu H: Effect of miR-21 on apoptosis in lung cancer cell through inhibiting the PI3K/Akt/NF-κB signaling pathway in vitro and in vivo. Cell Physiol Biochem 46: 999-1008, 2018.
64. Zhao MY, Wang LM, Liu J, Huang X, Liu J and Zhang YF: MiR-21 suppresses anoikis through targeting PDCD4 and PTEN in human esophageal adenocarcinoma. Curr Med Sci 38: 245-251, 2018.
65. Naro Y, Ankenbruck N, Thomas M, Tivon Y, Connelly CM, Gardner L and Deiters A: Small molecule inhibition of MicroRNA miR-21 rescues chemosensitivity of renal-cell carcinoma to topotecan. J Med Chem: 11 Jul, 2018 (Epub ahead of print).
66. Zhang R and Xia T: Long non-coding RNA XIST regulates PDCD4 expression by interacting with miR-21-5p and inhibits osteosarcoma cell growth and metastasis. Int J Oncol 51: 1460-1470, 2017.
67. He H, Dai J, Zhuo R, Zhao J, Wang H, Sun F, Zhu Y and Xu D: Study on the mechanism behind lncRNA MEG3 affecting clear cell renal cell carcinoma by regulating miR-7/RASL11B signaling. J Cell Physiol 233: 9503-9515, 2018.
68. Wang L, Meng L, Wang XW, Ma GY and Chen JH: Expression of RRM1 and RRM2 as a novel prognostic marker in advanced non-small cell lung cancer receiving chemotherapy. Tumour Biol 35: 1899-1906, 2014.
69. Zhang C, Liu Z, Wang L, Qiao B, Du E, Li L, Xu Y and Zhang Z: Prognostic significance of GPC5 expression in patients with prostate cancer. Tumour Biol 37: 6413-6418, 2016.
70. Yuan S, Yu Z, Liu Q, Zhang M, Xiang Y, Wu N, Wu L, Hu Z, Xu B, Cai T, *et al*: GPC5, a novel epigenetically silenced tumor suppressor, inhibits tumor growth by suppressing Wnt/β-catenin signaling in lung adenocarcinoma. Oncogene 35: 6120-6131, 2016.
71. Han L, Kong R, Yin DD, Zhang EB, Xu TP, De W and Shu YQ: Low expression of long noncoding RNA GAS6-AS1 predicts a poor prognosis in patients with NSCLC. Med Oncol 30: 694, 2013.