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Gene microarray analysis of the circular RNAs expression profile in human gastric cancer

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Abstract. Human gastric cancer is a common malignant neoplasm of the digestive system and represents a threat to human health worldwide. The mechanisms underlying gastric cancer germination and development are not yet fully understood. Circular RNAs (circRNAs) serve crucial roles in various physiological and pathological processes, particularly cancer. However, few studies have focused on the mechanisms involving circRNAs in gastric cancer. Therefore the present study set out to identify the differentially expressed circRNAs in gastric cancer. Three specimens of gastric cancer and normal gastric tissue were selected and circRNA microarray analysis was performed to detect the differentially expressed circRNAs. The changes in circRNAs were confirmed by reverse transcription-quantitative polymerase chain reaction analysis. A total of 347 upregulated and 603 downregulated circRNAs (fold-change, >2.0) were identified in gastric cancer compared with the normal gastric tissue. A total of 20 selected circRNAs were dysregulated during gastric cancer, which suggests their potential role in gastric cancer. The present study identified circRNAs in the expression profile of human gastric cancer that were potentially involved in the underlying molecular mechanisms of its development.

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Introduction

Human gastric cancer is the fourth most common malignancy and the second leading cause of cancer-related deaths worldwide (1). Adenocarcinoma is the most common pathological type of gastric cancer, while lymphoma, carcinoid, and sarcoma constitute <5% of the pathology (2). The pathogenesis of gastric cancer is complicated owing to the interaction of multiple factors, including *Helicobacter pylori* infection, environment, and heredity. The environmental factors play critical roles in the pathogenesis of gastric cancer; the major risk factors include smoking and diet (3). However, determining the molecular markers of gastric cancer is yet a great challenge.

Recently, the identification and characterization of circular RNA (circRNA) have revolutionized the field of RNA. CircRNAs have gained increasing attention in deciphering the complicated mechanisms underlying the malignant processes such as tumorigenesis, multidrug resistance, invasion, and metastasis. Although circRNAs have been reported as early as 20 years ago (4), they were mostly misinterpreted as splicing artefacts or gene rearrangements. Following high-throughput RNA sequencing and bioinformatics, thousands of different circRNAs have been rediscovered in the recent several years (5-8). Preliminary data revealed that circRNAs were abundantly expressed and evolutionarily conserved across the eukaryotes and functioned as miRNA sponges (5,6,9). Cdr1as (also known as ciRS-7), as the maximally studied circRNA, was reported as the miR-7 sponge or inhibitor (5,6).

ciRS-7 was highly expressed in a wide variety of cancer cell lines, and ciRS-7/miR-7 network suggested a therapeutic potential for carcinoma. This network might regulate the majority of the cancer pathways such as p21-activated kinase 1 (Pak1) (10), epidermal growth factor receptor (EGFR) (11), activated cdc42-associated kinase 1 (Ack1) (12), and phosphoinositide 3-kinase catalytic subunit delta (PIK3CD) (13). Increasing number of evidence indicated miR-7 as a potential tumor suppressor in several human cancers. Xiong *et al* reported that miR-7 selectively induced growth suppression and apoptosis of non-small cell lung cancer (NSCLC) by targeting B-cell lymphoma-2 (BCL-2) *in vitro* (14). Similarly, miR-7 was confirmed as a novel miRNA exhibiting tumor

suppression function in colon cancer (15). Although the majority of reports supported the tumor-enhancing effect of circRNAs, the converse was also reported. ciR-ITCH demonstrated an inhibitory effect on esophageal squamous cell carcinoma, acting as a sponge of miR-7, miR-17, and miR-214 via the regulation of the Wnt/β-catenin pathway (16). Altogether, these results suggested that the relationship between circRNA and cancer was complicated and precise mechanisms needed further investigation.

In this study, we presented the circRNAs' expression profile in normal gastric tissue and gastric adenocarcinoma through microarray technology in order to explore the function of circRNAs in gastric cancer for early diagnosis and treatment of cancer.

Materials and methods

Patient samples. The present study was approved by the Research Ethics Committee of the Affiliated Drum Tower Hospital of Nanjing University, Medical School, and all patients provided informed consent before the samples were collected. Gastric cancer was confirmed by histopathological diagnosis. Finally, 15 patients (eight men, seven women; mean age 64.1 years, range 48-81) were enrolled and 15 pairs of gastric carcinoma tissues and normal para-carcinoma samples were collected. All samples were rapidly frozen in liquid nitrogen and stored at -80˚C for subsequent investigation. For circRNA microarray analysis, a total of 3 gastric carcinoma and 3 normal para-carcinoma gastric tissues (control) were randomly selected for the study.

RNA extraction. Total RNA was extracted from the frozen tissue block that was homogenized (IKA Werke GmbH & Co. KG, Staufen, Staufen, Germany) and resuspended in TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Then, the total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Additionally, the RNA integrity was assessed by denaturing agarose gel electrophoresis.

RNA labeling and array hybridization. The sample labeling and microarray hybridization were performed by KangChen Biotech (Shanghai, China). Briefly, the circRNA was treated with RNase R (Epicenter Biotechnologies, USA) to remove the linear RNAs. Each sample was amplified and transcribed into fluorescent cRNA using a random priming method (Arraystar Super RNA Labeling kit; Arraystar Inc., Rockville, MD, USA). Subsequently, these labeled cRNAs were purified by RNeasy Mini kit (cat. no. 74106, Qiagen GmbH, Hilden, Germany Germany), and the concentration and specific activity were measured by NanoDrop (NanoDrop Technologies). Then, 1 μg of each labeled cRNA was fragmented by adding 5 μl of 10X blocking agent and 1 μl of 25X fragmentation buffer, followed by heating at 60˚C for 30 min. Then, 25 μl of 2X hybridization buffer was added to dilute the labeled cRNA, and 50 μl of the hybridization solution was dispensed into the gasket slide and assembled on the circRNA expression microarray slide. These slides were incubated for 17 h at 65˚C in a hybridization oven (Agilent). The hybridized arrays were washed, fixed, and scanned using the Axon GenePix 4000B microarray scanner (Molecular Devices, LLC, Sunnyvale, CA, USA).

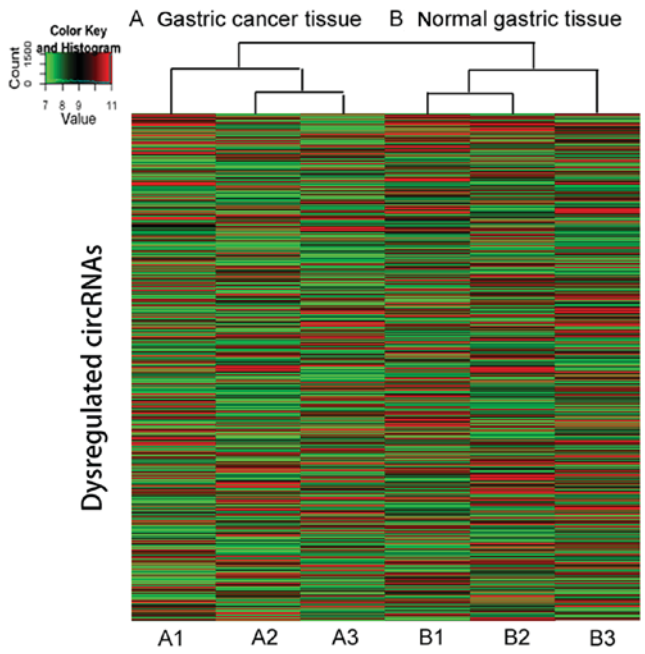

Figure 1. Differentially expressed circRNAs between (A) gastric cancer tissue and (B) adjacent normal gastric tissue. The result from hierarchical clustering analysis reveals circRNA expression variation among samples. The color scale reflects the $\log_2$ signal intensity and runs from green (low intensity), to black (medium intensity), to red (strong intensity). circRNA, circular RNA.

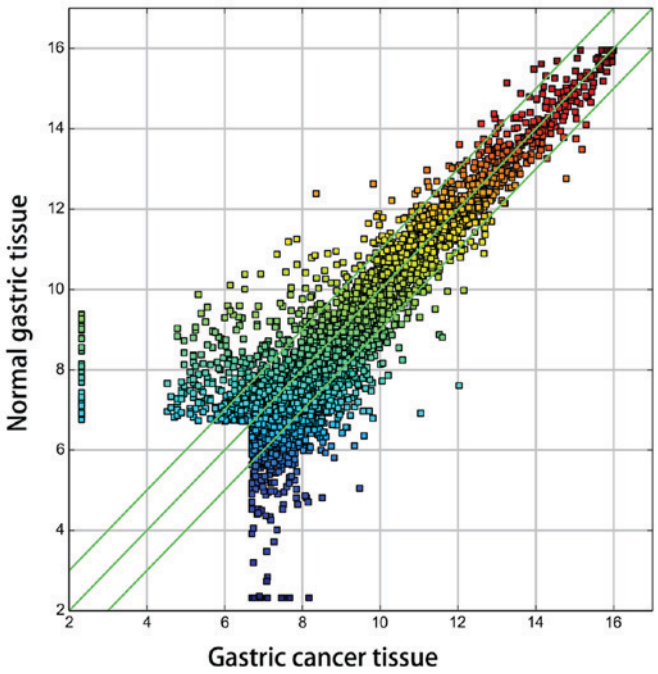

Figure 2. Scatter-plot of circRNA expression variation between the gastric cancer tissue (x-axis) and normal gastric tissue (y-axis). The values of the x-axis and y-axis in the Scatter-plot are the averaged normalized signal values of gastric cancer and normal gastric tissues ($\log_2$ scaled). The green lines are fold-change lines. CircRNAs in the Scatter-plot above the top green line and below the bottom green line indicate more than 2.0 fold-change of circRNAs between the two compared samples. circRNA, circular RNA.

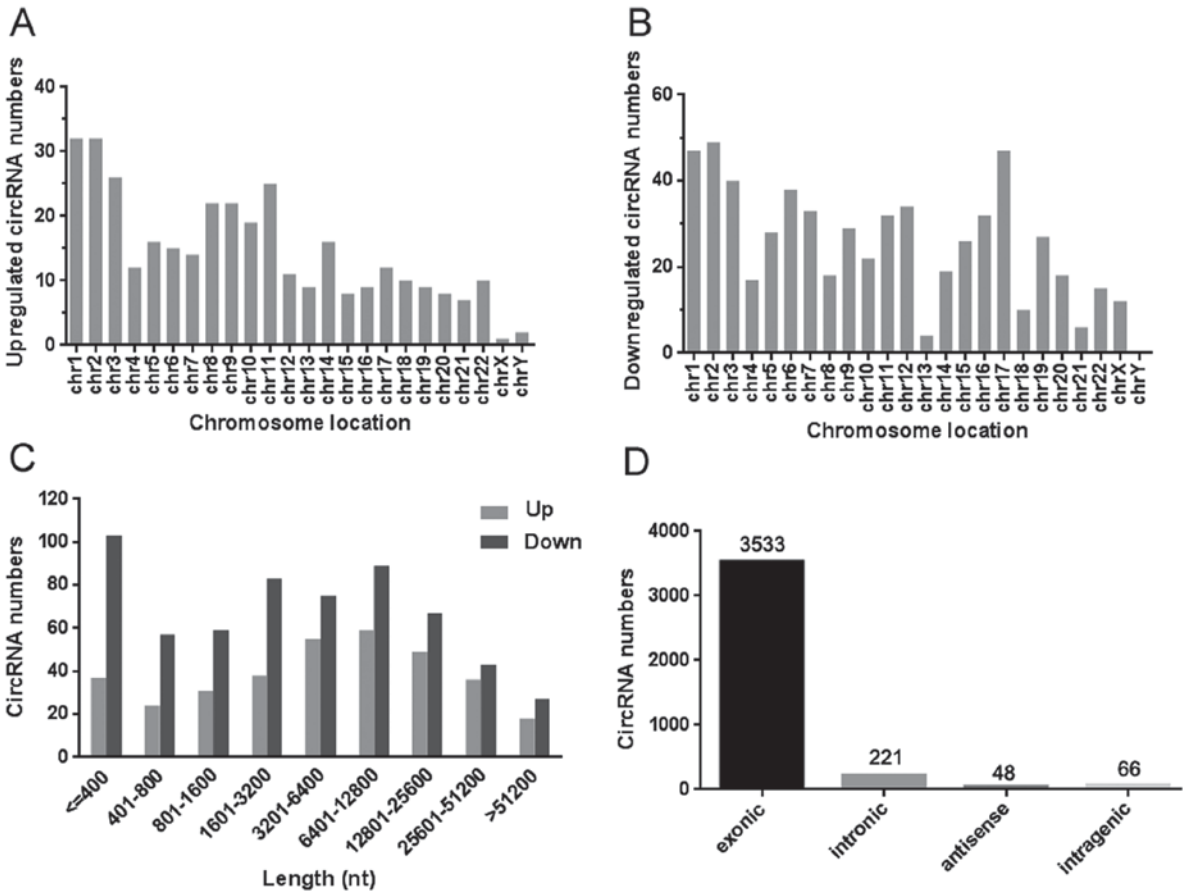

Figure 3. Annotation of differentially expressed circRNAs in gastric cancer tissues. (A) The location of upregulated circRNAs in human chromosomes. (B) The location of downregulated circRNAs in human chromosomes. (C) The length distribution of the dysregulated circRNAs. (D) The functional classification between the dysregulated circRNAs and their targets. circRNA, circular RNA.

Microarray analysis. The Arraystar Human Circular RNA Microarray V2.0 (Arraystar, Inc.) was designed for the purpose of profiling both circRNAs and protein-coding RNAs in the human genome. The differentially expressed circRNAs were identified through fold-change filtering and standard Student's t-test. The circRNAs are exhibiting a fold-change ≥2.0 and a P-value <0.05 were selected as significantly differentially expressed circRNAs.

Quantitative reverse transcription PCR. cDNA samples were prepared from total RNA of gastric tissues by reverse transcription. In total, 20 circRNAs were analyzed by SYBR Green I dye-based detection with specific primer sequences. The primer sequences were shown in Table I. The $2^{-\Delta\Delta Cq}$ method was performed in 18 samples and applied for the quantification of the relative expression of circRNAs that was normalized against the expression of the housekeeping gene, *GAPDH*.

Statistical analysis. The two groups were compared by the standard Student's t-test for the evaluation of the microarray analysis. The results were considered statistically significant at a P-value <0.05. The false discovery rate (FDR) was calculated to correct the P-value. Fold-change ≥2.0 and P-value <0.05 were used to identify the differentially expressed circRNAs.

Results

circRNA expression profiles in gastric cancer tissues relative to adjacent normal gastric tissues. Randomly, 3 gastric cancer and 3 adjacent normal gastric tissues were selected for a standard circRNA microarray independently. The circRNA expression patterns between gastric cancer and the adjacent normal gastric tissues were found to be significantly different. After scanning and normalization, a total of 950 circRNAs were found to be differentially expressed in the microarray (fold-change in expression ≥2.0, P<0.05), consisting of 347 upregulated circRNAs and 603 downregulated circRNAs (data not shown). The hierarchical clustering of circRNA expression described the variation in the expression between the groups of gastric cancer and normal gastric tissues (Fig. 1). Furthermore, the variation in circRNA expression among the samples was assessed by Scatter-plot visualization (Fig. 2).

Annotation of differentially expressed circRNAs in gastric cancer tissues. The general data, including the chromosome location, length distribution and functional classification of these differentially expressed circRNAs were summarized. Fig. 3A and B demonstrated that the up- and downregulated circRNAs were located in human chromosomes. The length data displayed two peaks that were distributed among these

Table I. Primer sequences for reverse transcription-polymerase chain reaction.

Gene	Direction	Primer sequence
hsa_circ_0001017	Forward	AGTGCGAAGTAATCTATGCCAGC
	Reverse	AGCCATTCTTTGCTGGGCTC
hsa_circ_0001772	Forward	GCCAGAGGAGGAGCAGCTTTA
	Reverse	GCTCTTCATCTGACAAATCCGAC
hsa_circ_0002346	Forward	GTGCAAACCAGTTTTCGGCG
	Reverse	TCCAGTTCTCATCTTGTTGGCA
hsa_circ_0000072	Forward	TTGGCAGCAAATGGAGTTCGT
	Reverse	GTGCCTGCCACCATTTCCTTA
hsa_circ_0003221	Forward	GATGCGGGGCAATGCACTA
	Reverse	ACCAGTACCCAGGTGAGTCTT
hsa_circ_0001865	Forward	GCTCCACAGACTTCCCAGAGT
	Reverse	GGCAAGTTCCAACGTCTCCT
hsa_circ_0003441	Forward	ACCACAGTTCTTGGTGGTGAAG
	Reverse	TGACTTTGTCTGGAGAGCTTGTG
hsa_circ_0008285	Forward	GCTGTTAACGGGAAAGGTTGAA
	Reverse	GCGTCTGTTGAAGTCGTGGA
hsa_circ_0023923	Forward	AGCACATCAAAGCTGCCCAA
	Reverse	TGCACTGAATTAAGTCTCCCCA
hsa_circ_0000347	Forward	GAAAAAGAACCAATGCAAAGAAGGT
	Reverse	GCACTGAATTAAGTCTCTGCAACT
hsa_circ_0046881	Forward	AAGTCAGGCAGCTTTGCTGG
	Reverse	CACAGTTGGTTAGCCACAGC
hsa_circ_0023940	Forward	ATGCTCCTGTTCAAAGATGCCA
	Reverse	TTTGAAGACCACCACCCAACT
hsa_circ_0023891	Forward	CCTGCTACTACACCAACAGGC
	Reverse	ACTGAATTAAGTCTGTGCTCCTGA
hsa_circ_0002433	Forward	TGAGCGTTTTATTCAGTATTTGGCT
	Reverse	GCACTGAATTAAGTCTTGCAATCCA
hsa_circ_0050278	Forward	AAGCCAGACCTGATCACTTGTC
	Reverse	TGTCAATGGTCCCTGTGGGT
hsa_circ_0000154	Forward	ACCAACGTTGAGCAAGATGC
	Reverse	TTCTCCAGTGTCATTCCAACAGA
hsa_circ_0075048	Forward	GGCCACATCGACAACTCCAT
	Reverse	GCTCGTTCACACTTGTTGATGC
hsa_circ_0001824	Forward	TGCATCAGCTCCAGGGCAAT
	Reverse	TTGAAAGAAATGTGGCATGTGAGA
hsa_circ_0000835	Forward	CAGCATGGTCATGGAGGATGG
	Reverse	ATGCTTGATGCCTATTGCCACT
hsa_circ_0000825	Forward	GAAAAGCGCGCTAAAGCTGA
	Reverse	TCCATCTCAGCACGGAGTTCA
hsa_circ_0009109	Forward	ATCTGGCTCAGATGACACCAA
	Reverse	TATGTTTGCTCGGTGCCCTG
hsa_circ_0087855	Forward	ACTTCCACACCTGCATCCAT
	Reverse	TGCTTTCACCTGTCAGTTGCT
hsa_circ_0001747	Forward	GACAAGCTGGTGTTGAAGGGT
	Reverse	AGCAGGCCTTTCGAGCTTTAG
hsa_circ_0009061	Forward	CCAAGCATCAGGTGTGGAGG
	Reverse	TCTCTGTACTCTACTGTGCGGT
hsa_circ_0000997	Forward	TGCACCACTGGATGTTGTTTACT
	Reverse	GTGGTCTCCACCTGTTTTGGAT

Table I continued.

Gene	Direction	Primer sequence
hsa_circ_0001073	Forward	ACTTGTTCCAACTCAAGTGCTATAC
	Reverse	GTAGCAAAACAATGCCGCCG
hsa_circ_0000085	Forward	TTTGGCAGACTTTTACCTGGTG
	Reverse	TGGATTGCTGCTTAAGCTTCCT
hsa_circ_0088021	Forward	GCTGAACAGGTGCCTGAACT
	Reverse	CAATTCCAGGTCTGCTGCCG
hsa_circ_0020353	Forward	GCAGACTCCTGCAAGTTCCC
	Reverse	GTGCTTATCCACAAGGGCCA

Table II. Upregulated circRNAs between gastric cancer and normal gastric tissues.

circRNA	P-value	FC	Log_2 FC	Regulation	circRNA type	Chrom	Strand	Best transcript	Gene symbol
hsa_circRNA_104804	<0.001	117.219	6.873	Up	Exonic	chr9	-	uc004amv.3	UBQLN1
hsa_circRNA_102678	<0.001	125.119	6.967	Up	Exonic	chr2	+	uc002rpd.3	CRIM1
hsa_circRNA_100261	0.011	41.319	5.368	Up	Exonic	chr1	-	uc001dex.4	ANKRD13C
hsa_circRNA_100927	0.002	70.919	6.148	Up	Exonic	chr11	-	uc001pbl.3	PICALM
hsa_circRNA_100924	0.001	88.219	6.463	Up	Exonic	chr11	-	uc001pbl.3	PICALM
hsa_circRNA_100922	<0.001	101.119	6.659	Up	Exonic	chr11	-	uc001pbl.3	PICALM
hsa_circRNA_102713	0.010	44.119	5.463	Up	Exonic	chr2	-	uc002rus.3	SRBD1
hsa_circRNA_102499	0.008	56.519	5.820	Up	Exonic	chr19	+	uc002npf.3	ZNF85
hsa_circRNA_102315	0.007	52.219	5.706	Up	Exonic	chr18	+	uc002ktp.3	MIB1
hsa_circRNA_100911	0.004	71.319	6.156	Up	Exonic	chr11	-	uc001pbl.3	PICALM
hsa_circRNA_104850	0.015	45.319	5.502	Up	Exonic	chr9	+	uc011lwa.2	RAD23B
hsa_circRNA_100925	0.002	75.269	6.233	Up	Exonic	chr11	-	uc001pbl.3	PICALM
hsa_circRNA_102293	0.010	49.919	5.641	Up	Exonic	chr18	+	uc002knq.2	CCDC165
hsa_circRNA_102737	<0.001	133.619	7.061	Up	Exonic	chr2	-	uc002sbj.3	XPO1
hsa_circRNA_104868	0.021	34.519	5.109	Up	Exonic	chr9	-	uc010muc.1	KIAA0368
hsa_circRNA_100381	0.013	48.619	5.603	Up	Exonic	chr1	+	uc001gev.3	DCAF6
hsa_circRNA_104707	<0.001	120.019	6.907	Up	Exonic	chr8	-	uc003yvs.3	PTK2
hsa_circRNA_104532	<0.001	132.219	7.046	Up	Exonic	chr7	+	uc003wme.3	RBM33
hsa_circRNA_104492	0.012	45.319	5.502	Up	Exonic	chr7	+	uc003vqs.3	MKLN1
hsa_circRNA_104689	0.009	53.719	5.747	Up	Exonic	chr8	-	uc003ysz.2	ASAP1
hsa_circRNA_100380	0.010	56.219	5.812	Up	Exonic	chr1	+	uc001gev.3	DCAF6
hsa_circRNA_104016	0.011	54.219	5.760	Up	Exonic	chr5	+	uc003mby.4	ERGIC1
hsa_circRNA_100715	0.021	34.419	5.105	Up	Exonic	chr10	-	uc001lif.4	CTBP2
hsa_circRNA_101270	<0.001	108.219	6.757	Up	Exonic	chr13	+	uc001vib.4	TDRD3
hsa_circRNA_102830	0.014	41.519	5.375	Up	Exonic	chr2	+	uc002twg.3	ACVR2A
hsa_circRNA_100241	<0.001	123.419	6.947	Up	Exonic	chr1	-	uc001cyx.1	OMA1
hsa_circRNA_104052	0.001	103.319	6.690	Up	Exonic	chr6	+	uc003mwi.3	CDYL
hsa_circRNA_102298	0.003	87.319	6.448	Up	Exonic	chr18	-	uc002kod.1	PPP4R1
hsa_circRNA_100097	0.011	44.619	5.479	Up	Exonic	chr1	+	uc001bgi.2	KDM1A

FC, fold change; chrom, chromosome; circRNA, circular RNA.

dysregulated circRNAs in ≤400 bp and 1,601-25,600 bp (Fig. 3C). Fig. 3D revealed the relationship between the mentioned circRNAs and their molecular targets including exonic, intronic, antisense and intragenic. Nevertheless, the exonic targets occupied the vast majority of all types of functional classification.

Table III. Downregulated circRNAs between gastric cancer and normal gastric tissues.

circRNA	P-value	FC	Log_2 FC	Regulation	circRNA type	Chrom	Strand	Best_transcript	Gene symbol
hsa_circRNA_103538	<0.001	-40.819	-5.351	Down	Exonic	chr3	+	uc003fpi.3	MAP3K13
hsa_circRNA_102016	0.038	-8.032	-3.005	Down	Exonic	chr17	-	uc002heo.1	SSH2
hsa_circRNA_102464	0.006	-17.362	-4.117	Down	Exonic	chr19	+	uc002myp.3	PKN1
hsa_circRNA_100109	0.036	-8.135	-3.024	Down	Exonic	chr1	+	uc001bmt.1	ARID1A
hsa_circRNA_103477	0.002	-20.819	-4.379	Down	Exonic	chr3	+	uc003eqt.3	EPHB1
hsa_circRNA_100801	0.007	-21.424	-4.421	Down	Exonic	chr11	+	uc001mxq.4	HSD17B12
hsa_circRNA_104984	0.028	-12.154	-3.603	Down	Exonic	chrX	-	uc004czk.2	MAP3K15
hsa_circRNA_104968	0.030	-9.387	-3.230	Down	Exonic	chr9	+	uc004coa.3	EHMT1
hsa_circRNA_101585	0.034	-11.713	-3.550	Down	Exonic	chr15	-	uc010biv.1	CELF6
hsa_circRNA_102540	0.035	-10.064	-3.331	Down	Exonic	chr19	+	uc002ohk.3	SIPA1L3
hsa_circRNA_103568	0.038	-9.790	-3.291	Down	Exonic	chr3	-	uc003fxp.2	DLG1
hsa_circRNA_100144	<0.001	-57.519	-5.845	Down	Exonic	chr1	+	uc001bui.3	TXLNA
hsa_circRNA_105041	0.005	-27.519	-4.782	Down	Exonic	chrX	-	uc004flx.1	G6PD
hsa_circRNA_103901	<0.001	-36.919	-5.206	Down	Exonic	chr5	-	uc003kfo.3	LHFPL2
hsa_circRNA_100061	0.004	-19.228	-4.265	Down	Exonic	chr1	-	uc001aub.3	DHRS3
hsa_circRNA_104046	0.003	-20.371	-4.348	Down	Exonic	chr6	+	uc003mtz.3	WRNIP1
hsa_circRNA_104351	0.040	-9.361	-3.226	Down	Exonic	chr7	-	uc011kbg.2	GLI3
hsa_circRNA_104601	0.035	-12.922	-3.691	Down	Exonic	chr8	-	uc003xpe.3	SLC20A2
hsa_circRNA_102489	<0.001	-35.219	-5.138	Down	Exonic	chr19	+	uc002nkf.3	UPF1
hsa_circRNA_102471	0.041	-8.065	-3.011	Down	Exonic	chr19	+	uc002nfj.1	MYO9B
hsa_circRNA_104280	0.002	-21.819	-4.447	Down	Exonic	chr7	-	uc003six.1	PRKAR1B
hsa_circRNA_101657	0.038	-9.248	-3.209	Down	Exonic	chr15	+	uc010urq.2	IGF1R
hsa_circRNA_104318	0.032	-11.642	-3.541	Down	Exonic	chr7	-	uc003sti.3	ANKMY2
hsa_circRNA_102212	0.038	-9.358	-3.226	Down	Exonic	chr17	-	uc002jwc.1	USP36
hsa_circRNA_100752	0.007	-24.319	-4.604	Down	Exonic	chr11	-	uc001maq.2	OR51B5
hsa_circRNA_104190	0.002	-23.098	-4.529	Down	Exonic	chr6	-	uc003qez.2	HBS1L
hsa_circRNA_001369	0.024	-10.828	-3.436	Down	Antisense	chr12	-	NM_000020	ACVRL1
hsa_circRNA_100677	0.005	-21.387	-4.418	Down	Exonic	chr10	-	uc009xxl.3	PCGF6

FC, fold change; circRNA, circular RNA; chrom, chromosome.

Reverse transcription-PCR validation of some differentially expressed circRNAs. We set a threshold as log_2 fold-change >5 in upregulated circRNAs, >3 in downregulated circRNAs as the previous study (17), and P-value <0.05, and found 29 upregulated (Table II) and 28 downregulated differentially expressed circRNAs (Table III). Next, we randomly selected 20 differentially expressed circRNAs, including 10 upregulated (102713, 100715, 100261, 100924, 104804, 104707, 102830, 102298, 100911, and 102293) and 10 downregulated circRNAs (103538, 104318, 103477, 104280, 100144, 104984, 001369, 103901, 100677, and 102464) for substantiation in the gastric tissue samples. The results of the microarray were in agreement with those of the real-time PCR; 6 selected upregulated circRNAs (Fig. 4A) and 5 selected downregulated circRNAs (Fig. 4B) were verified. However, the expressions of upregulated (Fig. 4C) and downregulated circRNAs (Fig. 4D) were not related to the expression of the host genes.

Discussion

Human gastric cancer is one of the most commonly known malignancies all over the world. A large number of studies have shown that the occurrence of gastric cancer involves several molecular mechanisms. However, the precise biological process of gastric cancer is not yet clearly elucidated. Several circRNAs have been recently discovered constituting a new specific class of endogenous non-coding RNAs. Hsa_circ_001569 promoted colorectal cancer in cell proliferation and invasion as a sponge of miR-145 (18). On the contrary, hsa_circ_002059 was found to be significantly down-regulated in gastric cancer as a typical circRNA, and its expression level was correlated with tumor metastasis and TNM stage (19). Thus, circRNAs might play a major role in the occurrence and development of gastric cancer; however, our understanding about the correlation between circRNAs expression and gastric cancer remains controversial due to the limited number of studies. Hence, the expression

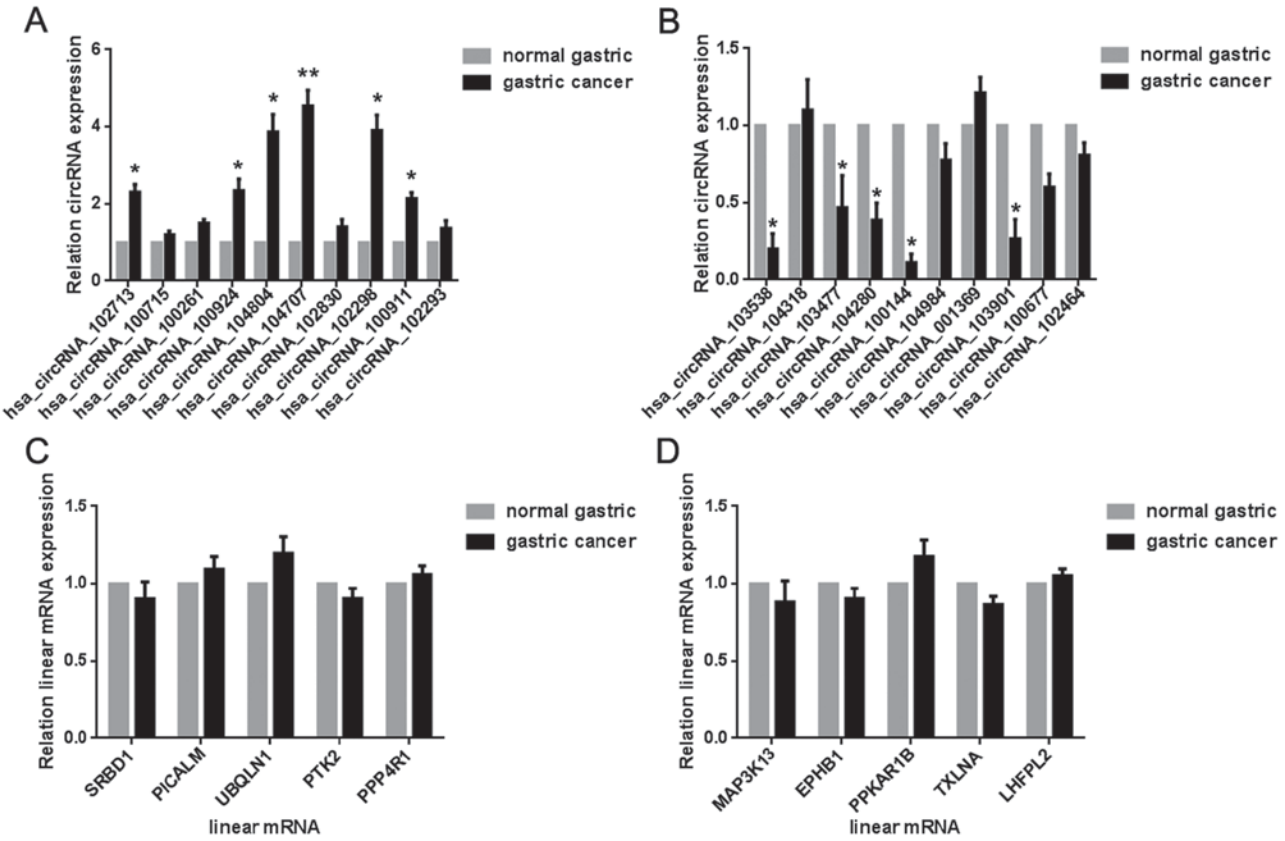

Figure 4. RT-PCR validation of some differentially expressed circRNAs and mRNAs from microarray analysis. (A) Selected upregulated circRNAs were verified by RT-PCR. (B) Selected downregulated circRNAs were verified by RT-PCR. (C) The relationship between the expression of upregulated circRNAs and host genes. (D) The relationship between the expression of downregulated circRNAs and host genes. Data shown are representative of 3 gastric cancer and 3 adjacent normal gastric tissues. Values are the mean ± standard deviation. *P<0.05, **P<0.01 vs. normal gastric tissue group. circRNA, circular RNA; RT-PCR, reverse transcription polymerase chain reaction.

profile of circRNAs in gastric cancer necessitates further exploration with respect to the potential mechanisms.

The specific repeated pattern of chromosomal aberrations is not associated with tumorigenesis and progression in gastric cancer. The current study conformed to the conclusion considering that all the chromosomes can experience unequable changes (20). However, there are inconsistencies regarding the chromosomal location of dysregulated circRNAs. Our experimental results revealed that chromosomal abnormalities were mainly distributed on chromosomes 1, 2, 3, 6, 9, 11, and 17, while previous studies designated chromosomes 8, 12, 15, 17, and 20 in gastric cancer (21,22). This phenomenon might be attributed to the following: firstly, the experimental method to detect chromosomal dysregulation in the past was FISH technology, while currently gene microarray is employed. Secondly, the sample size in the current study was small due to the high cost of gene microarray technology. Finally, the inherent differences in gastric cancer, such as the degree of pathological differentiation, stages, and grades, might also result in the differential distribution of chromosomal abnormalities.

Here, we reverse transcription-PCR verified the microarray analysis results. Recent evidence demonstrated that circRNAs play a crucial role in fine-tuning the level of miRNA-mediated regulation of gene expression via miRNA sequestration (23,24). In addition, several of the predicted binding sites of circRNAs on miRNAs are functional and appear to be under less selective pressure as compared to the corresponding miRNA binding sites in mRNAs (25). However, in the current study, the expression of circRNAs did not correlate with the expression of the host genes, suggesting an independent regulation of transcription vs. circRNA formation. We will verify the findings in further studies with larger sample size. Combining with previous studies (26,27), we currently propose that the circRNA-miRNA-mRNA axis may be the putative mechanism promoting the growth of the tumor, although the specific effect might not be deduced. Thus, further studies are essential for an insight into the exact mechanism.

In conclusion, we reported the profile of differentially expressed circRNAs between normal gastric and gastric cancer tissues. The network of differentially expressed numerous circRNAs was constructed and they found to be involved in the development and metabolism of gastric cancer with our and previous studies (27-29). Therefore, a further exploration of the biological processes and molecular mechanisms of the dysregulated circRNAs is imperative in order to clarify the pathogenesis of gastric cancer or provide a new therapeutic target via the regulation of the key circRNAs.

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Availability of data and materials

All data generated or analyzed during the study are included in this published article.

Authors' contributions

XZ and XL designed the research. YS, JZ, ZF, BZ, MC, XL and XZ performed the experiments. YS, JZ, BZ and MC analyzed the data. YS wrote the paper and ZF, XL and XZ critically revised the manuscript for important intellectual content.

Ethics approval and consent to participate

The present study was approved by the Research Ethics Committee of the Affiliated Drum Tower Hospital of Nanjing University, Medical School (Nanjing, China) and all patients provided informed consent prior to their inclusion within the study.

Consent for publication

All patients provided written informed consent for the publication of their data.

Competing interests

The authors declare that they have no competing interests.

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