Bioinformatic analysis and prediction of the function and regulatory network of long non-coding RNAs in hepatocellular carcinoma

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Abstract. Computational analysis and bioinformatics have significantly advanced the ability of researchers to process and analyze biological data. Molecular data from human and model organisms may facilitate drug target validation and identification of biomarkers with increased predictive accuracy. The aim of the present study was to investigate the function of long non-coding RNAs (lncRNAs) in hepatocellular carcinoma (HCC) using online databases, and to predict their regulatory mechanism. HCC-associated lncRNAs, their downstream transcription factors and microRNAs (miRNAs/miRs), as well as the HCC-associated target genes, were identified using online databases. HCC-associated lncRNAs, including HOX antisense intergenic RNA (HOTAIR) and metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) were selected based on established databases of lncRNAs. The interaction between the HCC-associated lncRNAs and miRNAs (hsa-miR-1, hsa-miR-20a-5p) was predicted using starBase2.0. Signal transducer and activator of transcription 1, hepatocyte nuclear factor 4α (HNF4A), octamer-binding transcription factor 4, Nanog homeobox (NANOG), caudal type homeobox 2 (CDX2), DEAD-box helicase 5, brahma-related gene 1, MYC-associated factor X and MYC proto-oncogene, bHLH transcription factor have been identified as the transcription factors for HOTAIR and MALAT1 using ChIPBase. Additionally, CDX2, HNF4A, NANOG, ETS transcription factor, Jun proto-oncogene and forkhead box protein A1 were identified as the transcription factors for hsa-miR-1 and hsa-miR-20a-5p. CDX2, HNF4A and NANOG were the transcriptional factors in common between the lncRNAs and miRNAs. Cyclin D1, E2F transcription factor 1, epithelial growth factor receptor, MYC, MET proto-oncogene, receptor tyrosine kinase and vascular endothelial growth factor A were identified as target genes for the HCC progression, two of which were also the target genes of hsa-miR-1 and hsa-miR-20a-5p using the miRwalk and OncoDN. HCC databases. Additionally, these target genes may be involved in biological functions, including the regulation of cell growth, cell cycle progression and mitosis, and in disease progression, as demonstrated using DAVID clustering analysis. The present study aimed to predict a regulatory network of lncRNAs in HCC progression using bioinformatics analysis.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies globally and the second-leading cause of mortality in China (1). There are ~62 million newly diagnosed cases of HCC annually worldwide (2). Numerous factors, including hepatitis B virus infection and alfatoxin induction, may contribute to the development of HCC. These factors may lead to abnormal gene expression, resulting in increased cancer cell proliferation and escape from immune surveillance (3-5). The majority of patients with HCC are diagnosed at advanced stages of the disease. Currently, there is no effective surgical or pharmaceutical treatment for HCC. Therefore, investigation of the molecular mechanisms underlying HCC development is necessary to develop therapeutic strategies for HCC.

With the development of sequencing technology and the in-depth studies of oncological diseases in previous years, long non-coding RNAs (lncRNAs), once considered to be the 'noise' in genomic transcription, have come to the attention of scholars (6-8). lncRNAs are non-protein-coding transcripts that are >200 nucleotides, which may interact with various biomolecules, including DNA, RNA and proteins, to regulate gene expression at transcriptional, post-transcriptional and epigenetic levels (9), serving

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important functions in a wide range of biological processes, including cell proliferation, survival, differentiation and chromatin remodeling (10,11). To date, the encyclopedia of DNA elements project (GENCODE v26) has conservatively annotated in humans close to 16,000 lncRNA genes that give rise to >28,000 distinct transcripts (12). In previous years, researchers have made notable advances in the study of lncRNA and diseases, particularly in the area of tumors (13-15). It has been suggested that the abnormal expression of lncRNA may be the major inducer and accelerant of tumorigenesis (16). However, the functions of lncRNA and their underlying molecular mechanisms are not well understood, meaning that further studies are required.

Increasingly, evidence demonstrates that a number of abnormally expressed lncRNAs are closely associated with the occurrence, invasion, metastasis and recurrence of HCC, and have great potential in its prediction, diagnosis and treatment (17). The regulatory mechanism of lncRNA is complex, and its role in HCC is diverse (18,19). The present study utilized bioinformatic analysis to investigate the regulatory network of HCC-associated lncRNAs and their target genes in order further understanding on the functions of lncRNA in the pathogenesis of HCC, which is also conducive to follow-up studies.

Materials and methods

Identification of HCC-associated lncRNAs. The lncRNA disease database (http://www.cuilab.cn/lncrnadisease) has identified >200 types of lncRNA-associated diseases and can be utilized as a bioinformatic tool to predict human lncRNA-associated diseases (20). In the present study, HCC-associated lncRNAs were screened and candidates lncRNAs were selected using the lncRNA disease database.

lncRNA-miRNA interaction analysis. The interaction between miRNAs (6-8mer sites) and lncRNAs was predicted from the crosslinking immunoprecipitation RNA sequencing (CLIP-Seq) data using the starBase platform (http://starbase.sysu.edu.cn/) (21).

Identification of HCC-associated miRNAs. HCC-associated miRNAs were mapped into the HMDD database (http://202.38.126.151/hmdd/mirna/md/) (22).

Target gene prediction. ChIPBase (http://deepbase.sysu.edu.cn/chipbase/) is a database for decoding the transcriptional regulation of lncRNAs and miRNAs (23). The candidate transcription factors for lncRNAs and miRNAs were predicted using ChIPBase and analyzed using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/).

Identification of the target genes for the candidate miRNAs. miRWalk (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/) is a comprehensive atlas of microRNA-target interactions, which provides the possible miRNA binding sites within the complete sequence of a gene (24). The target genes for the candidate miRNAs were identified using the miRWalk database.

Identification of the HCC-associated target genes. OncoDB. HCC (http://oncodb.hcc.ibms.sinica.edu.tw/index.htm) is the

Table I. Hepatocellular carcinoma-associated lncRNAs obtained from the lncRNA disease database.

lncRNA	Dysfunction type	Chromosome	Genbank
H19	Expression	11	NR_002196.1
	Expression	11	NR_002196.48
	N/A	11	NR_002196.17
	Expression	11	NR_002196.1
	Regulation	11	NR_002196.1
HEIH	Expression	5	NR_045680.1
HOTAIR	Expression	12	NR_047517.1
	Expression	12	NR_047517.10
	Expression	12	NR_047517.13
	Expression	12	NR_047517.3
	Expression	12	NR_047517.1
	Expression	12	NR_047517.15
	Regulation	12	NR_047517.1
HOTTIP	Expression	7	NR_037843.3
	Expression	7	NR_037843.3
	Expression	7	NR_037843.2
HULC	Expression	6	NR_004855.2
	Regulation	6	NR_004855.2
	Regulation	6	NR_004855.2
	Expression	6	NR_004855.3
	Expression	6	NR_004855.4
	Expression	6	NR_004855.6
	Expression	6	NR_004855.2
	Regulation	6	NR_004855.2
IGF2-AS	N/A	11	NR_028044.1
KCNQ1OT1	Mutation	11	NR_002728.3
MALAT1	Expression	11	NR_002819.2
	Expression	11	NR_002819.2
	Expression	11	NR_002819.7
	Expression	11	NR_002819.12
	Expression	11	NR_002819.17
	Mutation	11	NR_002819.5
	Expression	11	NR_002819.2
MEG3	Expression	14	NR_002766.2
	Expression	14	NR_002766.15
	Regulation	14	NR_002766.2
MINA	Expression	3	XR_241516.2
MIR7-3HG	Expression	19	NR_027148.1
NPTN-IT1	Expression	15	AK055007.1
	Expression	15	AK055007.1

HCC, hepatocellular carcinoma; lncRNA, long non-coding RNA; HEIH, hepatocellular carcinoma upregulated EZH2-associated long non-coding RNA HOTAIR, HOX transcript antisense RNA; HOTTIP, HOXA distal transcript antisense RNA; HULC, highly upregulated in liver cancer; IGF2-AS, insulin-like growth factor 2-antisense RNA; KCNQ1OT1, KCNQ1 overlapping transcript 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; MEG3, maternally expressed 3; MINA, MYC induced nuclear antigen; MIR7-3HG, MIR7-3 host gene; NPTN-IT1, NPTN intronic transcript 1; N/A, not available.
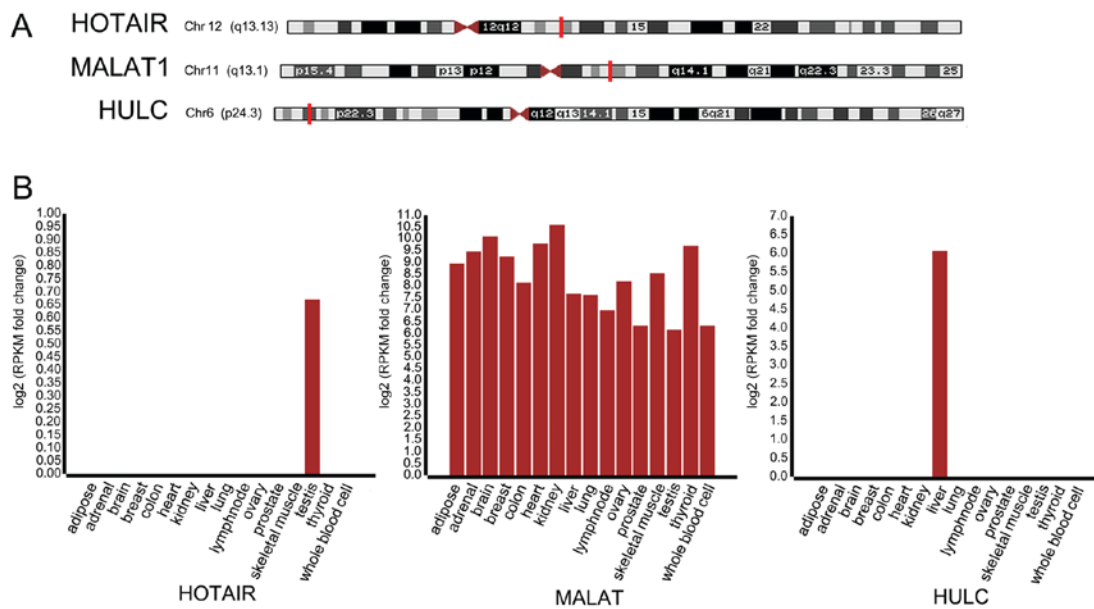
Figure 1. (A) Location of the target lncRNAs and their (B) expression pattern in different tissue. lncRNAs, long non-coding RNAs; HOTAIR, HOX antisense inter-genic RNA; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; Chr, chromosome; HULC, highly upregulated in liver cancer; wbc, whole blood cells.

first integrated oncogenomic database of hepatocellular carci-noma, which may aid identification aberrant cancer target genes and loci (25). Target genes that were not HCC-associated were further screened using the OncoDB.HCC database.

Functional enrichment analysis. DAVID (https://david.ncifcrf. gov/home.jsp) is a database that provides functional interpre-tation of a large list of genes derived from genomic studies. DAVID accelerates the analysis of genome-scale datasets by facilitating the transition from data collection to biological meaning (26). In the present study, miRNA target genes were further analyzed using DAVID, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and it was considered to be significant if the Benjamini-corrected P-value was <0.05.

Identification of transcription factors for the target lncRNAs and miRNAs. ConSite is a web-based tool for identifying *cis*-regulatory elements in genomic sequences (http://consite. genereg.net). Predictions are made based on the integration of binding site prediction generated with high-quality transcrip-tion factor models and cross-species comparison filtering (27). The transcription factors for each target gene were predicted using ConSite. The transcription factors that were associated with HCC were further screened using PubMed.

Construction of the regulatory network of lncRNAs in HCC. On the basis of the genomic information that was collected, the possible regulatory network of the selected lcnRNAs in HCC was constructed by Cytoscape software version 3.5.0 (http://www.cytoscape.org/index.html).

Results

HCC-associated lncRNAs. Of the 12 lncRNAs that were associated with HCC obtained from the lncRNA disease

database (Table I), HOX antisense intergenic RNA (HOTAIR), metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) and highly upregulated in liver cancer (HULC) were selected for further analysis.

Analyses of the target lncRNAs. The three target lncRNAs, HOTAIR, MALAT1 and HULC, were analyzed using UCSC Genome Browser. As presented in Fig. 1A, HOTAIR, MALAT1 and HULC were located on chromosome 12, 11 and 6, respectively. Additionally, MALAT1 and HULC were highly expressed in the liver, whereas HOTAIR was expressed in the liver at a lower level (Fig. 1B).

Prediction of the interaction between target lncRNAs and miRNAs. The miRNAs that were associated with HOTAIR, MALAT1 and HULC, were predicted using starBase2.0. A total of 32 miRNAs were predicted to interact with HOTAIR, and 113 miRNAs were predicted to interact with MALAT1. Among these miRNAs, miR-206, miR-1, miR-17-5p, miR-20a-5p, miR-93-5p, miR-106a-5p, miR-106b-5p, miR-613, miR-519d-5p, miR-217 and miR-20b-5p were overlapping (Fig. 2). However, no miRNA was identified to interact with HULC. Therefore, HULC was excluded from further analysis.

Identification of HCC-associated miRNAs. The HCC-associated miRNAs were obtained from HMDD and compared with the previously identified miRNAs. As presented in Fig. 3, miR-1, miR-17-5p, miR-20a-5p, miR-93-5p, miR-106a-5p, miR-106b-5p, miR-519d-5p, miR-217 and miR-20b-5p were involved in the progression of HCC and were identified to interact with HOTAIR and MALAT1. A previous study demonstrated the negative association between miRNA and lncRNA (28). Therefore, in the present study, miR-1 and miR-20a-5p were selected for further analysis, owing to their low expression in HCC.

Figure 2. Prediction of interaction between MALAT1/HOTAIR and miRNAs. HOTAIR, HOX antisense intergenic RNA; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; miRNA/miR, microRNA.

Figure 3. HCC-associated miRNAs. HCC, hepatocellular carcinoma; miRNA/miR, microRNA.

Figure 4. Prediction of transcription factors for the lncRNAs MALAT1 and HOTAIR. lncRNAs, long non-coding RNAs; HOTAIR, HOX antisense intergenic RNA; MALAT1, metastasis-associated lung adenocarcinoma transcript 1.

Prediction of the regulatory association between transcription factors and target lncRNAs/miRNAs. The transcription factors for lncRNAs (MALAT1 and HOTAIR) and miRNAs (hsa-miR-1 and hsa-miR-20a-5p) were separately predicted using ChIPBase. As presented in Fig. 4, a total of 78 and 17

transcription factors were identified for MALAT1 and HOTAIR, respectively. As presented in Fig. 5, a total of 13 and 59 transcription factors were identified for hsa-miR-1 and has-miR-20a-5p, respectively. A total of 7 overlaps were identified between hsa-miR-1 and has-miR-20a-5p (Fig. 5).
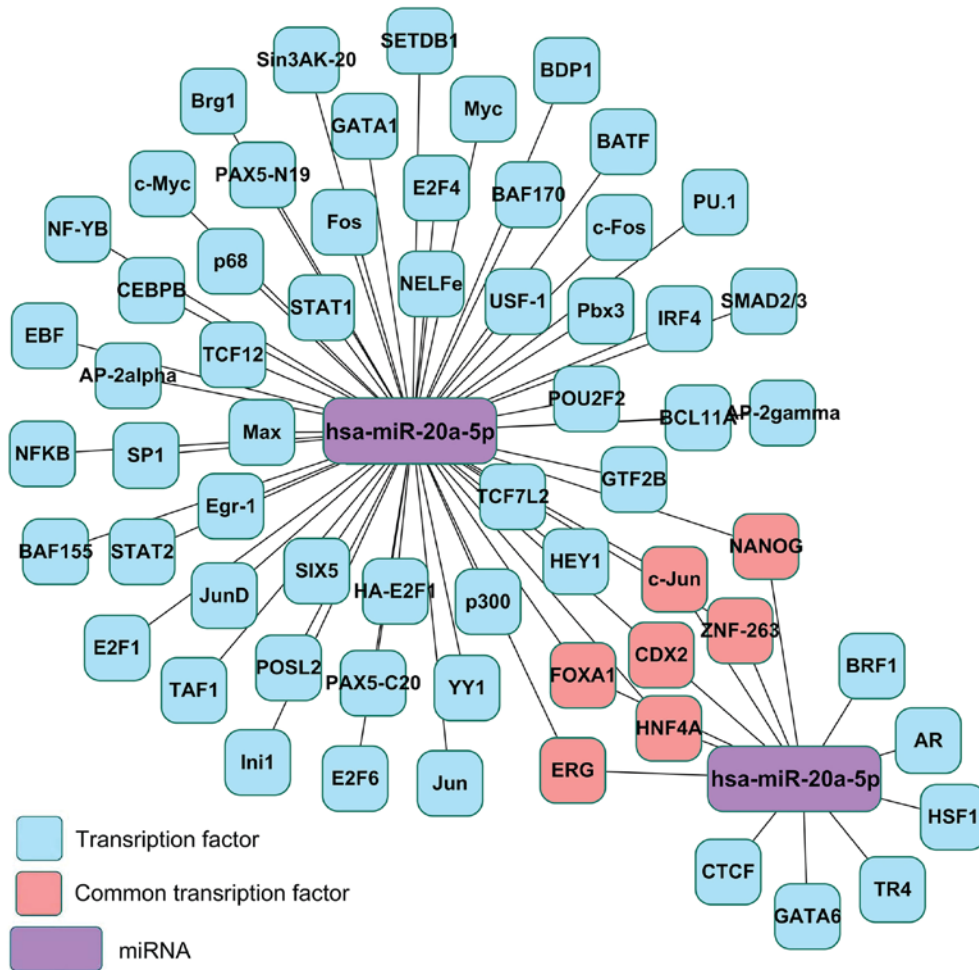
Figure 5. Prediction of transcription factors for the target miRNAs (hsa-miR-1 and hsa-miR-20a-5p). miRNA/miR, microRNA.
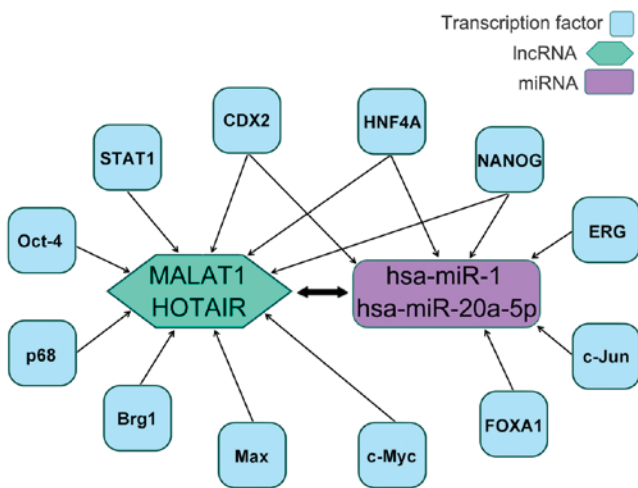

Figure 6. HCC-associated transcription factors for the target miRNAs and lncRNAs. miRNA/miR, microRNA; HCC, hepatocellular carcinoma; lncRNAs, long non-coding RNAs.

Identification of HCC-associated transcription factors. HCC-associated transcription factors were identified using PubMed. The results demonstrated that there were nine lncRNA transcription factors associated with HCC: Signal transducer and activator of transcription 1 (STAT1),

hepatocyte nuclear factor 4α (HNF4A), octamer-binding transcription factor 4 (Oct-4), Nanog homeobox (NANOG), caudal type homeobox 2 (CDX2), DEAD-box helicase 5 (p68), brahma-related gene 1 (Brg1), MYC-associated factor X (Max) and MYC proto-oncogene, BHLH transcription factor (c-Myc) (29-36). Additionally, CDX2, erythroblast transformation-specific-related gene (ERG), forkhead box protein A1 (FOXA1), HNF4A, c-Jun and NANOG were identified as the transcription factors for hsa-miR-1 and hsa-miR-20a-5p (30-32,37-39). CDX2, HNF4A and NANOG were identified to be common between the target lncRNAs and miRNAs (Fig. 6).

Identification of target genes for hsa-miR-1 and hsa-miR-20a-5p. The target genes for hsa-miR-1 and hsa-miR-20a-5p were predicted using the miRWalk database. A total of 879 and 101 target genes were identified for hsa-miR-1 and has-miR-20a-5p, respectively. A total of 32 overlaps were identified between these target miRNAs (Fig. 7).

Identification of the HCC-associated target genes for hsa-miR-1 and hsa-miR-20a-5p. The OncoDB.HCC database was used for the identification of the HCC-associated target genes for hsa-miR-1 and hsa-miR-20a-5p. As presented in

Table II, among the 32 target genes, 11 of them were involved in the progression of HCC. As miRNAs are single-stranded RNA molecules that bind to targets in a base pair-mediated manner, resulting in the degradation or inhibition of the expression and function of target genes, the expression of the target genes were inverse to that of the miRNAs (40), Then, seven target genes [cyclin D1, E2F transcription factor 1 (E2F1), epithelial growth factor receptor (EGFR), MYC, MET proto-oncogene, receptor tyrosine kinase (MET), nitric oxide synthase 2A (NOS2A) and vascular endothelial growth factor (VEGF)] with increased expression in HCC were selected for the further analysis.

GO and KEGG pathway enrichment analysis of the target genes. GO and KEGG pathway enrichment analysis of identified target genes was performed using DAVID. GO analysis revealed that six genes were involved in cellular functions such as cell growth, cycle and mitosis (Table III). Additionally, KEGG analysis revealed that six genes were involved in the cell cycle and focal adhesion, and in disease progression (Table IV). NOS2A was excluded from further analysis.

Prediction of the transcription factors for the HCC-associated miRNAs. The transcription factors for the HCC-associated miRNAs were predicted using ConSite (Fig. 8). The results demonstrated that snail family transcriptional repressor 1 (Snail) was a common transcription factor for the six target genes and was involved in the progression of HCC (Table V) (41).

Functional regulatory network of lncRNA in HCC. Fig. 9 presents the proposed regulatory network of lncRNAs in HCC.

Discussion

Following advances in proteomics and genomics, the function of numerous human ncRNAs has been investigated. On the basis of their length and biological function, ncRNAs can be divided into miRNAs, small nucleolar RNA, small nuclear RNAs, piwi-interacting RNAs, natural antisense transcripts, ribosomal RNAs, transfer RNAs and lncRNAs (42). A total of 270 lncRNAs have been documented in the lncRNAdb database (20). Several studies have demonstrated that lncRNAs may serve notable functions in cell physiological activities, in the development of various types of tumor and in epigenetics, transcriptional and post-transcriptional mechanisms (42-46).

Bioinformatics is a powerful tool for genomic research that facilitates the identification of candidate genes and nucleotide sequences, and predicts the biological functions and regulatory pathways of the target genes. At present, research into lncRNAs is limited. In the present study, HCC-associated lncRNAs (HOTAIR, MALAT1 and HULC) were identified using lncRNA disease database. According to data from the UCSC Genome Browser and lncRNA disease database, HOTAIR was located on human chromosome 12 (12649 bp) and exhibited a low expression in the liver, but increased expression in HCC (47). HOTAIR may inhibit the expression of interferon-γ and cell cycle-associated genes, thus promoting cellular invasion in HCC. Knockout of HOTAIR significantly decreased cellular proliferation and promoted apoptosis in HCC (48,49). The 3-year recurrence-free survival rate for patients with HCC that exhibited low expression of HOTAIR was significant increased compared
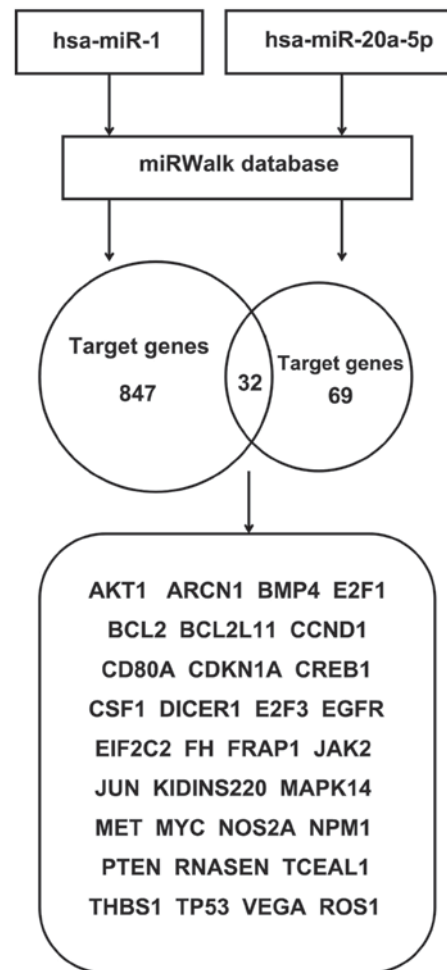

Figure 7. Shared target genes between hsa-miR-1 and hsa-miR-20a-5p obtained from the miRWalk database. miR, microRNA.

with that in patients with high expression of lncRNA following liver transplantation. Therefore, HOTAIR may be used as an independent indicator for tumor recurrence following liver transplantation (47). The results of the present study demonstrated that MALAT1 was located on human chromosome 11q13 (8708 bp), with a length of 870 bp. MALAT1 was identified in various tissues and exhibited increased expression levels in HCC (50). One previous study (51) demonstrated that MALAT1 may promote cellular proliferation in HCC, and patients with HCC with increased expression of MALAT1 exhibited an increased risk of tumor recurrence. Therefore, MALAT1 may be used for the prediction of HCC recurrence. MALAT1 may regulate the migration of HCC cells (52). The results of the present study demonstrated that HULC was located on human chromosome 6p24.3. The expression of HULC was increased in the liver but low in HCC tissues (53).

lncRNAs may interact with miRNAs (54). The results of previous studies have indicated that the interaction of lncRNAs and miRNAs serve a notable function in the progression of cancer (55-57). In the present study, starBase2.0 was used to predict the miRNAs that were associated with lncRNAs (HOTAIR, MALAT1 and HULC). However, no miRNA was identified to interact with HULC, so further analyses were performed on HOTAIR and MALAT1 only. A total of 11 overlaps were identified between HOTAIR and MALAT1, 9 of

Table II. HCC-associated miRNA target genes obtained from the OncoDB.HCC database.

Gene	Description	Evidence
CCND1	G1/S-specific cyclin-D1 (PRAD1 oncogene) (BCL-1 oncogene). [Source: Uniprot/SWISSPROT; Acc: P24385]	Arrays: C.R.01a, PNAS01, MBC 02 Experiments: upregulated (RT-PCR, IHC and western blotting)
E2F1	Transcription factor E2F1 (E2F-1) (Retinoblastoma-binding protein 3) (RBBP-3) (PRB-binding protein E2F-1) (PBR3) (Retinoblastoma-associated protein 1) (RBAP-1). [Source: Uniprot/SWISSPROT; Acc: Q01094]	Experiments: upregulated (RT-PCR)
EGFR	Epidermal growth factor receptor precursor (EC 2.7.10.1) (Receptor tyrosine-protein kinase ErbB-1). [Source: Uniprot/SWISSPROT; Acc: P00533]	Experiments: upregulated (IHC)
MYC	Myc proto-oncogene protein (c-Myc) (Transcription factor p64). [Source: Uniprot/SWISSPROT; Acc: P01106]	Arrays: PNAS01, Onc. 02, MBC 02 Experiments: upregulated (RT-PCR and IHC)
NOS2A	Nitric oxide synthase, inducible (EC 1.14.13.39) (NOS type II) (Inducible NO synthase) (Inducible NOS) (iNOS) (Hepatocyte NOS) (HEP-NOS). [Source: Uniprot/SWISSPROT; Acc: P35228]	Experiments: upregulated (RT-PCR)
VEGFA	Vascular endothelial growth factor A precursor (VEGF-A) (Vascular permeability factor) (VPF). [Source: Uniprot/SWISSPROT; Acc: P15692]	Experiments: upregulated (Northern blotting and IHC)
MET	Hepatocyte growth factor receptor precursor (EC 2.7.10.1) (HGF receptor) (Scatter factor receptor) (SF receptor) (HGF/SF receptor) (Met proto-oncogene tyrosine kinase) (c-Met). [Source: Uniprot/SWISSPROT; Acc: P08581]	Experiments: upregulated (RT-PCR and mutation)
CDKN1A	Cyclin-dependent kinase inhibitor 1 (p21) (CDK-interacting protein 1) (Melanoma differentiation-associated protein 6) (MDA-6). [Source: Uniprot/SWISSPROT; Acc: P38936]	Arrays: Gast01, Onc.02, J.H.03 Experiments: downregulated (IHC and RT-PCR)
NPM1	Nucleophosmin (NPM) (Nucleolar phosphoprotein B23) (Numatrin) (Nucleolar protein NO38). [Source: Uniprot/SWISSPROT; Acc: P06748]	Arrays: PNAS01, MBC02, CCR03, Prot05a, Prot05c
THBS1	Thrombospondin-1 precursor. [Source: Uniprot/SWISSPROT; Acc: P07996]	Experiments: downregulated (qPCR)
TP53	Cellular tumor antigen p53 (Tumor suppressor p53) (Phosphoprotein p53) (Antigen NY-CO-13). [Source: Uniprot/SWISSPROT; Acc: P04637]	Experiments: other (IHC, western blotting, mutation and methylation)

HCC, hepatocellular carcinoma; miR, microRNA; RT-PCR, reverse transcription-polymerase chain reaction; IHC, immunohistochemistry; qPCR, quantified polymerase chain reaction.

which were HCC-associated miRNAs according to the HMDD database. hsa-miR-1 and hsa-miR20a-5p were identified to be downregulated in HCC and selected for further analysis.

ChIPBase was used to predict the transcription factors for lncRNAs (MALAT1 and HOTAIR) and miRNAs (hsa-miR-1 and hsa-miR-20a-5p). The results demonstrated that 9 transcription factors for lncRNAs (STAT1, HNF4A, Oct-4, NANOG, CDX2, p68, Brg1, Max and c-Myc) were associated with HCC. Additionally, CDX2, ERG, FOXA1, HNF4A, c-Jun and NANOG were identified to associate with the target miRNAs and were associated with the progression of HCC. Among them, CDX2, HNF4A and NANOG were identified as the transcription factors in common for these lncRNAs and miRNAs.

In the present study, a total of 32 target genes were identified for hsa-miR-1 and hsa-miR-20a-3p using miRWalk. Among them, 11 genes were involved in the development of HCC, according to the OncoDB.HCC database. A total of seven target genes with increased expression in HCC (cyclin D1, E2F1, EGFR, MYC, MET, NOS2A, VEGFA) were selected for the further analysis. Additionally, GO and KEGG enrichment analysis was performed on the seven HCC-associated miRNA target genes using DAVID. The results demonstrated that six genes were involved in various cellular functions, including cell growth, cell cycle progression and mitosis. KEGG analysis revealed that six genes were involved in the cell cycle, focal adhesion and disease progression

Snail was predicted to be the transcription factor in common among the six target genes and was involved in the progression of HCC according to data from ConSite. A previous study demonstrated that upregulation of Snail may promote the proliferation and invasion of HCC cells (58).

Table III. GO enrichment analysis for the seven HCC-associated miRNA target genes.

GO ID	Term	Genes	Percentage[a]	P-value	Benjamini[b]
GO:0008283	Cell proliferation	E2F1, EGFR, MET, MYC, VEGFA	83.3	5.20×10^{-6}	2.40×10^{-3}
GO:0051726	Regulation of cell cycle	E2F1, CCND1, EGFR, MYC	66.7	1.40×10^{-4}	3.20×10^{-2}
GO:0008284	Positive regulation of cell proliferation	CCND1, EGFR, MYC, VEFA	66.7	2.70×10^{-4}	4.10×10^{-2}
GO:0044093	Positive regulation of molecular function	CCND1, EGFR, MET, MYC	66.7	7.60×10^{-4}	4.20×10^{-2}
GO:0009891	Positive regulation of biosynthetic process	E2F1, EGFR, MYC, VEGFA	66.7	1.20×10^{-3}	4.30×10^{-2}
GO:0022402	Cell cycle process	E2F1, CCND1, EGFR, MYC	66.7	6.80×10^{-4}	4.40×10^{-2}
GO:0051325	Interphase	E2F1, CCND1, EGFR	50	6.00×10^{-4}	4.50×10^{-2}
GO:0007346	Regulation of mitotic cell cycle	CCND1, EGFR, MYC	50	1.20×10^{-3}	4.60×10^{-2}
GO:0031328	Positive regulation of cellular biosynthetic process	E2F1, EGFR, MYC, VEGFA	66.7	1.20×10^{-3}	4.90×10^{-2}

[a]Percentage of target genes from current functional annotation that are total target genes; [b]Benjamini-corrected P-value. HCC, hepatocellular carcinoma; miR, microRNA; GO, gene ontology.

Table IV. KEGG pathway enrichment analysis for the seven HCC-associated miRNA target genes.

KEGG ID	Term	Genes	Percentage[a]	P-value	Benjamini[b]
hsa05219	Bladder cancer	E2F1, CCND1, EGFR, MYC, VEGFA	83.3	2.00×10^{-8}	7.00×10^{-7}
hsa05200	Pathways in cancer	E2F1, CCND1, EGFR, MET, MYC, VEGFA	100	1.10×10^{-6}	1.90×10^{-5}
hsa05218	Melanoma	E2F1, CCND1, EGFR, MET	66.7	2.60×10^{-5}	3.00×10^{-4}
hsa05212	Pancreatic cancer	E2F1, CCND1, EGFR, VEGFA	66.7	2.70×10^{-5}	2.30×10^{-4}
hsa05210	Colorectal cancer	CCND1, EGFR, MET, MYC	66.7	4.20×10^{-5}	3.00×10^{-4}
hsa04510	Focal adhesion	CCND1, EGFR, MET, VEGFA	66.7	5.70×10^{-4}	3.30×10^{-3}
hsa05213	Endometrial cancer	CCND1, EGFR, MYC	50	1.00×10^{-3}	5.00×10^{-3}
hsa05223	Non-small cell lung cancer	E2F1, CCND1, EGFR	50	1.10×10^{-3}	4.70×10^{-3}
hsa05214	Glioma	E2F1, CCND1, EGFR	50	1.50×10^{-3}	5.70×10^{-3}
hsa05220	Chronic myeloid leukemia	E2F1, CCND1, MYC	50	2.10×10^{-3}	7.30×10^{-3}
hsa05222	Small cell lung cancer	E2F1, CCND1, MYC	50	2.60×10^{-3}	8.30×10^{-3}
hsa05212	Prostate cancer	E2F1, CCND1, EGFR	50	2.90×10^{-3}	8.50×10^{-3}
hsa04110	Cell cycle	E2F1, CCND1, MYC	50	5.70×10^{-3}	1.50×10^{-2}

[a]Percentage of target genes from current functional annotation that are total target genes; [b]Benjamini-corrected P-value. HCC, hepatocellular carcinoma; miR, microRNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Therefore, in the present study a complete regulatory network of lncRNAs in HCC was proposed, whereby each one of the participants was either directly or indirectly associated and whose deregulation may result in HCC progression. Nevertheless, further research is required to confirm these regulatory associations.

In the present study the function and regulatory network of HCC-associated lncRNAs (HOTAIR and MALAT1) was predicted using bioinformatics analysis to understand the molecular mechanisms underlying the progression of HCC.

Table V. Prediction of the overlapping transcription factors for the six target genes using ConSite.

Transcription factor	Sequence	From (position)	To (position)	Score
Hunchback	TTTTTTATGC	6,433	6,442	12.824
AML-1	TTTGTGGTT	2,631	2,639	12.626
c-REL	GGGGTTTTCC	8,856	8,865	12.159
E74A	CCGGAAG	6,379	6,385	10.755
Snail	CAGGTG	2,151	2,156	10.744
Thing1-E47	GGTCTGGCTT	1,529	1,538	12.164

TF, transcription factor; AML-1, acute myeloid leukemia protein-1; c-REL, proto-oncogene c-Rel; E74A, ETS transcription factor E74A.
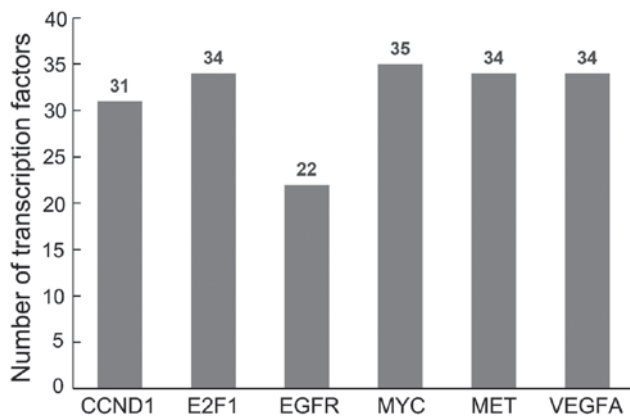
Figure 8. Prediction of the number of the transcription factors for the six target genes using ConSite. CCND1, cyclin D1; E2F1, E2F transcription factor 1; EGFR, epithelial growth factor receptor; MYC, MYC proto-oncogene, bHLH transcription factor; MET, MET proto-oncogene, receptor tyrosine kinase; VEGFA, vascular endothelial growth factor A.

Availability of data and materials

The data that support results of the present study are available from lncRNA disease database, starBase, HMDD, ChIPBase, PubMed, miRWalk, OncoDB.HCC, DAVID and ConSite (webpages cited in-text).

Authors' contributions

MRC drafted the article. ZPH, JML and JBZ performed the experiments. MRC and ZPH analysed the data and interpreted the results. YBL produced the figures and tables. MRC and ZPH revised the article. JHH and YGL conceived and designed the study. All authors read and approved the final article.

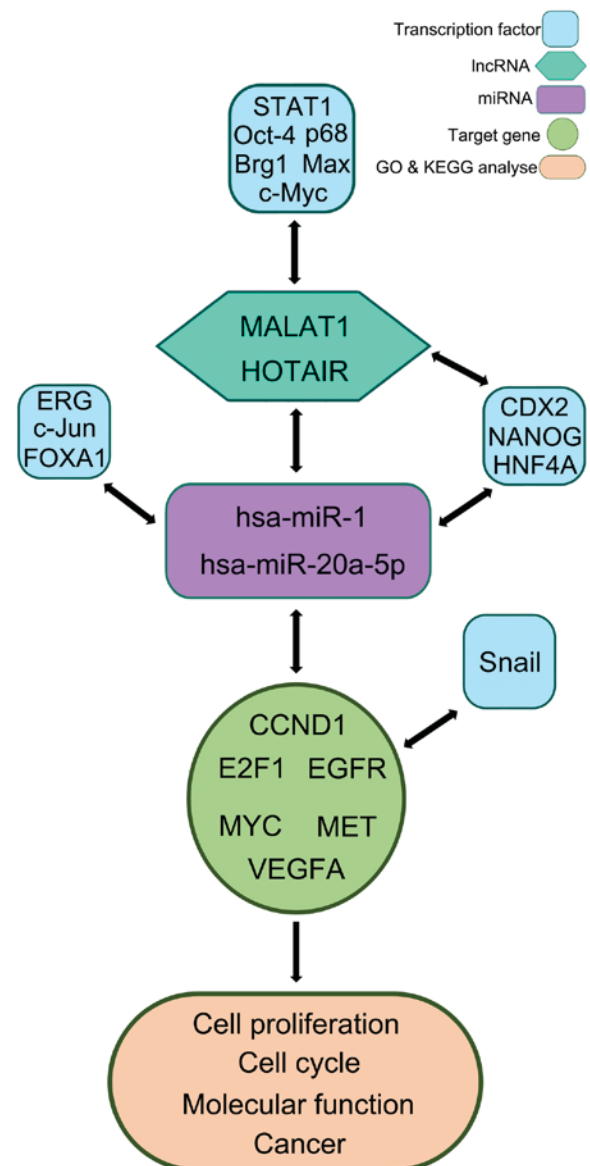**Ethics approval and consent to publish**

Not applicable.

Figure 9. Proposed functional regulatory network of lncRNA in HCC. miR, microRNA; HCC, hepatocellular carcinoma; lncRNA, long non-coding RNA.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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