Circular RNA hsa_circ_000780 has been identified by genomic expression profile analysis as a potential diagnostic marker for gastric cancer

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Abstract

Background : The present study attempted to detect a specific circular RNA (circRNA) for the early diagnosis of gastric cancer (GC).

Methods: We selected four patients with GC for this study. The total RNA of the malignant and adjacent tissues was extracted, and the circRNA was screened. The eight most upregulated and downregulated circRNAs with a statistically significant relation to GC and paired adjacent nontumorous tissues were identified using real-time fluorescent quantitative polymerase chain reaction (PCR). CircRNA expression was identified by quantitative reverse transcriptase PCR in 78 cases of GC and adjacent tissues, and in the gastric fluids of 30 patients with chronic nonatrophic gastritis, 30 patients with chronic atrophic gastritis, 21 with early GC, and 57 with advanced GC.

Results: A total of 445 circRNAs, including 69 upregulated and 376 downregulated circRNAs, showed significant aberrant expression in GC tissues. A majority of the differentially expressed circRNAs originated from chr1, chr3, chr4, chr6, and chr11. Hsa_circ_000780 was significantly downregulated in 80.77% of GC tissues, and its level in GC tissues correlated with the tumor size, tumor stage, T stage, venous invasion, carcinoembryonic antigen, and carbohydrate antigen 19-9 expression. A crucial observation was the presence of this circRNA in the gastric fluid of patients with early and advanced GC.

Conclusions: The present study uncovered a new hsa_circRNA expression profile in human GC, of which hsa_circ_000780 was significantly downregulated in GC tissues and in gastric fluid. It can be potentially used as a novel biomarker for early GC screening.

Background

Gastric cancer (GC) ranks third in global cancer mortality and is the most common cause of cancer mortality in China [1]. As GC is difficult to diagnose in the early stage, it is crucial to develop a noninvasive molecular diagnostic target for GC. Presently, the gold standard for the early diagnosis of GC is still gastroscopy. However, China has a large population, inadequate awareness of cancer prevention, low compliance of gastroscopy screening, and the number of digestive endoscopists cannot meet the needs of the general population for gastroscopy screening. In recent years, rapid progress in human genome sequencing, epigenetics, circular RNA (circRNA), and other molecular biological techniques have enabled the search for molecular diagnostic targets for GC. Gene molecular targets are widely distributed in the human body (blood, urine, feces, and various body fluids), the samples are easily obtainable, and the detection technology is mature. Among the various methods for studying gene mutations, circRNAs are a promising target for the molecular diagnosis of GC [2-7].

CircRNA is a closed circular genetic structure with no 3'-end poly-A structure and 5'-end cap structure. CircRNAs range from hundreds to thousands of base pairs in length. They are not degraded by RNA exonuclease, are stable, and exist widely in the biological community with evolutionary conservatism [8]. Studies have reported that while circRNAs are widely considered to be miRNA sponges [9], not many of

them own more predicted miR-binding sites than expected [10, 11]. Recent studies have shown abnormal circRNA expression in various tumor cells can influence tumor occurrence, proliferation, and invasion [12-14]. Due to their considerable stability, CircRNAs and their role in cancer have been the focus of extensive research in recent years as potential tumor molecular targets [15-17], especially in GC [18]. Some researchers have observed that circ_002059, circ_0000745, circ_00000181, circ_0047905, circ_0014717, circ_0001017, and circ_0061276 were significantly downregulated in patients with GC, and have better sensitivity and specificity for the diagnosis of GC [19-24]. Additionally, the role of microRNAs has been highlighted in the development and maintenance of drug resistance in GC, which is the most vital cause of GC treatment failure. CircRNA acts as an miRNA sponge and influences gene regulation and expression [25, 26]. Although the global circRNA expression profile in human GC continues to be studied, no circRNA molecular target with clinical application value in GC has been reported. Moreover, the role of circRNAs in the early diagnosis of GC is not fully understood. Therefore, the present study attempted to identify a specific circRNA for the early diagnosis of GC.

Methods

Sample collection

A total of 82 patients with GC admitted to the Cancer Hospital Affiliated to Hainan Medical College and examined in the endoscopy center from January 2017 to December 2018 were recruited in the study after institutional ethics clearance. Patients younger than 80 years of age with complete clinical data, who were undergoing selective GC surgery, who did not receive chemotherapy or other adjuvant treatment before operation, and those without active gastrointestinal bleeding or obstruction were included in the study. Patients with uncontrolled diabetes or hypertension, coronary heart disease, stroke, cardiovascular, and cerebrovascular diseases; with severe basic diseases such as pulmonary, liver, and kidney dysfunction; and those requiring resection of other organs were excluded from the study. Patients admitted to the Cancer Hospital Affiliated to Hainan Medical College and examined in the endoscopy center from January 2017 to December 2018 were recruited to this study following written informed consent and in accordance with the local ethics committee approval. Of the 82 patients, four patients were selected for the circRNA chip screening study. Of these, there were two men (one with T3N1M0, moderately differentiated adenocarcinoma; one with T3N2M0, poorly differentiated adenocarcinoma), and two women (one with T3N1M0, moderately differentiated adenocarcinoma; one with T3N2M0, poorly differentiated adenocarcinoma). The average age, weight, and height of the four patients were 56.7 years, 58.3 kg, and 168 cm, respectively. Another 78 patients with GC (Table 1) were selected to obtain endoscopic biopsy and gastric fluid samples. These patients were included in the validation study of differential circRNA expression. The diagnostic criteria for early GC (EGC) and advanced GC (AGC) were according to the National Comprehensive Cancer Network clinical practice guidelines in oncology (version 3.2016). Additionally, 30 patients with chronic nonatrophic gastritis (CNAG) and 30 patients with chronic atrophic gastritis (CAG) were randomly selected as the control group. The diagnostic criteria for CNAG and CAG were according to the consensus opinion of the 2012 Chinese Chronic Gastritis of Gastroenterology Branch of the Chinese Medical Association.

The GC specimens were obtained by cutting 0.5 cm³ of the whole layer of the GC tissue, whereas the paracancerous tissue specimens were obtained by cutting 0.5 cm³ of the mucosa at least 5 cm away from the tumor body. The samples were separated from the body, quickly sliced to the required size, and placed into frozen storage tubes stored in liquid nitrogen.

The endoscopic tissue samples and gastric juices were extracted from 78 patients with GC (21 patients with EGC and 57 patients with AGC), 30 patients with CNAG, and 30 patients with CAG. Table 1 illustrates basic characteristics of the patient and control groups. All the specimens were collected and pretreated according to the previously described protocol and preserved at -80°C until RNA extraction [27].

Total RNA extraction and reverse transcription

Total tissue RNA and gastric fluids were extracted using TRIzol reagent (Invitrogen, Life Technologies Inc., Germany). RNA concentration was measured by measuring the absorbance at 260 nm (OD₂₆₀) using a NanoDrop ND-1000 instrument (Thermo Fisher Scientific, DE, USA). Integrity of the RNA was verified by denatured agarose electrophoresis. Finally, the total RNA was transcribed to the cDNA through the GoScript Reverse Transcription (RT) system (Promega, WI, USA) following the manufacturer's protocol.

Microarray hybridization of circRNAs

GC tissues and their matched adjacent nontumorous tissues were selected to analyze the circRNA expression profile using Human circRNA Array v2 (Arraystar, MD, USA). Total RNA was digested with RNase R (20 U/μL, Epicentre, Inc., Madison, WI, USA) to remove linear RNAs and enrich circRNAs. The enriched circRNAs were amplified and transcribed into fluorescent cRNA through a random priming method (Super RNA Labelling Kit; Arraystar). Labeled cRNAs were hybridized onto Human circRNA Array v2 (8 × 15 K, Arraystar). Slides were incubated for 17 h at 65°C in a hybridization oven (Agilent, CA, USA). After washing the slides, the arrays were scanned through an Agilent Scanner (G2505C). The scanned images were then imported into the Agilent Feature Extraction software for grid alignment and data extraction. Quantile normalization and subsequent data processing were performed using the R software package. The expression profile of circRNA, identified through volcano plot filtering, between GC and paired adjacent nontumorous tissues was statistically significant [fold change (FC) ≥ 2.0 and $P \leq 0.05$]. Hierarchical clustering was performed to exhibit the distinguishable expression pattern of circRNAs among samples. The circRNA/microRNA interaction was predicted using TargetScan [28] & miRanda [29].

Quantitative reverse transcription–polymerase chain reaction

The eight most upregulated and downregulated circRNAs exhibiting the greatest difference in expression between groups were selected for quantitative reverse transcription–polymerase chain reaction (qRT–PCR) verification in the four GC specimens and their adjacent tissues. qRT–PCR was performed using the GoTaq qPCR Master Mix (Promega) on an Mx3005P Real-Time PCR System (Stratagene, CA, USA) in accordance with the manufacturer's protocols. Divergent primers of the top eight upregulated and downregulated circRNAs and convergent primers of β-actin (H) were designed and synthesized by

Aksomics (Shanghai) Biotechnology Co. Ltd. The use of divergent primers could only amplify circRNA and differentiate the contamination from its linear isoforms. Table 2 lists the circRNA primer sequences used for this procedure.

RT-PCR was performed as follows: 40 PCR cycles of 95°C for 10 s and 60°C for 60 s for amplification; followed by annealing at 95°C for 10 s, 60°C for 60 s, and 95°C for 15 s with slow heating from 60°C to 99°C (at 0.05°C/s).

The target and housekeeping genes of each sample were analyzed by RT-PCR. According to the gradient dilution DNA standard curve, the concentration results of the target and the housekeeping gene of each sample were directly generated using an Applied Biosystems ViiA™ 7 Real-Time PCR System (ThermoFisher Scientific, USA). The target gene concentration of each sample divided by its housekeeping gene concentration was considered the corrected relative content of this sample gene.

Statistical analysis

Statistical analyses were performed using the SPSS 22.0 software (SPSS, IL, USA). When comparing the GC and paired nontumorous tissue groups for profile differences, the “FC” (the ratio of the group averages) between the groups for each circRNA was computed. The statistical significance of the difference was estimated by the *t* test. CircRNAs having FCs ≥ 2.0 were considered as significantly differentially expressed. The analysis outputs were filtered, and the differentially expressed circRNAs were ranked according to characteristics such as the FC value, *P* value, and chromosome location. The differences in the hsa_circ_000780 levels between the GC and paired adjacent nontumorous tissues were assessed using the *t* test for paired data and among multiple groups (CNAG, CAG, EGC, and AGC) using one-way analysis of variance with the LSD post-hoc test. The correlations between hsa_circ_000780 levels and clinicopathological factors were further analyzed by the Analyze–Correlate–Bivariate menu of SPSS 22.0. A *P* value < 0.05 was considered statistically significant.

Results

Profiles of circRNAs in GC

A total of 13,617 circRNAs were analyzed in the GC and paired nontumorous samples through the circRNA microarray analysis. Among them, 445 circRNAs were significantly aberrantly expressed ($P < 0.05$ and $FC > 2.0$) between the GC and paired nontumorous tissues. FC filtering (Fig. 1A) or volcano plot filtering (Fig. 1B) was used to identify circRNAs whose differential expression was statistically significant. Hierarchical clustering was performed to exhibit the distinguishable circRNA expression pattern among samples (Fig. 1C). Of the 445 circRNAs, 69 (15.51%) were significantly upregulated and 376 (84.49%) were significantly downregulated. The eight most upregulated and downregulated circRNAs in the GC tissues are listed in Table 3. Most of the differentially expressed circRNAs originated from chr1, chr3, chr4, chr6, and chr11, whereas a few circRNAs originated from chr13, ChrX, and chrY (Fig. 1D).

Expression of hsa_circ_000780 in GC

The sample size in this study was expanded to 78 patients with GC and their matched adjacent nontumorous tissues to validate the accuracy of the microarray results and qRT-PCR. The expression levels of hsa_circ_000780 in these tissues were measured by the qRT-PCR method. The relative concentrations of hsa_circ_000780 in GC and their matched adjacent nontumorous tissues were $6.87 \times 10^{-4} \pm 3.12 \times 10^{-4}$ and $11.67 \times 10^{-4} \pm 2.29 \times 10^{-4}$ ($P < 0.001$). Taking the mean value of hsa_circ_000780 in paracancerous tissues as the critical value of GC diagnosis, the downregulation rate of hsa_circ_000780 expression in the GC group was 80.77% (63/78), and that in the paracancerous group was 7.69% (6/78) ($P < 0.001$). Moreover, bioinformatics analysis predicted that hsa_circ_000780 could interact with hsa_miR_522-3p, hsa_miR_381-3p, hsa_miR_300, and hsa_miR_15a-3p (Fig. 2). Subsequently, the correlation between the hsa_circ_000780 expression level and the clinicopathological characteristics of the patients were analyzed. As shown in Table 4, the hsa_circ_000780 expression levels in GC tissues were significantly related to the tumor size ($P = 0.020$), tumor stage ($P = 0.001$), T stage ($P = 0.029$), venous invasion ($P = 0.042$), carcinoembryonic antigen (CEA) ($P = 0.001$), and carbohydrate antigen 19-9 (CA19-9) ($P = 0.001$) expression. However, they were not significantly associated with other clinicopathological factors such as sex, age, tumor location, pathological diagnosis, lymphatic metastasis, distal metastasis, and cell differentiation ($P > 0.05$).

Detection of hsa_circ_000780 in gastric juice

The hsa_circ_000780 level in the gastric fluid of 30 patients with CNAG, 30 patients with CAG, 21 patients with EGC, and 57 patients with AGC were tested through qRT-PCR. The values in CNAG, CAG, EGC, and AGC were $(15.63 \pm 2.44) \times 10^{-4}$, $(12.59 \pm 2.13) \times 10^{-4}$, $(4.28 \pm 0.98) \times 10^{-4}$, and $(4.39 \pm 1.15) \times 10^{-4}$, respectively (Fig. 3). The expression level of hsa_circ_000780 significantly differed between the CNAG and CAG groups compared with the EGC and AGC groups ($P < 0.001$). The hsa_circ_000780 levels were significantly decreased in the gastric fluid of the GC group. No significant difference in hsa_circ_000780 levels was found between both the AGC and EGC groups ($P > 0.05$) or between the CNAG and CAG groups ($P > 0.05$).

Discussion

Several studies have demonstrated the relation of circRNAs with the proliferation, apoptosis, invasion, and metastasis of human tumors [19, 24, 30]. Huang et al. [31] discovered 16 circRNAs that were upregulated and 84 circRNAs that were downregulated in GC. Of these, only hsa_circ_0000026 expression was downregulated by a fold change of 2.8 in GC as detected by qRT-PCR, and this difference was significant. Dang et al. [32] screened the expression profiles of five pairs of GC and matched non-GC tissues and found that 713 circRNAs were differentially expressed in GC, of which 191 and 522 were upregulated and downregulated, respectively. Shen et al. [33] performed circRNA microarray analysis and stated that 347 upregulated and 603 downregulated circRNAs were observed in GC compared with normal gastric tissue. Of 20 randomly selected circRNAs, 10 were verified to have differential expression.

Results of the circRNA microarray in the present study revealed a new circRNA expression profile in human GC, and the differentially expressed circRNA detected in this study showed a significant difference compared to those reported in other studies [24, 32]. This study showed that 445 circRNAs were significantly dysregulated in GC. Of these 445 circRNAs, 15.51% were upregulated and 84.49% were downregulated. The trend of downregulated circRNA expression observed in this study was similar to that reported in other studies. Results of a literature search retrieved from PubMed (<https://www.ncbi.nlm.nih.gov/PubMed/>) before November 8, 2019, did not report the top eight upregulated and downregulated circRNAs in GC. This study is the first to report the eight most upregulated and downregulated circRNAs in GC. These results suggested the genetic heterogeneity of GC. In addition, the distribution of differentially expressed circRNAs on human chromosomes in this study exhibited that most of them were transcribed from chr1, chr3, chr4, chr6, and chr11. Shao et al [24] observed that the differentially expressed circRNAs were mainly transcribed from chr1 and chr3, suggesting that despite the great heterogeneity in the genetic mechanism of GC, there remained to be overlaps in the expression of circRNAs. This observation may provide a direction for the further study of GC pathogenesis and diagnostic targets.

The circRNA expression profiles in GC further confirmed that circRNAs were closely associated with GC. However, only few circRNAs were ascertained to regulate carcinogenesis in GC [34-36]. In the present study, hsa_circ_000780 was selected as a targeted circRNA to validate the accuracy of the microarray results. The results revealed that hsa_circ_000780 was significantly downregulated in 80.77% of GC tissues. Bioinformatics analysis predicted that hsa_circ_000780 could interact with hsa_miR_522-3p, hsa_miR_381-3p, hsa_miR_300, and hsa_miR_15a-3p. Additionally, the hsa_circ_000780 expression level in GC was significantly related to the tumor size, stage, degree of invasion, and CEA and CA19-9 expression, suggesting that hsa_circ_000780 had the potential to predict clinical prognosis.

Gastric juices is a good sample for use in the diagnosis of gastric diseases. In the present study, we further studied the expression of hsa_circ_000780 in the gastric juices of patients with CNAG, CAG, EGC, and AGC. Although the hsa_circ_000780 levels in the gastric juices of GC patients was obviously decreased, there was no significant difference in the hsa_circ_000780 level between the EGC and AGC groups. This finding indicates that hsa_circ_000780 could exist in gastric juices, and has the potential for use as a biomarker for early GC screening.

Conclusions

In conclusion, the present study found a new expression profile of circRNAs in GC. Among them, hsa_circ_000780 was significantly downregulated in GC tissues, suggesting that it might be involved in the occurrence of GC. The level of this circRNA was related to some clinicopathological characteristics of patients with GC. However, its role and mechanism in the occurrence of GC must be studied further. hsa_circ_000780 could be detected in gastric juices in early GC and was significantly different than that in the control group. Therefore, this circRNA has the potential to be used as novel biomarkers for the screening of early GC.

Abbreviations

circRNA: circular RNA; GC: gastric cancer; PCR: polymerase chain reaction; EGC: early gastric cancer; AGC: advanced gastric cancer; NCCN: National Comprehensive Cancer Network; CNAG: chronic nonatrophic gastritis; RT: Reverse Transcription; FC: fold change; qRT-PCR: quantitative reverse transcription–polymerase chain reaction.

Declarations

Ethics approval and consent to participate

The study was approved by the human medicine research ethics committee of Hainan Cancer Hospital (No. 2017011003). All participants provided written informed consent.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JS was responsible for the project design, main research issues, and paper writing; SY and DZ were responsible for the project experiment guidance; WY, ZJ, GY, PL, RZ, YL, GZ, and ZC were responsible for the project material supply, experiment, and data collation and analysis. All authors have read and approved the manuscript

Acknowledgments

Not applicable

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Tables

Table 1. Basic characteristics of patients

Characteristic	CNAG (<i>n</i> = 30)	CAG (<i>n</i> = 30)	EGC (<i>n</i> = 21)	AGC (<i>n</i> = 57)
Male	15	15	11	29
Female	15	15	10	28
Age (years)	51.7 ± 9.6	58.9 ± 9.5	59.6 ± 10.3	58.8 ± 8.6
BMI (kg/m ²)	25.5 ± 2.5	24.8 ± 1.8	25.8 ± 2.3	23.3 ± 1.6
CEA (ng/ml)	/	/	4.56 ± 1.5	35.7 ± 3.5
CA199 (u/ml)	/	/	25.7 ± 3.5	78.6 ± 9.5

CNAG: chronic nonatrophic gastritis, CAG: chronic atrophic gastritis, EGC: early gastric cancer, AGC: advanced gastric cancer.

Table 2. Primer sequences of circRNAs

Gene	Primers
β -actin (H)	F: 5'-GTGGCCGAGGACTTTGATTG-3'
	R: 5'-CCTGTAACAACGCATCTCATATT-3'
Hsa_circ_000102	F: 5'-AACGTATGAGGGTAGAAGAGAGA-3'
	R: 5'-TCAGGTCTATAATCAATTTTCATCTC-3'
Hsa_circ_000320	F: 5'-AATCTTAAGGGGCCAAAATTG-3'
	R: 5'-TCCATTTTGGGTCCTTTGATT-3'
Hsa_circ_000324	F: 5'-GTAAGTAAGTGCCCGCACCATA-3'
	R: 5'-CAGCGTGTTAGCAACAGAACC-3'
Hsa_circ_000780	F: 5'-TAGGAAACCTGCTGTGGAGTG-3'
	R: 5'-AAGGGAAC TATACAAGGAAATGC-3'
Hsa_circ_007738	F: 5'-ACATTGAGGAAGAAGGGCAGTA-3'
	R: 5'-TTCAAGAGGGCTTACCTGGTA-3'
Hsa_circ_047478	F: 5'-CAGGAAGCCTAAAGGATTTTG-3'
	R: 5'-CTTCAATAAACGGGAGGTGGT-3'
Hsa_circ_049637	F: 5'-ATTAAATTTTGTGTCTCCGCG-3'
	R: 5'-CCTTTAAAACGACCCTCCG-3'
Hsa_circ_102411	F: 5'-CACCAACGACCATGAGAAGGTG-3'
	R: 5'-AAGGACAGCAGGACGCAGAC-3'
Hsa_circ_103128	F: 5'-CAAGCACCAAAAGCAAGAAA-3'
	R: 5'-CAGCGGCAAAACTATAACACC-3'
Hsa_circ_104293	F: 5'-GCACAGATCTGATTCTGAACGT-3'
	R: 5'-TCATTGGATATGTCCTGATAGTCC-3'
Hsa_circ_404798	F: 5'-CTTCCGAATGCAAGAAAGATTG-3'

	R: 5'-CCCTTACGGTACTTGAGGACC-3'
Hsa_circ_000250	F: 5'-GGGAGTGGCTGTGGATAAGT-3'
	R: 5'-AGCATTTTTTGTGAAATGGTGC-3'
Hsa_circ_018497	F: 5'-GTGATGGATATGATGGTGCAT-3'
	R: 5'-AGTCCACGAAGTCGTACTGTC-3'
Hsa_circ_008882	F: 5'-TGGCAGCCTAGCATTAGC-3'
	R: 5'-AGGGAGGTTGAAGTGAGAGGT-3'
Hsa_circ_002699	F: 5'-TGAGCACTGCTTTAATAGGGG-3'
	R: 5'-GCCTTCATTATGAGAGGTTTATC-3'

Table 3. The eight most upregulated and downregulated circRNAs in GC

CircRNA ID	Chromosome	Regulation	Fold change	Strand	GeneSymbol	<i>P</i> value
Has_circ_047478	chr18	Up	2.5070284	+	KIAA1328	0.017366625
Has_circ_104293	chr7	Up	2.0857679	-	FBXL18	0.021932803
Has_circ_000324	chr11	Up	2.0957677	+	NEAT1	0.045278792
Has_circ_000102	chr1	Up	2.4795408	-	AKNAD1	0.039757867
Has_circ_007738	chr9	Up	2.9836255	-	SHC3	0.037385496
Has_circ_018497	chr10	Up	2.0093889	+	HNRNPH3	0.046702283
Has_circ_002699	chr7	Up	2.7422086	+	MET	0.026582751
Has_circ_000250	chr18	Up	2.2513738	-	SMAD7	0.036879883
Has_circ_049637	chr19	Down	4.6708359	+	CALR	0.017328184
Has_circ_404798	chr10	Down	2.6790388	-	DNMBP	0.018477575
Has_circ_000780	chr10	Down	2.3500864	-	FAM107B	0.021222425
Has_circ_000320	chr11	Down	2.4551818	-	AHNAK	0.035942593
Has_circ_405324	chr15	Down	3.2099669	+	STARD9	0.026064961
Has_circ_008882	chrM	Down	6.0137146	+	MTND5	0.013967832
Has_circ_102411	chr19	Down	2.7435361	-	MFSD12	0.024725222
Has_circ_103128	chr21	Down	2.5589795	+	DYRK1A	0.049179211

Table 4. Correlation of the expression levels of hsa_circ_000780 with the clinicopathological characteristics of GC tissues

Characteristics	No. of patients (<i>n</i> = 78, %)	Expression levels of has_circ_000780 (Mean ± SD, ×10 ⁻⁴)	<i>P</i> value
Age (year)			
≥ 60	45 (57.7)	5.97 ± 1.65	1.000
< 60	33 (42.3)	5.97 ± 2.12	
Sex			
Male	40 (51.3)	6.32 ± 3.09	0.112
Female	38 (48.7)	7.45 ± 3.09	
Tumor location			
Sinuses ventriculi	39 (50.0)	6.20 ± 3.29	0.418
Cardia	17 (21.8)	7.80 ± 3.04	
Corpora ventriculi	13 (16.7)	6.90 ± 2.57	
Others	9 (11.5)	7.98 ± 2.96	
Diameter (cm)			
≥5	38 (48.7)	6.03 ± 3.16	0.020
<5	40 (51.3)	7.67 ± 2.91	
Differentiation			
Well	9 (11.5)	7.29 ± 2.55	0.303
Moderate	36 (46.2)	7.38 ± 3.04	
Poor	32 (41.3)	6.20 ± 3.20	
Stage			
Early	21 (26.9)	5.08 ± 2.13	0.001
Advanced	57 (73.1)	7.53 ± 3.19	
Pathologic diagnosis			
Signet ring cell cancer	11 (14.1)	5.21 ± 2.80	0.055
Adenocarcinoma	67 (85.9)	7.14 ± 3.11	
T stage			

T1 and T2	25 (32.1)	5.78 ± 2.84	0.029
T3 and T4	53 (67.9)	7.38 ± 3.14	
Lymphatic metastasis			
N0	28 (56.0)	6.63 ± 3.44	0.625
N1-2	50 (44.0)	7.01 ± 2.96	
Distal metastasis			
M0	68 (87.2)	5.81 ± 3.74	0.345
M1	10 (12.8)	7.03 ± 3.02	
Venous invasion			
Absent	41 (51.3)	6.19 ± 3.20	0.042
Present	37 (51.3)	7.62 ± 2.89	
Carcinoembryonic antigen			
Positive	25 (52.6)	5.22 ± 2.63	0.001
Negative	53 (47.1)	7.65 ± 3.05	
CA19-9 (Tissue)			
Positive	21 (26.9)	4.67 ± 2.14	0.001
Negative	57 (73.1)	7.68 ± 3.05	
Bold values: $P < 0.05$.			

Figures

Figure 1

Differences and characterizations of the circRNA expression profile between GC and paired adjacent nontumorous tissues. (a) Scatter plots were used to evaluate the difference in the circRNA expression between GC and paired adjacent nontumorous tissues. (b) Volcano plots were used to visualize the differential expression of circRNA between GC and paired adjacent nontumorous tissues. The red points in the plot represent the differentially expressed circRNAs with statistical significance. (c) Hierarchical

cluster analysis of expressed circRNAs in GC tissues (blue bars) and paired adjacent nontumorous tissues (red bar). (d) Chromosomal distribution of differentially expressed circRNAs in GC tissues and paired adjacent nontumorous tissues.

Figure 2

Bioinformatics analysis predicting that hsa_circ_000780 interacted with (A) hsa_miR_522-3p, (B) hsa_miR_381-3p. (C) hsa_miR_300, and (D) hsa_miR_15a-3p.

Figure 3

The corrected relative concentration of hsa_circ_000780 in gastric juices. Hsa_circ_000478 levels in gastric juices at various stages of GC, including patients with CNAG (n = 30), CAG (n = 30), EGC (n = 21), and AGC (n = 57), were detected by qRT-PCR (**P < 0.001).