Screening of up- and downregulation of circRNAs in HBV-related hepatocellular carcinoma by microarray

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Abstract. The present study describes circular RNA (circRNA) profiles in three pairs of hepatocellular carcinoma (HCC) tissues and the corresponding adjacent non-tumorous tissues (NTs) by microarray. circRNA is a type of endogenous RNA that serve a crucial role in disease development and aberrantly express in a number of types of cancer. In the present study, 3 paired HCC tissues and paired adjacent NTs were collected from HCC surgical specimens from 3 hepatitis B virus-infected patients with HCC. With abundant and varied probes accounting for 5,396 circRNAs, a large number of circRNAs are able to be quantitatively determined. Based on the microarray data, 222,567,556 upregulated circRNAs and 125,439,219 downregulated circRNAs were identified respectively. Further analysis revealed 24 upregulated and 23 downregulated significantly circRNAs (fold-change ≥2; P≤0.05) in HCC tissues compared with NTs. By means of computer analysis and database inquiring, the microRNA (miRNA) response elements associated with the abnormally expressed circRNAs were annotated. The present study showed novel evidence determining genome-wide circRNA expression patterns in HCC using microarray analysis. The results demonstrated that clusters of circRNAs were aberrantly expressed in HCC compared with NTs. These circRNAs may be involved in the occurrence and development of HCC. Therefore, the results of the present study may provide a novel approach for improving the understanding of the molecular basis of HCC. Furthermore, the identified circRNAs may be potential biomarkers for the diagnosis of HCC.

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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer worldwide, ranking fifth and eighth in males and females, respectively, and exhibits one of the highest mortality rates (1). Hepatitis B virus (HBV) and hepatitis C virus (HCV) are primary causes of HCC. Chronic HBV infection is a dominant risk factor in the majority of areas of Asia and Sub-Saharan Africa that have a high incidence of HCC (2). The majority of patients with HCC whoexperience HBV infectionexhibit cirrhosis, secondary to the chronic necroinflammation (3). HBV, an oncogenic virus, promotes HCC via indirect (necroinflammation and regeneration injury) and direct (integration of its DNA in the host genome) pathways (4). The aberrant expression of genes and regulatory RNA moleculesare key nodes for the occurrence and development of HCC.

Circular RNAs (circRNA/ciR), initially observed in RNA viruses in the 1970s, have been identified as unique non-coding RNA molecules (5). CircRNAsare a type of endogenous RNA with a stable structure and tissue-specific expression (6) and are widely present in the cytoplasm of eukaryotic organisms, in the circular form (7). CircRNA forms a covalently closed continuous loop by means of unique non-canonical 'head-to-tail' splice without a free 3' or 5' end (8-10). CircRNAs derive from non-linear reverse splicing or gene rearrangement and circRNAs dominate the total spliced transcripts (11). High-throughput sequencing has enabled >25,000 types of circRNAs to be discovered in human fibroblasts (12). In addition, circRNAs may be formed in exons and introns, and cirRNAs with either origin may function in the regulation of gene expression (13).

A previous study demonstrated that circRNA served a role in the level of miRNA-mediated regulation of gene expression by sequestering the miRNAs. Furthermore, circRNAs are able to regulate gene expression by acting as competing endogenous RNAs and also termed miRNA 'sponges' (14). CircRNAs contain multiple, tandem miRNA binding sites. CircRNAs adsorb and sequester miRNAs to terminate the suppression of their targets, and to modulate the expression levels of other associated RNA molecules which share the same miRNA response elements (MREs) (14-16). The interaction between circRNAs and disease-associated miRNAs indicates that circRNAs are important for disease regulation (17).

CircRNAs serve crucial roles in the development of diseases, including nervous system disorders and atherosclerosis (18,19). In addition, circRNAs have been demonstrated to be involved in the neoplastic process (20); however, the molecular mechanisms underlying the association of circRNAs with cancer remain unclear (21).

To the best of our knowledge, a large-scale microarray screening of HCC and the focus of circRNAs as biomarkers of HCC has not been previously reported. The present study screened dysregulated circRNAs expression in HCC tissues using a microarray and annotated them for circRNA/microRNA (miRNA/miR) interactions.

Patients and methods

Patients and clinical specimens. The total three paired HCC tissues and adjacent non-tumorous tissues (NTs; Table I) were collected from HCC surgical specimens between June 2012 and December 2013 at Beijing YouAn Hospital, Capital Medical University (Beijing, China). All tissue specimens were immediately preserved in RNA-fixer reagent (BioTeke Corporation, Beijing, China) following removal from the body and were stored at -80˚C until use. The corresponding adjacent NTs were taken 5 cm from the edge of the cancer and contained no obvious tumor cells, as evaluated by an experienced pathologist.

All three HCC patients were diagnosed with HBV infection. Tumors were staged according to the tumor-node-metastasis (TNM) staging system (22). The three patients were diagnosed with T1N0M0, T1N0M0, and T3aN0M0, respectively (Table I). No radiotherapy, chemotherapy or targeted therapy was administered prior to surgery.

The present study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Beijing YouAn Hospital, Capital Medical University (Beijing, China). Written informed consent was obtained from all participants.

Total RNA extraction, labeling, hybridization, and array scanning. Total tissue RNA was extracted from the HCC tissues and paired adjacent NTs using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), following the manufacturer's protocol. CircRNAs were treated with RNase R (Epicentre; Illumina, Inc., San Diego, CA, USA) to remove linear RNAs, according to the manufacturer's protocol. Each sample was amplified and transcribed into fluorescent complementary RNA utilizing a random priming method (Arraystar Super RNA Labeling kit; Arraystar, Inc., Rockville, MD, USA). The labeled circRNAs were hybridized onto the Arraystar Human circRNA Array (5,396 human circRNA probes; cat. no. 6x7K; Arraystar, Inc.).

The labeled circRNAs were purified using an RNeasy Mini kit (Qiagen, Inc., Valencia, CA, USA). The concentration and specific activity of the labeled circRNAs (pmol Cy3/μg circRNA) were measured using NanoDrop ND-1000 spectrophotometer (NanoDrop; Thermo Fisher Scientific, Inc., Wilmington, DE, USA). A total of 1 μg of each labeled circRNA was dispensed into the gasket slide and assembled to the circRNA expression microarray slide. The slides were incubated for 17 h at 65˚C in an Agilent Hybridization Oven (Agilent Technologies, Inc., Santa Clara, CA, USA). The hybridized arrays were washed, fixed and scanned using the Axon GenePix 4000B microarray scanner (Molecular Devices, Sunnyvale, CA, USA).

Detection of expression profiling data and differentially expressed data. Scanned images were imported into GenePix Pro version 6.0 software (Axon; Molecular Devices) for grid alignment and raw data extraction. Quantile normalization of raw data and subsequent data processing were performed using the R software package (version 3.1.2; Lucent Technologies, Inc.; Nokia, Espoo, Finland). Low-intensity filtering was performed, and the circRNAs with ≥2 of the 6 samples having 'flags expressed' (≥2 times background standard deviation) were retained for further analysis. The analysis outputs were filtered and the differentially expressed circRNAs were ranked according to fold-change and P-value. Differentially expressed circRNAs were filtered and illustrated as a volcano plot. Hierarchical clustering was performed to reveal the distinguishable circRNAs expression pattern among samples.

Annotation for circRNA/miRNA interaction. The circRNA/miRNA interaction was predicted using Target Scan (www.targetscan.org/vert_71) and Miranda (www.microrna.org/microrna/home.do). All differentially expressed circRNAs were annotated in detail using the circRNA/miRNA interaction information.

Statistical analysis. All data were analyzed using SPSS (version 21.0; IBM Corp., Armonk, NY, USA) and all results were presented as the mean ± standard deviation. Differences between two groups were estimated using the Student's t-test, and fold-change ≥2.0 and P≤0.05 were considered to indicate a statistically significant difference.

Results

circRNA expression profiles. A total of 5,396circRNAs were scanned and the array image of each sample was demonstrated. Quantile normalization of raw data and subsequent data processing were performed using the R software package. The data demonstrated that 222,567,556 circRNAs were upregulated (fold-change ≥2) and 125,439,219 circRNAs were downregulated (fold-change ≥2; Fig. 1).

Differentially expressed circRNAs. The differentially expressed circRNAs with statistical significance between the two groups (HCC tissues group vs. NT group) were identified through using volcano plot filtering. A total of 24 upregulatedcircRNAs and 23 downregulated circRNAs were identified to be significant in HCC tissues compared with NTs (fold-change ≥2; P≤0.05; Fig. 2; Tables II and III). The top five upregulated circRNAs were hsa_circRNA_104351, hsa_circRNA_102814, hsa_circRNA_103489, hsa_circRNA_102109 and hsa_circRNA_100381. Furthermore, the top five downregulated circRNAs were hsa_circRNA_100327, hsa_circRNA_101764, hsa_circRNA_101092, hsa_circRNA_001225 and hsa_circRNA_102904.

Table I. Clinical parameters of the three patients with hepatitis B-associated HCC.

Patient	Sex	Age, years	HBV-DNA, IU/ml	Cirrhosis	Differentiation	HCC stage, TNM
1	M	63	$4.01x10^6$	Yes	Middle	T1N0M0
2	M	44	$9.85x10^2$	Yes	Poor	T3aN0M0
3	F	45	$1.98x10^3$	Yes	Middle	T1N0M0

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; TNM, tumor-node-metastasis; M, male; F, female.

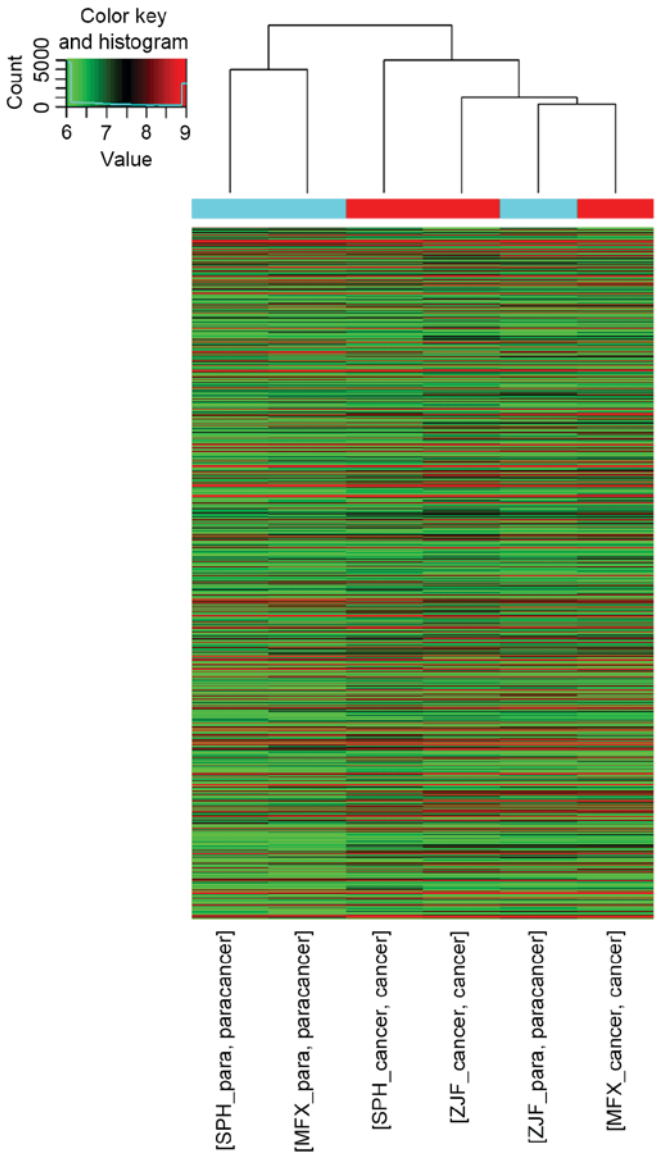

Figure 1. Hierarchical clustering of circRNA expression profiles. The results demonstrate a distinguishable circRNA expression profiling among 6 different samples. The dendrogram indicates the associations between the expression levels of samples. A total of 222,567,556 upregulated circRNAs and 125,439,219 downregulated circRNAs (fold-change ≥2) were identified. circRNA, circular RNA.

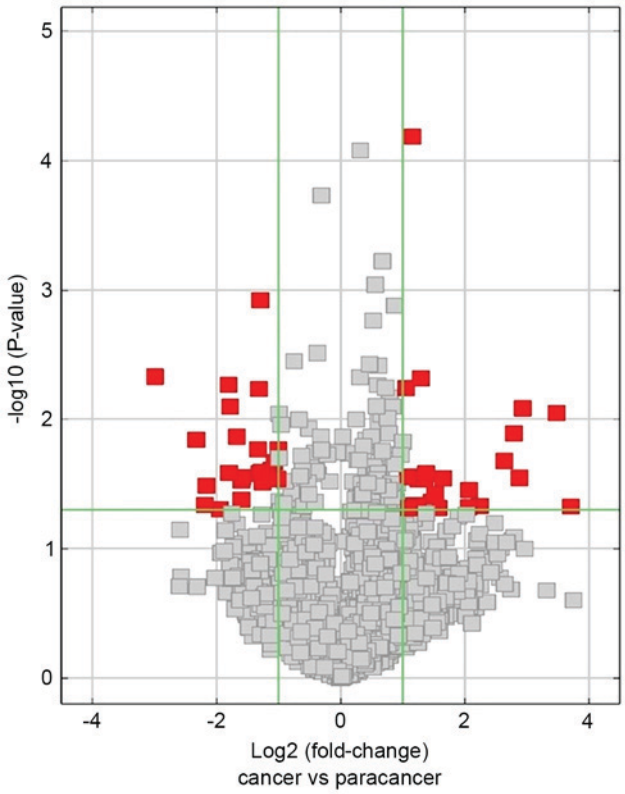

Figure 2. Volcano plot of the expression profiles of dysregulated circRNAs in HCC vs. NT. The vertical green lines correspond to 2-fold up- and downregulation; and the horizontal line represents, P=0.05. The red points in the plot represent the differentially expressed circRNAs in HCC tissues with statistical significance, compared with NTs (fold-change ≥2; P≤0.05). A total of 24 up- and 23 downregulated circRNAs were identified. circRNAs, circular RNAs; NTs, non-tumorous tissues.

Annotation for circRNA/miRNA interactions. The circRNA/miRNA interaction was predicted using the miRNA target prediction software. All differentially expressed circRNAs (fold-change ≥2; P≤0.05) were annotated in detail using the circRNA/miRNA interaction information (Tables IV and V). The most upregulated circRNA, hsa_circRNA_104351, adjusts its MREs: hsa-miR-490-5p, hsa-miR-876-5p, hsa-miR-619-3p, hsa-miR-619-3p, hsa-miR-331-3p and hsa-miR-411-3p. Similarly, the most downregulated circRNA, hsa_circRNA_100327, targets the following MREs: Hsa-miR-637, hsa-miR-326, hsa-miR-330-5p, hsa-miR-646 and hsa-miR-24-3p.

Discussion

Previously, circRNAs have been identified to serve a role in a number of types of disease, including cancer. The majority of circRNAs exhibit distinct tissue/developmental-stage and diseases-specific expression in the process of organismal

Table II. Significantly upregulated circRNAs in HCC vs. NTs.

circRNA	Alias[a]	Gene symbol	FC[b]	P-value[c]	circRNA_type[d]	Chromosome	Strand
hsa_circRNA_103436	hsa_circ_0006884	TIMMDC1	2.3799788	0.02920409	Exonic	chr3	+
hsa_circRNA_103920	hsa_circ_0001513	LNPEP	2.1170474	0.02782662	Exonic	chr5	+
hsa_circRNA_101842	hsa_circ_0007669	SLC7A6	2.9740602	0.048252979	Exonic	chr16	+
hsa_circRNA_104497	hsa_circ_0008419	EXOC4	2.108166	0.028206035	Exonic	chr7	+
hsa_circRNA_103792	hsa_circ_0003037	TRIO	2.7598056	0.043619449	Exonic	chr5	+
hsa_circRNA_102814	hsa_circ_0003789	TSN	11.121925	0.008936648	Exonic	chr2	+
hsa_circRNA_101169	hsa_circ_0028630	RFC5	2.0770889	0.005712231	Exonic	chr12	+
hsa_circRNA_102583	hsa_circ_0003449	MEIS3	2.221169	0.00006516883	Exonic	chr19	-
hsa_circRNA_102541	hsa_circ_0050834	RYR1	2.2713685	0.045762338	Exonic	chr19	+
hsa_circRNA_103588	hsa_circ_0068925	FAM193A	4.725986	0.046621193	Exonic	chr4	+
hsa_circRNA_101828	hsa_circ_0039787	C16orf70	2.4441344	0.004790339	Exonic	chr16	+
hsa_circRNA_104543	hsa_circ_0083172	DNAJB6	2.5453667	0.028933433	Exonic	chr7	+
hsa_circRNA_000596	hsa_circ_0000661	ADAMTS17	3.1358992	0.028697139	Antisense	chr15	+
hsa_circRNA_102189	hsa_circ_0006942	ATP5H	6.2062939	0.020961504	Exonic	chr17	-
hsa_circRNA_102230	hsa_circ_0046215	HGS	4.1868338	0.03517735	Exonic	chr17	+
hsa_circRNA_100021	hsa_circ_0009456	NPHP4	2.5935132	0.026262605	Exonic	chr1	-
hsa_circRNA_100381	hsa_circ_0009109	DCAF6	6.9136901	0.012898864	Exonic	chr1	+
hsa_circRNA_102109	hsa_circ_0003650	KPNB1	7.3568855	0.028283407	Exonic	chr17	+
hsa_circRNA_100770	hsa_circ_0021506	ANO5	2.3549632	0.04635107	Exonic	chr11	+
hsa_circRNA_103616	hsa_circ_0069380	TBC1D19	4.1619716	0.048093616	Exonic	chr4	+
hsa_circRNA_104351	hsa_circ_0008537	GLI3	13.0357676	0.04722754	Exonic	chr7	-
hsa_circRNA_103489	hsa_circ_0067717	RNF13	7.5996366	0.008247639	Exonic	chr3	+
hsa_circRNA_102630	hsa_circ_0003287	NBAS	2.866929	0.038026903	Exonic	chr2	-
hsa_circRNA_101539	hsa_circ_0005402	ANXA2	2.141776	0.048215125	Exonic	chr15	-

[a]Alias, circRNA ID in circBase (circbase.mdc-berlin.de). [b]FC, fold-change, the absolute ratio (no log scale) of normalized intensities between two conditions (threshold, 2.0). [c]P-value calculated using a paired t-test (threshold, 0.05). [d]CircRNAs were classified into five types: Exonic, circRNA arising from the exons of the linear transcript; intronic, circRNA arising from an intron of the linear transcript. Antisense represents circRNA whose gene locus overlap with linear RNA but transcribed from the opposite strand; intragenic represents circRNA transcribed from the same gene locus as the linear transcript but not classified into 'exonic' and 'intronic'; intergenic represents circRNA located outside a known gene locus. CircRNA, circular RNA; hsa, homo sapiens; chr, chromosome; +, positive strand; -, negative strand.

Table III. Significantly downregulated circRNAs in HCC vs. NTs.

circRNA	Alias[a]	Gene symbol	FC[b]	P-value[c]	circRNA_type[d]	Chromosome	Strand
hsa_circRNA_101405	hsa_circ_0003670	NEK9	2.4741971	0.026208247	Exonic	chr14	-
hsa_circRNA_101764	hsa_circ_0038645	PRKCB	5.0118456	0.014366467	Exonic	chr16	+
hsa_circRNA_104342	hsa_circ_0003162	BBS9	2.5248442	0.017053943	Exonic	chr7	+
hsa_circRNA_103627	hsa_circ_0069559	APBB2	2.485444	0.005793933	Exonic	chr4	-
hsa_circRNA_104044	hsa_circ_0075447	GMDS	2.3901855	0.030706961	Exonic	chr6	-
hsa_circRNA_102446	hsa_circ_0049356	CARM1	3.1862585	0.013653006	Exonic	chr19	+
hsa_circRNA_104865	hsa_circ_0004928	LPAR1	3.0272178	0.029853247	Exonic	chr9	-
hsa_circRNA_001225	hsa_circ_0000305	CELF1	4.4818374	0.032503965	Intronic	chr11	-
hsa_circRNA_100327	hsa_circ_0014022	TARS2	7.9507428	0.004638981	Exonic	chr1	+
hsa_circRNA_100147	hsa_circ_0004240	EIF3I	2.1647679	0.024721895	Exonic	chr1	+
hsa_circRNA_000963	hsa_circ_0001644	BCLAF1	2.4269392	0.025372306	Intronic	chr6	-
hsa_circRNA_103137	hsa_circ_0061817	C2CD2	3.0063191	0.0279151	Exonic	chr21	-
hsa_circRNA_100904	hsa_circ_0007767	ALG8	2.0097943	0.016978441	Exonic	chr11	-
hsa_circRNA_104349	hsa_circ_0079929	CDK13	3.4763303	0.026028214	Exonic	chr7	+
hsa_circRNA_102904	hsa_circ_0008459	KANSL1L	3.8502422	0.049696658	Exonic	chr2	-
hsa_circRNA_100883	hsa_circ_0005918	FCHSD2	3.028108	0.042033201	Exonic	chr11	-
hsa_circRNA_101092	hsa_circ_0008594	SRGAP1	4.566634	0.045816812	Exonic	chr12	+
hsa_circRNA_100705	hsa_circ_0008898	OAT	2.4566529	0.001192501	Exonic	chr10	-
hsa_circRNA_103361	hsa_circ_0001296	SMARCC1	3.50005	0.005363723	Exonic	chr3	-
hsa_circRNA_100395	hsa_circ_0015278	KLHL20	2.0964102	0.021512811	Exonic	chr1	+
hsa_circRNA_100748	hsa_circ_0020926	STIM1	3.4384778	0.007965023	Exonic	chr11	+
hsa_circRNA_102600	hsa_circ_0000958	PPP1R12C	2.2008434	0.027502872	Exonic	chr19	-
hsa_circRNA_102261	hsa_circ_0046534	TBCD	2.0251354	0.028926837	Exonic	chr17	+

[a]Alias, circRNA ID in circBase (circbase.mdc-berlin.de). [b]FC, fold-change, the absolute ratio (no log scale) of normalized intensities between two conditions (threshold, 2.0). [c]P-value calculated using a paired t-test (threshold, 0.05). [d]CircRNAs were classified into five types: Exonic, circRNA arising from the exons of the linear transcript; intronic, circRNA arising from an intron of the linear transcript. Antisense, represents circRNA whose gene locus overlaps with linear RNA but transcribed from the opposite strand; intragenic represents circRNA transcribed from the same gene locus as the linear transcript but not classified into 'exonic' and 'intronic'; intergenic represents circRNA located outside a known gene locus. CircRNA, circular RNA; hsa, homo sapiens; chr, chromosome; +, positive strand; -, negative strand.

Table IV. Upregulated circRNAs annotated with circRNA/miRNA interaction information.

circRNA	MRE1	MRE2	MRE3	MRE4	MRE5
hsa_circRNA_103436	hsa-miR-363-3p	hsa-miR-32-5p	hsa-miR-501-3p	hsa-miR-600	hsa-miR-502-3p
hsa_circRNA_103920	hsa-miR-1298-3p	hsa-miR-29b-1-5p	hsa-miR-452-3p	hsa-miR-34c-5p	hsa-miR-9-5p
hsa_circRNA_101842	hsa-miR-489-3p	hsa-miR-892b	hsa-miR-449c-5p	hsa-miR-665	hsa-miR-138-5p
hsa_circRNA_104497	hsa-miR-139-5p	hsa-miR-141-5p	hsa-miR-597-3p	hsa-miR-632	hsa-miR-648
hsa_circRNA_103792	hsa-miR-215-3p	hsa-miR-627-5p	hsa-miR-532-3p	hsa-miR-181b-5p	hsa-miR-630
hsa_circRNA_102814	hsa-miR-516a-5p	hsa-miR-224-5p	hsa-miR-501-5p	hsa-miR-429	hsa-miR-500a-5p
hsa_circRNA_101169	hsa-miR-591	hsa-miR-1264	hsa-miR-517b-3p	hsa-miR-517a-3p	hsa-miR-22-5p
hsa_circRNA_102583	hsa-miR-762	hsa-miR-23b-3p	hsa-miR-765	hsa-miR-675-5p	hsa-miR-423-5p
hsa_circRNA_102541	hsa-miR-486-3p	hsa-miR-328-5p	hsa-miR-125a-3p	hsa-miR-296-5p	hsa-miR-873-5p
hsa_circRNA_103588	hsa-miR-127-3p	hsa-miR-452-5p	hsa-miR-22-5p	hsa-miR-219a-2-3p	hsa-miR-509-5p
hsa_circRNA_101828	hsa-miR-619-3p	hsa-miR-877-5p	hsa-miR-520f-3p	hsa-miR-452-5p	hsa-miR-490-3p
hsa_circRNA_104543	hsa-miR-196b-3p	hsa-miR-1298-3p	hsa-miR-345-3p	hsa-miR-1-3p	hsa-miR-1224-3p
hsa_circRNA_000596	hsa-miR-647				
hsa_circRNA_102189	hsa-miR-588	hsa-miR-659-3p	hsa-miR-490-3p	hsa-miR-152-5p	hsa-miR-330-3p
hsa_circRNA_102230	hsa-miR-588	hsa-miR-10b-3p	hsa-miR-657	hsa-miR-150-5p	hsa-miR-636
hsa_circRNA_100021	hsa-miR-593-5p	hsa-miR-93-3p	hsa-miR-30b-3p	hsa-miR-766-3p	hsa-miR-484
hsa_circRNA_100381	hsa-miR-525-5p	hsa-miR-544a	hsa-miR-345-3p	hsa-miR-577	hsa-miR-587
hsa_circRNA_102109	hsa-miR-1301-3p	hsa-miR-20b-3p	hsa-miR-505-5p	hsa-miR-616-3p	hsa-miR-761
hsa_circRNA_100770	hsa-miR-19b-2-5p	hsa-miR-19b-1-5p	hsa-miR-767-3p	hsa-miR-506-5p	hsa-miR-550a-3p
hsa_circRNA_103616	hsa-miR-629-3p	hsa-miR-761	hsa-miR-603	hsa-miR-150-5p	hsa-miR-186-5p
hsa_circRNA_104351	hsa-miR-490-5p	hsa-miR-876-5p	hsa-miR-619-3p	hsa-miR-331-3p	hsa-miR-411-3p
hsa_circRNA_103489	hsa-miR-654-3p	hsa-miR-511-5p	hsa-miR-632	hsa-miR-643	hsa-miR-889-5p
hsa_circRNA_102630	hsa-miR-19b-2-5p	hsa-miR-105-5p	hsa-miR-29b-1-5p	hsa-miR-15b-3p	hsa-miR-29a-5p
hsa_circRNA_101539	hsa-miR-224-5p	hsa-miR-182-5p	hsa-miR-208a-5p	hsa-miR-9-5p	hsa-miR-33b-5p

CircRNA, circular RNA; hsa, homo sapiens; MRE, miRNA response element; miR/miRNA, microRNA.

Table V. Downregulated circRNAs annotated with circRNA-miRNA interaction information.

circRNA	MRE1	MRE2	MRE3	MRE4	MRE5
hsa_circRNA_101405	hsa-miR-646	hsa-miR-452-5p	hsa-miR-504-5p	hsa-miR-661	hsa-miR-383-3p
hsa_circRNA_101764	hsa-miR-181b-5p	hsa-miR-181c-5p	hsa-miR-181d-5p	hsa-miR-181a-5p	hsa-miR-329-5p
hsa_circRNA_104342	hsa-miR-411-5p	hsa-miR-520c-5p	hsa-miR-526a	hsa-miR-518d-5p	hsa-miR-518f-5p
hsa_circRNA_103627	hsa-miR-215-3p	hsa-miR-105-3p	hsa-miR-877-3p	hsa-miR-605-5p	hsa-miR-615-5p
hsa_circRNA_104044	hsa-miR-188-3p	hsa-miR-335-3p	hsa-miR-580-5p	hsa-miR-659-5p	hsa-miR-139-5p
hsa_circRNA_102446	hsa-miR-377-5p	hsa-miR-658	hsa-miR-889-5p	hsa-miR-23b-5p	hsa-let-7i-5p
hsa_circRNA_104865	hsa-miR-135a-3p	hsa-miR-7-5p	hsa-miR-588	hsa-miR-383-5p	hsa-miR-620
hsa_circRNA_001225	hsa-miR-30b-3p	hsa-miR-887-5p	hsa-miR-26b-3p	hsa-miR-485-5p	hsa-miR-363-5p
hsa_circRNA_100327	hsa-miR-637	hsa-miR-326	hsa-miR-330-5p	hsa-miR-646	hsa-miR-24-3p
hsa_circRNA_100147	hsa-miR-219a-1-3p	hsa-miR-486-3p	hsa-miR-493-3p	hsa-miR-92a-2-5p	hsa-miR-495-3p
hsa_circRNA_000963	hsa-miR-324-3p	hsa-miR-214-5p	hsa-miR-423-3p		
hsa_circRNA_103137	hsa-miR-497-5p	hsa-miR-487b-3p	hsa-miR-25-3p	hsa-miR-92a-3p	hsa-miR-92b-3p
hsa_circRNA_100904	hsa-miR-627-3p	hsa-miR-539-5p	hsa-let-7i-5p	hsa-miR-190b	hsa-miR-98-5p
hsa_circRNA_104349	hsa-miR-212-5p	hsa-miR-26a-1-3p	hsa-miR-26a-2-3p	hsa-miR-639	hsa-miR-1271-3p
hsa_circRNA_102904	hsa-miR-519c-3p	hsa-miR-148a-3p	hsa-miR-519a-3p	hsa-miR-567	hsa-miR-205-3p
hsa_circRNA_100883	hsa-miR-217	hsa-miR-376a-2-5p	hsa-miR-92b-3p	hsa-miR-511-5p	hsa-miR-92a-3p
hsa_circRNA_101092	hsa-miR-631	hsa-miR-612	hsa-miR-221-5p	hsa-miR-889-3p	hsa-miR-1298-3p
hsa_circRNA_100705	hsa-miR-365b-3p	hsa-miR-365a-3p	hsa-miR-670-3p	hsa-miR-34b-5p	hsa-miR-101-3p
hsa_circRNA_103361	hsa-miR-449c-3p	hsa-miR-582-5p	hsa-miR-509-3p	hsa-miR-510-5p	hsa-miR-369-5p
hsa_circRNA_100395	hsa-miR-141-3p	hsa-miR-588	hsa-miR-660-3p	hsa-miR-136-5p	hsa-miR-200a-3p
hsa_circRNA_100748	hsa-miR-136-3p	hsa-miR-598-3p	hsa-miR-556-3p	hsa-miR-335-3p	hsa-miR-499a-3p
hsa_circRNA_102600	hsa-miR-214-3p	hsa-miR-324-3p	hsa-miR-770-5p	hsa-miR-484	hsa-miR-513a-5p
hsa_circRNA_102261	hsa-miR-510-5p	hsa-miR-1271-3p	hsa-miR-604	hsa-miR-339-5p	hsa-miR-146b-3p

CircRNA, circular RNA; hsa, homo sapiens; MRE, miRNA response element; miR/miRNA, microRNA.

differentiation, development and diseases (6). CircRNAs regulate cancer development via a number of mechanisms, including miRNA sponges, modulating the Wnt signaling pathway and epithelial-mesenchymal transition (23). The abnormal expression levels of circRNAs have been observed in a number of types of cancer (24), including: glioma (25-27), renal cell carcinoma (28), bladder carcinoma (29,30), laryngeal cancer (31), lung cancer (32,33), breast cancer (34,35), esophageal cancer (36,37), gastric cancer (38-40), colorectal cancer (41-46), pancreatic ductal adenocarcinoma (47), cutaneous squamous cell carcinoma (48), basal cell carcinoma (49) and ovarian cancer (50).

Abnormal circRNAs have been identified to be involved in the occurrence and development of HCC (51). In a previous study, the RNA-seq data from 50 paired HCC tissues and NTs were analyzed to identify the function of circRNAs in HCC (51). Protein-coding genes (PCGs) associated with the 2091 circRNAs were identified to be enriched predominantly on liver/cardiovascular-related diseases, and participated in a number of metabolic processes (51). A total of 45 circRNAs and 23 PCGs exhibited significant expression alterations between HCC and normal tissues (51).

Representative circRNA, antisense to cerebellar degeneration-related protein 1 (Cdr1as, also termed ciRS-7), has been identified to act as an oncogene through targeting miR-7 in HCC (52). Cdr1as expression was upregulated and miR-7 expression was downregulated in HCC tissues. Knockdown of Cdr1as downregulated the expression of miR-7, and inhibited the expression of Cyclin E1 (CCNE1) and phosphatidylinositol 3-kinase catalytic subunit delta (PIK3CD), resulting in the suppression of proliferation and invasion of HCC cells through targeting miR-7 (52). Increased Cdr1as expression was significantly associated with hepatic microvascular invasion (MVI), alpha-fetoprotein (AFP) level, younger age and deterioration of HCC. The expression of Cdr1as in HCC tissues with concurrent MVI was inversely associated with miR-7 and positively associated with two miR-7-targeted genes (PIK3CD and p70S6K) (53).

The expression of circZKSCAN1 (zinc finger with KRAB and SCAN domains 1) and ZKSCAN1mRNA was significantly decreased in the HCC samples compared with matched adjacent non-tumorous tissues. The circZKSCAN1 levels varied in patients with tumor numbers, cirrhosis, vascular invasion and the tumor grade. ZKSCAN1mRNA primarily regulated cellular metabolism, whereas circZKSCAN1 mediated a number of cancer-related signaling pathways, suggesting a non-redundant role for ZKSCAN1mRNA and circZKSCAN1 (54).

CircRNAs are abundant, evolutionally conserved and relatively stable in the cytoplasm and therefore may be valuable for cancer diagnosis (12,14). A previous study demonstrated that a total of 174 and 353 circRNAs were upregulated and downregulated in HCC tissues, respectively, according to microarray analysis (55). hsa_circ_0004018 may be involved in cancer-related pathways via interactions with miRNAs (55). hsa_circ_0004018 is downregulated in HCC tissues and HCC lines, and a decreased hsa_circ_0004018 level is associated with serum AFP level, tumor diameters, differentiation, Barcelona Clinic Liver Cancer stage (56) and TNM stage. An additional study identified that hsa_circ_0001649 expression was significantly downregulated in HCC tissues, using the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) (57). hsa_circ_0001649 expression was associated with tumor size and the occurrence of tumor embolus in HCC; therefore, hsa_circ_0001649 may function in the tumorigenesis and metastasis of HCC and serve as a novel potential biomarker (57). In addition, GO analysis revealed that hsa_circ_0005075 may participate in cell adhesion during HCC development. Upregulated hsa_circ_0005075 exhibited an association with HCC tumor size and revealed diagnostic potential (58).

The present study compared the circRNA expression profiles between HCC tissue and adjacent NTs using microarray analysis with 5,396 circRNA probes. On the basis of the microarray data, 222,567,556 upregulated circRNAs and 125,439,219 downregulated circRNAs were identified in HCC tissues compared with adjacent NTs. Further analysis identified 24 upregulated and 23 downregulated significantly circRNAs (fold-change ≥ 2; $P \leq 0.05$) in HCC tissues compared with NTs. The results of the present study demonstrated that the circRNA expression profiles of HCC tissues differ from that of NTs.

Computer and database analysis annotated the MREs associated with the abnormally expressed circRNAs. The upregulated circRNAs may suppress miRNA expression. On the contrary, downregulated circRNAs may increase miRNA expression. The circRNAs, as a sponge for miRNAs, may be associated with the occurrence and progression of HCC, and may provide a novel approach to identify the underlying molecular basis of HCC. Furthermore, the identified differentially expressed circRNAs may be used as biomarkers for HCC.

In the present study, the abnormal expression levels of three paired HCC tissues were analyzed. Additional studies, with larger cohorts and using RT-qPCR, are required to validate the results from the present study. The data from the present study indicated that abnormal expression of certain circRNAs in HCC tissues and abnormal circRNAs may be novel biomarkers for the diagnosis of HCC.

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