Genomic circular RNA expression profile analysis indicated hsa_circRNA_000780 as a diagnostic marker for gastric cancer

Jian Song (✉ songjian0532@sina.com)

The Affiliated cancer Hospital of Hainan Medical University <https://orcid.org/0000-0002-6507-3230>

Shuyong Yu

The Affiliated cancer Hospital of Hainan Medical University

Dunjing Zhong

The Affiliated cancer Hospital of Hainan Medical University

Weizhong Yang

The Affiliated Second Hospital of Hainan Medical University

Zhen Jia

The Affiliated cancer Hospital of Hainan Medical University

Guihong Yuan

The Affiliated cancer Hospital of Hainan Medical University

Ping Li

The Affiliated cancer Hospital of Hainan Medical University

Ronglin Zhang

The Affiliated cancer Hospital of Hainan Medical University

Yini Li

The Affiliated cancer Hospital of Hainan Medical University

Guobing Zhong

The Affiliated cancer Hospital of Hainan Medical University

Zhaowei Chen

The Affiliated cancer Hospital of Hainan Medical University

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Abstract

Background: This study aimed to find the specific circular RNA (circRNA) for gastric cancer (GC) and lay the foundation for the early diagnosis of GC.

Methods: Four patients with GC were selected for this study. The total RNA of their cancer tissues and adjacent tissues was extracted, and the circRNA was screened. The top eight upregulated and downregulated circRNAs with statistical significance between GC and paired adjacent nontumorous tissues were identified using real-time fluorescent quantitative polymerase chain reaction (PCR). The expression level of circRNA was identified by quantitative reverse transcription PCR in 78 cases of GC and its adjacent tissues, and in the gastric juice of 30 patients with chronic nonatrophic gastritis, 30 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, were found to be significantly aberrantly expressed in GC tissues. Most of the differentially expressed circRNAs originated from chr1, chr3, chr4, chr6, and chr11. Hsa_circRNA_000780 was significantly downregulated in 80.77% of GC tissues. Its level in GC tissues correlated with the tumor size, tumor stage, T stage, venous invasion, carcinoembryonic antigen, and carbohydrate antigen 19-9 expression. More importantly, hsa_circRNA_000780 was found in the gastric juice of early and advanced GC.

Conclusions: This study uncovered the new hsa_circRNA expression profile in human GC. Among them, hsa_circRNA_000780 was significantly downregulated in GC tissues and gastric juice. It had the potential to be used as a novel biomarker for the screening of early GC.

Background

Gastric cancer (GC) ranks third in the global cancer mortality and first in China [1]. GC is difficult to diagnose in the early stage. Therefore, it is very important to develop a noninvasive molecular diagnostic target for GC. At present, the gold standard of early diagnosis for GC is still gastroscopy; however, in China, the population is large, the awareness of cancer prevention is insufficient, the compliance of gastroscopy screening is low, and the number of digestive endoscopists cannot meet the needs of the general population for gastroscopy screening. In recent years, the rapid progress of human genome sequencing, epigenetics, circular RNA (circRNA), and other molecular biology techniques has enabled the search of molecular diagnostic targets for GC. Gene molecular targets are widely distributed in human body (blood, urine, feces, various body fluids, and so forth), samples are easy to obtain, detection technology is mature, and so forth. Among various methods for studying gene mutations, circRNAs is a promising target for GC molecular diagnosis [2-7].

CircRNA is a closed circular structure, which has no 3'-end polyA structure and 5'-end cap structure, and its length ranges from hundreds to thousands. It is not degraded by RNA exonuclease, and it is stable and widely exists in the biological community, with evolutionary conservatism [8]. In recent years, the abnormal expression of circRNA in various tumor cells has been found to play an important role in tumor occurrence, proliferation, and invasion [9-11]. It has become a research hotspot of tumor molecular targets because of its good stability. The study of circRNA in GC has increased largely in recent years. Some researchers found that circ_002059, circ_0000745, circ_00000181, circ_0047905, circ_0014717, circ_0001017, and circ_0061276 were significantly

downregulated in patients with GC, having better sensitivity and specificity for the diagnosis of GC [12-17]. However, the global circRNA expression profile in human GC has been less studied, and no circRNA molecular target has been reported with clinical application value in GC. Moreover, the role of circRNAs in early diagnosis of GC is not fully understood. Therefore, this study aimed to find the specific circRNA for GC and lay the foundation for the early diagnosis of GC.

Methods

Sample collection

The 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. The inclusion criteria of patients with GC were as follows: (1) patients younger than 80 years, undergoing selective GC surgery, did not receive chemotherapy or other adjuvant treatment before operation, and without active gastrointestinal bleeding or obstruction; (2) patients with complete clinical data; and (3) the experiment was approved by the hospital ethics committee, and the patients and their families signed the informed consent form. The exclusion criteria were as follows: (1) patients with uncontrolled diabetes or hypertension, coronary heart disease, stroke, cardiovascular and cerebrovascular diseases, and severe basic diseases such as pulmonary, liver, and kidney dysfunction; and (2) patients requiring resection of other organs. Four patients with GC were selected for the study of circRNA chip screening. Among them, two were men (one with T3N1M0, moderately differentiated adenocarcinoma, and one with T3N2m0, poorly differentiated adenocarcinoma), and two were women (one with T3N1M0, moderately differentiated adenocarcinoma, and one with T3N2m0, poorly differentiated adenocarcinoma). The average age, weight, and height of these four patients were 56.7 years, 58.3 kg, and 168 cm, respectively. Another 78 patients with GC (Table 1) were selected, and their endoscopic biopsy samples and gastric juice samples were included in the validation study of the differential expression of circRNA. The diagnostic criteria of early gastric cancer (EGC) and advanced gastric cancer (AGC) were according to the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in oncology GC (version 3.2016). Meanwhile, 30 patients with chronic nonatrophic gastritis (CNAG) and 30 patients with chronic atrophic gastritis (CAG) were randomly included in the control study. The diagnostic criteria of CNAG and CAG were according to the consensus opinion of the 2012 Chinese Chronic Gastritis of Gastroenterology Branch of Chinese Medical Association.

The specimens of GC were obtained as follows. The diameter of GC tissue was about 0.5 cm, obtained by cutting the whole layer of the tumor body; the diameter of paracancerous tissue was about 0.5 cm, obtained by cutting the mucosa at least 5 cm away from the tumor body. The sample was separated from the body, quickly sliced to the required size, and put into the frozen storage tube stored in liquid nitrogen for standby.

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. The basic characteristics of the patient and control groups are shown in Table 1. All specimens were collected and pretreated in accordance with the previously described protocol and preserved at -80°C until RNA extraction [18].

Total RNA extraction and reverse transcription

The total RNA of tissue and gastric juice was extracted using TRIzol reagent (Invitrogen, Life Technologies Inc., Germany). The RNA concentration was measured from OD260 using a NanoDrop ND-1000 instrument (Thermo Fisher Scientific, DE, USA). The RNA integration was verified by denatured agarose electrophoresis. Finally, the total RNA was transcribed to the cDNA using 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 the Arraystar Human circRNA ArrayV2 (Arraystar, MD, USA). The total RNAs were digested with RNase R (20 U/ μ L, Epicentre, Inc. Madison WI 53713, USA) to remove linear RNAs and enrich circRNAs. The enriched circRNAs were amplified and transcribed into fluorescent cRNA using a random priming method (Super RNA Labelling Kit; Arraystar). The labeled cRNAs were hybridized onto the Human circRNA ArrayV2 (8 × 15 K, Arraystar). The slides were incubated for 17 h at 65°C in a hybridization oven (Agilent, CA, USA). After washing the slides, the arrays were scanned using the 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. CircRNAs differentially expressed with statistical significance between GC and paired adjacent nontumorous tissues [fold change (FC) $\geq$ 2.0 and $P \leq$ 0.05] were identified through volcano plot filtering. Hierarchical clustering was performed to show the distinguishable expression pattern of circRNAs among samples. The circRNA/microRNA interaction was predicted using TargetScan [19] & miRanda [20].

Quantitative reverse transcription–polymerase chain reaction

According to the results of circRNA chip screening, the top eight upregulated and downregulated circRNAs with the greatest difference were selected for quantitative reverse transcription–polymerase chain reaction (qRT-PCR) verification in four GC and its adjacent tissues, which were studied using the Arraystar Human circRNA ArrayV2. The qRT-PCR was performed using the GoTaq qPCR Master Mix (Promega) on an Mx3005P Real-Time PCR System (Stratagene, CA, USA) following 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. The primer sequences of circRNA are listed in Table 2.

RT-PCR was performed as follows: 40 PCR cycles (95°C, 10 s; 60°C, 60 s). To establish the fusion curve of PCR products, the procedures (95°C, 10 s; 60°C, 60 s; 95°C, 15 s) were performed after the amplification reaction, with slow heating from 60°C to 99°C (the ramp rate of the instrument was 0.05°C/s).

The target gene and housekeeping gene of each sample were analyzed by RT-PCR. According to the gradient dilution DNA standard curve, the concentration results of the target gene and the housekeeping gene of each sample were directly generated using a machine. The target gene concentration of each sample divided by the concentration of its housekeeping gene was the corrected relative content of this gene of the sample.

Statistical analysis

Statistical analyses were performed using the SPSS 22.0 software (SPSS, IL, USA). When comparing GC and paired nontumorous tissue groups of profile differences, the “fold change” (the ratio of the group averages) between the groups for each circRNA was computed. The statistical significance of the difference was conveniently estimated by the *t* test. CircRNAs having FCs ≥ 2.0 were selected as significantly differentially expressed. The analysis outputs could be filtered, and the differentially expressed circRNAs were ranked according to FC, *P* value, chromosome location, and so forth, using Microsoft Excel’s Data/Sort and Filter functionalities. The differences in the levels of hsa_circ_000780 between GC tissues 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. The correlations between hsa_circ_000780 levels and clinicopathological factors were further analyzed by analyze–correlated–bivairate 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 detected in GC and paired nontumorous samples by circRNA microarray analysis. Among them, 445 circRNAs were significantly aberrantly expressed ($P < 0.05$ and FC was > 2.0) between GC tissues and paired nontumorous tissues. Differentially expressed circRNAs with statistical significance were identified through FC filtering (Fig. 1A) or volcano plot filtering (Fig. 1B). Hierarchical clustering was performed to show 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 top eight upregulated circRNAs in GC (hsa_circRNA_047478, hsa_circRNA_104293, hsa_circRNA_000324, hsa_circRNA_000102, hsa_circRNA_007738, hsa_circRNA_018497, hsa_circRNA_002699, and hsa_circRNA_000250) and downregulated circRNAs (hsa_circRNA_049637, hsa_circRNA_404798, hsa_circRNA_000780, hsa_circRNA_000320, hsa_circRNA_405324, hsa_circRNA_008882, hsa_circRNA_102411, and hsa_circRNA_103128) are listed in Table 3. Most of the differentially expressed circRNAs originated from chr1, chr3, chr4, chr6, and chr11, and few from chr13, ChrX, and chry (Fig. 1D).

Expression of hsa_circ_000780 in GC

The sample size in this study was expanded to 78 GC and its matched adjacent nontumorous tissues to validate the accuracy of microarray results and qRT-PCR. Its expression levels in 78 GC and their matched adjacent nontumorous tissues were measured by the qRT-PCR method. The relative contents 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) (GC vs control, $P < 0.001$). 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 expression level of hsa_circ_000780 and the clinicopathological characteristics of the patients were analyzed. As shown in Table 4, its 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 antigen19-9 (CA19-9; $P = 0.001$) expression. However, they were not associated with other clinicopathological factors such as sex, age, tumor location, pathological diagnosis, lymphatic metastasis, distal metastasis, and cell differentiation.

Detection of hsa-circ-000780 in gastric juice

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

Discussion

A large number of studies demonstrated that circRNAs were strongly related to the proliferation, apoptosis, invasion, and metastasis of human tumors [12, 17, 21]. Huang et al. [22] discovered that 16 circRNAs were upregulated whereas 84 circRNAs were downregulated in GC. Among these circRNAs, only the expression of hsa_circ_0000026 was significantly downregulated by 2.8-FC in GC as detected by qRT-PCR. Dang et al. [23] revealed that 713 circRNAs showed differential expression in GC tissues as screened by the expression profiles of five pairs of GC and matched non-GC tissues. Of these circRNAs, 191 and 522 were upregulated and downregulated, respectively. Shen et al. [24] performed a circRNA microarray analysis and confirmed that 347 upregulated and 603 downregulated circRNAs were found in GC compared with the normal gastric tissue. Ten out of 20 randomly selected circRNAs were verified to have differential expression. The results of the circRNA microarray in the present study showed a new expression profile of circRNA in human GC, and a significant difference was found in the differentiated circRNA between this study and previous studies [17, 23]. This study showed that 445 circRNAs were significantly dysregulated in GC. Among them, the upregulated ones accounted for 15.51% and the downregulated ones for 84.49%. In GC, the downregulated expression trend of circRNA was mainly the same as that in previous studies. The literature from PubMed (<https://www.ncbi.nlm.nih.gov/pubmed/>) before November 8, 2019, did not report the top eight upregulated and top eight downregulated circRNAs in GC found in this study. These results suggested the genetic heterogeneity of GC. In addition, the distribution of differentially expressed circRNAs on human chromosomes showed that most of them were transcribed from chr1, chr3, chr4, chr6, and chr11 in this study. Compared with the studies by Shao et al [17], it was found that the differentially expressed circRNAs were mainly from chr1 and chr3 chromosomes, suggesting that despite the great heterogeneity in the genetic mechanism of GC, the expression of circRNAs still had common points. This provided the direction and clue for the further study of GC pathogenesis and diagnostic targets.

The expression profiles of circRNAs in GC further confirmed that circRNAs were closely associated with GC. However, only a small number of circRNAs were ascertained to regulate carcinogenesis in GC [25-27]. In this study, hsa_circ_000780 was selected as a targeted circRNA to validate the accuracy of microarray results. The results showed 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. In addition, the expression level of hsa_circ_000780 in GC 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.

The gastric juice is a good sample for diagnosing gastric diseases. In this study, the expression of hsa_circ_000780 in the gastric juice of patients from CNAG, CAG, EGC, and AGC was further studied. The hsa_circ_000780 levels in the gastric juice from patients with GC obviously decreased. No significant difference in the level of hsa_circ_000780 was found between the EGC and AGC groups. This implied that hsa_circ_000780 could exist in gastric juice, and had the potential to be used as a biomarker for early GC screening.

Conclusions

In conclusion, this study found a new expression profile of circRNAs in GC. Among them, hsa_circRNA_000780 was significantly downregulated in GC tissues, which was related to some clinicopathological characteristics of patients with GC, suggesting that it might be involved in the occurrence of GC. However, its role and mechanism in the occurrence of GC need to be studied further. In addition, hsa_circRNA_000780 could be detected in the gastric juice of early GC, and had a significant difference with the control group. It had 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; CAG: chronic atrophic gastritis; 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 analysed during the current study are not publicly available due [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

SJ was responsible for the project design, main research issues and paper writing; YS and ZD were responsible for the project experiment guidance; YW, JZ, YG, LP, ZR, LY, ZG, and CZ were responsible for the project material supply, experiment and data collation and analysis. All authors have read and approved the manuscript

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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	51.7 ± 9.6	58.9 ± 9.5	59.6 ± 10.3	58.8 ± 8.6
BMI	25.5 ± 2.5	24.8 ± 1.8	25.8 ± 2.3	23.3 ± 1.6
CEA	/	/	4.56 ± 1.5	35.7 ± 3.5
CA199	/	/	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_circRNA_000102	F: 5'-AACGTATGAGGGTAGAAGAGAGA-3' R: 5'-TCAGGTCTATAATCAATTTTCATCTC-3'
Hsa_circRNA_000320	F: 5'-AATCTTAAGGGGCCAAAATTG-3' R: 5'-TCCATTTTGGGTCCTTTGATT-3'
Hsa_circRNA_000324	F: 5'-GTAAGTAAGTGCCCGCACCATA-3' R: 5'-CAGCGTGTTAGCAACAGAACC-3'
Hsa_circRNA_000780	F: 5'-TAGGAAACCTGCTGTGGAGTG-3' R: 5'-AAGGGAACCTATACAAGGAAATGC-3'
Hsa_circRNA_007738	F: 5'-ACATTGAGGAAGAAGGGCAGTA-3' R: 5'-TTCAAGAGGGCTTACCTGGTA-3'
Hsa_circRNA_047478	F: 5'-CAGGAAGCCTAAAGGATTTTG-3' R: 5'-CTTCAATAAACGGGAGGTGGT-3'
Hsa_circRNA_049637	F: 5'-ATTAAATTTTGTGTCTCCGCG-3' R: 5'-CCTTTAAAACGACCCTCCG-3'
Hsa_circRNA_102411	F: 5'-CACCAACGACCATGAGAAGGTG-3' R: 5'-AAGGACAGCAGGACGCAGAC-3'
Hsa_circRNA_103128	F: 5'-CAAGCACCAAAAGCAAGAAA-3' R: 5'-CAGCGGCAAACTATAACACC-3'
Hsa_circRNA_104293	F: 5'-GCACAGATCTGATTCTGAACGT-3' R: 5'-TCATTGGATATGTCCTGATAGTCC-3'
Hsa_circRNA_404798	F: 5'-CTTCCGAATGCAAGAAAGATTG-3' R: 5'-CCCTTACGGTACTTGAGGACC-3'
Hsa_circRNA_000250	F: 5'-GGGAGTGGCTGTGGATAAGT-3' R: 5'-AGCATTTTTGTGAAATGGTGC-3'
Hsa_circRNA_018497	F: 5'-GTGATGGATATGATGGTGCAT-3' R: 5'-AGTCCACGAAGTCGTACTGTC-3'
Hsa_circRNA_008882	F: 5'-TGGCAGCCTAGCATTAGC-3' R: 5'-AGGGAGGTTGAAGTGAGAGGT-3'

Hsa_circRNA_002699 F: 5'–TGAGCACTGCTTTAATAGGGG-3'
R: 5'–GCCTTCATTATGAGAGGTTTATC-3'

Table 3. Top eight upregulated and downregulated circRNAs in GC

CircRNA ID	Chromosome	Regulation	Fold change	Strand	GeneSymbol	P value
hsa_circRNA_047478	chr18	Up	2.5070284	+	KIAA1328	0.017366625
hsa_circRNA_104293	chr7	Up	2.0857679	–	FBXL18	0.021932803
hsa_circRNA_000324	chr11	Up	2.0957677	+	NEAT1	0.045278792
hsa_circRNA_000102	chr1	Up	2.4795408	–	AKNAD1	0.039757867
hsa_circRNA_007738	chr9	Up	2.9836255	–	SHC3	0.037385496
hsa_circRNA_018497	chr10	Up	2.0093889	+	HNRNPH3	0.046702283
hsa_circRNA_002699	chr7	Up	2.7422086	+	MET	0.026582751
hsa_circRNA_000250	chr18	Up	2.2513738	–	SMAD7	0.036879883
hsa_circRNA_049637	chr19	Down	4.6708359	+	CALR	0.017328184
hsa_circRNA_404798	chr10	Down	2.6790388	–	DNMBP	0.018477575
hsa_circRNA_000780	chr10	Down	2.3500864	–	FAM107B	0.021222425
hsa_circRNA_000320	chr11	Down	2.4551818	–	AHNAK	0.035942593
hsa_circRNA_405324	chr15	Down	3.2099669	+	STARD9	0.026064961
hsa_circRNA_008882	chrM	Down	6.0137146	+	MTND5	0.013967832
hsa_circRNA_102411	chr19	Down	2.7435361	–	MFSD12	0.024725222
hsa_circRNA_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 circRNA expression profile between GC and paired adjacent nontumorous tissues. (A) Scatter plots were used to evaluate the difference in the expression of circRNA 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 and paired adjacent nontumorous tissues. (D) Chromosomal distributions of differentially expressed circRNAs in GC tissues and paired adjacent nontumorous tissues.

Figure 2

Bioinformatics analysis predicted 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

Corrected relative concentration of hsa_circ_000780 in gastric juice. The hsa_circ_000478 levels in gastric juices in 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.)