Computational identification of microRNAs and their targets in liver cirrhosis

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Abstract. Previous studies have revealed that the deregulation of circulating miRNAs is associated with liver cirrhosis. The present study aimed to identify reliable candidate biomarkers to improve the early detection of liver cirrhosis. An integrated analysis of expression profiles of microRNAs (miRNAs/miRs) and mRNAs in liver cirrhosis tissues from the GEO database was performed. Next, the regulatory targets of the differentially expressed miRNAs in liver cirrhosis tissues were predicted. In addition, a regulatory network of miRNA-target genes was constructed. A total of 4 eligible mRNA expression profiling studies and 2 miRNA expression profiling studies met the inclusion criteria, and were thus included. A total of 48 differentially expressed miRNAs and 1,773 differentially expressed genes were identified in liver cirrhosis tissues compared with normal tissues. There were 240 miRNA-target pairs whose expression was negatively correlated. In the miRNA-target regulatory network, overexpression of miR-21 and miR-199a-3p was suggested to be closely associated with the progression of liver cirrhosis. In addition, functional enrichment analysis of the target genes indicated that cell cycle was the most significantly enriched pathway, and the dysregulation of leukemia inhibitory factor, cancerous inhibitor of protein phosphatase 2A and retinoblastoma-associated protein 1 clearly suggested their importance in the development of liver cirrhosis. We hypothesized that miR-21 and miR-199a-3p may be promising non-invasive diagnostic biomarkers for the early diagnosis of liver cirrhosis. The miRNA-target regulatory network may provide additional insight into the current data regarding the role of miRNAs in liver cirrhosis.

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Introduction

Liver cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous connective tissue in response to chronic liver disease (1). Generally, liver cirrhosis is asymptomatic and unsuspected. Liver biopsy is considered as the gold standard for diagnosis of cirrhosis, followed by assessment of risk progression according to sequential histological grading of inflammation and staging of fibrosis. However, biopsy is prone to considerable sampling variability in all liver diseases (2-5). Considering the limitations of liver biopsy, developing a reliable non-invasive and convenient method for liver cirrhosis has become an urgent requirement for early diagnosis (6-8).

MicroRNAs (miRNAs/miRs) are a class of evolutionarily conserved small (18-24 nucleotides) single-stranded non-coding RNAs, which regulate target gene expression at the post-transcriptional level by the degradation of target mRNA or the suppression of mRNA translation subsequent to its specific binding to target mRNA (9,10). In addition, miRNAs are highly stable in serum and plasma, providing the possibility of evaluating circulatory miRNA as a biomarker (11,12). Dysregulated miRNA expression has been reported in nearly all types of human cancer, including hepatocellular carcinoma (HCC) (13-15). Several miRNAs were identified as potential biomarkers for liver injury (16-20). A previous study also identified frequent and extensive dysregulation of miRNA in liver adenoma and cirrhosis, and found that different liver cancer stages may affect the dysregulation of miRNA expression (21).

Expression microarray and next-generation sequencing may reveal more differentially regulated miRNAs. However, due to the heterogeneity of the disease, variations of sample sources and diversity of analysis methods, the results of these studies are inconsistent (13). In the present study, the miRNA and mRNA expression profiles for liver cirrhosis tissues from the Gene Expression Omnibus (GEO) database were integrated. In addition, differentially expressed miRNA target genes were predicted, and a miRNA-target regulatory network was constructed. Certain key miRNAs were established

as biomarkers for diagnosis, prognosis and prediction of therapeutic response.

Materials and methods

Gene expression profiles. The miRNA and mRNA expression profiles for liver cirrhosis tissues were obtained from the public repository of GEO database (http://www.ncbi.nlm.nih.gov/geo) (22). The following key words were used: 'Liver cirrhosis' and '*Homo sapiens*'. Previous studies that compared gene expression profiling between liver cirrhosis and normal tissues or cultured cells were included in the present study. The type of study was defined as 'expression profiling by array' or 'non-coding RNA profiling by array'.

Screening of differentially expressed miRNAs and mRNAs. Raw data were preprocessed via background correction and normalization. The Limma package in R (23) was used to identify the differentially expressed miRNAs and mRNAs between liver cirrhosis tissues and controls with an unpaired t-test, then a P-value was calculated. P-values from multiple studies were combined using Fisher's exact test, and the random effects model was used to combine effect size from multiple studies. The miRNAs and mRNAs with a false discovery rate of <0.01 were regarded as differently expressed miRNAs and mRNAs.

Identification of regulatory targets for differently expressed miRNA. As the miRNAs recognize their regulatory targets through base pairing, computational methods have been invaluable for identifying these targets. The target genes for human miRNAs were downloaded from miRTarBase (http://mirtarbase.mbc.nctu.edu.tw), and the transcriptional targets of the identified miRNAs in liver cirrhosis tissues were predicted. In the present study, the differentially expressed target genes whose expression was inversely correlated with that of miRNAs were subjected to subsequent study. The miRNA-target gene interaction network in liver cirrhosis tissues was then constructed, and the network was visualized using Cytoscape (http://www.cytoscape.org) (24).

Functional enrichment analysis of differentially expressed target genes. The Database for Annotation, Visualization and Integrated Discovery (25) is the most common tool to analyze the functional enrichment of genes. To gain insights into the biological functions of miRNA target genes, Gene Ontology (GO) (26) classification and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (27) enrichment analysis were performed. P<0.05 was selected as the cutoff criterion.

Clinical specimens. The blood samples were obtained from 5 patients with liver cirrhosis and 3 healthy volunteers. Written consent was obtained from all of the study participants, and the project was approved by the committee of Ditan Hospital (Beijing, China) for the screening, inspection and data collection from the patients.

RNA preparation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted using the RNAiso Plus reagent (Takara Bio, Inc., Otsu, Japan) according to the manufacturer's protocol. Total RNA (1 μg) was reverse-transcribed using a cDNA Synthesis kit (Beijing Transgen Biotech Co., Ltd., Beijing, China), and the resulting cDNA was used as the template for qPCR. qPCR was performed in a BIO-RAD IQ5 Real-Time PCR system with a PCR SYBR Green Master mix reagent kit (Beijing Transgen Biotech Co., Ltd.). Following an initial denaturing for 2 min at 94˚C, qPCR was performed with 45 cycles of 94˚C for 20 sec and 60˚C for 34 sec. All reactions were performed in triplicate. The results were analyzed using the Cq method, using Data Assist Software version 3.0 (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The human β-actin gene was used as an endogenous control. The sequences of the PCR primers used are provided in Table I. The universal primer used for miR-21, miR-199a-3p and U6 was obtained from the miRcute Plus miRNA qPCR Detection kit (SYBR-Green) (cat. no. FP411; Tiangen Biotech Co., Ltd.). Data were analyzed with a one-way analysis of variance and Tukey's test using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Differentially expressed miRNAs and mRNAs in liver cirrhosis tissues. In total, 4 mRNA expression profiling studies and 2 miRNA expression profiling studies that met the inclusion criteria were included. The characteristics of each study are summarized in Table II. Of these studies, 166 cases of liver cirrhosis and 64 controls were analyzed.

Following normalization of the original miRNA and mRNA expression datasets, a total of 48 differentially expressed miRNAs were identified, including 5 that were upregulated and 43 that were downregulated (Table III). Using integrated analysis, a set of 1,773 differentially expressed genes (DEGs) were identified in liver cirrhosis tissues compared with normal tissues, including 1,037 upregulated and 736 downregulated genes.

Regulatory network of miRNAs and targets in liver cirrhosis. The miRTarBase database was used to predict the putative transcriptional targets of upregulated or downregulated miRNAs in liver cirrhosis tissues. Subsequent to comparing the putative targets with DEGs in liver cirrhosis tissues, a total of 240 miRNA-target gene pairs, whose expression was inversely correlated, were obtained. According to the miRNA-target gene pairs, a miRNA-target gene regulatory network was constructed (Fig. 1). In the network, the top three miRNAs (miR-21, miR-18a and miR-375) regulated the majority of the target genes. Leukemia inhibitory factor (LIFR) was identified as a target of miR-21, whereas cancerous inhibitor of protein phosphatase 2A (KIAA1524) and E2F transcription factor 1 (E2F1) were identified as targets of miR-181b.

Functional enrichment analysis. GO enrichment analysis of all target genes was performed to understand their biological functions. It was identified that the significantly enriched GO terms for molecular functions were protein binding, nucleotide binding and DNA binding. The significantly enriched GO

Table I. Primers used in the present study.

Primers	Sequences (5'-3')
KIAA1524	
Forward	GCCACTCTGGGAAGCCATACTAAA
Reverse	GCAGCAGAAGGGTCACAAAACG
β-actin	
Forward	ACTTAGTTGCGTTACACCCTT
Reverse	GTCACCTTCACCGTTCCA
hsa-miR-21 forward	TAGCTTATCAGACTGATGTTG
hsa-miR-199a-3p forward	CCCAGTGTTCAGACTACCTGTTC
U6 forward	CTGCGCAAGGATGACACGCAAATT

hsa, *Homo sapiens*; miR, microRNA; KIAA1524, cancerous inhibitor of protein phosphatase 2A.

terms for cellular components were nucleus, cytoplasm and cytosol, while those for biological processes were mitotic cell cycle, cell division and DNA replication (Table IV).

The results of KEGG pathway enrichment analysis indicated that the most significantly enriched pathway was the cell cycle, which may have a significant effect on liver cirrhosis (Table V).

RT-qPCR validation. To validate the associated between miRNAs and their target genes in the regulatory network, the blood samples from 5 pairs of patients with liver cirrhosis and normal controls were used. hsa-miR-21 (P=0.1678), hsa-miR-199a-3p (P=0.0001) and KIAA1524 (P=0.0028) were selected for RT-qPCR. The RT-qPCR results indicated that the expression was consistent with the integrated analysis data (Fig. 2).

Discussion

An increasing number of studies have focused on the role of miRNAs in regulating cancer progression and metastasis, including proliferation, invasion, migration, angiogenesis and apoptosis (28,29). Emerging evidence suggests that circulating miRNAs may function as stable, reliable and non-invasive diagnostic biomarkers for cancer (30). In the present study, 2 miRNA expression profiling studies and 4 mRNA expression profiling studies of liver cirrhosis tissues were integrated, and a set of 48 differentially expressed miRNA and 1,773 DEGs were identified. By bioinformatics prediction, a total of 240 miRNA-target gene pairs were obtained, whose expression was inversely correlated. The miRNA-targets regulatory network was constructed for liver cirrhosis tissues. In the miRNA-targets regulatory network, it was identified that the top ten upregulated and downregulated miRNAs were hsa-miR-212, hsa-miR-21, hsa-miR-199a-3p, hsa-miR-142-5p, hsa-miR-142-3p, hsa-miR-132, hsa-miR-139-5p, hsa-miR-181b, hsa-miR-21* and hsa-miR-18a, and the majority of them had previously been identified to be closely associated with alcoholic liver

Table II. Characteristics of mRNA and miRNA expression profiles of liver cirrhosis.

Author	Year	Country	GEO ID	Platform	Samples (N:C)	PMID	(Refs.)
mRNA expression profile							
Yildiz *et al*	2013	Turkey	GSE17548	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	0:20	23691139	(50)
Archer *et al*	2009	USA	GSE17967	GPL571 [HG-U133A_2] Affymetrix Human Genome U133A 2.0 Array	0:47	19861515	(51)
Mas *et al*	2009	USA	GSE14323	GPL96 [HG-U133A] Affymetrix Human Genome U133A Array	19:41	19098997	(52)
Caillot *et al*	2008	France	GSE10356	GPL5215 INSERM *Homo sapiens* 14K array Liverpool3	24:21	19140229	(53)
miRNA expression profile							
Wojcicka *et al*	2014	Poland	GSE63046	GPL11154 Illumina HiSeq 2000	9:15	24875649	(54)
Vuppalanchi *et al*	2013	USA	GSE49012	GPL17470 Thermo Scientific Dharmacon microRNA human array	12:22	24058572	(55)

miRNA, microRNA. N, normal; C, cirrhosis.

Table III. List of differentially expressed miRNAs in liver cirrhosis.

miRNAs	Log (fold-change)	P-value
Upregulated miRNAs		
hsa-miR-212	4.45E-01	6.29×10^{-4}
hsa-miR-142-5p	9.23E-01	1.88×10^{-3}
hsa-miR-142-3p	9.39E-01	2.38×10^{-3}
hsa-miR-199a-3p	1.07E+00	8.38×10^{-4}
hsa-miR-21	1.09E+00	4.15×10^{-4}
Downregulated miRNAs		
hsa-miR-132	-9.37E-01	7.20×10^{-4}
hsa-miR-139-5p	-9.18E-01	4.80×10^{-4}
hsa-miR-181b	-8.57E-01	2.22×10^{-4}
hsa-miR-21*	-8.36E-01	3.55×10^{-4}
hsa-miR-18a	-8.31E-01	4.50×10^{-4}
hsa-let-7d	-8.30E-01	2.10×10^{-4}
hsa-miR-20b	-8.28E-01	4.43×10^{-4}
hsa-miR-660	-8.26E-01	4.15×10^{-4}
hsa-miR-376c	-8.16E-01	1.65×10^{-3}
hsa-miR-192;	-7.74E-01	1.99×10^{-4}
hsa-miR-377	-7.67E-01	3.60×10^{-3}
hsa-miR-18b	-7.67E-01	6.73×10^{-4}
hsa-miR-29c*	-7.66E-01	1.21×10^{-3}
hsa-miR-375	-7.66E-01	6.19×10^{-4}
hsa-miR-148b	-7.63E-01	5.17×10^{-4}
hsa-miR-127-3p	-7.62E-01	1.64×10^{-3}
hsa-miR-136*	-7.59E-01	5.34×10^{-4}
hsa-miR-200c	-7.59E-01	1.93×10^{-3}
hsa-miR-342-3p	-7.45E-01	5.11×10^{-3}
hsa-miR-923	-7.31E-01	8.61×10^{-4}
hsa-miR-151a-5p	-7.30E-01	7.01×10^{-4}
hsa-miR-191	-7.27E-01	1.06×10^{-3}
hsa-miR-450a	-7.14E-01	1.15×10^{-3}
hsa-miR-151a-3p	-7.13E-01	8.95×10^{-4}
hsa-miR-454	-6.99E-01	3.24×10^{-3}
hsa-miR-141	-6.97E-01	4.59×10^{-3}
hsa-let-7f-1*	-6.89E-01	2.58×10^{-3}
hsa-miR-204	-6.87E-01	2.84×10^{-4}
hsa-miR-128	-6.71E-01	3.74×10^{-3}
hsa-miR-30a*	-6.59E-01	4.98×10^{-3}
hsa-miR-92b	-6.46E-01	8.45×10^{-3}
hsa-miR-103	-6.30E-01	6.62×10^{-4}
hsa-miR-582-5p	-6.26E-01	2.76×10^{-3}
hsa-miR-455-5p	-6.23E-01	3.55×10^{-3}
hsa-miR-519e*	-6.13E-01	3.10×10^{-4}
hsa-miR-99a	-6.12E-01	7.53×10^{-3}
hsa-miR-22	-6.05E-01	2.12×10^{-3}
hsa-miR-122*	-5.79E-01	5.61×10^{-3}
hsa-miR-99b	-5.73E-01	1.43×10^{-3}
hsa-miR-552	-5.53E-01	5.33×10^{-3}
hsa-miR-219-1-3p	-4.92E-01	3.57×10^{-3}
hsa-miR-548a-3p	-3.93E-01	7.99×10^{-3}
hsa-miR-491-5p	-3.52E-01	4.61×10^{-3}

hsa, *Homo sapiens*; miR/miRNA, microRNA.

disease, hepatic fibrogenesis, liver cirrhosis or hepatocellular carcinoma (31-34).

Several miRNAs are involved in hepatic fibrogenesis (20,35,36). It was previously suggested that miR-21 was upregulated at the onset of fibrosis in the human liver, and that it may promote fibrogenic activation of fibroblasts (37,38) and be involved in the amplification of certain important cellular signaling pathways (39,40). A recent study also indicated that a significant elevation of hepatic miR-21 expression is associated with mitogen-activated protein kinase 3 signaling and epithelial-mesenchymal transition in liver fibrosis (34). The miR-199 family is associated with liver fibrosis. By comprehensive analysis, Murakami *et al* (41) identified that the high expression of 4 miRNAs (miR-199a, miR-199a*, miR-200a and miR-200b) were closely associated with the progression of liver fibrosis in humans and mice. Consistent with this, the present study revealed that miR-21 and miR-199a-3p were also significantly upregulated in liver cirrhosis tissues, implying that they may serve an important role in the progression of liver cirrhosis and that they may be promising biomarkers for the early diagnosis of liver cirrhosis.

A previous study demonstrated that the other two miRNAs, miR-142-3p and miR-142-5p, were significantly downregulated in HCC, and that they may cooperatively regulate cell movement (42). Conversely, the results of the present study indicated that miR-142-5p and miR-142-3p were significantly upregulated in liver cirrhosis tissues compared with normal tissues, implying that the expression pattern of these 2 miRNAs may be particular to liver cirrhosis, and that they may serve different roles in liver cirrhosis and HCC by a currently unknown mechanism.

In addition, Wang *et al* (43) identified that the expression of miR-181b may be induced by transforming growth factor-β1, which serves an important role in liver cirrhosis. miR-132 expression may be induced following alcohol intake, and it was previously shown to be increased in alcoholic liver disease (31). It was indicated that serum miR-18a was significantly higher in patients infected with hepatitis B virus (HBV) with HCC compared with healthy controls, and it may be used in discriminating HBV-associated HCC from chronic hepatitis or cirrhosis (32). Notably, in the present study, the aforementioned 3 miRNAs (miR-181b, miR-132 and miR-18a) were significantly downregulated in liver cirrhosis tissues compared with normal tissues, indicating that they may exhibit potential for diagnostic and therapeutic applications in liver cirrhosis.

In order to additionally investigate the role of miRNAs in liver cirrhosis, the transcriptional targets of the identified miRNAs in liver cirrhosis tissues were predicted, and several targets were noted, including LIFR, KIAA1524 and E2F1. It was observed that the LIFRβ subunit is intensely expressed in reactive bile ductductuules in the cirrhotic liver, in which bile duct proliferation occurs frequently (44). Previous studies have demonstrated that KIAA1524 is a cellular inhibitor of protein phosphatase 2A, which is an important tumor-suppressor protein, and that the inhibition of KIAA1524 may determine the apoptotic effects of erlotinib on HCC cells (45-47). An additional important target was E2F1, which is considered a novel fibrogenic gene, and was identified to be highly upregulated in human liver cirrhotic specimens (48). Additionally, experiments in

Table IV. Top 15 GO terms of differentially expressed microRNA target genes.

GO ID	GO term	Count	P-value	False discovery rate
Biological process				
GO:0000278	Mitotic cell cycle	25	5.14×10^{-21}	4.38×10^{-18}
GO:0051301	Cell division	20	1.17×10^{-15}	4.99×10^{-13}
GO:0006260	DNA replication	14	5.92×10^{-13}	1.68×10^{-10}
GO:0000082	G1/S transition of mitotic cell cycle	13	5.73×10^{-12}	1.22×10^{-9}
GO:0007067	Mitosis	13	1.42×10^{-10}	2.42×10^{-8}
GO:0000075	Cell cycle checkpoint	11	5.14×10^{-10}	7.29×10^{-8}
GO:0007049	Cell cycle	16	1.16×10^{-8}	1.42×10^{-6}
GO:0000086	G2/M transition of mitotic cell cycle protein kinase activity	9	3.69×10^{-8}	3.93×10^{-6}
GO:0000079	Regulation of cyclin-dependent	7	4.26×10^{-8}	4.03×10^{-6}
GO:0000077	DNA damage checkpoint	5	4.59×10^{-7}	3.91×10^{-5}
GO:0000236	Mitotic prometaphase	7	8.88×10^{-7}	6.31×10^{-5}
GO:0031100	Organ regeneration	6	8.27×10^{-7}	6.41×10^{-5}
GO:0000087	M phase of mitotic cell cycle	7	1.89×10^{-6}	1.24×10^{-4}
GO:0051726	Regulation of cell cycle	6	3.35×10^{-6}	2.04×10^{-4}
GO:0000083	Regulation of transcription involved in G1/S phase of mitotic cell cycle	4	5.85×10^{-6}	3.32×10^{-4}
Cellular component				
GO:0005634	Nucleus	99	4.92×10^{-27}	1.02×10^{-24}
GO:0005737	Cytoplasm	96	7.53×10^{-26}	7.79×10^{-24}
GO:0005829	Cytosol	51	1.68×10^{-17}	1.16×10^{-15}
GO:0005654	Nucleoplasm	32	6.06×10^{-16}	3.14×10^{-14}
GO:0005730	Nucleolus	30	5.18×10^{-9}	2.14×10^{-7}
GO:0005813	Centrosome	14	1.28×10^{-8}	4.41×10^{-7}
GO:0000785	Chromatin	8	1.87×10^{-8}	5.53×10^{-7}
GO:0005694	Chromosome	11	7.36×10^{-7}	1.90×10^{-5}
GO:0005856	Cytoskeleton	19	1.10×10^{-6}	2.28×10^{-5}
GO:0000776	Kinetochore	6	1.03×10^{-6}	2.38×10^{-5}
GO:0000775	Chromosome, centromeric region	6	1.57×10^{-6}	2.96×10^{-5}
GO:0000794	Condensed nuclear chromosome	4	2.38×10^{-5}	4.10×10^{-4}
GO:0000777	Condensed chromosome kinetochore	5	3.49×10^{-5}	5.56×10^{-4}
GO:0048471	Perinuclear region of cytoplasm	11	5.25×10^{-5}	7.76×10^{-4}
GO:0000922	Spindle pole	5	9.46×10^{-5}	1.31×10^{-3}
Molecular function				
GO:0005515	Protein binding	100	6.85×10^{-35}	2.18×10^{-32}
GO:0000166	Nucleotide binding	41	3.15×10^{-11}	5.00×10^{-9}
GO:0003677	DNA binding	31	1.26×10^{-7}	1.34×10^{-5}
GO:0005524	Adenosine triphosphate binding	26	1.32×10^{-6}	1.05×10^{-4}
GO:0019899	Enzyme binding	9	2.50×10^{-6}	1.59×10^{-4}
GO:0019904	Protein domain specific binding	8	6.99×10^{-6}	3.70×10^{-4}
GO:0016301	Kinase activity	9	1.10×10^{-5}	5.01×10^{-4}
GO:0019901	Protein kinase binding	8	1.05×10^{-4}	4.19×10^{-3}
GO:0043425	Basic helix-loop-helix transcription factor binding	3	1.39×10^{-4}	4.93×10^{-3}
GO:0042393	Histone binding	4	3.25×10^{-4}	1.03×10^{-2}
GO:0003700	Sequence-specific DNA binding transcription factor activity	15	4.07×10^{-4}	1.08×10^{-2}
GO:0042826	Histone deacetylase binding	4	4.00×10^{-4}	1.16×10^{-2}
GO:0051082	Unfolded protein binding	5	8.39×10^{-4}	2.05×10^{-2}
GO:0008565	Protein transporter activity	4	9.22×10^{-4}	2.09×10^{-2}
GO:0008139	Nuclear localization sequence binding	2	1.28×10^{-3}	2.54×10^{-2}

GO, gene ontology.

Table V. KEGG pathways of differentially expressed microRNA target genes.

KEGG ID	KEGG term	Count	False discovery rate
hsa04110	Cell cycle	15	1.47×10^{-13}
hsa05222	Small cell lung cancer	6	7.27×10^{-4}
hsa04115	Tumor protein 53 signaling pathway	5	2.13×10^{-3}
hsa00240	Pyrimidine metabolism	5	7.56×10^{-3}
hsa04114	Oocyte meiosis	5	1.31×10^{-2}
hsa00072	Synthesis and degradation of ketone bodies	2	1.85×10^{-2}
hsa03030	DNA replication	3	2.05×10^{-2}
hsa05215	Prostate cancer	4	2.15×10^{-2}
hsa03013	RNA transport	5	2.25×10^{-2}
hsa05162	Measles	5	2.32×10^{-2}
hsa04914	Progesterone-mediated oocyte maturation	4	2.42×10^{-2}
hsa05200	Pathways in cancer	7	3.61×10^{-2}

KEGG, Kyoto Encyclopedia of Genes and Genomes; hsa, *Homo sapiens*.

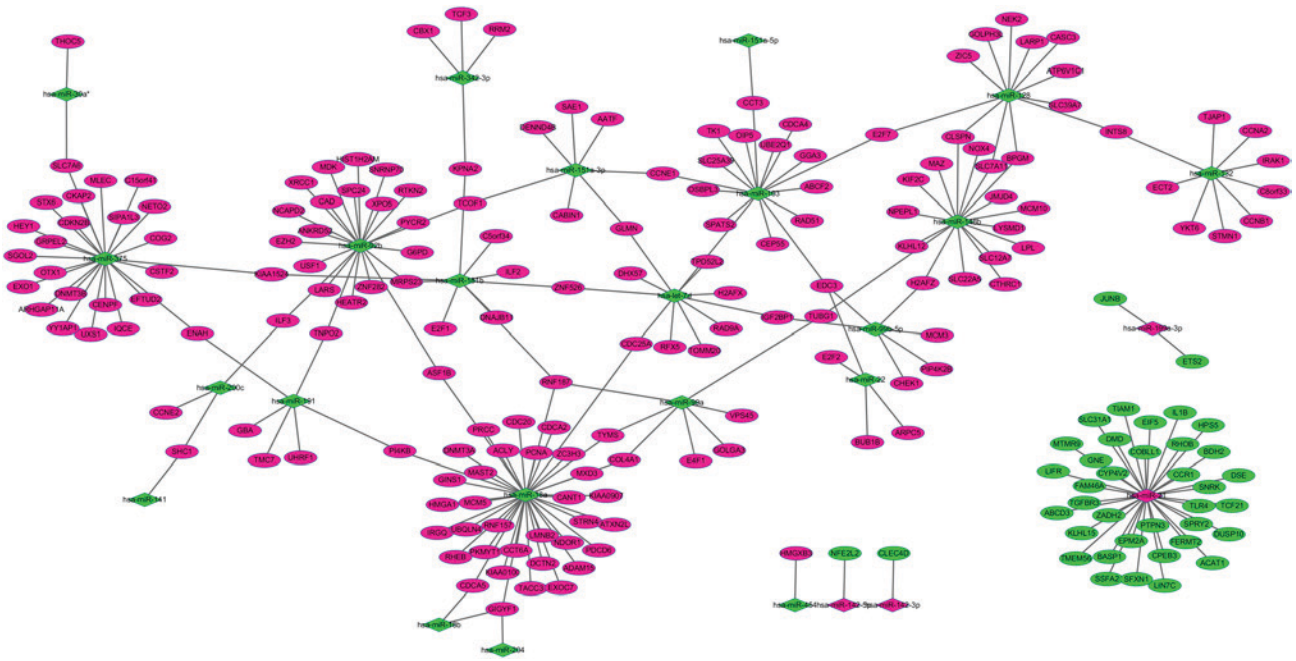

Figure 1. Regulatory network between miRNAs and target genes in liver cirrhosis tissues. The diamonds and ellipses represent the miRNAs and targets, respectively. The pink and green colors represent the relative upregulation and downregulation, respectively. miR/miRNA, microRNA; hsa, *Homo sapiens*.

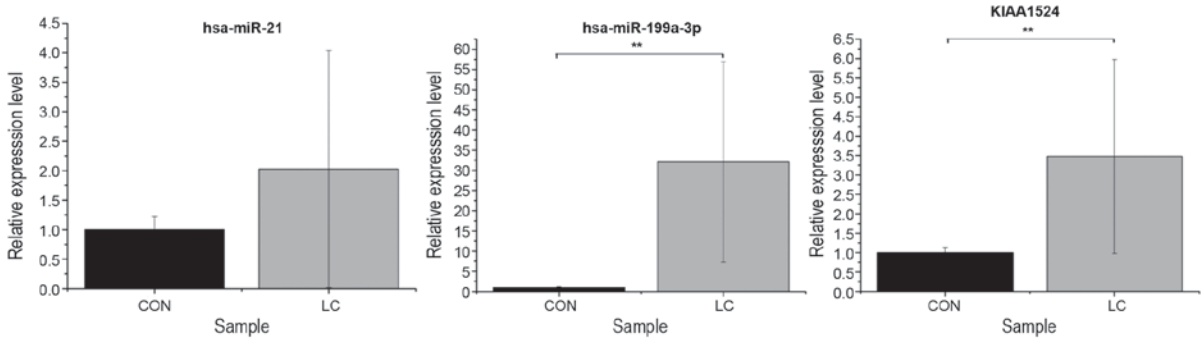

Figure 2. Reverse transcription-quantitative polymerase chain reaction validation of microRNAs and targets in 5 pairs of blood samples from patients with liver cirrhosis and healthy volunteers. **P<0.01. LC, liver cirrhosis; CON, control; hsa, *Homo sapiens*; miR, microRNA; KIAA1524, cancerous inhibitor of protein phosphatase 2A.

mice demonstrated that E2F1-deficiency may largely inhibit the development of biliary liver fibrosis (48). In the present study, the dysregulation of LIFR, KIAA1524 and E2F1 in the cirrhotic liver clearly suggested their importance in the development of liver cirrhosis. The results of the function enrichment analysis of the target genes indicated that the cell cycle was the most significantly enriched pathway. This result is consistent with the importance of the cell cycle in various types of cancer (49).

In conclusion, a set of 48 differentially expressed miRNAs and 1,773 DEGs were identified in liver cirrhosis tissues compared with normal tissues. A total of 240 miRNA-target gene pairs whose expression was inversely correlated were identified. Additionally, a global miRNAs-target regulatory network was constructed, which was expected to improve the understanding of the regulatory mechanisms of liver cirrhosis pathology, and to provide a clearer biomarker for diagnosis and safer therapeutic strategies of liver cirrhosis. Additional functional studies are required to confirm the regulative associations between the miRNAs and their predictive targets.

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