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ORIGINAL RESEARCH

Biomarker and competing endogenous RNA potential of tumor-specific long noncoding RNA in chromophobe renal cell carcinoma

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Background: Accumulating evidence suggests long noncoding RNAs (lncRNAs) play important roles in the initiation and progression of cancers. However, their functions in chromophobe renal cell carcinoma (chRCC) are not fully understood.

Methods: We analyzed the expression profiles of lncRNA, microRNA, and protein-coding RNA, along with the clinical information of 59 primary chRCC patients collected from The Cancer Genome Atlas database to identify lncRNA biomarkers for prognosis. We also constructed an lncRNA–microRNA–mRNA coexpression network (competitive endogenous RNAs network) by bioinformational approach.

Results: One hundred and forty-two lncRNAs were found to be differentially expressed between the cancer and normal tissues (fold change ≥1.5, $P<0.001$). Among them, 12 lncRNAs were also differentially expressed with the corresponding clinical characteristics (fold change ≥1.5, $P<0.01$). Besides, 7 lncRNAs (COL18A1-AS, BRE-AS1, SNHG7, TMEM51-AS1, C21orf62-AS1, LINC00336, and LINC00882) were identified to be significantly correlated with overall survival (log-rank $P<0.05$). A competitive endogenous RNA network in chRCC containing 16 lncRNAs, 18 miRNAs, and 168 protein-coding RNAs was constructed.

Conclusion: Our results identified specific lncRNAs associated with chRCC progression and prognosis, and presented competing endogenous RNA potential of lncRNAs in the tumor.

Keywords: long noncoding RNA, chromophobe renal cell carcinoma, biomarker, competing endogenous RNA network

Introduction

Renal cell carcinoma (RCC) is one of the most common genitourinary cancers worldwide.[1] An estimated 61,560 new cases of RCC were expected in the US in 2015.[2] Chromophobe renal cell carcinoma (chRCC) is a relatively rare subtype of RCC, accounting for approximately 5% of all patients.[3] Compared to other RCC subtypes, chRCC has significantly higher cancer-specific survival probabilities. Prognosis for patients with chRCC has improved in past decades due to technological advances in early detection and intervention.[4] Even so, the clinical behavior and long-term outcomes of chRCC are still highly variable. Hence, identifying novel molecular biomarkers and studying the detailed molecular mechanism of chRCC are necessary.

Noncoding RNAs with length greater than 200 nucleotides are cataloged as long noncoding RNAs (lncRNAs).[5] LncRNAs are usually short of meaningful open reading frames (ORFs) and not translated into proteins, but they can regulate the gene expression in the form of RNA in many aspects.[6,7] Competitive endogenous RNA (ceRNA) hypothesis was proposed by Salmena et al in 2011. They pointed out

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that some messenger RNAs and noncoding RNAs such as pseudogene, lncRNAs, and circular RNAs can regulate the target genes by competitive binding to the same microRNA (miRNA)-binding sites through miRNA response elements (MREs), so the inhibition of target genes by miRNA can be released or lessened.[8] This is to say that lncRNA–miRNA–mRNA may form a large and subtle regulatory RNA network in tumors. To date, various lncRNA and miRNA interactions with significant functions have been identified in many cancers.[9–11] In RCC, lncRNA MALAT1 was found to function as a competing endogenous RNA to regulate epithelial–mesenchymal transition-related proteins by sponging miR-200s and miR-205, and HOTAIR was proved to promote the proliferation and invasion of renal clear cell adenocarcinoma cells 786-O by interacting with miR-141.[12–15] However, more functions of lncRNA in chRCC remain to be elucidated.

In this study, we analyzed the expression data of lncRNA, miRNA, and protein-coding RNA and the corresponding clinical information of 59 chRCC patients selected from The Cancer Genome Atlas (TCGA) database to explore the differential expression profiles of lncRNAs in different clinical statuses and to identify tumor-specific lncRNAs' competing endogenous RNA potential in the tumor.

Methods

Data collection

Fifty-nine chRCC patients selected from the TCGA database were enrolled in our study. The inclusion criteria were set as follows: 1) the tumor histological type was chRCC; 2) the patient did not have a history of other malignancies; 3) the patient had not received neoadjuvant therapy; and 4) the clinical information was complete. Among these 59 patients (Cohort T), 23 patients provided the adjacent nontumor tissues (Cohort M). Their corresponding RNA expression data (level 3) were downloaded from TCGA data portal (http://cancergenome.nih.gov, up to Jan 20, 2016). These gene expression profiles were produced by using Illumina HiSeq 2000 sequencer platforms (Illumina Inc., San Diego, CA, USA). The raw expression data of lncRNAs and mRNAs which were generated from RNA sequencing raw reads by RNASeqV2 postprocessing pipelines were normalized as RNA-Seq by Expectation-Maximization. The raw expression data of miRNAs were standardized as reads per million by the TCGA project. Patient data were collected and processed following the data access policies approved by the Ethics Committee of The Cancer Genome Atlas Program. The authors downloaded all the data from the TCGA database and performed this study in line with the TCGA publication guidelines (http://cancergenome.nih.gov/publications/publicationguidelines). All patients enrolled in the program were well informed. Therefore, no further ethical approval was required for this study. We analyzed these expression profiles with BRB-Array tools (version 4.4.0) developed by Dr Richard Simon and the BRB-Array Tools Development Team.[16]

Construction of lncRNA-associated ceRNA network

LncRNA-associated ceRNA network was constructed based on the "ceRNA hypothesis" that lncRNAs can regulate the expression of mRNAs which contain common MREs by combining the miRNAs competitively. We identified differentially expressed lncRNAs and miRNAs (fold change ≥ 5.0, $P<0.001$) in the tumor. Predicted human miRNA–lncRNA interactions were collected from starBase v2.0[17] and miRcode.[18] Experimentally validated miRNA–target mRNA interactions were retrieved from the miRTarBase.[19] Differentially expressed miRNAs were set as hub nodes. The lncRNAs and mRNAs were connected with these hub nodes according to their interactions. Maximal information coefficient (MIC) algorithm was used to identify the robustness of pair-wise relationships of miRNA–lncRNA and miRNA–mRNA (MIC >0.15, MIC-$\rho 2 >0.15$).[20] Cytoscape v3.0[21] was applied to construct and visualize the network graph.

Functional enrichment analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the coding RNAs involved in the ceRNA network was conducted using Database for Annotation, Visualization, and Integrated Discovery.[22] We did the analysis with default parameters. The whole human genome was set as background; functional categories with P-value <0.05 were regarded as statistically significant.

Statistical analysis

Clinical category variables were presented as counts and percentages. The chi-square test was applied to analyze differences of distribution between Cohort M and Cohort T. RNA expression data were presented as mean ± standard deviation. Paired sample t-test was used to examine differences in lncRNA and miRNA expression between cancerous and matched adjacent tissues (significant P-value was set as 0.001). Unpaired t-test was conducted to find out the difference in lncRNA expression levels between different clinicopathological groups (significant P-value was set as 0.01). Unsupervised hierarchical cluster analysis was used to generate tree clusters for the separation of different classes with lncRNA expression profiles. Univariate Cox proportional

hazards regression was applied to identify the lncRNAs associated with overall survival; Kaplan–Meier survival analyses and log-rank test were performed to study the relations of lncRNA expression states (cutoff point: median value) and survival time (significant *P*-value was set as 0.05). All statistical analyses were performed by the SPSS 19 (IBM Corporation, Armonk, NY, USA) and BRB-Array Tools 4.0.

Results

Patient characteristics

A total of 59 chRCC patients were enrolled in our study. Among them (Cohort T), 23 patients provided adjacent tissues (Cohort M). Their demographic characteristics and clinical information are summarized in Table 1.

Differential expression analysis of lncRNAs

We identified 605 lncRNAs from the TCGA level 3 RNASeqV2 data according to the classification of HUGO Gene Nomenclature Committee (HGNC) (http://www.genenames.org). A total of 143 lncRNAs were found to be expressed differentially between the cancer and the paired adjacent tissues (fold change ≥1.5, $P<0.001$) (Table S1). Unsupervised hierarchical clustering could clearly discriminate cancer and normal class with these differentially expressed lncRNAs (Figures S1 and S2). In consideration of the fold change, 43 of them had an absolute fold change ≥5.0, and they were selected to build the ceRNA network (Table 2). Furthermore, among these 143 differentially expressed

Table 1 Clinical characteristics of patients with chromophobe renal cell carcinoma

Category	Cohort M (n=23) (%)	Cohort T (n=59) (%)	*P*-value
Age, mean ± SD	52.6±13.3	51.0±14.2	0.647
Gender, n (%)			0.623
Female	12 (52.2)	26 (44.1)	
Male	11 (47.8)	33 (55.9)	
AJCC stages, n (%)			0.594
Stage I	9 (39.2)	17 (28.9)	
Stage II	8 (34.8)	23 (39.0)	
Stage III	3 (13.0)	14 (23.7)	
Stage IV	3 (13.0)	5 (8.4)	
Tumor size, n (%)			0.790
T1	9 (39.1)	17 (28.8)	
T2	8 (34.8)	23 (39.0)	
T3	5 (21.7)	14 (23.7)	
T4	1 (4.4)	5 (8.5)	
Lymph node, n (%)			0.382
N0	11 (47.8)	38 (64.4)	
N1+N2	2 (8.7)	4 (6.8)	
NX	10 (43.5)	17 (28.8)	
Metastasis status, n (%)			0.947
M0	18 (78.3)	48 (81.4)	
M1	1 (4.4)	2 (3.4)	
MX	4 (17.3)	9 (15.2)	
Tumor status, n (%)			0.783
Tumor free	19 (82.6)	50 (84.7)	
With tumor	3 (13.0)	8 (13.6)	
NA	1 (4.4)	1 (1.7)	

Abbreviations: AJCC, American Joint Committee on Cancer; NA, not applicable; SD, standard deviation.

Table 2 Forty-three cancer specific lncRNAs in ceRNA network construction

LncRNA	Entrez ID	Chromosome	Expression change (T vs N)
LINC00588	26138	Chr8	Upregulation
SLC26A4-AS1	286002	Chr7	Upregulation
BAALC-AS2	157556	Chr8	Upregulation
LINC00265	349114	Chr7	Upregulation
UCKL1-AS1	100113386	Chr20	Upregulation
LINC00239	145200	Chr14	Upregulation
PART1	25859	Chr5	Upregulation
PACRG-AS1	285796	Chr6	Upregulation
KRTAP5-AS1	338651	Chr11	Upregulation
CDKN2B-AS1	100048912	Chr9	Upregulation
LINC00889	158696	ChrX	Upregulation
LINC00669	647946	Chr18	Upregulation
LINC00930	100144604	Chr15	Upregulation
LINC00598	646982	Chr13	Upregulation
NR2F1-AS1	441094	Chr5	Downregulation
LINC00882	100302640	Chr3	Downregulation
LINC00242	401288	Chr6	Downregulation
LINC01554	202299	Chr5	Downregulation
CASC2	255082	Chr10	Downregulation
LINC00312	29931	Chr3	Downregulation
TINCR	257000	Chr19	Downregulation
LINC00092	100188953	Chr9	Downregulation
HCG4	54435	Chr6	Downregulation
HNF1A-AS1	283460	Chr12	Downregulation
LOC145837	145837	Chr15	Downregulation
MEG3	55384	Chr14	Downregulation
LINC00839	84856	Chr10	Downregulation
LOC285768	285768	Chr6	Downregulation
ADORA2A-AS1	646023	Chr22	Downregulation
GATA3-AS1	399717	Chr10	Downregulation
LINC00924	145820	Chr15	Downregulation
BRE-AS1	100302650	Chr2	Downregulation
UCA1	652995	Chr19	Downregulation
EGOT	100126791	Chr3	Downregulation
LINC00908	284276	Chr18	Downregulation
LINC00671	388387	Chr17	Downregulation
LINC00271	100131814	Chr6	Downregulation
COL18A1-AS1	378832	Chr21	Downregulation
LINC01550	388011	Chr14	Downregulation
WT1-AS	51352	Chr11	Downregulation
LINC01139	339535	Chr1	Downregulation
LINC00473	90632	Chr6	Downregulation
LHFPL3-AS2	723809	Chr7	Downregulation

Notes: The names, Entrez IDs and chromosomal locations of theses lncRNAs were obtained from the Entrez Gene database http://www.ncbi.nlm.nih.gov/gene.[38]
Abbreviations: ceRNA, competing endogenous RNA; lncRNA, long noncoding RNA; N, normal; T, tumor.

Table 3 LncRNAs associated with the progression of chromophobe renal cell carcinoma

Comparisons	Downregulated	Upregulated
Gender (female vs male)	CHKB-AS1, LOC285768	XIST
Age at diagnosis (≥51 vs <51)		LINC01119
AJCC stage (III+IV vs I+II)	TMEM51-AS1	LINC00242, CHKB-AS1
AJCC T (T3+T4 vs T1+T2)	TMEM51-AS1	LINC00242, CHKB-AS1
Tumor status (with tumor vs tumor free)	PSMD5-AS1, ADORA2A-AS1, INE2	CDKN2B-AS1, LINC00669

Abbreviations: AJCC, American Joint Committee on Cancer; lncRNA, long noncoding RNA.

lncRNAs, 12 cancer-specific lncRNAs were also identified to be differentially expressed in different clinical features (fold change ≥1.5, $P<0.01$) with 3 for gender, 1 for age, 5 for tumor status, and 3 for American Joint Committee on Cancer stage and tumor size (Table 3). Because the number of patients with metastasis status M1 and lymph node status N1+N2 was too small, class comparison analyses were not conducted for them.

LncRNAs in relation to patient prognosis

Among differentially expressed lncRNAs, 7 lncRNAs (COL18A1-AS, BRE-AS1, SNHG7, TMEM51-AS1, C21orf62-AS1, LINC00336, and LINC00882) were identified to be associated with the overall survival of chRCC by univariate Cox regression analysis. Kaplan–Meier survival curves indicated that COL18A1-AS1 (P=0.009), BRE-AS1 (P=0.011), SNHG7 (P=0.014), TMEM51-AS1 (P=0.024), C21orf62-AS1 (P=0.027), and LINC00336 (P=0.037) were positively correlated with overall survival, while the remaining LINC00882 (P=0.047) was negatively associated with overall survival (Figure 1).

LncRNA-associated ceRNA network

Thirty-one miRNAs identified to be expressed differentially between the cancer and adjacent tissues with absolute fold change higher than 5 ($P<0.001$) (Table S2) were selected to construct the ceRNA network. In a ceRNA network, miRNAs interact with lncRNAs through MREs, and we used miRcode and starBase v2.0 to find the potential MREs of these miRNAs in tumor-specific lncRNAs, as described in Table 2. The result demonstrated that 18 of 31 cancer-specific miRNAs might interact with 16 of 43 cancer-specific lncRNAs (Table 4). Subsequently, 167 experimentally validated target genes of miRNAs described in Table 4 were identified by using miRTarBase (Table 5), and all these miRNA–mRNA interactions were validated by reporter assay, Western blot, and qPCR. Then, an lncRNA–miRNA–mRNA network was established based on the above-mentioned data (Tables 4 and 5). The MIC algorithm was applied to test pair-wise correlations based on their expression levels. To enhance the robustness of the ceRNA network, only those pair-wise interactions with MIC >0.15 and MIC-ρ2 >0.15 were included in the ceRNA network (Figure 2).

KEGG pathway enrichment analysis

To explore the biological functions of these protein-coding RNAs involved in the ceRNA network, KEGG pathway enrichment analysis was conducted using Database for Annotation, Visualization, and Integrated Discovery. As summarized in Table 6, 12 cancer-related pathways were enriched, including those for prostate cancer, melanoma, pancreatic cancer, chronic myeloid leukemia, colorectal cancer, bladder cancer, glioma, RCC, small cell lung cancer, endometrial cancer, and acute myeloid leukemia, and 6 non-cancer-related pathways were enriched, including those for focal adhesion, adherens junction, cell cycle, neurotrophin signaling pathway, ErbB signaling pathway, and p53 signaling pathway.

Discussion

RCC has various histological subtypes, of which clear-cell RCC (about 70%), papillary RCC (about 10%–15%), and chRCC (about 5%) are the most prevalent.[3] These subtypes have diverse genetic and clinical features, and the identification of molecular mechanisms behind their oncogenesis and progression comprises an important area of cancer research.[4,23] In the present study, we focused on exploring the prognostic roles and the competing endogenous RNA potential of lncRNAs in chRCC. By analyzing the clinical information and large-scale sequencing data pertaining to a chRCC patient cohort, we identified tumor-specific lncRNAs in chRCC and investigated their distribution in different clinical features and prognoses. Besides, we constructed an lncRNA-related ceRNA network of chRCC consisting of lncRNAs, miRNAs, and protein-coding RNAs.

As a highly heterogeneous group of noncoding RNAs, lncRNAs can regulate the gene expression by means of diverse mechanisms and are involved in various biological processes.[5,24] Mounting evidences suggest lncRNAs have key roles in regulation of tumor development and progression.[10,25] These aberrantly expressed lncRNAs could

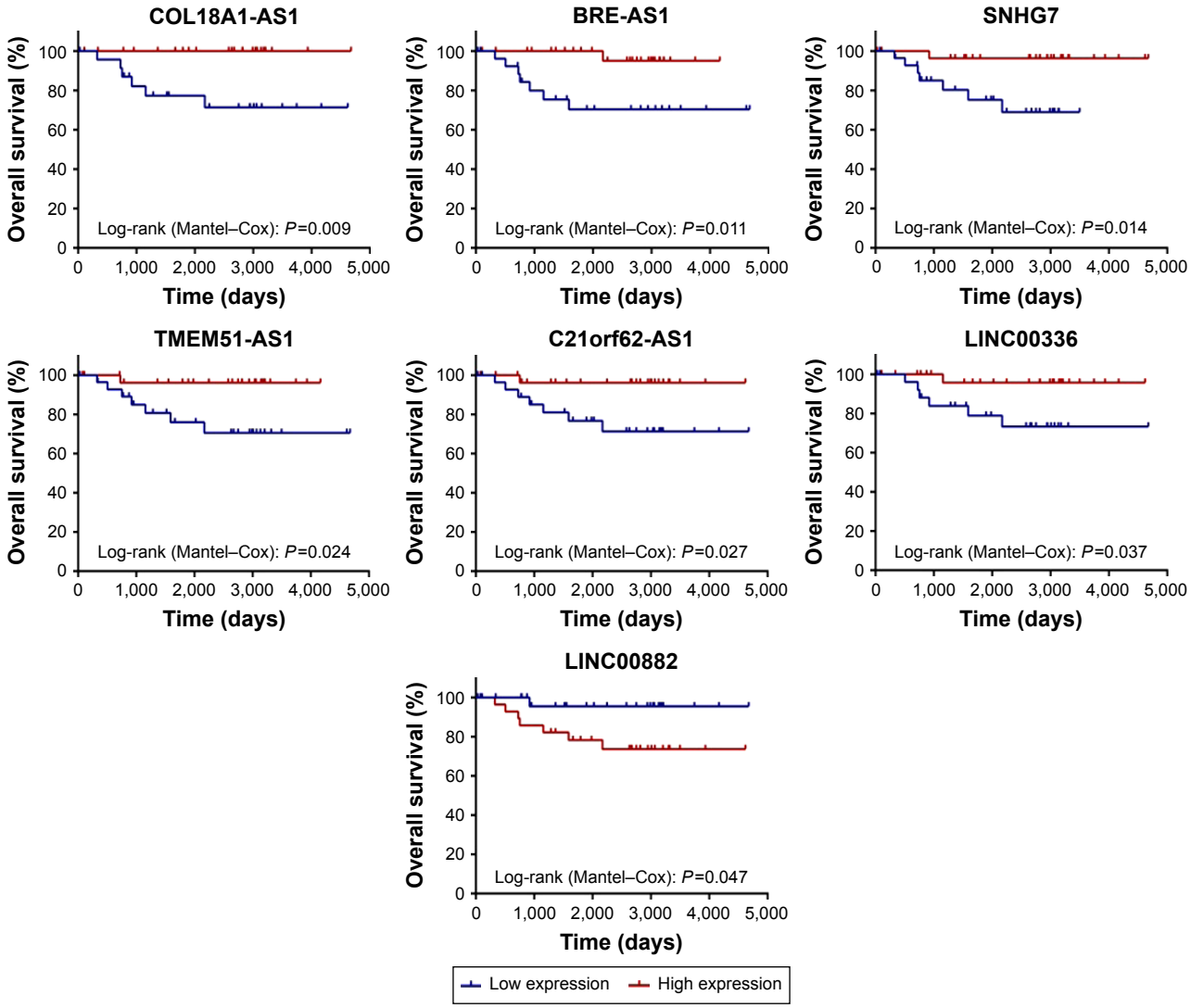

Figure 1 Kaplan–Meier survival curves for 7 prognosis-related lncRNAs.
Notes: Horizontal axis: overall survival time; vertical axis: survival function; cutoff point: median value.
Abbreviation: LncRNA, long noncoding RNA.

be tracked in the migration, apoptosis, proliferation, and drug resistance patterns of tumor cells, which implies that lncRNAs could serve as potential therapeutic targets and biomarkers.[26–29] Numerous studies have documented that lncRNAs could affect the expression of cancer-related proteins by interacting with miRNAs, somewhat validating the ceRNA hypothesis.[14,15] In order to gain more insight about their effects in tumors, lncRNA profiling has become a major method to study the widespread dysregulated lncRNAs, and their coexpression networks with mRNAs and miRNAs have been constructed in various tumors.[30–32] However, such lncRNAs-related ceRNA networks in RCC are still poorly explored.

Hence, we conducted the present study with the aim to identify lncRNA biomarkers of prognosis and construct an lncRNA–miRNA–mRNA coexpression network in chRCC. By analyzing the lncRNA expression profiles of 59 primary chRCC patients, we identified 142 differentially expressed lncRNAs between cancer and adjacent tissues, 43 of which had a more than a fivefold change in expression levels. In those upregulated lncRNAs, CDKN2B-AS1 has previously been reported to be able to promote cell proliferation. Furthermore, its high expression has been linked to poor prognosis in prostate and gastric cancer.[33,34] The expression level of SLC26A4-AS1 was found to be significantly associated with overall higher survival of gastric cancer patients, but the mechanism was not elaborated.[35] In those downregulated lncRNAs, CASC2 was found to be aberrantly expressed in glioma and non-small-cell lung cancer. Increase in CASC2 expression could inhibit cell proliferation of the 2 tumors, and

Table 4 Putative miRNAs that may target cancer-specific lncRNAs by MREs

lncRNA	miRNAs
LINC00473	hsa-mir-199a-1/2, hsa-mir-199b
WT1-AS	hsa-mir-199a-1/2, hsa-mir-199b, hsa-mir-221, hsa-mir-9-1, hsa-mir-96
COL18A1-AS1	hsa-mir-187, hsa-mir-196a-1
LINC00271	hsa-mir-192
EGOT	hsa-mir-183
UCA1	hsa-mir-182, hsa-mir-190, hsa-mir-455, hsa-mir-96
LINC00839	hsa-mir-130a
MEG3	hsa-mir-182, hsa-mir-192, hsa-mir-199a-1/2, hsa-mir-199b, hsa-mir-204, hsa-mir-217, hsa-mir-221, hsa-mir-455, hsa-mir-9-1, hsa-mir-96
HNF1A-AS1	hsa-mir-183, hsa-mir-194-1/2, hsa-mir-199a-1/2, hsa-mir-199b, hsa-mir-217, hsa-mir-455, hsa-mir-9-1
HCG4	hsa-mir-217, hsa-mir-96
LINC00312	hsa-mir-190, hsa-mir-192, hsa-mir-9-1
CASC2	hsa-mir-130a, hsa-mir-192, hsa-mir-194-1/2
LINC00242	hsa-mir-204, hsa-mir-217, hsa-mir-221, hsa-mir-222
PART1	hsa-mir-9-1
LINC00265	hsa-mir-182, hsa-mir-217
SLC26A4-AS1	hsa-mir-130a

Abbreviations: lncRNA, long noncoding RNA; miRNA, microRNA; MREs, microRNA response elements.

CASC2 was proved to be an independent predictor of overall survival for non-small-cell lung cancer patients.[36] In addition, 12 tumor-specific lncRNAs were found to be abnormally expressed in different clinical features. Dysregulated lncRNAs identified by tumor stage or size are identical because patients' distributions in their different groups are common. As the number of patients with metastasis status M1 and lymph node status N1 + N2 was too small, class comparison analyses were not conducted. In consideration of the relationship between cancer-specific lncRNAs and prognosis, we identified 7 lncRNAs that were associated with chRCC overall survival, and they may serve as prognosis prediction tools or candidate drug targets for chRCC management. Among the 6 protective lncRNAs, SNHG7 was reported to be involved in the cellular response to radiation-induced oxidative stress.[37] The functions of the other 5 protective and 1 risky lncRNA are still unknown.

For further analyzing the interactions between lncRNA, miRNA, and mRNA in chRCCs, we constructed a ceRNA network by bioinformational methods. This ceRNA network contained 16 tumor-specific lncRNAs, 18 tumor-specific miRNAs, and 168 protein-coding RNAs. To improve the prediction accuracy of the coexpression network, pair-wise relationships of lncRNA–miRNA–mRNA were filtered based on their expression levels by the MIC algorithm which could detect novel associations in complex datasets. Through KEGG analysis, we found that those ceRNA network-involved genes were mainly enriched in cancer-related pathways, further indicating that lncRNAs may play a vital role in tumor molecular regulatory networks. The ceRNA network we constructed reveals an unknown ceRNA regulatory network in chRCC and gives some new perspectives of lncRNAs' functions in gene regulation. However, some issues should

Table 5 Experimentally validated miRNA targets

miRNA	mRNAs targeted by miRNA
hsa-mir-130a	HOXA5, ATXN1, MEOX2, PPARG, GJA1, TNF
hsa-mir-182	FOXO1, CDKN1A, MITF, RECK, FLOT1, PTEN, GSK3B, ANUBL1, CYLD, BCL2, CCND2, PDCD4, SATB2, CHL1, CADM1, TP53INP1, TCEAL7, ULBP2
hsa-mir-183	FOXO1, EZR, PDCD4, AKAP12, GSK3B, SMAD4, ZFPM1, DKK3, BMI1, ZEB1, SNAI2, PPP2CB, PPP2R4
hsa-mir-187	TNF, CD276
hsa-mir-190	IGF1, PHLPP1, MARK2, KCNQ5
hsa-mir-192	ALCAM, CDC7, CUL5, ERCC3, LMNB2, MAD2L1, ERCC4, RB1, WNK1, DICER1, CAV1
hsa-mir-194-1/2	IGF1R, CDH2, RAC1, HBEGF, PTPN12, PTPN13, ITGA9, SOCS2, DNMT3A, SOX5, BMI1, RBX1, BMP1
hsa-mir-199a-1/2	MET, MTOR, CAV1, GSK3B, FZD4, WNT2, JAG1, CD44, IKBKB, KL, CDH1, HIF1A, SMARCA2, MAPK1, DDR1, MAP3K11, FUT4, CAV2, ERBB2, SIRT1, PTGS2, HSPA5, ATF6, ERN1, HGF, WNK1, NFKB1, ACVR1B
hsa-mir-199b	HES1, SET, PODXL, JAG1, DDR1, ERBB2, SETD2
hsa-mir-204	BCL2, THRB, BIRC2, EZR, M6PR, RAB22A, RAB40B, SERP1, TCF12, SOX4, CDC42, RUNX2, EFNB2, SIRT1, NTRK2, USP47, ANKRD13A, TMPRSS3, CDH1, VIM, BDNF, HMX1
hsa-mir-217	SIRT1, ROBO1, EZH2, DACH1, FOXO3, GPC5
hsa-mir-221	CDKN1B, DDIT4, KIT, CDKN1C, BBC3, BNIP3L, FOS, BNIP3, MBD2, BMF, FOXO3, TMED7, ESR1, TICAM1, PTEN, TRPS1, WEE1, HECTD2, ASZ1, MDM2, ETS1, IMP3, DIRAS3, CERS2, ZEB2, RB1, APAF1, ANXA1, CTCF, RAB1A, RECK, SIRT1
hsa-mir-222	CDKN1B, SOD2, MMP1, KIT, FOS, PTEN, STAT5A, FOXO3, CDKN1C, ESR1, BBC3, TRPS1, VGLL4, ETS1, TIMP3, DIRAS3, CERS2, DKK2
hsa-mir-455	MUC1, NCSTN
hsa-mir-9-1	RAB34, ONECUT2, FOXO1, NFKB1, NR2E1, AP3B1, CCNG1, DICER1, SIRT1, STMN1, CREB1, NF1, ELAVL1, CXCR4, FOXP1, PRTG, ACAT1, MTHFD1, BCL2L11
hsa-mir-96	FOXO1, CDKN1A, KRAS, FOXO3, GSK3B, RECK, REV1, RAD51, ALK, ZEB1, SNAI2

Abbreviation: miRNA, microRNA.

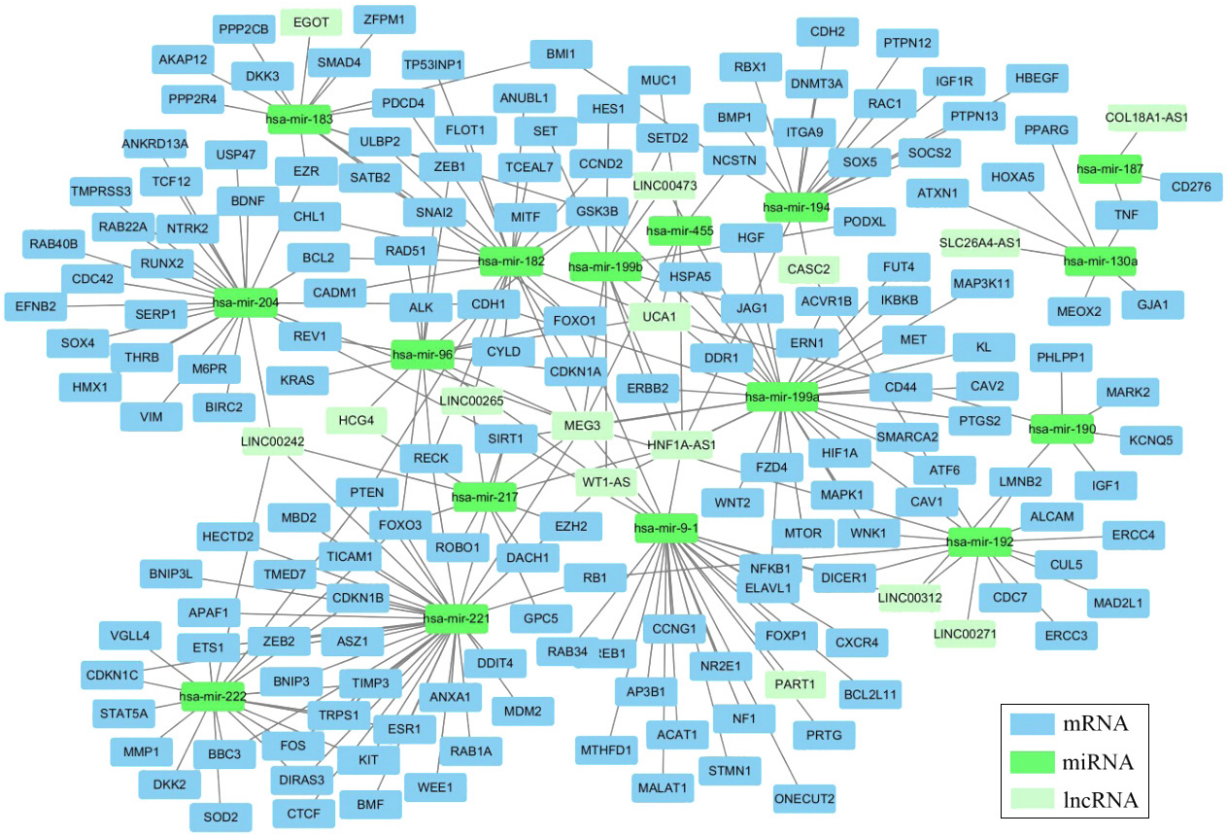

Figure 2 Cancer-specific lncRNA associated ceRNA network presented by Cytoscape.[21]
Abbreviations: lncRNA, long noncoding RNA; miRNA, microRNA.

Table 6 KEEG pathways enriched by the protein-coding genes involved in ceRNA network with $P<0.001$

Pathway type	KEGG pathways	Number of genes	*P*-value
Cancer-related pathways	Pathways in cancer	37	1.25571E–19
	Prostate cancer	17	3.53254E–12
	Melanoma	12	5.47354E–08
	Pancreatic cancer	11	6.62018E–07
	Chronic myeloid leukemia	11	9.7726E–07
	Colorectal cancer	11	2.83419E–06
	Bladder cancer	8	1.00607E–05
	Glioma	9	1.83661E–05
	Renal cell carcinoma	9	4.02163E–05
	Small cell lung cancer	9	0.000149891
	Endometrial cancer	7	0.000369377
	Acute myeloid leukemia	7	0.000671143
Noncancer-related pathways	Focal adhesion	16	3.59367E–06
	Adherens junction	10	1.07149E–05
	Cell cycle	12	1.72817E–05
	Neurotrophin signaling pathway	11	9.0498E–05
	ErbB signaling pathway	9	0.000191896
	p53 signaling pathway	8	0.000243417

Note: The *P*-value is corrected for multiple hypothesis testing using the Benjamini–Hochberg method.
Abbreviations: ceRNA, competing endogenous RNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

be acknowledged in interpreting this ceRNA network. The network was constructed in silico and could serve as a reference for further research. For validation of the lncRNA/miRNA/mRNA pathway, additional biological experiments need to be conducted.

Conclusion

By analyzing an independent chRCC patient cohort extracted from the TCGA database, we screened differentially expressed lncRNAs under different clinical features and constructed an lncRNA-related ceRNA network. Our study suggests that some lncRNAs are associated with chRCC progression and prognosis, and they may function as ceRNAs in a complex ceRNA network.

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Disclosure

The authors report no conflicts of interest in this work.

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