Competing endogenous RNA analysis identified lncRNA DSCR9 as a novel prognostic biomarker associated with metastasis and tumor microenvironment in renal cell carcinoma

YING LIU[1*], JIAN LUO[2*], JING ZENG[3], WEIPENG LIU[3], BIN FU[3] and JING XIONG[3]

[1]Department of Preventive Medicine, School of Public Health, Hubei University of Medicine, Shiyan, Hubei 442000; [2]Department of Laboratory Medicine, The First People's Hospital of Yichang, The People's Hospital of China Three Gorges University, Yichang, Hubei 443000; [3]Department of Urology, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi 330000, P.R. China

Received November 4, 2022; Accepted March 23, 2023

DOI: 10.3892/ol.2023.13876

Abstract. Distant metastasis is the main cause of death in patients with clear cell renal carcinoma (ccRCC). The dysregulation of the tumor microenvironment is responsible for tumorigenesis and metastasis in ccRCC. The role of long non-coding RNAs in the tumor immune of ccRCC remains unclear. The present study screened differentially expressed protein-coding genes and non-coding genes between ccRCC and normal tissues based on three datasets. The commonly deregulated genes were used to identify distant metastasis-related long non-coding RNAs (lncRNAs) and prognostic lncRNAs. Pearson correlation analysis was used to identify immune-related lncRNAs. A competing endogenous RNA network was constructed and hub lncRNAs were identified. A total of 1650 coding genes, 821 lncRNAs and 62 miRNAs were commonly deregulated in the three datasets. A total of 408 lncRNAs associated with the overall survival of patients with ccRCC were identified. Among them, 82 lncRNAs were distant metastasis-related. Further analysis identified 52 lncRNAs associated with the immune pathway. Functional analyses concordantly demonstrated the role of the 52 lncRNAs in metastasis and tumor immunology. The ceRNA network analysis indicated lncRNA DSCR9 as the key lncRNA regulator. Univariate and multivariate analysis in two independent cohorts validated that DSCR9 could be an independent risk factor for the progression-free survival of patients with ccRCC. Further analyses indicated that DSCR9 might be associated with the immunotherapeutic response. reverse transcription-quantitative PCR demonstrated that the RNA expression level of DSCR9 was upregulated in ccRCC compared with normal kidney samples. The present study demonstrated the potential of LncRNA DSCR9 in assessing the prognosis and developing future immunotherapy for patients with metastatic ccRCC.

Correspondence to: Dr Jing Xiong, Department of Urology, The First Affiliated Hospital of Nanchang University, 17 Yongwai Street, Nanchang, Jiangxi 330000, P.R. China
E-mail: alex7023@126.com

*Contributed equally

Key words: competing endogenous RNA, long non-coding RNA, immune, metastasis, renal cell carcinoma, prognosis

Introduction

In 2020, ~73,750 new cases of kidney cancer were diagnosed in the United States, with >20% of patients succumbing to the disease (1). Patients with distant metastasis demonstrated a 5-year survival of ~10% (1). Renal cell carcinoma (RCC) is the most common type of kidney cancer, and the most common histologic subtype of RCC is clear cell RCC (ccRCC) (2). The prognosis and treatment of metastatic ccRCC is a current problem (2). Existing knowledge of the mechanisms of metastatic ccRCC has indicated the critical role of the immune-related pathways (3). Immunotherapy a promising treatment strategy alternative to targeted therapy for metastatic renal cancer (3).

Long non-coding (lnc)RNA is involved in the regulation of gene expression at epigenetic, transcriptional and post-transcriptional levels at all stages of tumorigenesis and cancer progression. lncRNAs serve a key role in a variety of cellular processes and molecular signaling pathways. For example, the activation of the lncRNA gene EPIC1 enhances tumorigenesis by promoting the binding of MYC to its target genes (e.g., CDKN1A), which indicates that it has a carcinogenic effect (4), while the inactivation of the lncRNA gene growth arrest specific 5 promotes cell proliferation and tumor formation in cancer, which indicates its tumor inhibitory effect (5). Moreover, lncRNA also participates in tumor progression and metastasis, which is closely related to prognosis (6). lncMX1-215 is upregulated by IFNα and inhibits the proliferation and metastasis of head and neck squamous carcinoma cells (7). lncMX1-215 has been reported to negatively regulate PD-L1 expression to inhibit immune escape by the suppression of H3K27 acetylation via binding to H3K27 acetylase GCN5 (also known as

KAT2A, lysine acetyltransferase 2A) (7). Moreover, lncRNA HOXA-AS2 promoted proliferation, invasion and migration of nasopharyngeal carcinoma by promoting hypoxia inducible factor-1α and PD-L1 expression via the direct targeting of miR-519 (8). However, the role of long non-coding RNAs (lncRNA) in metastatic ccRCC remains unclear.

Competing endogenous RNA (ceRNA) is a type of regulatory network. It is known that microRNAs (miRNAs) can cause gene silencing by binding to mRNA, while lncRNAs can regulate gene expression by competitively binding miRNA (9). Previous studies have reported that the ceRNA mechanism exists in a wide variety of signaling pathways and serves an important role in metastatic ccRCC (10). However, the immune-related ceRNA regulatory networks are poorly understood. The lncRNAs could be biomarkers for the prognosis of ccRCC and could pave the way for further investigation of the immune-related mechanisms and therapeutic potentials of these lncRNAs in ccRCC.

Materials and methods

Transcriptome data. The transcriptome data were downloaded from The Cancer Genome Atlas project (https://portal.gdc.cancer.gov/) and Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) databases. The raw (.CEL) files of microarray (Affymetrix Human Genome U133 Plus 2.0 Array platform) datasets GSE53757 (11) and GSE66270 (12) were downloaded. A total of 72 tumor samples and 72 normal samples were downloaded in the GSE53757 dataset, and 14 tumor samples and 14 normal samples were downloaded in the GSE66270 dataset. Moreover, the read count expression data and the corresponding clinical data of the TCGA-KIRC (n=603) dataset were downloaded from the Genomic Data Commons data portal (https://portal.gdc.cancer.gov/repository). The RNA-Seq data (read count) and the corresponding clinical information for ccRCC (557 donors, accession number KIRC-US) from the International Cancer Genome Consortium (ICGC) project were downloaded from the Xena data hub (https://xena.ucsc.edu/public). The annotation file (.gtf) from the GENCODE (v23) database was used for the gene annotation for TCGA-KIRC dataset and ICGC KIRC-US dataset.

Differential expression analysis. The edgeR package (13) and limma package (14) in R software (version 3.6.1; R core team) were used to perform differential expression (DE) analysis for the read count data and microarray expression data, respectively. The genes and probes with Benjamini-Hochberg (BH) adjusted P<0.05 were considered as statistically significant. The DE lncRNAs, DE miRNAs and DE coding genes commonly screened from all three datasets were identified as high-confidence DE genes.

ceRNA network analysis. The DE lncRNAs, DE miRNAs and DE coding genes were used to construct the ceRNA network based on the strategy previously reported by Zhang *et al* (15). Based on the ceRNA theory, lncRNA may interact with miRNA to prevent the interaction of miRNA with its targets. This strategy identified the lncRNAs and miRNAs with interactions. lncRNA-miRNA regulation networks were predicted using the miRcode database (16). Similarly, miRNA-mRNA regulation networks were predicted using the miRDB database (17). The predicted networks based on miRcode and miRDB databases were used to construct the ceRNA networks. The ceRNA network was constructed using Cytoscape (version 3.7.4; https://cytoscape.org/).

Identification of prognostic, metastasis-related and immune-related lncRNAs. We identified metastasis-related lncRNAs based on a previously reported algorithm (18), which leveraged the change in gene differential expression status, in different stages of the disease of interest, to identify the significant dynamic expression changes of genes. Specifically, the delta value of each lncRNA was calculated. The lncRNAs with the absolute value of delta >0 were determined as metastasis-related. To identify immune-related lncRNAs, the gene sets of immune pathways (https://www.kegg.jp/kegg/pathway.html; pathways of '5.1 immune system') from the Kyoto Encyclopedia of Genes and Genomes (KEGG) (19) were downloaded. The prognostic lncRNAs were identified using univariate and multivariate Cox regression analysis. To determine whether the lncRNA was an independent prognostic factor, multivariate analysis was performed using the clinical parameters as covariates. Both the overall survival (OS) and progression-free survival (PFS) were evaluated. The log-rank test was used to assess whether the lncRNA was prognosis-related.

To identify immune-related lncRNAs, for each lncRNA, Pearson correlation analysis was first performed to identify all associated protein-coding genes in the KEGG database. Then, pathway enrichment analysis was performed to determine whether these coding genes were overrepresented in the immune pathway. The Pearson correlation was performed between lncRNAs expression and the KEGG genes. Gene pairs with coefficient $|r|>0.4$ and BH-adjusted $P<0.05$ were considered significant.

Functional enrichment analysis. Function analysis of lncRNAs was performed based on the correlated protein-coding genes. The significant lncRNA-coding gene correlation was defined by the expression correlation (Pearson correlation $|r|\geq0.4$ with BH-adjusted $P<0.05$). KEGG and Gene Ontology (GO) terms were analyzed.

Reverse transcription-quantitative PCR (RT-qPCR). RT-qPCR was used to assess the relative expression of β-actin and DSCR9 on commercial cDNA chips to compare DSCR9 gene expression between cancer and adjacent samples. A total of 30 pairs of ccRCC samples were analyzed. Total RNA was extracted from tissues or cells using the Eastep® Super Total RNA Extraction Kit (Promega Corporation), and the reverse transcription was performed using the PrimeScript™ RT Master Mix (Takara Biotechnology Co., Ltd.) by Shanghai Outdo Biotech Co., Ltd., according to the manufacturer's instructions. The ccRCC samples were collected by Shanghai Outdo Biotech Co., Ltd. and the cDNA of ccRCC sample tissues generated by Shanghai Outdo Biotech Company was purchased and used in the present study. qPCR was performed using the SYBR Green PCR Kit (Bio-Rad Laboratories, Inc.) in triplicate in three independent experiments using the $2^{-\Delta\Delta Cq}$ method (20). The qPCR conditions were as follows: 95˚C for

Table I. Univariate and multivariate Cox regression model.

A, TCGA_KIRC n=434

	Univariate regression		Multivariate regression	
Variable	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)
DSCR9 (high vs. low)	0.000049	2.11 (1.46-3.05)	0.000012	2.32 (1.57-3.36)
Age (ref, ≤59 years)				
>59	0.083	1.36 (0.96-1.94)	0.11	1.36 (0.93-1.99)
Gender (ref, female)				
Male	0.060	1.46 (0.98-2.17)	0.039	1.58 (1.02-2.45)
Grade (ref, G1)				
G2	0.66	1.57 (0.21-11.56)	0.79	0.76 (0.10-5.78)
G3	0.20	3.60 (0.49-26.03)	0.69	1.50 (0.19-11.33)
G4	0.0079	14.79 (2.02-107.97)	0.28	3.10 (0.39-24.17)
Unknown	0.79	1.44 (0.09-23.15)	0.79	1.46 (0.08-24.51)
Stage (ref=I)				
II	0.037	2.13 (1.04-4.36)	0.085	4.97 (0.80-30.84)
III	$1.39x10^{-8}$	4.38 (2.61-7.20)	0.037	5.69 (1.10-29.31)
IV	$1.39x10^{-30}$	18.17 (11.08-29.80)	$8.67x10^{-6}$	180.68 (18.29-1784.09)
Unknown	0.99	NA	0.99	NA
pM (ref, M0)				
M1	$1.72x10^{-29}$	8.66 (5.95-12.61)	0.040	0.15 (0.02-0.92)
Unknown	0.87	0.90 (0.28-2.88)	0.98	1.02 (0.26-3.97)
pN (ref, N0)				
N1	0.00001	4.90 (2.40-9.99)	0.0069	3.38 (1.39-8.22)
Unknown	0.15	0.76 (0.53-1.18)	0.14	0.74 (0.57-1.10)
pT (ref, T1)				
T2	0.00007	3.26 (1.82-5.84)	0.18	0.31 (0.05-1.64)
T3	$1.45x10^{-15}$	6.16 (3.96-9.64)	0.33	0.45 (0.09-2.18)

B, ICGC_KIRC n=384

	Univariate regression		Multivariate regression	
Variable	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)
DSCR9 (high vs. low)	0.003	1.72 (1.2-2.47)	0.0009	1.89 (1.29-2.76)
Age (ref, ≤59)				
>59	0.090	1.28 (0.91-1.99)	0.040	1.49 (1.01-2.18)
Gender (ref, female)				
Male	0.067	1.41 (0.99-2.04)	0.16	1.36 (0.88-2.10)
Grade (ref, G1)				
G2	0.68	1.55 (0.32-10.25)	0.91	0.89 (0.11-6.78)
G3	0.30	3.51 (0.47-21.25)	0.61	1.68 (0.22-12.77)
G4	0.0092	12.14 (2.32-71.96)	0.27	3.18 (0.40-24.81)
Unknown	0.80	1.41 (0.09-16.39)	0.83	1.36 (0.08-23.02)
Stage (ref, I)				
II	0.041	2.13 (1.08-4.36)	0.13	4.23 (0.65-27.19)
III	$1.10x10^{-5}$	4.33 (2.61-7.20)	0.035	5.98 (1.13-31.46)
IV	$1.22x10^{-25}$	18.17 (11.08-29.80)	0.00004	112.98 (11.81-1080.33)
Unknown	0.99	NA	0.99	NA

Table I. Continued.

B, ICGC_KIRC n=384

	Univariate regression		Multivariate regression	
Variable	P-value	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)
pM (ref, M0)				
M1	$1.72x10^{-25}$	8.10 (4.97-10.5)	0.13	0.25 (0.04-1.44)
Unknown	0.95	0.90 (0.36-2.87)	0.70	0.74 (0.16-3.37)
pN (ref, N0)				
N1	0.00005	4.20 (2.54-8.65)	0.014	3.15 (1.26-7.87)
Unknown	0.18	0.88 (0.61-1.14)	0.32	0.82 (0.56-1.21)
pT (ref, T1)				
T2	0.00009	3.01 (1.54-5.12)	0.36	0.44 (0.07-2.4)
T3	$1.36x10^{-12}$	5.69 (3.24-8.21)	0.32	0.44 (0.09-2.17)

The median expression of the gene was used as the cut-off to divide patients into the High or Low group. CI, confidence interval; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; pM, pathological M stage; pN, pathological N stage; pT, pathological T stage.

30 sec, followed by 39 cycles at 95˚C for 5 sec and 60˚C for 30 sec. The relative expression of DSCR9 was normalized to β-actin and assessed using a RT-PCR Quantitation Kit (cat. no. E21006; Shanghai GenePharma Co., Ltd.). Informed consent was obtained from all participating patients by Shanghai Outdo Biotech Co., Ltd. Ethical approval for the use of ccRCC samples was provided. The levels of DSCR9 were assessed by qPCR on the Step One Plus Real-Time PCR system, and β-actin was used as endogenous control. The primer sequences used for qPCR were as follows: DSCR9 forward (F), 5'-AGGAAGGAACTGAGAACACC-3' and reverse (R), 5'-CAGTCCATTTCTACCGTCAC-3'; and β-actin F, 5'-CCTTCCTGGGCATGGAGTC-3' and R, 5'-TGATCTTCATTGTGCTGGGTG-3'.

Statistical analysis. The BH adjustment was performed for multiple tests in differential expression analysis, enrichment analysis and expression correlation analysis. Cox regression analysis was performed to investigate the correlation between lncRNAs and patient survival. Log-rank test was used and hazard ratios were calculated using survival/R package (version 3.6.1; https://www.r-project.org/). The statistical test used for two-group comparison in differential expression of TCGA samples, using the edgeR package in R (version 3.6.1) was empirical Bayes quasi-likelihood F-tests. The statistical test for two-group comparison of the qPCR results was a two-tailed Student's t-test. P<0.05 was considered to indicate a statistically significant diffference.

Results

Differential expression analysis. Differential expression analysis was performed to screen ccRCC-related genes in three independent datasets (TCGA-KIRC, GSE53757 and GSE66270). The miRNAs, lncRNAs, and coding genes were annotated using the GENCODE database. Commonly deregulated genes with BH-adjusted P<0.01 were retained. A total of 1650 coding genes, 821 lncRNAs and 62 miRNAs were identified as differentially expression genes between normal and tumor samples (Fig. 1A). A volcano plot and heatmap of the deregulated genes in the TCGA dataset were generated (Fig. 1B and C).

Identification of prognosis-related and metastasis-related lncRNAs. To identify lncRNAs associated with patient survival, univariate and multivariate Cox regression analyses were performed based on the TCGA dataset. The candidate lncRNAs considered in this step were obtained from the aforementioned differential expression analysis. A total of 522 lncRNAs associated with OS or PFS were identified (Table SI). Among the identified lncRNAs, multivariate analysis based on the lncRNA expression and clinical parameters indicated that 408 lncRNAs were independent factors for the prognosis of ccRCC patients. Whether the prognosis-related lncRNAs were associated with distant metastasis of ccRCC was evaluated by calculating the delta values (|deltal>0), which indicated that 82 of the lncRNAs were distant metastasis-related (Fig. 2). The 82 lncRNAs were assessed using multivariate models of OS or PFS (Fig. 3A and B). The Kaplan Meier plots of the top 5 lncRNAs associated with OS (CAHM, RP11.93H24.3, RP11.383I23.2, RP11.670E13.6 and U47924; Fig. 3C) and the top 5 lncRNAs associated with PFS (DSCR9, AC067959.1, RP1.86C11.7, LINC00652 and RP11.670E13.6; Fig. 3D) were presented.

ceRNA networks based on immune-related lncRNAs. To evaluate the functions of the distant metastasis-related lncRNAs, Pearson correlation analysis was performed to screen the correlated protein-coding genes. Then, pathway enrichment analysis was performed for each lncRNA to identify the involved pathways. The results demonstrated that the lncRNAs

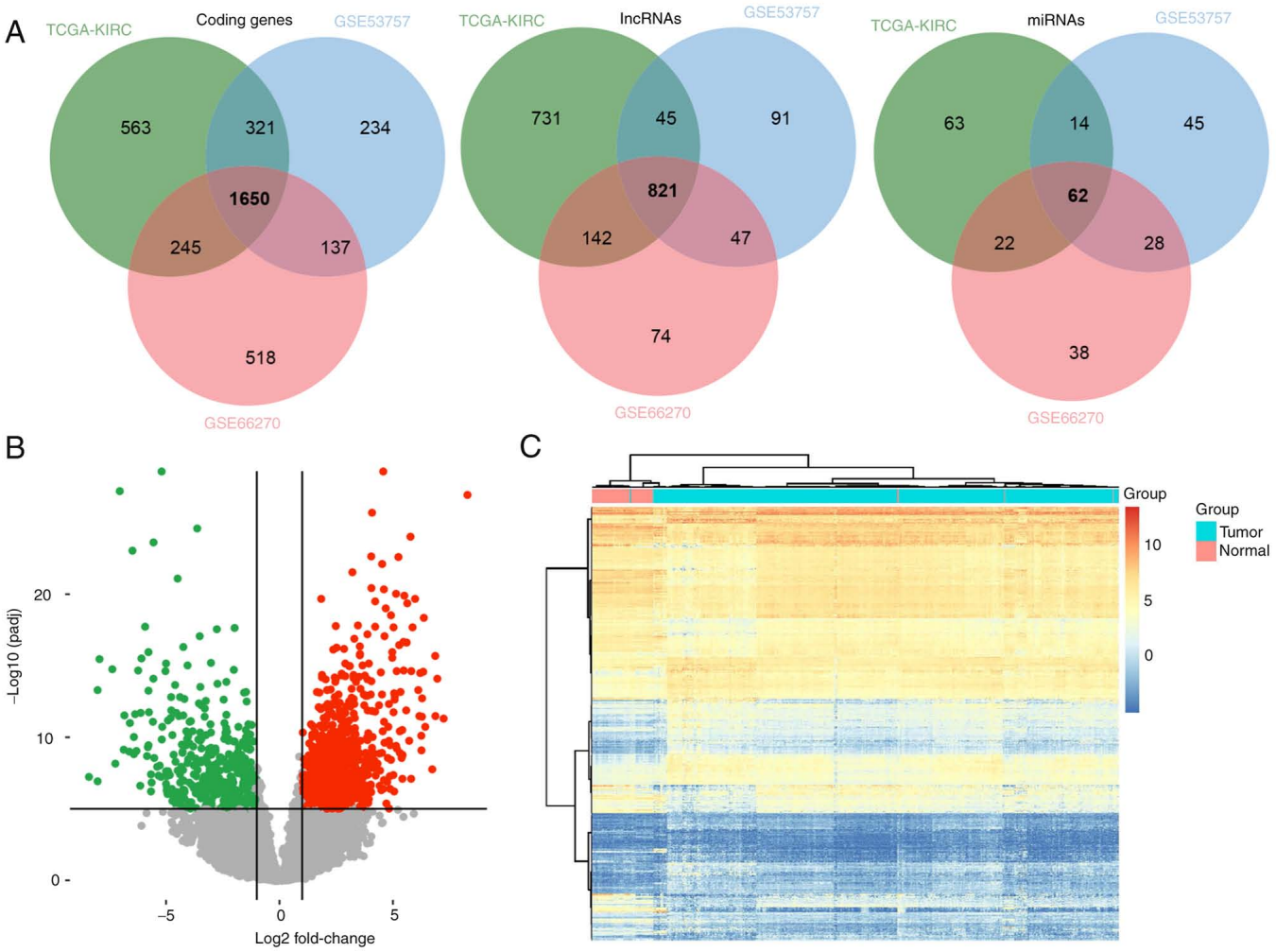

Figure 1. Differential expression analysis. (A) Differentially expressed protein-coding genes, lncRNAs and miRNAs in the three independent datasets. (B) Volcano plot and (C) heatmap of differentially expressed genes in The Cancer Genome Atlas. For the heatmap, the Euclidean distance metric with ward linkage was used. lncRNA, long non-coding RNA; miRNA, micro RNA; padj, P-values adjusted by Benjamini-Hochberg method.

were associated with cell adhesion and cytoskeleton-related pathways, such as tight junction, focal adhesion, regulation of actin cytoskeleton and cell adhesion molecule cams (Fig. 4), which supported the association of these lncRNAs and metastasis. Moreover, it was observed that most of the lncRNAs were involved in immune-related pathways, such as the T cell receptor signaling pathway, NK cell-mediated cytotoxicity and chemokine signaling pathway. Thus, a group of 52 lncRNAs were identified (bottom, 52 lncRNAs from right to left, Fig. 4). To evaluate the function of lncRNAs, GO enrichment analysis was performed for each lncRNA based on the lncRNA-correlated proteins. This demonstrated that DSCR9 was involved in T cell activation regulation (biological process), protein complexes (such as inflammasome complex and cell adhesion-related complex; cellular components) and ATPase activity (molecular function; Fig. 5A).

A ceRNA network was constructed based on the 52 lncRNAs, 62 DE miRNAs and 1,650 DE mRNAs. To improve the confidence of the regulatory network, only the lncRNA-miRNA pairs and the miRNA-mRNA pairs with opposite DE directions were retained. This resulted in ceRNA networks which included 14 lncRNAs, 13 miRNAs, and 107 mRNAs (Fig. 5).

DSCR9 could be an independent prognostic biomarker. In the ceRNA networks, lncRNA DSCR9 demonstrated a significant association with PFS of patients with ccRCC (Fig. 6A). Therefore, another independent cohort was used to validate the prognostic value of DSCR9 in ccRCC. The univariate and multivariate Cox analyses concordantly demonstrated that DSCR9 was an independent risk factor for PFS of ccRCC patients (Figs. 3B and 6B; Table I). Considering that the clinical stage was evaluated based on the TNM stage, we used the TNM stage instead of the clinical stage in the multivariate analysis based on the methods previously reported (21). The subnetwork of DSCR9 was extracted (Fig. 6C). DSCR9 was associated with the immune pathway, so the correlation between DSCR9 and immunotherapeutic markers was assessed. This demonstrated that DSCR9 was significantly associated with programmed cell death protein 1 (PDCD1, also known as PD-1) and correlated with CTLA4 (Fig. 6D), which implied that DSCR9 might be related to immunotherapeutic response. However, no significant association was demonstrated between DSCR9 and CD274 (P=0.45).

DSCR9 was upregulated in ccRCC tissues. lncRNA DSCR9 was further assessed to validate it's expression in tumor and

Figure 2. Identification of distant metastasis-related lncRNAs based on Delta values. lncRNAs with delta not equal to zero were identified as distant metastasis-related. The negative delta value indicated downregulation in metastatic ccRCC and the positive delta value indicated upregulation in metastatic ccRCC. lncRNA, long non-coding RNA.

normal tissues. This was because DSCR9 was identified in the top 5 lncRNAs associated with PFS and had the highest hazard ratio (HR) (HR=2.15). Moreover, further analysis of regulatory network showed that DSCR9 was the only one of the top 5 PFS-related lncRNAs to be included in the final network.

A total of 30 pairs of ccRCC tissues and normal kidney samples were used to evaluate the expression of DSCR9 in ccRCC. The results demonstrated that the RNA expression level of DSCR9 was significantly upregulated in ccRCC compared with normal kidney samples (Fig. 7).

Discussion

The present study identified the key immune-related lncRNAs in the process of the distant metastasis of ccRCC and constructed ceRNA networks based on the immune-related lncRNAs. First, differentially expressed lncRNAs, miRNAs and mRNAs between ccRCC and normal samples were screened using three independent datasets, which identified 408 prognostic lncRNAs and 82 distant metastasis-related lncRNAs. Pathway analysis demonstrated that the 82 lncRNAs were mainly involved in immune-related pathways. Based on

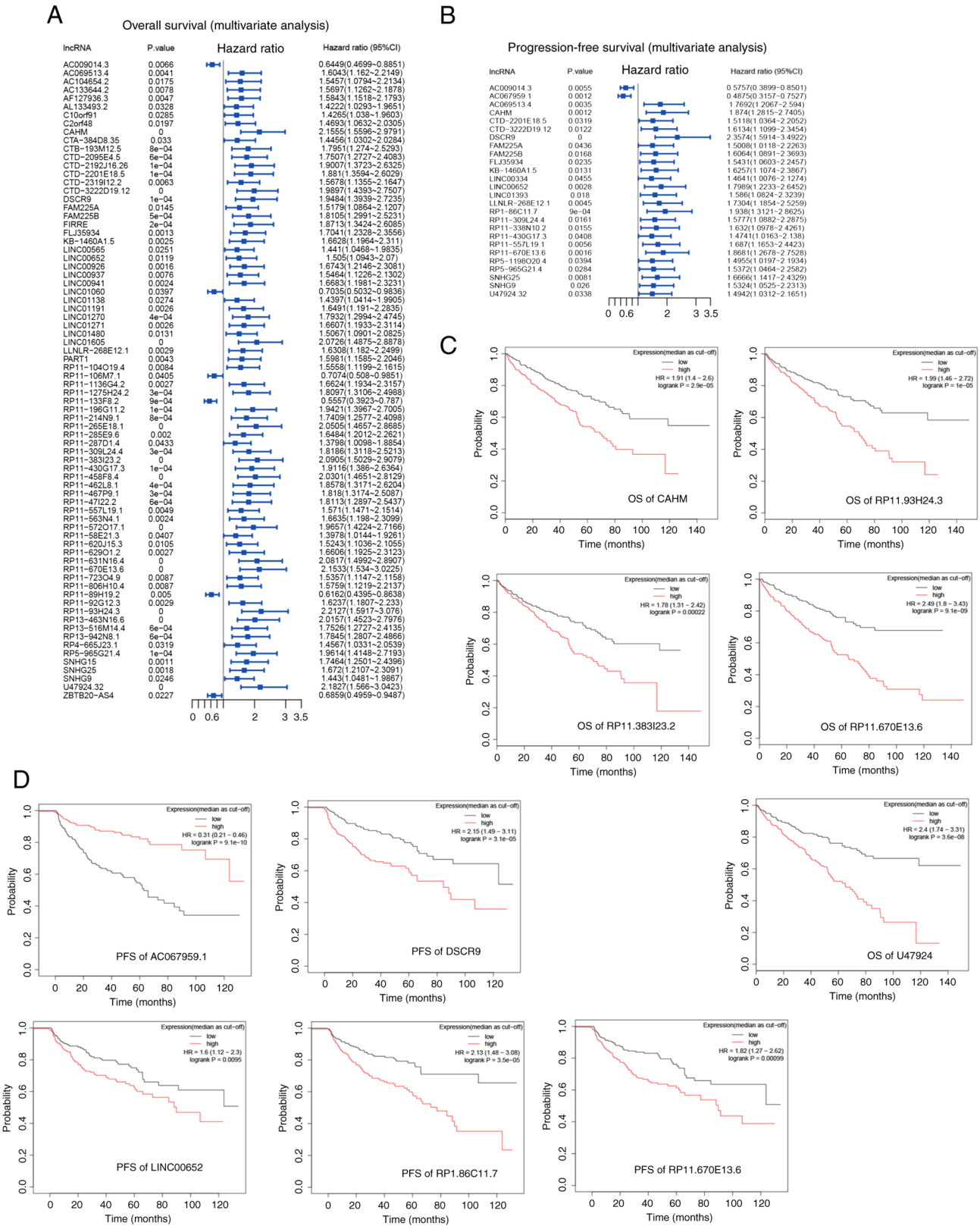

Figure 3. Identification of prognostic lncRNAs. (A) Model parameters of the multivariate analyses using patient clinical characteristics as covariates for OS. (B) Model parameters of the multivariate analyses using patient clinical characteristics as covariates for PFS. (C) Kaplan Meier curves of the top 5 lncRNAs associated with OS. (D) Kaplan Meier curves of the top 5 lncRNAs associated with PFS. lncRNA, long non-coding RNA; OS, overall survival; PFS, progression-free survival.

the clustering, 52 immune-related lncRNAs were identified. Finally, ceRNA networks including 14 lncRNAs, 13 DE miRNAs and 107 DE mRNAs were constructed. DSCR9 may serve an important role in the regulatory network. It was demonstrated that DSCR9 could be an independent risk factor for the PFS prognosis of patients with ccRCC. Further

Figure 4. Identification of immune-related long non-coding RNAs. The Benjamini-Hochberg adjusted P-values of enrichment analysis were presented as different colors in the heatmap. To aid visualization, the P-values were transformed to -10log$_{10}$ (P-value).

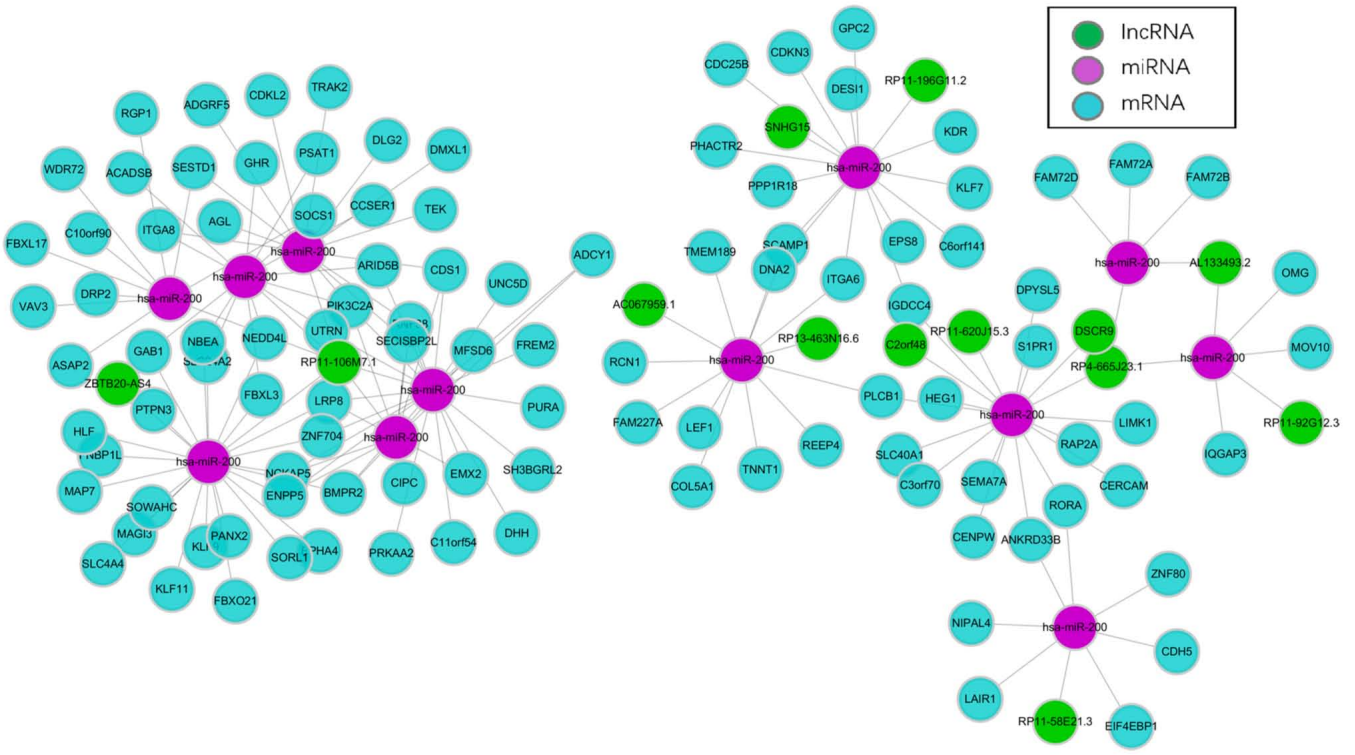

Figure 5. Functional enrichment and the ceRNA network of lncRNAs. (A) Results of Gene Ontology enrichment analysis for lncRNA DSCR9. (B) The ceRNA network was constructed based on 14 lncRNAs, 13 miRNAs and 107 mRNAs. All gene pairs in the network demonstrated opposite deregulation directions. lncRNA, long non-coding RNA; miRNA, micro RNA; ceRNA, competing endogenous RNA.

analyses indicated DSCR9 might be associated with immunotherapeutic response, and the association between DSCR9 and immunotherapy response could be an area for future study. The present study demonstrated a significant association between DSCR9 and ccRCC prognosis based on Cox regression analysis. Furthermore, it was demonstrated that DSCR9 was an unfavorable prognostic factor of PFS for ccRCC (log-rank $P<3.1x10^{-5}$; HR=2.15; 95% CI of HR, 1.49-3.11). The association between DSCR9 and immune-related pathways was demonstrated based on correlation analysis and pathway enrichment analysis. The expression of DSCR9 was positively associated with the expression of the immune checkpoint blockade therapy target PDCD1 and positively correlated with CTLA-4. These data implied that DSCR9 might play a role in immune response, which could be a future direction of study.

Gene expression in most cancers is a dynamic process involving all stages of tumor progression. Genes might have different expression statuses (upregulated or downregulated) in different stages of cancer progression. For example, loss of the H3K36me3 demethylase SETD2 has been reported in both primary ccRCC and metastases of ccRCC (22), while decreased methylation in regional H3K36me3 was only reported in lesions of distant metastases (23), to the best of our knowledge, which indicated that identification of dynamically expressed genes is crucial for understanding ccRCC metastasis. Thus, the present study applied a previously reported algorithm (18) to identify distant metastasis-related lncRNA events, including lncRNAs with reversed expression change and lncRNAs with consistent expression change. For example, LINC00652 (Delta=-3) demonstrated upregulation in non-distant metastatic ccRCC and downregulation in distant metastatic ccRCC. Further studies should be conducted to investigate the mechanism of LINC00652 in in non-distant metastatic ccRCC and distant metastatic ccRCC in the future. Another lncRNA, DSCR9 (Delta=1), demonstrated consistent upregulation in both non-metastatic and metastatic ccRCC. These examples indicated the dynamic behaviors of the prognosis-related modulators in ccRCC distant metastasis. The present study identified 82 distant metastasis-related lncRNAs, certain of which were also associated with patient OS and PFS in ccRCC. For example, lncRNAs FAM225A/B, SNHG9 and SNHG25 (24) were unfavorable prognostic factors, while lncRNAs AC067959.1, DSCR9 and AC009014.3 were favorable prognostic factors.

Enrichment analysis is usually used to evaluate the main functions and pathways of a set of non-coding genes. In the present study, this method was used to distinguish whether a single lncRNA was associated with immune-related signaling pathways. It was demonstrated that the 82 lncRNAs were mainly involved in immune-related pathways, such as T-cell receptor signaling pathway, NK cell-mediated cytotoxicity, cytokine-cytokine receptor interactions and chemokine signaling pathway. A recent study reported the role of lncRNA in T-cell and NK-cell immunology; the lncRNA HOTAIR facilitated the induction of IκBα phosphorylation by suppressing the expression of the NF-κB upstream protein UBXN1, which promoted NF-κB phosphorylation and nuclear translocation in gliomas. *In vivo*, HOTAIR reduction decreased PD-L1 protein expression, which indicated that cells may have been targeted by immune T cells (25). lncRNA NCAL1 enhanced the cytotoxicity of NK cells toward tumor cells through the GAB2-PI3K-AKT pathway (26). Moreover,

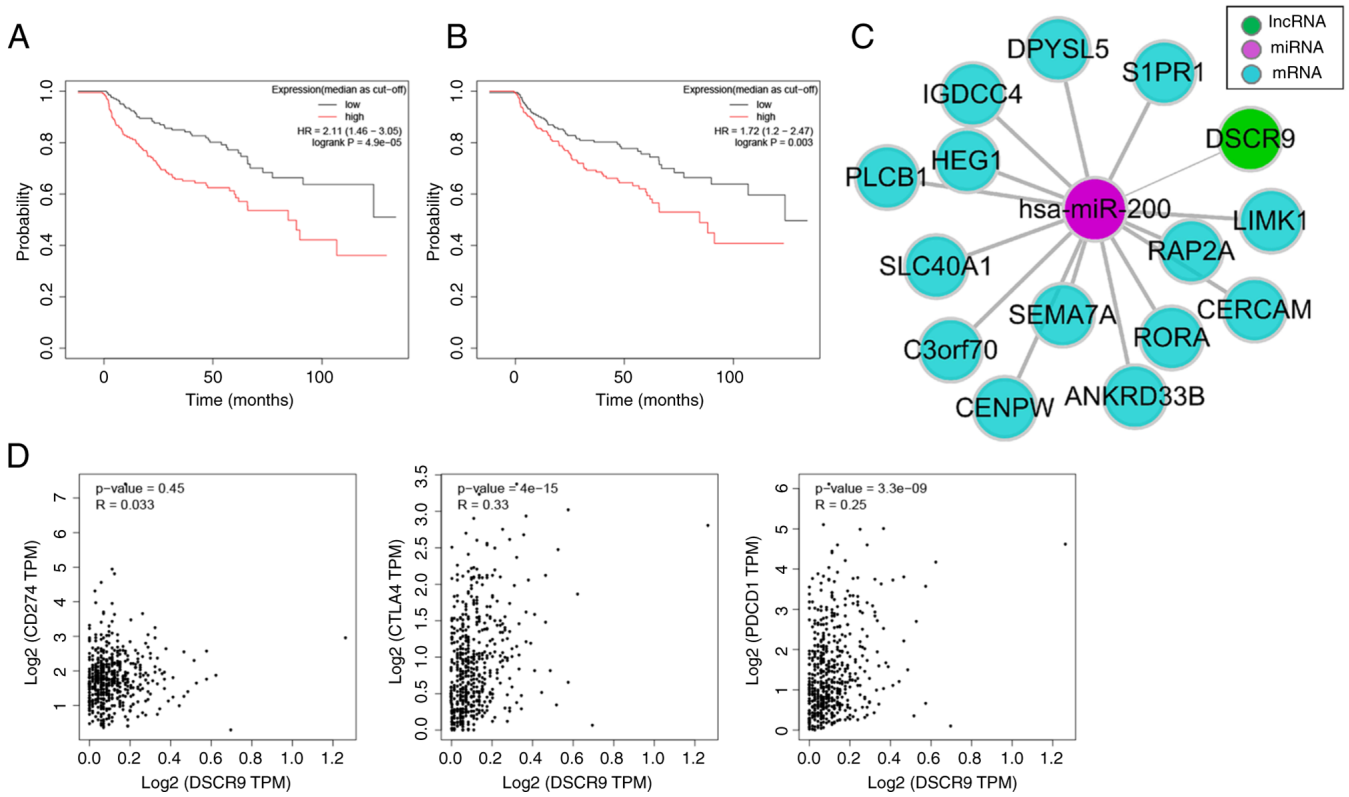

Figure 6. DSCR9 could be a novel prognostic marker. Kaplan Meier curves of PFS and DSCR9 in the (A) TCGA cohort and (B) ICGC cohort. (C) The sub-network of DSCR9 from the competing endogenous RNA network. (D) Pearson correlation of DSCR9 and three immunotherapeutic markers, CD274 (also known as PD-L1; P=0.45), CTLA4 ($P=4x10^{-15}$) and PDCD1 (also known as PD-1; $P=3.3x10^{-9}$), respectively from left to right. lncRNA, long non-coding RNA; miRNA, micro RNA; OS, overall survival; PFS, progression-free survival; TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; TPM, transcripts per million; PDCD1, programmed cell death protein 1.

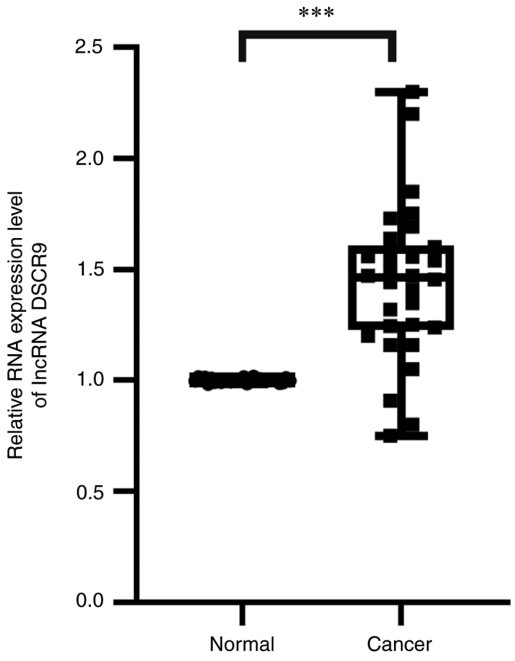

Figure 7. RNA expression levels of DSCR9 in ccRCC tumors and normal samples (n=60). Paired Student's t-test was used. ***P<0.001.

the results of the present study also demonstrated that the 82 lncRNAs were distant-metastasis-related because numerous metastasis-related functions and pathways, such as cell junction/adhesions and CAMs, were identified. Furthermore, ceRNA regulatory networks were inferred based on the immune-related lncRNAs using miRcode (16) and miRDB (17) databases. The miR-200-centered regulation network was identified. mir-200 is a critical miRNA in the progression of ccRCC (27). The specific mechanism of DSCR9 in regulating miR-200 in ccRCC needs to be elucidated in the future.

In the constructed ceRNA networks, two cohorts were used to validate that DSCR9 was an independent unfavorable factor for PFS in patients with ccRCC. DSCR9 was first reported to express preferentially in testis with unknown function in 2002 (28). Studies in subsequent years reported that it was expressed in numerous tissues, such as the kidney (29), prostate (30), testis (29) and breast (31). The genetic locus of DSCR9 (21q22.13) was linked to the human eye color phenotype (32). DSCR9 was also reported to be associated with prostate cancer (30), Down's syndrome (33) and patients with rheumatoid arthritis (34). Recent studies reported that DSCR9 was significantly associated with immune infiltration and survival, in conditions including pancreatic cancer (35) and triple-negative breast cancer (31). The present study demonstrated the DSCR9 was associated with CTLA4 and PDCD1, which indicated that it may be related to immunotherapeutic responses.

In the present study, correlation between lncRNA DSCR9 and tumor metastasis and immune-related pathways was demonstrated based on a series of data analyses. It is demonstrated that DSCR9 could be an independent risk factor for the

PFS prognosis of patients with ccRCC. Patients with a higher level of DSCR9 demonstrated worse PFS. It is essential to determine whether more lncRNAs could serve as biomarkers and therapeutic targets. Moreover, how DSCR9 regulates ccRCC metastasis, for example, by the T cell receptor signaling pathway and the specific molecular mechanism require further study and elucidation. Addressing these points is crucial for understanding the biological function of DSCR9 in ccRCC metastasis.

The limitations of the present study should be disclosed. First, although the PFS prognostic potential of DSCR9 in ccRCC was discovered, validation using further independent cohorts and prospective clinical trials are needed in the future. Second, the predicted regulation network implied a role for DSCR9 in ccRCC progression potentially by interacting with miRNAs. However, to validate the role of DSCR9, fluorescence *in situ* hybridization dual-luciferase reporter experiments should be performed in the future to explore the specific binding site of DSCR9 with miR-200.

Acknowledgements

Not applicable.

Funding

The study was supported by the Natural Science Foundation of Jiangxi (grant no. 20212BAB206038) and the Jiangxi Provincial Health Technology Project (grant no. 202210339).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available as follows: GSE53757 (https://www.ncbi.nlm.nih.gov/geo), GSE66270 (https://www.ncbi.nlm.nih.gov/geo), TCGA-KIRC (https://portal.gdc.cancer.gov/) and KIRC-US dataset of ICGC downloaded from UCSC Xena (https://xenabrowser.net/hub/).

Authors' contributions

JX and WL conceived and designed the study. BF, YL, JL, JZ and LY downloaded, processed, analyzed and interpreted the data. JX and YL confirm the authenticity of all the raw data. WL and LY provided constructive advice for conception and data analyses. JX wrote the manuscript. BF revised the manuscript critically for important intellectual content. JX supervised the study. All authors were responsible for reviewing the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Informed consent was obtained from all participating patients by Shanghai Outdo Biotech Co., Ltd. Ethical approval for the use of ccRCC samples was provided.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2020. CA Cancer J Clin 70: 7-30, 2020.
2. Jonasch E, Walker CL and Rathmell WK: Clear cell renal cell carcinoma ontogeny and mechanisms of lethality. Nat Rev Nephrol 17: 245-261, 2021.
3. Terry S, Dalban C, Rioux-Leclercq N, Adam J, Meylan M, Buart S, Bougoüin A, Lespagnol A, Dugay F, Moreno IC, *et al*: Association of AXL and PD-L1 expression with clinical outcomes in patients with advanced renal cell carcinoma treated with PD-1 blockade. Clin Cancer Res 27: 6749-6760, 2021.
4. Wang Z, Yang B, Zhang M, Guo W, Wu Z, Wang Y, Jia L, Li S; Cancer Genome Atlas Research Network; Xie W and Yang D: lncRNA epigenetic landscape analysis identifies EPIC1 as an oncogenic lncRNA that interacts with MYC and promotes cell-cycle progression in cancer. Cancer Cell 33: 706-720.e9, 2018.
5. Yang X, Xie Z, Lei X and Gan R: Long non-coding RNA GAS5 in human cancer. Oncol Lett 20: 2587-2594, 2020.
6. Yang Z, Li X, Yang Y, He Z, Qu X and Zhang Y: Long noncoding RNAs in the progression, metastasis, and prognosis of osteosarcoma. Cell Death Dis 7: e2389, 2016.
7. Ma H, Chang H, Yang W, Lu Y, Hu J and Jin S: A novel IFNα-induced long noncoding RNA negatively regulates immunosuppression by interrupting H3K27 acetylation in head and neck squamous cell carcinoma. Mol Cancer 19: 4, 2020.
8. Wang S, You H and Yu S: Long non-coding RNA HOXA-AS2 promotes the expression levels of hypoxia-inducible factor-1α and programmed death-ligand 1, and regulates nasopharyngeal carcinoma progression via miR-519. Oncol Lett 20: 245, 2020.
9. Braga EA, Fridman MV, Moscovtsev AA, Filippova EA, Dmitriev AA and Kushlinskii NE: LncRNAs in ovarian cancer progression, metastasis, and main pathways: ceRNA and alternative mechanisms. Int J Mol Sci 21: 8855, 2020.
10. Zhao K, Zhang Q, Wang Y, Zhang J, Cong R, Song N and Wang Z: The construction and analysis of competitive endogenous RNA (ceRNA) networks in metastatic renal cell carcinoma: A study based on the cancer genome atlas. Transl Androl Urol 9: 303-311, 2020.
11. von Roemeling CA, Radisky DC, Marlow LA, Cooper SJ, Grebe SK, Anastasiadis PZ, Tun HW and Copland JA: Neuronal pentraxin 2 supports clear cell renal cell carcinoma by activating the AMPA-selective glutamate receptor-4. Cancer Res 74: 4796-4810, 2014.
12. Wotschofsky Z, Gummlich L, Liep J, Stephan C, Kilic E, Jung K, Billaud JN and Meyer HA: Integrated microRNA and mRNA signature associated with the transition from the locally confined to the metastasized clear cell renal cell carcinoma exemplified by miR-146-5p. PLoS One 11: e0148746, 2016.
13. 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.
14. Wu Z, Irizarry RA, Gentleman R, Martinez-Murillo F and Spencer F: A model-based background adjustment for oligonucleotide expression arrays. J Am Stat Assoc 99: 909-917, 2004.
15. Zhang H, Chen X, Zhang D, Liu L, Song J, Xu Y and Tian J: Identification of a novel six-long noncoding RNA signature for molecular diagnosis of dilated cardiomyopathy. DNA Cell Biol: Nov 3, 2020 (Epub ahead of print).
16. 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.
17. Wong N and Wang X: miRDB: An online resource for microRNA target prediction and functional annotations. Nucleic Acids Res 43 (Database Issue): D146-D152, 2015.
18. Song J, Song F, Liu K, Zhang W, Luo R, Tang Y and Ran L: Multi-omics analysis reveals epithelial-mesenchymal transition-related gene FOXM1 as a novel prognostic biomarker in clear cell renal carcinoma. Aging (Albany NY) 11: 10316-10337, 2019.
19. Kanehisa M: The KEGG database. Novartis Found Symp 247: 91-103, 119-128, 244-252, 2002.

20. 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.
21. Jiang Y, Han D, Zhao Y, Zhang C, Shi X and Gu W: Multi-omics analysis of the prognosis and biological function for TRPV channel family in clear cell renal cell carcinoma. Front Immunol 13: 872170, 2022.
22. Hakimi AA, Ostrovnaya I, Reva B, Schultz N, Chen YB, Gonen M, Liu H, Takeda S, Voss MH, Tickoo SK, *et al*: Adverse outcomes in clear cell renal cell carcinoma with mutations of 3p21 epigenetic regulators BAP1 and SETD2: A report by MSKCC and the KIRC TCGA research network. Clin Cancer Res 19: 3259-3267, 2013.
23. Ho TH, Park IY, Zhao H, Tong P, Champion MD, Yan H, Monzon FA, Hoang A, Tamboli P, Parker AS, *et al*: High-resolution profiling of histone h3 lysine 36 trimethylation in metastatic renal cell carcinoma. Oncogene 35: 1565-1574, 2016.
24. Yang W, Zhang K, Li L, Ma K, Hong B, Gong Y and Gong K: Discovery and validation of the prognostic value of the lncRNAs encoding snoRNAs in patients with clear cell renal cell carcinoma. Aging (Albany NY) 12: 4424-4444, 2020.
25. Wang Y, Yi K, Liu X, Tan Y, Jin W, Li Y, Zhou J, Wang H and Kang C: HOTAIR up-regulation activates NF-κB to induce immunoescape in gliomas. Front Immunol 12: 785463, 2021.
26. Niu C, Li M, Chen Y, Zhang X, Zhu S, Zhou X, Zhou L, Li Z, Xu J, Hu JF, *et al*: LncRNA NCAL1 potentiates natural killer cell cytotoxicity through the Gab2-PI3K-AKT pathway. Front Immunol 13: 970195, 2022.
27. Gao C, Peng FH and Peng LK: MiR-200c sensitizes clear-cell renal cell carcinoma cells to sorafenib and imatinib by targeting heme oxygenase-1. Neoplasma 61: 680-689, 2014.
28. Takamatsu K, Maekawa K, Togashi T, Choi DK, Suzuki Y, Taylor TD, Toyoda A, Sugano S, Fujiyama A, Hattori M, *et al*: Identification of two novel primate-specific genes in DSCR. DNA Res 9: 89-97, 2002.
29. Rodriguez-Sanchez IP, Garza-Rodríguez ML, Tejero ME, Cole SA, Comuzzie AG and Barrera-Saldaña HA: DSCR9 gene simultaneous expression in placental, testicular and renal tissues from baboon (Papio hamadryas). BMC Res Notes 5: 298, 2012.
30. Yegnasubramanian S, Wu Z, Haffner MC, Esopi D, Aryee MJ, Badrinath R, He TL, Morgan JD, Carvalho B, Zheng Q, *et al*: Chromosome-wide mapping of DNA methylation patterns in normal and malignant prostate cells reveals pervasive methylation of gene-associated and conserved intergenic sequences. BMC Genomics 12: 313, 2011.
31. Liu Z, Mi M, Li X, Zheng X, Wu G and Zhang L: lncRNA OSTN-AS1 may represent a novel immune-related prognostic marker for triple-negative breast cancer based on integrated analysis of a ceRNA network. Front Genet 10: 850, 2019.
32. Liu F, Wollstein A, Hysi PG, Ankra-Badu GA, Spector TD, Park D, Zhu G, Larsson M, Duffy DL, Montgomery GW, *et al*: Digital quantification of human eye color highlights genetic association of three new loci. PLoS Genet 6: e1000934, 2010.
33. Chen M, Wang J, Luo Y, Huang K, Shi X, Liu Y, Li J, Lai Z, Xue S, Gao H, *et al*: Identify down syndrome transcriptome associations using integrative analysis of microarray database and correlation-interaction network. Hum Genomics 12: 2, 2018.
34. Wen J, Liu J, Jiang H, Wan L, Xin L, Sun Y, Zhang P, Sun Y, Zhang Y, Du X, *et al*: lncRNA expression profiles related to apoptosis and autophagy in peripheral blood mononuclear cells of patients with rheumatoid arthritis. FEBS Open Bio 10: 1642-1654, 2020.
35. Zhuang H, Huang S, Zhou Z, Ma Z, Zhang Z, Zhang C and Hou B: A four prognosis-associated lncRNAs (PALnc) based risk score system reflects immune cell infiltration and predicts patient survival in pancreatic cancer. Cancer Cell Int 20: 493, 2020.