Construction and analysis of circular RNA molecular regulatory networks in clear cell renal cell carcinoma

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Abstract. Increasing evidence has indicated that circular (circ)RNAs participate in carcinogenesis; however, the specific regulatory mechanisms underlying the effects of circRNAs, microRNAs (miRNAs/miRs) and genes on the development of clear cell renal cell carcinoma (CCRCC) remain unclear. In the present study, RNA microarray data from CCRCC tissues and control samples were downloaded from the Gene Expression Omnibus and The Cancer Genome Atlas, in order to identify significantly dysregulated circRNAs, miRNAs and genes. The Cancer-Specific circRNA Database was used to explore the interactions between miRNAs and circRNAs, whereas TargetScan and miRDB were employed to predict the mRNA targets of miRNAs. Functional enrichment and prognostic analyses were conducted in R. The results revealed that 324 circRNAs were downregulated, whereas 218 circRNAs were upregulated in cancer. In addition, a circRNA-miRNA-mRNA interaction network was constructed. Gene Ontology analysis of the upregulated genes revealed that these genes were enriched in biological processes, including 'flavonoid metabolic process', 'cellular glucuronidation' and 'T cell activation'. The downregulated genes were mainly enriched in biological processes, such as 'nephron development', 'kidney development' and 'renal system development'. The hub genes, including membrane palmitoylated protein 7, aldehyde dehydrogenase 6 family member A1, transcription factor AP-2α, collagen type IV α 4 chain, nuclear receptor subfamily 3 group C member 2, plasminogen, Holliday junction recognition protein, claudin 10, kinesin family member 18B and thyroid hormone receptor β, and the hub miRNAs, including miR-21-3p, miR-155-3p, miR-144-3p, miR-142-5p, miR-875-3p, miR-885-3p, miR-3941, miR-224-3p, miR-584-3p and miR-138-1-3p, were significantly associated with CCRCC survival. In conclusion, these results suggested that the significantly dysregulated circRNAs, miRNAs and genes identified in this study may be considered potential biomarkers of the carcinogenesis of CCRCC and the survival of patients with this disease.

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Introduction

Renal cell carcinoma (RCC) is a common type of cancer that is derived from renal tubular epithelial cells (1). Clear cell RCC (CCRCC) has been reported to be the most common histological subtype of RCC (2,3). As for clinical treatment, RCC is often resistant to radiotherapy, chemotherapy and hormonal treatments (4). Although surgical resection can effectively treat CCRCC, 20-40% of patients develop local recurrence or distant metastasis following surgery (5). The observed survival rate of advanced CCRCC is very low, which poses an obstacle in treating and managing patients with CCRCC (6). As CCRCC is a highly aggressive cancer with concomitant poor prognosis, reliable biomarkers for predicting the susceptibility and survival of patients with CCRCC are urgently required.

Circular RNAs (circRNAs) represent a series of endogenous RNAs that modulate the expression of genes and do not encode proteins (7). circRNAs are commonly characterized by their stabilized structure and tissue specificity, and are widely expressed in a variety of eukaryotic cells (8,9). CircRNAs also have tissue specificity and their expression is tissue specific in the eukaryotic transcriptome (10). An investigation into the regulation of competing endogenous RNAs (ceRNAs) by Meng *et al* (9) provided insight into the complex post-transcriptional interaction network of various circRNAs and long non-coding RNAs; these molecules function as microRNA (miRNA/miR) sponges, suppressing their effects via miRNA response elements. Emerging evidence has suggested that circRNAs may be considered robust biomarkers and potential therapeutic targets in several diseases, including cancer (11).

Numerous studies have confirmed the existence of the regulatory role of ceRNAs in the circRNA-miRNA-mRNA network within various diseases, including renal cancer (12,13). For example, the novel circRNA circHIAT1 has been reported to be downregulated in CCRCC tissues compared with normal tissues. Analysis of androgen receptor-inhibited circHIAT1 revealed the aberrant expression of miR-195-5p/29a-3p/29c-3p, which induced cell division cycle 42 expression, promoting the migration and invasion of CCRCC cells (12). Furthermore, a previous study demonstrated that knockdown of circRNA_0001451 significantly enhanced tumor proliferation *in vitro*; the levels

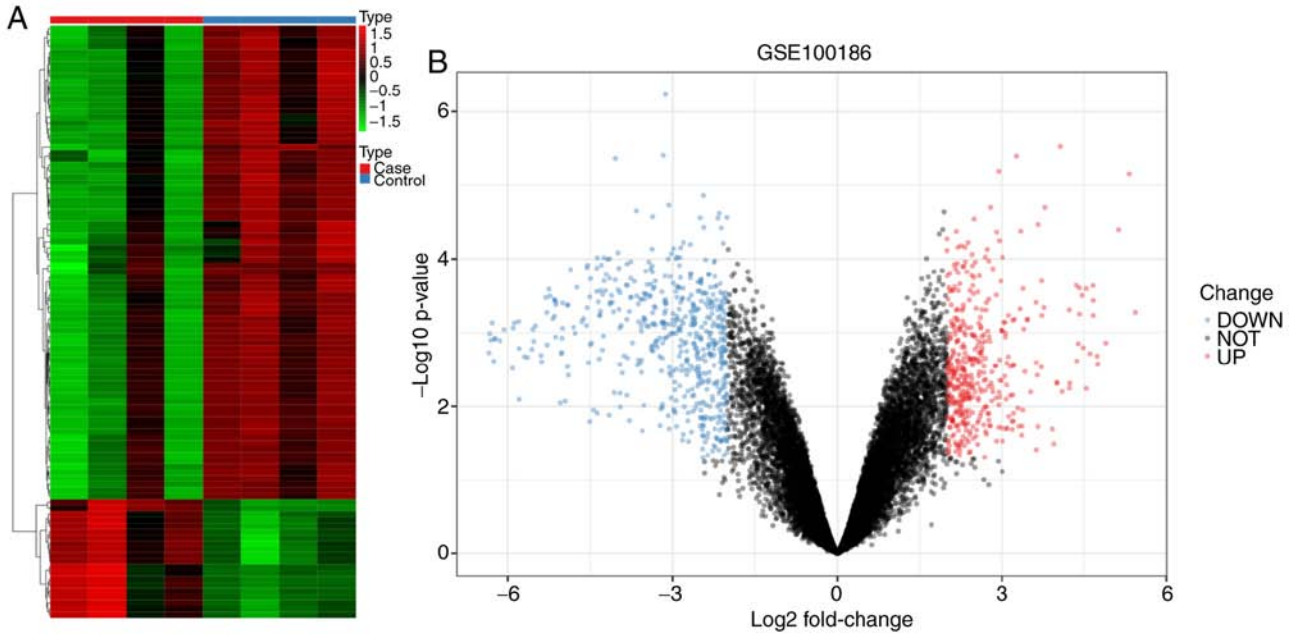

Figure 1. Differential expression analysis of microarray GSE100186. (A) Heatmap of GSE100186, in the heatmap, the green color represents low expression while the red color represents high expression. (B) Volcano plot of GSE100186. Compared with the normal group, red represents upregulated genes in the cancer group, whereas blue represents downregulated genes in the cancer group. NOT represents no change in the differential expression analysis.

of circRNA_0001451 were associated with the differentiation grade of patients with CCRCC (13). In addition, circRNA ZNF609 has been reported to serve as a ceRNA in modulating the expression of Forkhead box P4 via sponging miR-138-5p in renal cancer; high circ-ZNF609 expression was determined to enhance the growth and invasive characteristics of renal cancer cells (14).

Despite improved understanding of the association between circRNA expression and various types of human cancer, the role of circRNAs in renal cancer remains unclear. The present study identified several differentially expressed circRNAs, miRNAs and genes by analyzing datasets of the Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/gds/) and The Cancer Genome Atlas (TCGA, https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) for CCRCC. Additionally, a circRNA-miRNA-mRNA regulatory network was constructed using bioinformatics tools. The present findings may improve understanding of the mechanisms underlying the carcinogenesis of CCRCC.

Materials and methods

Microarray database. To identify datasets, 'renal cellular cell carcinoma' and 'circRNA' were used as keywords to search the GEO; datasets including cancer and normal groups was the main inclusion criterion. The data were downloaded from the GEO of the National Center for Biotechnology Information repository. The GSE100186 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE100186) circRNA expression microarray dataset of CCRCC was used, which contained data from four CCRCC samples and four normal samples. Arraystar circRNA microarray (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL21825) analysis was used to examine the expression of circRNAs in CCRCC and matched non-tumor tissues. mRNA expression and miRNA profiling of TCGA CCRCC data was performed to identify differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMs) between cancer and normal tissues. TCGA data were downloaded from UCSC XENA (https://xena.ucsc.edu/).

Data processing. The limma package in R (version 3.6.0, https://www.r-project.org/) was used to analyze differentially expressed circRNAs (DECs) between the groups. The 'edge R' package (version 3.26.8; https://www.bioconductor.org/packages/release/bioc/html/edgeR.html) was employed to analyze the DEGs and DEMs between the groups. $P<0.05$ and $|\log \text{fold change}|>2$ were applied to determine statistical significance.

circRNA-miRNA-mRNA regulatory network. The Cancer-Specific circRNA Database (CSCD; http://gb.whu.edu.cn/CSCD) can be used to predict interactions between circRNAs and miRNAs (15). Using the CSCD, miRNAs that interact with DECs were predicted. Subsequently, the DECs that interact with the miRNAs were identified as CCRCC-specific miRNAs. TargetScan 7.2 (http://www.targetscan.org/vert_72/) and miRDB 2.0 (http://www.mirdb.org/) were used to predict the target genes of DEMs, which were matched to genes with a mRNA expression profile that opposed the miRNA profile; the expression of miRNAs is often inversely associated with that of the target mRNA. Subsequently, the top five circRNAs with the highest degrees of connectivity were selected as the hub circRNAs. The circRNA-miRNA-mRNA regulatory network was constructed using Cytoscape 3.7.0 (https://cytoscape.org/).

Gene Ontology (GO) and pathway enrichment analyses. GO analysis is a useful bioinformatics strategy for annotating genes or gene products, and comprises three categories: Cellular component (CC), molecular function (MF) and biological

process (BP) (16). Kyoto Encyclopedia of Genes and Genomes (KEGG) is a collection of databases, which contain comprehensive information regarding genomes, biological pathways, diseases and chemical substances (17). In the present study, GO and KEGG enrichment analyses were conducted using R package clusterprofiler (version 3.12.0; https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html). P<0.05 was considered to indicate a statistically significant difference.

Prognostic value of circRNA-regulated DEGs and DEMs. TCGA contains the survival information of patients with various types of cancer. Using the survival 2.44 package (https://cran.r-project.org/web/packages/survival/index.html) in R, the prognostic value of circRNA-regulated DEGs and DEMs was assessed. Additionally, a survival curve was plotted using survminer 0.4.6 (https://cran.r-project.org/web/packages/survminer/index.html) package in R.

Results

Identification of DECs. Following analysis of differential expression, a total of 324 circRNAs in the GSE100186 dataset were downregulated in the cancer group, whereas 218 circRNAs were upregulated in the cancer group (Fig. 1 and Table SI). The top ten most significant circRNAs according to P-value included hsa_circ_0031594, hsa_circ_0001968, hsa_circ_0003596, hsa_circ_0058794, hsa_circ_0001873, hsa_circ_0003748, hsa_circ_0003997, hsa_circ_0000223, hsa_circ_0092367 and hsa_circ_0092360.

circRNA-miRNA-mRNA regulatory network. According to the CSCD datasets, 2,363 miRNAs were reported to interact with the identified DECs. After identifying the DECs that interact with the 2,363 miRNAs, 42 miRNAs were selected as CCRCC-specific miRNAs, including 32 upregulated miRNAs and 10 downregulated miRNAs. Using TargetScan, miRDB and TCGA-DEGs, a total of 244 downregulated genes and 85 upregulated genes were selected as circRNA-regulated genes. Subsequently, the circRNAs were sorted based on the degree of connectivity; the top five circRNAs were selected as hub circRNAs in the regulatory network of DEGs (Table I).

Construction of the circRNA-miRNA-upregulated mRNA network. As presented in Fig. 2A, a hub circRNA-miRNA-upregulated mRNA network was built. The results of enrichment analysis of the 85 upregulated genes are presented in Fig. 2B and Table II. The upregulated genes were mainly enriched in BP, including 'flavonoid metabolic process', 'cellular glucuronidation' and 'T cell activation'. In addition, these DEGs were enriched in MF, including 'glucuronosyltransferase activity', 'UDP-glycosyltransferase activity' and 'protein heterodimerization activity'. KEGG pathway analysis suggested that DEGs were associated with 'ascorbate and aldarate metabolism', 'pentose and glucuronate interconversions', 'steroid hormone biosynthesis' and 'retinol metabolism'.

Construction of the circRNA-miRNA-downregulated mRNA interaction network. As shown in Fig. 3A, a hub circRNA-miRNA-downregulated mRNA network was built.

Table I. Information regarding the hub circRNAs.

Author, year	Alias	LogFC	Position	Strand	Genomic length (bp)	Spliced length (bp)	Gene symbol	(Refs.)
Salzman *et al*, 2013	hsa_circ_0031594	5.320375	chr14:34398281-34400421	-	2,140	257	EGLN3	(35)
Salzman *et al*, 2013; Jeck *et al*, 2013; Rybak *et al*, 2015	hsa_circ_0001968	4.893003	chr11:68359043-68367962	+	8,919	407	PPP6R3	(35-37)
Jeck *et al*, 2013	hsa_circ_0003596	4.73989	chr9:137716445-137717750	+	1,305	369	COL5A1	(36)
Rybak *et al*, 2015; Salzman *et al*, 2013	hsa_circ_0058794	4.668168	chr2:236626200-236659132	+	32,932	451	AGAP1	(35,37)
Memczak, 2013	hsa_circ_0001873	4.666399	chr9:93637042-93639999	-	2,957	2,957	SYK	(38)
Jeck *et al*, 2013; Rybak *et al*, 2015; Salzman *et al*, 2013	hsa_circ_0003748	-6.26453	chr3:48726970-48728915	-	1,945	352	IP6K2	(35-37)
Jeck *et al*, 2013; Rybak *et al*, 2015	hsa_circ_0003997	-6.29312	chr11:122953792-122955421	-	1,629	493	CLMP	(36,37)
Memczak *et al*, 2013	hsa_circ_0000223	-6.31392	chr10:17754818-17754937	+	119	119	STAM	(38)
Zhang *et al*, 2013	hsa_circ_0092367	-6.33682	chr15:25325262-25326442	+	1,180	1,180	SNORD116-14	(39)
Zhang *et al*, 2013	hsa_circ_0092360	-6.34906	chr17:27047048-27047688	+	640	640	RPL23A	(39)

circRNA, circular RNA; FC, fold change.

Table II. Top five GO terms and KEGG pathways enriched in the circRNA-miRNA-upregulated mRNA network.

Term/pathway	ID	Description	Gene ratio	P-value	Count
BP	GO:0009812	Flavonoid metabolic process	5/81	5.62×10^{-9}	5
BP	GO:0052695	Cellular glucuronidation	5/81	1.15×10^{-8}	5
BP	GO:0006063	Uronic acid metabolic process	5/81	4.80×10^{-8}	5
BP	GO:0019585	Glucuronate metabolic process	5/81	4.80×10^{-8}	5
BP	GO:0009410	Response to xenobiotic stimulus	7/81	1.20×10^{-6}	7
MF	GO:0015020	Glucuronosyltransferase activity	5/78	4.05×10^{-7}	5
MF	GO:0046982	Protein heterodimerization activity	10/78	7.20×10^{-5}	10
MF	GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	9/78	9.24×10^{-5}	9
MF	GO:0008194	UDP-glycosyltransferase activity	5/78	0.000492	5
MF	GO:0005172	Vascular endothelial growth factor receptor binding	2/78	0.001068	2
KEGG	hsa00053	Ascorbate and aldarate metabolism	5/42	3.24×10^{-7}	5
KEGG	hsa00040	Pentose and glucuronate interconversions	5/42	1.09×10^{-6}	5
KEGG	hsa00860	Porphyrin and chlorophyll metabolism	5/42	3.21×10^{-6}	5
KEGG	hsa00140	Steroid hormone biosynthesis	5/42	1.91×10^{-5}	5
KEGG	hsa00830	Retinol metabolism	5/42	3.29×10^{-5}	5

BP, biological process; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function.

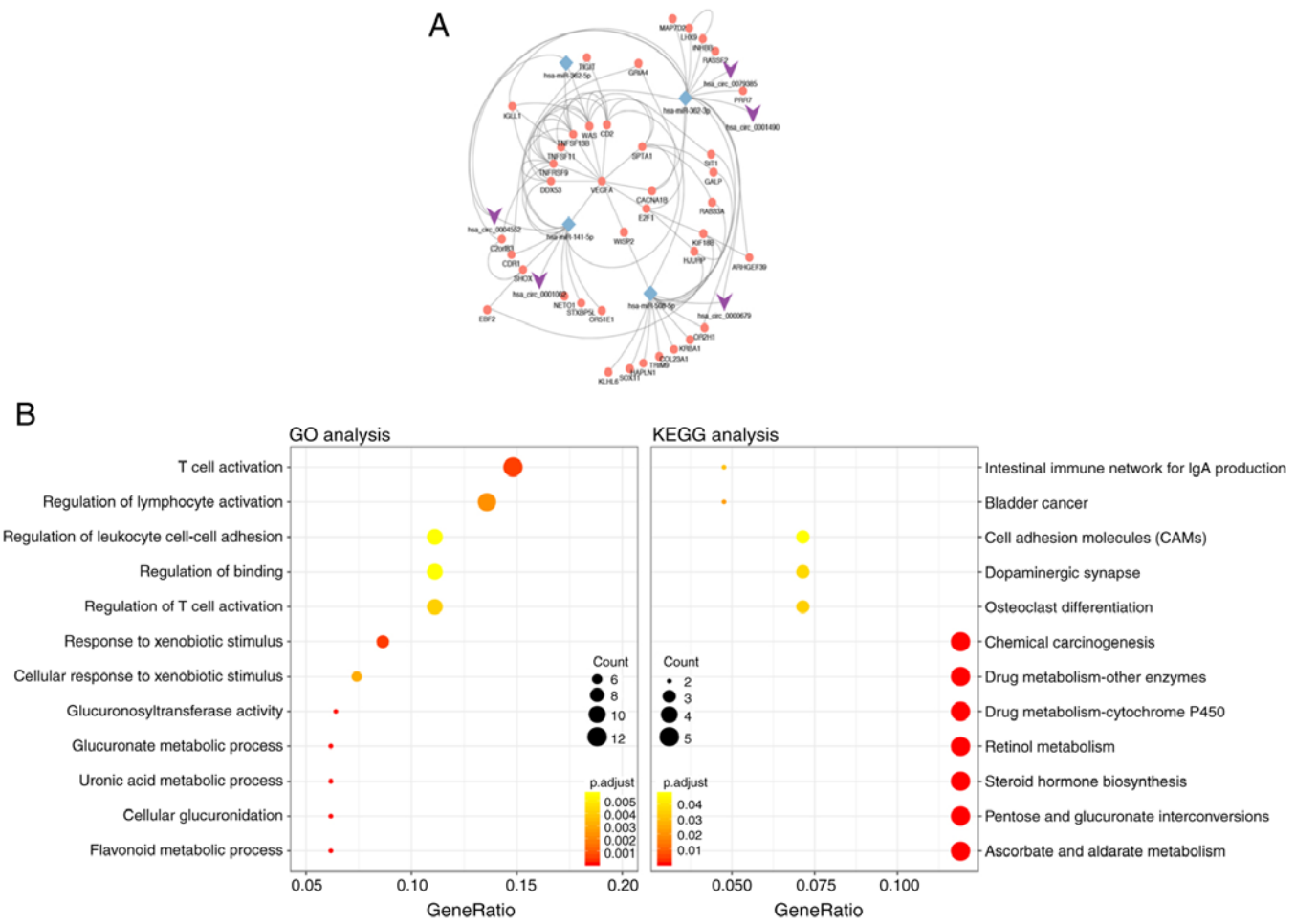

Figure 2. Enrichment analysis of the circRNA-miRNA-up-regulated mRNA network. (A) Regulatory network of hub circRNAs. Arrowheads represent circRNAs, diamonds represent miRNAs and circles represent genes. (B) Dotplot of GO and KEGG enrichment analyses. circRNA, circular RNA; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNA, microRNA.

Table III. Top five GO terms and KEGG pathways enriched in the circRNA-miRNA-downregulated mRNA network.

Term/pathway	ID	Description	Gene ratio	P-value	Count
BP	GO:0072006	Nephron development	14/230	4.20×10^{-9}	14
BP	GO:0001822	Kidney development	18/230	1.50×10^{-8}	18
BP	GO:0072001	Renal system development	18/230	3.72×10^{-8}	18
BP	GO:0072073	Kidney epithelium development	13/230	4.06×10^{-8}	13
BP	GO:0072009	Nephron epithelium development	11/230	2.11×10^{-7}	11
CC	GO:0043025	Neuronal cell body	20/240	1.12×10^{-6}	20
CC	GO:0044297	Cell body	20/240	8.82×10^{-6}	20
CC	GO:0044420	Extracellular matrix component	9/240	2.67×10^{-5}	9
CC	GO:0005604	Basement membrane	8/240	2.80×10^{-5}	8
CC	GO:0098644	Complex of collagen trimers	4/240	0.000113	4
MF	GO:0034483	Heparan sulfate sulfotransferase activity	4/228	3.54×10^{-5}	4
KEGG	hsa00534	Glycosaminoglycan biosynthesis-heparan sulfate/heparin	4/113	0.000418	4
KEGG	hsa04514	Cell adhesion molecules (CAMs)	8/113	0.001462	8
KEGG	hsa04974	Protein digestion and absorption	6/113	0.00233	6
KEGG	hsa04512	ECM-receptor interaction	5/113	0.007853	5
KEGG	hsa00590	Arachidonic acid metabolism	4/113	0.014923	4

BP, biological process; CC, cellular component; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function.

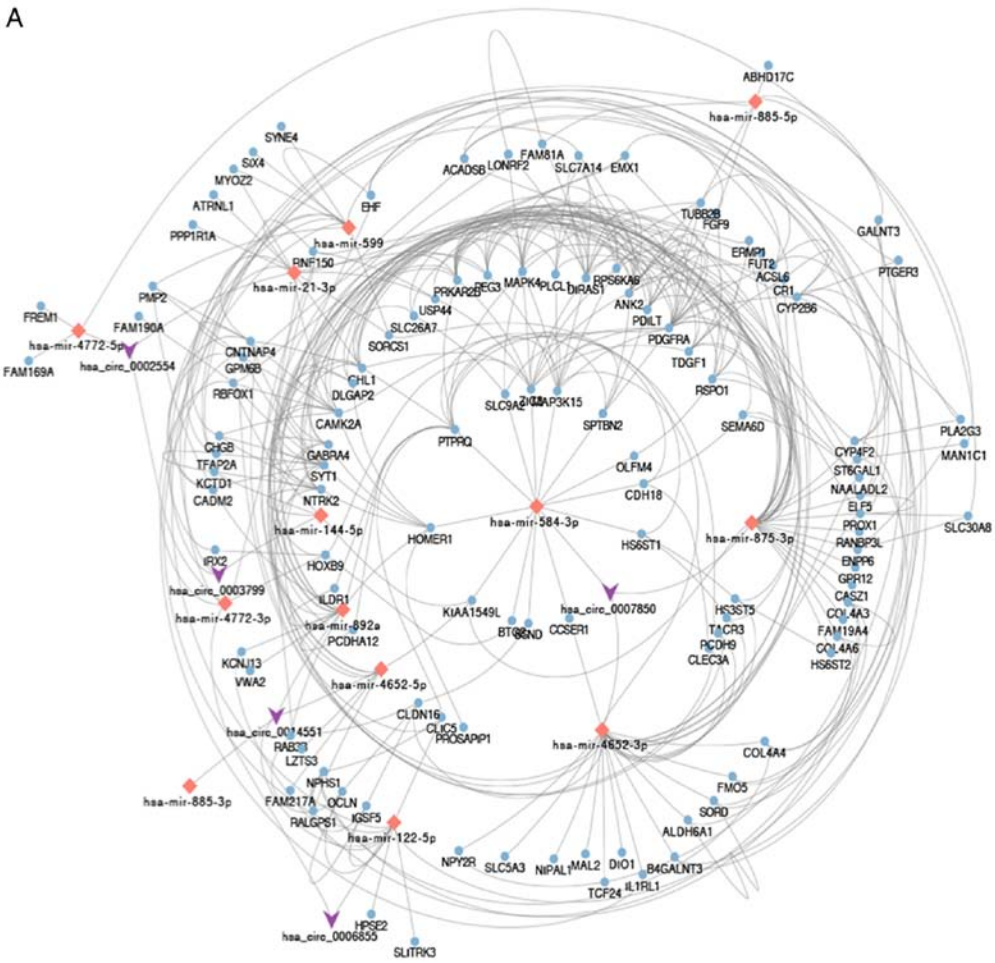

Figure 3. Enrichment analysis of the circRNA-miRNA-downregulated mRNA network. (A) Regulatory network of hub circRNAs. Arrowheads represent circRNAs, diamonds represent miRNAs and circles represent genes.

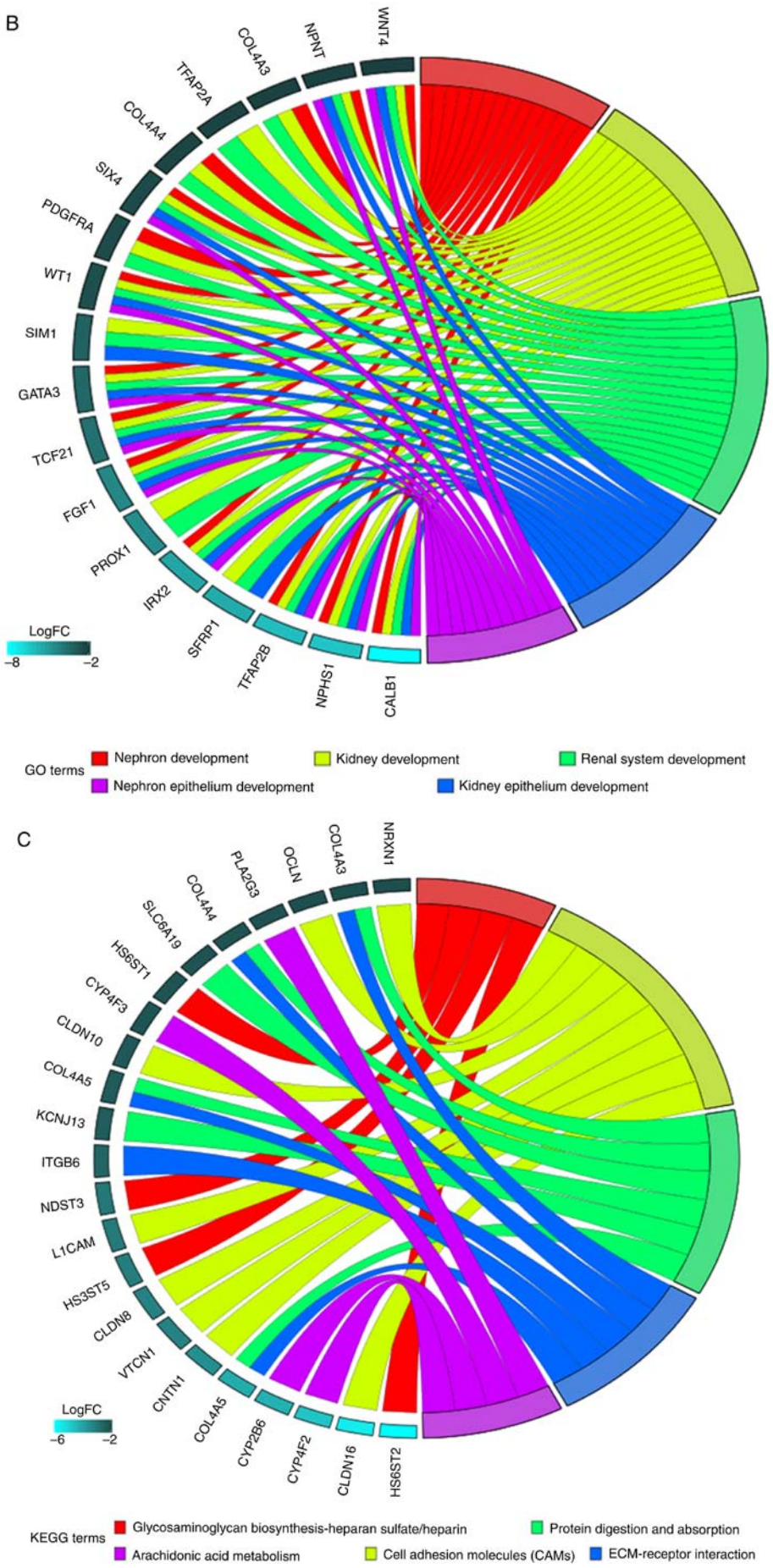

Figure 3. Continued. (B) Top 5 GO terms and (C) top 5 KEGG pathways, as determined by enrichment analysis. circRNA, circular RNA; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNA, microRNA.

Enrichment analysis was performed on the 244 downregulated genes. As presented in Fig. 3B and Table III, DEGs were enriched in BP, including 'nephron development', 'kidney development' and 'renal system development'. CC analysis suggested that DEGs were associated with 'neuronal cell body', 'cell body' and 'extracellular matrix component'. In addition, these DEGs were significantly enriched in MF, including 'heparan sulfate sulfotransferase activity'. KEGG enrichment pathway analysis revealed DEGs to be involved in 'ECM-receptor interaction', 'glycosaminoglycan biosynthesis-heparan sulfate/heparin' and 'cell adhesion molecules (CAMs)' (Fig. 3C and Table III).

Prognostic value of miRNAs and mRNAs regulated by circRNAs. To identify potential prognostic indicators of CCRCC, the miRNAs and mRNAs regulated by circRNAs were analyzed. Following prognostic analysis, 125 genes and 10 miRNAs were associated with the prognosis of CCRCC. Subsequently, as shown in Fig. 4 and Table IV, the genes and miRNAs were sorted based on P-value, after which the top 10 genes and miRNAs were selected as hub genes [membrane palmitoylated protein 7 (MPP7), aldehyde dehydrogenase 6 family member A1 (ALDH6A1), transcription factor AP-2α (TFAP2A), collagen type IV α 4 chain (COL4A4), nuclear receptor subfamily 3 group C member 2 (NR3C2), plasminogen (PLG), Holliday junction recognition protein (HJURP), claudin 10 (CLDN10), kinesin family member 18B (KIF18B) and thyroid hormone receptor β (THRB)] and hub miRNAs (hsa-miR-21-3p, hsa-miR-155-3p, hsa-miR-144-3p, hsa-miR-142-5p, hsa-miR-875-3p, hsa-miR-885-3p, hsa-miR-3941, hsa-miR-224-3p, hsa-miR-584-3p and hsa-miR-138-1-3p).

Table IV. Prognostic analysis of hub genes and miRNAs.

Gene	HR	95% CI	P-value
MPP7	0.397	0.293-0.538	1.42×10^{-9}
ALDH6A1	0.388	0.287-0.525	1.70×10^{-9}
TFAP2A	2.649	1.961-3.578	3.63×10^{-9}
COL4A4	0.398	0.295-0.538	7.88×10^{-9}
NR3C2	0.396	0.293-0.536	1.79×10^{-8}
PLG	0.411	0.304-0.556	3.15×10^{-8}
HJURP	2.455	1.817-3.319	5.84×10^{-8}
CLDN10	0.432	0.319-0.584	8.22×10^{-8}
KIF18B	2.415	1.787-3.265	9.02×10^{-8}
THRB	0.441	0.326-0.596	9.42×10^{-8}
hsa-mir-21-3p	2.745	2.029-3.712	7.12×10^{-11}
hsa-mir-155-3p	1.702	1.262-2.295	5.72×10^{-4}
hsa-mir-144-3p	0.625	0.463-0.843	0.002
hsa-mir-142-5p	1.587	1.176-2.14	0.003
hsa-mir-875-3p	0.626	0.462-0.849	0.004
hsa-mir-885-3p	0.649	0.481-0.876	0.005
hsa-mir-3941	0.71	0.526-0.958	0.025
hsa-mir-224-3p	1.371	1.017-1.849	0.039
hsa-mir-584-3p	0.734	0.544-0.989	0.045
hsa-mir-138-1-3p	1.35	1.001-1.822	0.048

miR/miRNA, microRNA.

Discussion

ceRNAs are involved in a complex regulatory network associated with the transcriptome; the present findings may improve understanding of the regulatory mechanism underlying gene expression. The present findings, along with other studies (9,11,18), suggest the importance of ceRNAs in the carcinogenesis of various types of cancer. The present study analyzed GEO and TCGA datasets to identify DECs, DEMs and DEGs in CCRCC. In addition, ceRNA regulatory networks in CCRCC were constructed using these circRNAs, miRNAs and genes. Additionally, the prognostic value of miRNAs and genes regulated by circRNAs was determined to identify indicators that may predict the prognosis of CCRCC.

By analyzing the data from a circRNA expression microarray of CCRCC (GSE100186), which contained four CCRCC samples and four normal samples, 324 downregulated and 218 upregulated circRNAs were reported in the cancer group. The mRNA and miRNA expression profiles of TCGA CCRCC dataset were used to identify DEGs and DEMs between cancer and normal samples. Using the CSCD, TargetScan and miRDB, a circRNA-miRNA-mRNA regulatory network was constructed in CCRCC based on the ceRNA theory.

The results of enrichment analysis of the 85 upregulated genes in the circRNA-miRNA-upregulated mRNA network suggested associated BP terms, including 'flavonoid metabolic process', 'cellular glucuronidation', 'uronic acid metabolic process', 'glucuronate metabolic process' and 'response to xenobiotic stimulus'. According to the results, CCRCC was associated with various metabolic processes, including flavonoid, uronic acid and glucuronate metabolism. The exact role of the various metabolic pathways in the initiation, progression and treatment of CCRCC requires further investigation. KEGG enrichment analysis indicated the importance of 'ascorbate and aldarate metabolism', 'pentose and glucuronate interconversions', 'steroid hormone biosynthesis' and 'retinol metabolism'. Steroid hormones serve a critical role in the regulation of metabolism, inflammation, immune functions, salt and water balance, the development of sexual characteristics, and the ability to withstand illness and injury (19). It has previously been reported that glucocorticoids inhibit the development of renal cancer by increasing the levels of Na and the expression of K-ATPase β-1 subunit, which suggests the possible benefits of glucocorticoids as a supplementary treatment in RCC management (20). In addition, aldosterone mediates the metastatic spread of renal cancer via its G protein-coupled estrogen receptor (GPER); therefore, GPER inhibitors may be considered promising therapeutic agents for inhibiting metastatic spread (21). Further study into the specific molecular mechanisms underlying the effects of steroid hormones on CCRCC development is required.

In the present study, enrichment analysis was performed on the 244 downregulated genes associated with the circRNA-miRNA-downregulated mRNA network. The results indicated critical BP terms, including 'nephron development', 'kidney development' and 'renal system development'. In addition, MF analysis revealed 'heparan sulfate sulfotransferase activity' was enriched in this network, whereas KEGG

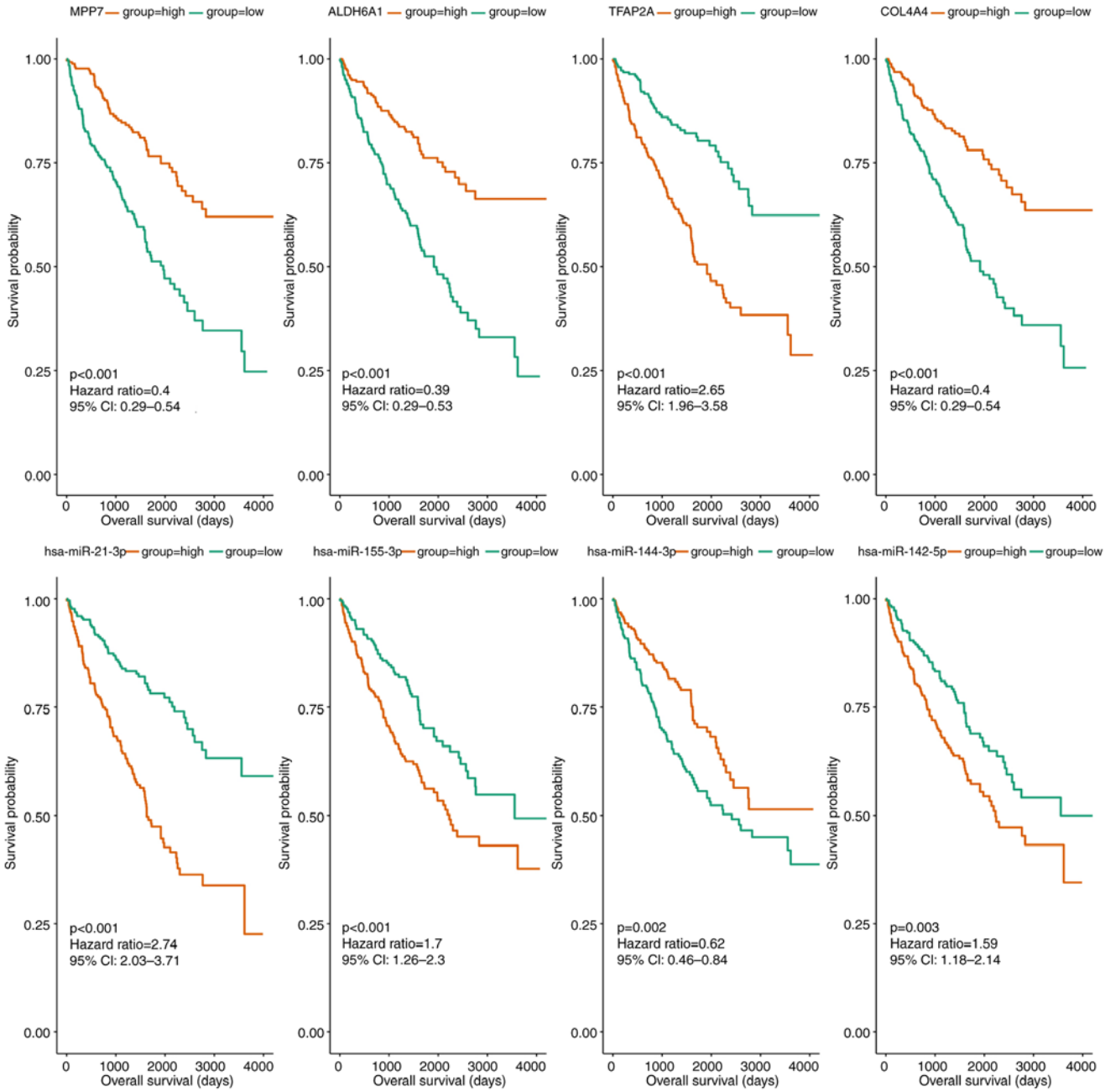

Figure 4. Prognostic analysis of the top four genes and miRNAs in relation to clear cell renal cell carcinoma survival. ALDH6A1, aldehyde dehydrogenase 6 family member A1; COL4A4, collagen type IV α 4 chain; miR/miRNA, microRNA; MPP7, membrane palmitoylated protein 7; TFAP2A, transcription factor AP-2 α.

pathway enrichment analysis suggested the importance of 'ECM-receptor interaction', 'glycosaminoglycan biosynthesis-heparan sulfate/heparin' and 'cell adhesion molecules (CAMs)', 'ECM-receptor interaction' and 'arachidonic acid metabolism'. CAMs are a subset of proteins that maintain cellular polarity and inhibit tumor growth (22). Cell adhesion molecule 4, which is one of the immunoglobulin-superfamily CAM proteins, has been proposed to be involved in suppressing tumor invasion and formation in CCRCC and nude mice (23). In addition, dysregulated methylation and suppression of the tumor inhibitor cell adhesion molecule 2 (CADM2) have been linked to human renal cell carcinogenesis; therefore, CADM2 could be a possible therapeutic target (24). These findings suggested that analysis of biological terms may provide novel insight into the complex mechanisms underlying the development and progression of CCRCC.

In order to identify prognostic indicators of CCRCC, the miRNAs and genes regulated by circRNAs were investigated. According to the analysis, the top 10 genes and miRNAs were selected as hub genes (MPP7, ALDH6A1, TFAP2A, COL4A4, NR3C2, PLG, HJURP, CLDN10, KIF18B and THRB) and hub miRNAs (miR-21-3p, miR-155-3p, miR-144-3p, miR-142-5p, miR-875-3p, miR-885-3p, miR-3941, miR-224-3p, miR-584-3p and miR-138-1-3p). The tumor suppressor gene TFAP2A has been reported to be hypermethylated and markedly downregulated in RCC. Therefore, analysis of TFAP2A methylation in cells obtained from urine or blood samples may be valuable in early diagnosis (25). The suppressive role of NR3C2 has

been reported in various types of cancer; low NR3C2 expression levels are correlated with aggressive characteristics and poorer survival in non-distant metastatic CCRCC (26). In a study investigating the role of gene copy number variation in relation to the clinical parameters of metastatic CCRCC, the loss of PLG was associated with advanced tumor stage and Fuhrman grade (27). As for the hub miRNAs, miR-21 has been widely studied in renal cancer for its regulatory roles in cellular proliferation and metastasis (28-30). In addition, the hub miRNA, miR-155, was determined to regulate the growth and invasion of CCRCC cells by interacting with E2F transcription factor 2 (31). miR-155 has also been suggested to modulate the proliferation, invasion and apoptosis of renal carcinoma cells by altering the glycogen synthase kinase-3β/β-catenin pathway (32). Furthermore, miR-144-3p could promote cell proliferation and migration in CCRCC by downregulating AT-rich interactive domain-containing protein 1A (33), which was also regarded as a possible novel plasma biomarker for the diagnosis of CCRCC (34). This study identified numerous ceRNAs that may serve a critical role in the development of CCRCC; however, the specific mechanism as to how these ceRNAs function requires further investigation.

In the present study, a series of circRNAs, miRNAs and genes, which may be implicated in CCRCC, were identified. In addition, the ceRNA regulatory network of circRNAs-miRNAs-genes was constructed, and could serve as a wide-scale profile of the complex regulation underlying the development of CCRCC. These findings may not only provide insight into the etiology of CCRCC, but could aid developments into the treatment of this disease.

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Availability of data and materials

The dataset generated and/or analyzed during the current study are available in TCGA (https://cancergenome.nih.gov/) and GEO (https://www.ncbi.nlm.nih.gov/gds/).

Authors' contributions

CM, JQ and XLW analysed the data, CM, JPZ and DJW wrote the manuscript, and XNL designed the study and wrote and revised the article manuscript. JPZ and DJW identified the databases and reviewed the data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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