

Integrated analysis of differentially expressed mRNAs and miRNAs between hepatocellular carcinoma and their matched adjacent normal liver tissues

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Abstract. Hepatocellular carcinoma has a high mortality rate, thus, there is a need for improvement of prognosis of such patients. The aim of the present study was to identify differentially expressed mRNAs and miRNAs between hepatocellular carcinoma tissues and their matched adjacent normal liver tissues, and to carry out a bioinformatics analysis. Agilent 8x60K microarray technology was used to detect the changes of mRNA and miRNA expression between hepatocellular carcinoma tissues and their matched adjacent normal liver tissues. To select differentially expressed mRNAs and miRNAs, gene ontology (GO) and pathway analysis were performed using bioinformatics methods. qPCR was used to verify the microarray data. As a result, 924 mRNAs and 21 miRNAs exhibited a higher expression in the hepatocellular carcinoma tissue than their matched adjacent normal liver tissue. In comparison with the adjacent normal tissue, the carcinoma tissue showed a downregulated expression of 1,770 mRNAs and 12 miRNAs. The GO and pathway analysis showed that these RNAs were involved in the transcription process, REDOX, signal transduction, ion transport, immune response, cell adhesion and binding functions. A total of 572 target genes of 14 miRNAs were identified, most of which were involved in tumors. The results of qPCR were in concordance with the microarray results. In summary, the differentially expressed mRNAs and miRNAs that include signal transduction, immune response and many other key links may provide novel targets for early diagnosis and therapy of hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) is the third most frequent cause of cancer mortality, together with gastric and esophageal carcinoma. HCC is also the fifth most common solid cancer with >500,000 new patients diagnosed with HCC annually worldwide (1). A large number of patients are asymptomatic with early stage of HCC, and due to the lack of early diagnostic indicators, prognosis of HCC is poor with a 5-year survival rate (2). Surgery, liver transplantation, topical treatment and chemotherapy are currently the main treatments offered to HCC patients. However, resistance to drugs and recurrence lead to the inefficacy of conventional radiotherapy and chemotherapy (3,4). Thus, it is important to identify the molecular changes involved in HCC to improve the prognosis of HCC patients.

The regulation of gene expression involving miRNAs has attracted much attention (5-7). miRNAs are small endogenous non-coding RNA molecules of 18-24 nucleotides in length that can regulate a variety of biological processes (8-10). Since the first miRNA was identified in nematodes, several studies have shown that miRNAs are closely associated with various human diseases, especially the development of cancer (11-13). It has been suggested that miRNAs modulate tumors by affecting apoptosis, transcription process, REDOX, signal transduction, immune response, cell adhesion and other associated biological process (14-16). We predicted that some miRNAs may be involved in the invasion and metastatic processes of tumors and it is possible that confirmation of the involvement of these miRNAs may provide new biomarkers for the early diagnosis and specific-targeted gene therapy of HCC.

As previously identified, microarrays have been used for the detection of differentially expressed mRNAs or miRNAs (17-19). However, to the best of our knowledge, few reports focus on the correlation analysis of miRNA and mRNA expression. In the present study, we collected 30 HCC tissues and their matched adjacent normal liver tissues. Using Agilent 8x60K microarray technology, we detected the changes of mRNA and miRNA expression. A total of 2,694 mRNAs and 33 miRNAs were differentially expressed in the HCC tissues compared with their matched adjacent normal liver tissues. Subsequently, three mRNAs and three miRNAs were verified

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Table I. Clinicopathological characteristics of patients with hepatocellular carcinoma. [n (%)].

Parameters	Description	Minimum	Maximum	Reference values
Age (years)	46.3±10.37	26	67	
Gender				
Male	23 (76.67)			
Female	7 (23.33)			
Tumor number				
Single	26 (86.66)			
Multiple	4 (13.33)			
Tumor size (cm)	6.6±2.81	3	15	
AFP (ng/ml)				
≤400	11 (36.67)			
>400	19 (63.33)			
Hepatocirrhosis				
With hepatocirrhosis	12 (40)			
Without hepatocirrhosis	18 (60)			
ALB (g/l)	40.85±8.15	29.7	75	35-50
GLO (g/l)	30.45±6.69	20.9	47.1	25-35
ALT (U/l)	41.66±18.66	14	95	0-40
AST (U/l)	46.16±18.82	17	85	0-40
ALP (U/l)	64.16±18.75	33	99	25-135
T-BIL (μmol/l)	12.48±5.7	5.6	28.6	3.4-20.5
D-BIL (μmol/l)	4.72±2.33	1.3	10	0-6.8
I-BIL (μmol/l)	7.7±3.78	2.7	19	2-12

by qPCR in 30 HCC tissues and their matched adjacent normal liver tissues. The differentially expressed mRNAs and miRNAs were then analyzed by bioinformatics tools including the Kyoto Encyclopedia of Genes and Genomes (KEGG), GenMAPP and BioCarta. Our results may provide potential insight into the early diagnosis and specific-targeted gene therapy of HCC.

Materials and methods

Tissue samples. In the present study, 30 pairs of HCC tissues and their matched adjacent normal liver tissues were obtained between January 2012 and December 2013 from the Affiliated Tumor Hospital of Guangxi Medical University. The participants involved in the study provided written informed consent. None of the subjects had undergone chemotherapy or radiotherapy prior to surgery. All the tissues were diagnosed independently by pathologists after liver resection and were snap-frozen in liquid nitrogen and stored at -80°C immediately. The present study was approved by the Ethics Committee of the Tumor Hospital. The clinicopathological characteristics of the tissues are provided in Table I.

Total RNA extraction. Total RNA of tissues was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The extracted total RNA was purified with the NucleoSpin® RNA Clean-up kit (Macherey-nagel, Dueren Germany). The NanoDrop 2000

spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to ensure the quality of total RNA. Agarose gel electrophoresis confirmed the integrity of the extracted total RNA. RNA was stored at -80°C for the microarray assay and subsequent qPCR assays.

Gene microarray assay. The samples of high quality RNA were marked with Cy3 fluorescent dye, then they were hybridized using the Agilent 8x60K microarray and scanned using an Agilent G2565CA microarray scanner (both from Agilent Technologies, Santa Clara, CA, USA). To obtain hybridization images, Feature Extraction software was used to analyze the images and image signals were changed into digital signals. To filter differentially expressed genes, the original data were processed using GeneSpring GX software. A normalization process was carried out using the percentile shift method. The filtered differentially expressed genes with an absolute ≥2-fold change were identified and recorded. The bioinformatics analysis was carried out using bioinformatics tools including KEGG, GenMAPP and BioCarta.

miRNA microarray assay. High quality RNA was mixed with Cy3 fluorescent dye after dephosphorylation and then hybridized using the Agilent 8x60K microarray and scanned using the Agilent G2565CA microarray scanner to obtain the hybridization images. The Agilent Feature Extraction (v10.7) was used to analyze the data and these were normalized to obtain the

Table II. Primers of mRNA qPCR.

Gene	GenBank accession number	Primers
GAPDH	NM_002046	Forward: 5'-GCACCGTCAAGGCTGAGAAC-3' Reverse: 5'-TGGTGAAGACGCCAGTGGA-3'
GPC3	NM_001164617	Forward: 5'-CCTTTGCTGGAATGGACAAGAAC-3' Reverse: 5'-CAGCTCATGGAGATTGAACTGG-3'
CTHRC1	NM_138455	Forward: 5'-GAGTCCTGGACACCCAACACTACAAG-3' Reverse: 5'-AGCCGAAGTGAGCCACTGAA-3'
GLS2	NM_013267	Forward: 5'-CCATGTGGATCGCATCTTTGA-3' Reverse: 5'-CCACAGGTCTGGGTTTGACTTG-3'

differentially expressed genes using the Agilent GeneSpring software.

qPCR. To validate the reliability of the microarray data, a two-step reaction process was used to detect the levels of differentially expressed genes including three mRNAs and three miRNAs that may be associated with the development of HCC. For the mRNA qPCR, 1 μ g of total RNA per sample was reverse transcribed into cDNA using the PrimeScript RT reagent kit (Takara Biotechnology, Dalian, China) according to the manufacturer's instructions. The products of RT were analyzed using an Agilent MX 3000 with the SYBR[®] Premix Ex Taq[™] II (Takara Biotechnology) according to the manufacturer's instructions. Each sample was analyzed in triplicate. *GAPDH* was used as the housekeeping gene, and the primers of real-time qPCR were designed and produced by Takara Biotechnology. The primer sequences are shown in Table II. For the miRNA real-time qPCR, 1 μ g of total RNA per sample was reverse transcribed into cDNA with miScript II RT kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The products of RT were analyzed using an Agilent Mx3000 with the miScript SYBR-Green PCR kit (Qiagen) according to the manufacturer's instructions. Each sample was analyzed in triplicate. U6 was used as the housekeeping gene, and the qPCR primers were designed and produced by Qiagen. qPCR reactions results were calculated using the $2^{-\Delta\Delta CT}$ method (20).

Results

Total RNA quality control. An RNA purity of $2.0 \geq A_{260}/A_{280} \geq 1.80$ and RNA mass of $\geq 1 \mu$ g were used for the microarray experiments. The integrity of the total RNA was examined by agarose gel electrophoresis. The results showed that the band of sample 3 was clear, with a brightness of 28S:18S rRNA $> 2:1$, and that the sample was within the limits allowed for the microarray experiment. The band of the other samples was also clear, with a brightness of 28S:18S rRNA $> 1:1$, and the sample was within the limits allowed for the microarray experiment (Fig. 1).

Analysis of differentially expressed mRNA. An mRNA expression profiling analysis was carried out between HCC tissues and

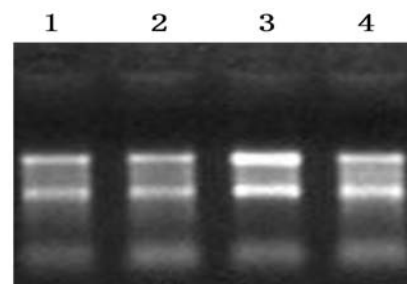


Figure 1. Agarose gel electrophoresis of total RNA (lanes 1 and 2 show hepatocellular carcinoma tissues, while lanes 3 and 4 show the adjacent normal liver tissues).

their matched adjacent normal liver tissues. A differential level ≥ 2.0 -fold change was regarded as significant. The expression levels of 2,694 mRNAs did not change significantly. Of these mRNAs, 924 mRNAs were upregulated and 1,770 were down-regulated in HCC tissues and their matched adjacent normal liver tissues (Fig. 2A). To conduct the analysis of gene function in detail, an analysis for the differentially expressed genes was performed. Several biological processes, molecular functions and cell components were taken into consideration. Concerning biological processes, transcription oxidation-reduction potentials, signal transduction, ion transport, immune response and cell adhesion can be involved. Regarding molecular functions, the processes of binding, protein binding, nucleotide binding, ion binding, ATP binding, transfer activity, oxidation-reduction activity and electron carrier activity were considered. Cell components, the extracellular region, cytoplasm, plasma membrane, endoplasmic reticulum and mitochondria were also considered to play a role (Fig. 3). Previous studies (28,29) showed that signaling pathways are closely associated with the development of HCC. Bioinformatics analysis was performed via analysis of the signaling pathway data using the KEGG, GenMAPP and BioCarta databases. As a result, the data suggested that the of Wnt, Jak-STAT, MAPK, ERBB2, mTOR and VEGF signaling pathways were involved in the development of HCC (Table III).

Analysis of differentially expressed miRNA. A miRNA expression profiling analysis was carried out between HCC tissues and their matched adjacent normal liver tissues. A total of 33 miRNAs were altered significantly, of which 21

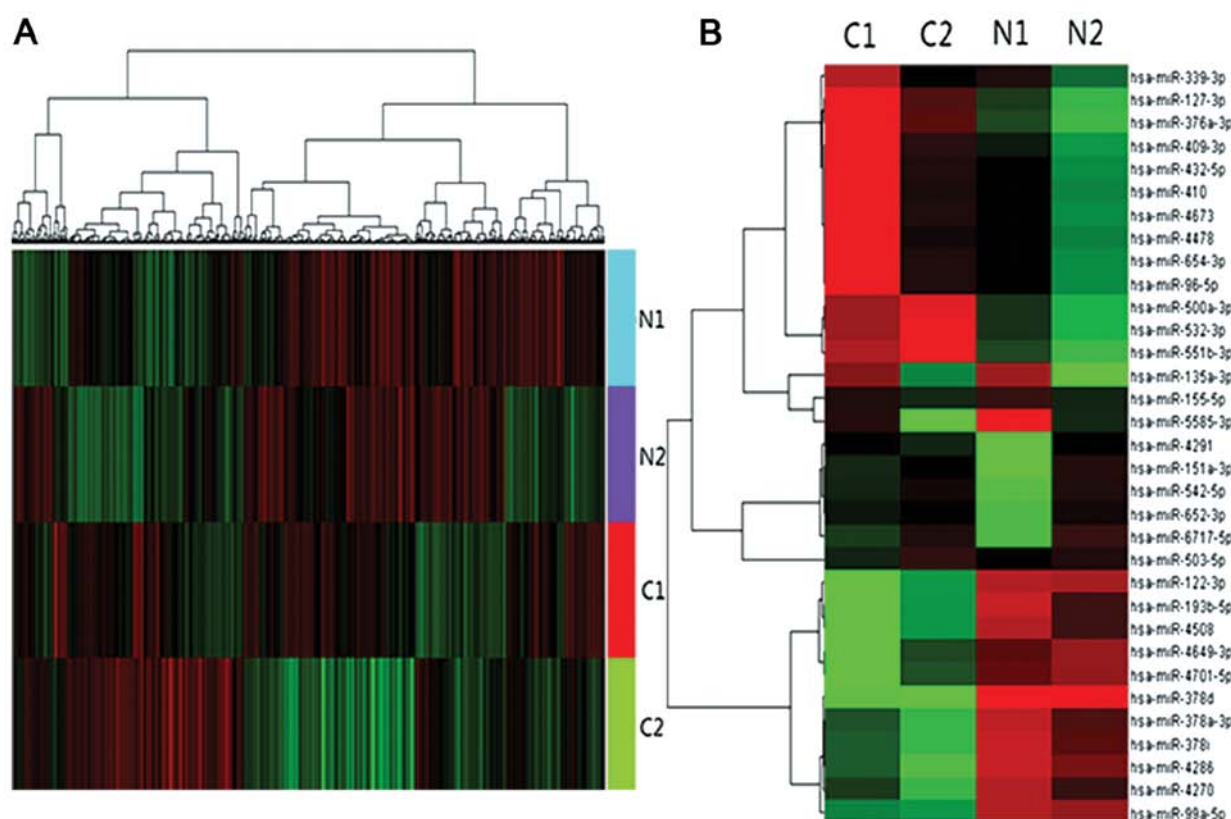


Figure 2. Differentially expressed mRNAs and miRNAs between hepatocellular carcinoma tissues and their adjacent normal liver tissues. (A) mRNA and (B) miRNA expression. C1 and C2 are hepatocellular carcinoma tissues, while N1 and N2 are their adjacent normal liver tissues. The colors show differentially expressed levels above, equal and below the mean (red, black and green, respectively).

Table III. Differentially expressed genes involved in Wnt signaling pathway in microarray analysis.

GenBank accession no.	Gene symbol	Fold change	Regulation direction
NM_003508	FZD9	2.21	Downregulation
NM_006238	PPARD	2.38	Upregulation
NM_002739	PRKCG	2.47	Downregulation
NR_028062	PRKY	2.63	Upregulation
NM_001198531	TCF7L2	3.12	Downregulation
NM_005052	RAC3	3.42	Downregulation
NM_001198531	TCF7	3.62	Downregulation
ENST00000394822	PPP3CB	3.94	Upregulation
NM_003506	FZD6	4.84	Upregulation
NM_172082	CAMK2B	4.99	Downregulation
NM_002752	MAPK9	6.75	Upregulation
NM_016269	LEF1	8.96	Upregulation
NM_003014	SFRP4	14.41	Upregulation
NM_014420	DKK4	16.91	Downregulation
NM_003015	SFRP5	54.29	Downregulation

miRNAs were upregulated and 12 downregulated in the HCC tissues compared with their matched adjacent normal liver tissues (Fig. 2B). The results of the mRNA gene microarray and miRNA microarray showed that differentially expressed mRNAs and miRNAs may be involved in the development of

HCC. In previous studies, mRNA was often found to be negatively regulated in relation to miRNA (39,40). We predicted the target genes of 14 differentially expressed miRNAs by DIANA microT v3.0. As a result, 1,288 target genes from 14 miRNAs were obtained. To confirm these results, we compared the

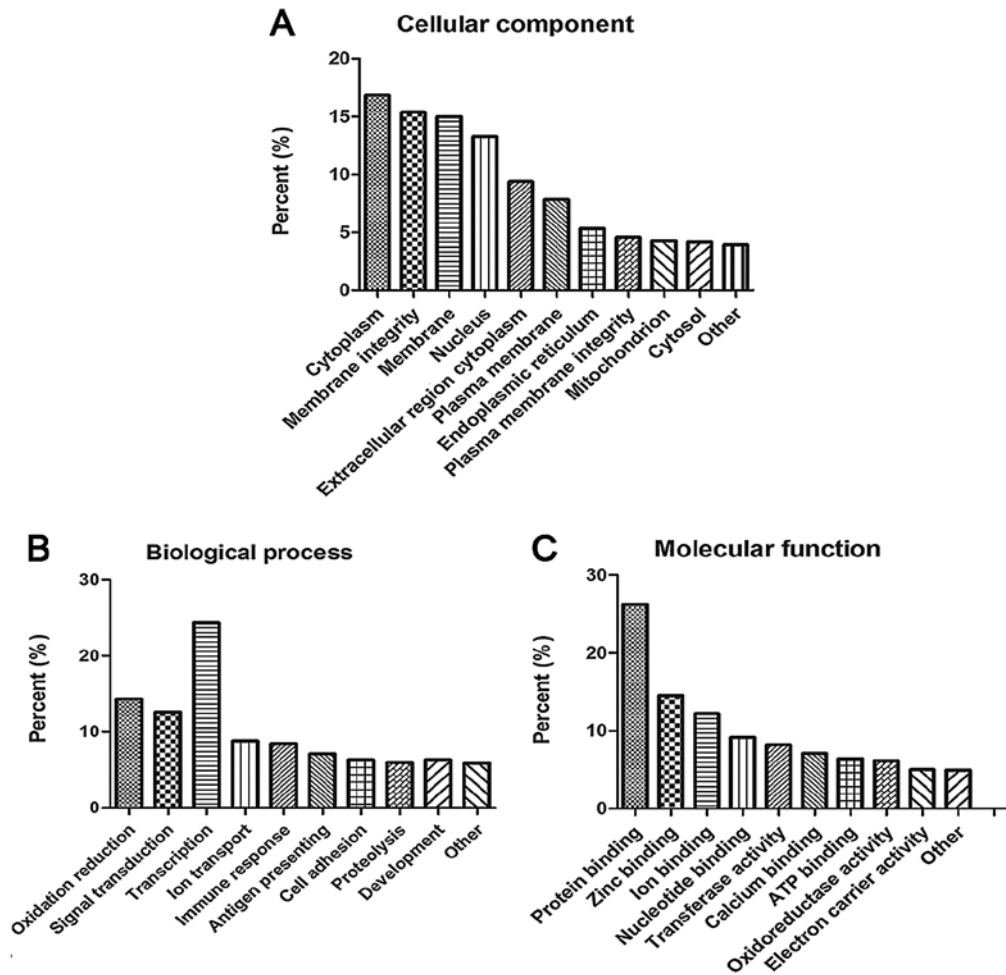


Figure 3. GO analysis of differentially expressed genes. (A) Cell components, (B) biological processes and (C) molecular functions.

obtained target genes with the results of the microarray. A total of 572 target genes were identified that were differentially expressed in the microarray (Table IV). qPCR was then performed to confirm the obtained target genes.

Validation of microarray data. To validate the microarray results, three mRNAs and three miRNAs were selected from the differentially expressed genes and miRNAs in the HCC tissues as compared with their matched normal liver tissues by qPCR were assessed. The results indicated that GPC3, CTHRC1, miR-155 and miR-96 were significantly upregulated, whereas GLS2 and miR-99a were significantly downregulated, which was consistent with the microarray results (Fig. 4).

Discussion

To the best of our knowledge, few studies have focused on the correlation analysis of miRNA and mRNA expression and the prediction of miRNA target genes. In the present study, we identified 2,694 mRNAs and 33 miRNAs that were differentially expressed between the HCC tissues and their matched adjacent normal liver tissues by microarray. Most of our identified mRNAs and miRNAs were also found to be involved in many biological processes, especially in the development and progression of tumors. miR-96 can be increased

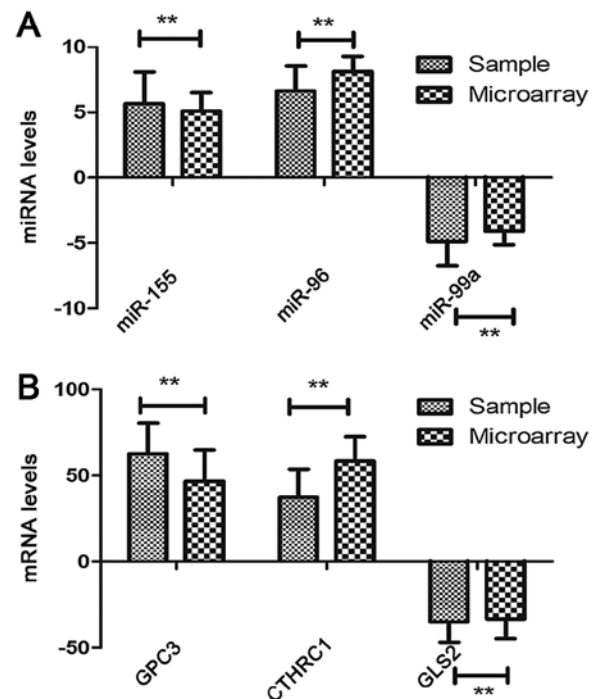


Figure 4. qPCR results of samples comparison with microarray (A) miRNA changes and (B) mRNA changes. **P<0.01.

Table IV. Target genes of 14 differentially expressed miRNA.

miRNA microarray		Gene microarray	
miRNA	Expression	Target genes	Expression
hsa-miR-127	Upregulation	C7orf10, GADD45G, LRRC27, SMPD3, SOX8, SYT7, TNFRSF10D, USP47, ZBTB7A	Downregulation
hsa-miR-96	Upregulation	ITPR2, FOXO1, PROSC, SDC2, TSKU, KLHDC9, ZNF577, SLC6A13, ACO1, FAM134B, OLFM1, PGM1, SNAI2, SLC1A2, GHR, KLF12, CBFA2T3, NCAM1, EPB41L4B, NRXN1, TMEM50B, TP53INP2, ACADSB, AKR1D1, AQP9, AVPI1, C1orf116, CAPN5, CASP10, CBLN3, CES3, CXorf38, CYP3A43, DBT, EFHA2, GIPR, HAPLN4, IGSF3, ITGB8, IYD, LPA, LZTS1, MASP1, MBP, MCART6, MMAB, NF1, PADI2, PIK3R1, PTC3, RASSF6, RCAN1, RNF43, SEC14L2, SH3BGRL2, SHMT1, SLC16A2, SLC23A2, SLC45A3, SLC47A1, SLC7A2, SMOC1, SPTB, USH2A, USP15, VAPA	Downregulation
hsa-miR-155	Upregulation	KALRN, VAPA, PCDH9, ZNF236, CYP2C9, EPB41L4B, GALC, HAL, KBTBD11, MCART6, SCAMP1, USH2A, MUT, CYR61, ACVR1C, SNX29, ETNK2, KPNA1, ACADSB, ACSM5, ARG1, ATF3, ATXN10, BCL2L15, BHMT2, CA13, CARD11, CASP10, EGFR, EML5, FOS, GABRP, GLS, GPD1, GRIA4, HCN3, IL28RA, MBP, MEIG1, NBPFF3, PEBP1, PIK3R1, PLN, PTC3, PTPRD, RCAN1, SH3RF2, SLC16A2, SLC1A2, SLC20A2, SLC7A2, SYT15, WNK3, ZNF577	Downregulation
hsa-miR-654	Upregulation	KLF12, MYO6, PPARGC1A, RNF128, USP15, ACADSB, AKR1D1, AQP9, ATXN10, BCKDHB, BCL2L15, BHMT, C1orf116, CAPN6, CASP10, CDC14B, CUX2, CXorf38, DBT, EFHA2, FOXO1, GLS, GRAMD1C, HEY2, HFE2, HLF, HNRNPA2B1, IL12RB2, ITGB8, KBTBD11, KPNA1, LPIN3, LRRC1, LRRK2, MBP, MCART6, MMAB, MSRA, MSRB2, NRXN1, OLFM1, PLN, PRRG4, PTPRD, RASSF6, RPL28, SCAMP1, SDC2, SEC14L3, SERPIND1, SH2D4A, SH3BGRL2, SLC1A2, SLC23A2, SLC2A2, SNX29, SPTB, SRD5A2, SSTR1, SULT1E1, TMC07, TUB, ZNF236, ZNF395	Downregulation
hsa-miR-339	Upregulation	BDH2, C10orf114, CROCC, GIPR, GREM2, KPNA1, KRBA2, PIK3R1, PSD3, RPL28, SALL3, SOX8, SSTR1, TRPV6	Downregulation
hsa-miR-432	Upregulation	MASP1, PTC3, SLC04C1, ADI1, ARMC5, PDE4DIP, SAMD4A, ABTB2, FAM134B, ITPR2, MACROD2, SPTB, ACVR1C, ANKS1A, CXCL2, GLYAT, KBTBD11, PPM1K, RPH3AL, SS18L1, TCF7, AQP9, BHMT2, BZRAP1, CDC14B, CES3, CUX2, CXCL12, CXCL14, CYP2B6, DBT, EFHA2, EGFR, EPB41L4B, FAM123B, FMO4, FOS, FOXO1, GABRP, GCHFR, GHR, GIPR, IGSF3, ITGB8, KMO, LNP1, LRRC1, LZTS1, NCAM1, NF1, PTPRD, RCL1, SH2D4A, SLC30A4, SLC7A2, SMPD3, TSKU, TSPAN12, TUB, VWCE, XPNPEP2, ZBTB7C	Downregulation
hsa-miR-410	Upregulation	ACADSB, AIM1, AMDHD1, EFHA2, EPB41L4B, HSD11B1, ITGB8, ITPR2, LRRK2, PLN, RGS16, SCAMP1, SLC1A2, SLC7A2, SRD5A1, TMEM50B, TNFRSF10D, ZNF577, ME1, ABCG2, ADI1, AQP9, ATM, BCL2L15, C1orf116, C7orf58, CA13, CMBL, CXCL12, CXCL14, DBT, FAM123B, FOSB, GALC, GFRA1, GIPC2, HS3ST3B1, IYD, KPNA1, MYO9A, NF1, OAT, PIK3R1, PSD3, PTPRD, PUS10, RASSF6, SH3BGRL2, SIVA1, SLC20A2, SLC30A4, SS18L1, SSTR1, TDRD6, TGDS, TSPAN12, USP15, VAPA, ZNF131, ZNF236	Downregulation
hsa-miR-99a	Downregulation	KBTBD8, CD93, RASA3, ST6GALNAC4	Upregulation

Table IV. Continued.

miRNA microarray		Gene microarray	
miRNA	Expression	Target genes	Expression
hsa-miR-409	Upregulation	EML5, ACVR1C, CYP3A4, KLF12, ME1, MPDZ, PTCD3, SALL3, SLC38A2, TUBE1, ASPA, EGFR, GREM2, ACADSB, ALDH5A1, BCL2L15, C7orf58, CACNA2D4, CAPN5, CXCL2, DBT, EFHA2, FAM123B, GALC, GHR, GRIA4, ITGB8, KCNB1, MBP, MCART6, MMAB, MMP19, PIK3R1, POU4F1, PPARGC1A, PTPN2, RASSF6, RNF128, SH2D4A, SH3RF2, SIRPB1, SLC30A4, SLC7A2, SNX29, TBX3, TDRD6, VAPA, WNK3, ZDHHC23, ZNF236, ZNF425	Downregulation
hsa-miR-542	Upregulation	IL28RA, USP15, DES, IER5L, DAK, FOSB, GIPR, GPR146, GUCA2B, HAPLN4, KLF12, MASP1, MORN1, MTPP, NF1, PKLR, RGS16, SH2D4A, SH3RF2, SLC16A2, SLC1A2, SMPD3, SNX29, SOX8, SRD5A2, SYT15, TP53INP2, VWA1	Downregulation
hsa-miR-652	Upregulation	KPNA1, LRRC1, BCL2L15, IL28RA, KCNB1, PPIL2, SNX29, TNFRSF10D	Downregulation
hsa-miR-551	Upregulation	ANKS1A, CACNA2D4, IYD, KBTBD11, UPB1	Downregulation
hsa-miR-532	Upregulation	CACNA2D4, CMBL, ETNK2, IL6R, KIAA1161, NF1, PRRG4, SH3RF2, SLC7A2, SMOC1, ZNF425, ABCA8, ACACB, ACADSB, ACO1, AKR1D1, ANKS1A, APOA5, AQP9, AZGP1, BDH2, C1orf116, C1orf187, C22orf36, CAPN6, CBLN3, CDC14B, CXCL12, CYP2A13, CYP3A4, DBT, DES, DIRAS3, FAM123B, FNDC5, FOSB, FOXO1, GCHFR, GLYAT, GPD1, GREM2, HAPLN4, HCN3, HLF, IGSF3, IYD, KANK4, KATNAL2, KCNK5, LPIN3, LRRC27, MBP, MMAB, MMP19, MSRA, NBPFF3, NCAM1, OASL, PI16, PIK3R1, POU4F1, PPARGC1A, PPP1R3F, PRKCG, PSD3, PTCD3, RCAN1, RGS16, SART1, SCAMP1, SEC14L2, SH3BGRL2, SHMT1, SLC20A2, SMPD3, SNX29, SOX8, SPTB, SS18L1, SYT7, TBCD, TCAP, TDRD6, TMEM105, TNFRSF10D, TNFRSF14, TRH, TUB, WFDC5, ZBTB7C, ZDHHC11, ZNF577	Downregulation
hsa-miR-503	Upregulation	C7orf58, GADD45G, CAPN6, PCDH9, SLC20A2, WNK3, ACADL, ALDH1A3, ANKS1A, C14orf180, CCDC25, IL28RA, ITGB8, PTCD3, SNX29, ACADSB, AIM1, BDH2, CACNA2D4, CBFA2T3, CDADC1, CDC14B, CIDEB, CLEC4G, EML5, EPB41L4B, FAM123B, GABRP, GALC, GHR, GPD1, GREM2, HAPLN4, IYD, KCNK5, LHPP, MBP, MCART6, MMAB, MPDZ, NF1, PIK3R1, PRKCG, PTPRD, RCL1, RNF43, RHBG, SALL3, SH3BGRL2, SLC7A2, SMPD3, TCF7, TUB, USH2A, ZDHHC23	Downregulation

with hypoxia in prostate cancer and stimulated autophagy by mTOR (21). Downregulation of miR-122 (22), miR-99a (23) and miR-193 (24) and upregulation of miR-127 (25) and miR-155 (26) have been reported to be involved in the development and progression of tumors. The results of the present study show that, miR-135a, miR-151a, miR-155, miR-96 and miR-410 were upregulated, and miR-122, miR-99a, miR-193b and miR-378a were downregulated in HCC tissues compared with their matched adjacent normal liver tissues. It is probable that the differentially expressed mRNAs and miRNAs may be involved in the development and progression of HCC.

Signaling pathways may be important in the development and progression of tumors (27-29). Thus, we carried out the bioinformatics analysis by analyzing the data of signaling

pathways using the KEGG, GenMAPP and BioCarta databases. As a result, the signaling pathways of Wnt, Jak-STAT, MAPK, ERBB2, mTOR and VEGF were found to be involved in the development of HCC. In the present study, several genes were found to be involved in signaling pathways, especially the Wnt signaling pathway. The Wnt signaling pathway is evolutionarily conserved and plays an important role in cell proliferation and embryonic development (30,31). The Wnt signaling pathway includes the signaling protein (Wnt protein), transmembrane receptors, cytoplasmic protein, transcription factors and related target genes. Activation of β -catenin can lead to activation of the Wnt signaling pathway (32,33). When there is no Wnt signal, GSK-3 β combines with APC and axin to phosphorylate β -catenin in the cytoplasm. β -catenin is then

degraded through the ubiquitin-proteasome pathway resulting in the Wnt signaling pathway being closed with a low level of β -catenin (34,35). The Wnt signaling pathway is activated when the above homeostasis was broken resulting in an excess of β -catenin in hepatocytes. β -catenin also binds to transcription factors such as TCF/LEF to form a heterodimer and this regulates related target genes such as MMP-9, cyclin D1, c-myc and COX-2 (36-38). Thus, β -catenin regulates hepatocyte proliferation through the above process.

Identification of miRNA target genes is crucial for the determination of biological functions of miRNAs (39,40). As predicted, 572 target genes from 14 miRNAs were identified that were differentially expressed in the microarray. To validate the results of microarray, three mRNAs and three miRNAs were subjected to qPCR. The results show that GPC3, CTHRC1, miR-155 and miR-96 were significantly upregulated, whereas GLS2 and miR-99a were significantly downregulated. The results of qPCR were in concordance with the microarray data. Of note, miR-155 was significantly upregulated and GLS2 was significantly downregulated in all the HCC tissues compared with their matched adjacent normal liver tissues. miR-155 has been regarded as a potential target for therapeutic intervention and acts as a ubiquitous oncogene in human cancers (41). Gao *et al* (42) found that a high expression of miR-155 potentially served as a valuable marker for non-small cell lung cancer patients in stage III. Gasparini *et al* (43) found that the expression level of miR-155 was significantly upregulated in breast cancer patients, and miR-155 was able to regulate DNA repair activity. In addition, the results of the study by Lao *et al* (44) showed that miR-155 regulated the expression of LKB1 to promote the proliferation of cervical cancer cells. According to the findings of Yang *et al* (45), miR-155 may be closely associated with tumor stage, and a high expression of miR-155 represented a new biomarker for rectal carcinoma. In the present study, upregulation of miR-155 indicated that it may be involved in the infinite proliferation of HCC cells. GLS2, the target gene of miR-155, has been known to play a significant role in energy metabolism. Giacobbe *et al* (46) found that GLS2 was upregulated as the target gene of TAp63 in colon adenocarcinoma, and the regulation mechanism of TAp63 and GLS2 was very important for physiological and pathological processes. The findings of Xiang *et al* (47) showed that GLS2 may be involved in glutamine metabolism, and they explored the potential mechanism of GLS2 in cervical carcinoma. Findings of those authors showed that GLS2 may play a significant role in cervical cancer patients. Zhang and his colleagues (48) considered that GLS2 played a significant role in energy metabolism, and was downregulated in HCC patients. Furthermore, they found that GLS2 probably promoted hypermethylation by epigenetic silencing, and that GLS2 seemed to be a tumor suppressor in colon adenocarcinoma and HCC patients. Martín-Rufián and his colleagues (49) found that the upregulation of GLS2 inhibited the induction of glioma cells, and ROS may inhibit cell migration by the overexpression of GLS2. The abovementioned studies suggest that miR-155 and its target gene GLS may be strongly linked with the proliferation and metastasis of HCC patients. Future studies should examine the potential mechanism of miR-155 and its target gene *GLS2*, which may provide reliable evidence for the specific-targeted gene therapy of HCC.

Our results have shown that, 15 differentially expressed genes were associated with the Wnt signaling pathway, in which 7 genes were upregulated and 8 genes downregulated. This suggests that the activation of these differentially expressed genes may lead to the infinite proliferation of HCC cells.

In the present study, we found 2,694 mRNAs and 33 miRNAs that were differentially expressed between HCC tissues and their matched adjacent normal liver tissues by microarray. The results of the GO and pathway analysis, biological processes, such as transcription, oxidation reduction, signal transduction, ion transport, immune response, the signaling pathways of Wnt, Jak-STAT, MAPK, ERBB2, mTOR and VEGF may be involved in the development of HCC. Results of the bioinformatics analysis may provide insight into the early diagnosis and specific-targeted gene therapy of HCC.

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