Circular RNA _0008278 acts as a sponge of miR-378 and promotes proliferation and metastasis in gastric cancer

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Abstract

Background: Circular RNA (circRNA) is rising as an indispensable regulatory molecule in the progression of various kinds of malignant growth. However, little is known about the capacity and instruments of circRNA_0008727 in gastric cancer (GC). Our point was to recognize a novel circRNA-microRNA-mRNA useful system in gastric cancer.

Method: CircRNA_0008278 was identified in three paired cancer specimens and adjacent normal tissues by RNA sequencing and genome-wide bioinformatic analysis and verified by quantitative real-time PCR (qRT-PCR). Knockdown or exogenous expression of circRNA_0008278 combined with in vitro and in vivo assays were performed to prove the functional significance of circRNA_0008278. The molecular mechanism of circRNA_0008278 was demonstrated by searching the CircNet database and confirmed by RNA in vivo precipitation assays, western blotting, luciferase assays and rescue experiments.

Results: CircRNA_0008278 was frequently upregulated in GC tissues, and high circRNA_0008278 expression was associated with poor prognosis, lymph node metastasis and poor TNM stage in GC patients. Functionally, circRNA_0008278 overexpression promoted GC cell proliferation and tumourigenicity in vitro and in vivo. Furthermore, circRNA_0008278 over-expression enhanced GC cell migration and invasion in vitro and tumour metastasis in vivo. In addition, we demonstrated that circRNA_0008278 could sponge miR-378, thus indirectly regulating the YY1 expression and contributing to GC tumourigenesis.

Conclusion: Our findings demonstrate that circRNA_0008278 functions as a tumour promoter in GC, and a new pathway circRNA_0008278/miR-378/YY1 which may be potential method for gastric cancer treatment.

Background

Gastric cancer (GC) is the most widely recognized tumor in the world. It showed that approximately 679,000 GC cases were occurred and 498,000 cases were died annually in China[1]. GC remains the third leading reason of malignant-related deaths and the fourth most elevated rate of malignant growth worldwide[2]. As we have known, the mortality of GC is high associated with the stage of GC. Several studies in Europe reported the detection rate early GC found during GC resections was 11–15% [3, 4], which was lower in China. There are still a large numbers of GC patients with poor prognosis, especially for those diagnosed at advanced stage[5]. Therefore, it is fundamental to found the molecular mechanisms for diagnosis and potential focuses for GC treatment.

Circular RNA (circRNA), a newly-identified noncoding RNA, form from the linkage of the 3' and 5' ends to a loop structures derived from exons, introns or intergenic regions[6]. In the last studies, it showed that several properties of circRNAs that are created by backsplicing, so it was considerably more steady than liner RNA. The most important function of circRNA is modulating gene transcription and interacting with RNA binding proteins as miRNA sponges[7, 8]. Several studies revealed that circRNAs participated in the

event and procedure of illnesses including cancers. For example, circRNA_001569 serves as miR-145 sponge regulating the development of colorectal cancer[9] . CircHIPK3 serves as miR-124 sponge regulating the expression of ELF3 in nasopharyngeal carcinoma[10] .

In our studies, we showed the expression of circRNA_0008278, miR-378 and Yin Yang 1 (YY1) were abundantly expressed in gastric cancer tissues and cells. Then, we aimed to identify the interaction of these genes and demonstrated whether circRNA_0008278/miR-378/YY1 axis involving in GC. These results may contribute to the pathogenesis of GC.

Materials And Methods

CircRNAs expression profiles in gastric cancer

Human circRNA microarray analysis was performed by Yunxu Biotechnology (Shanghai). Total RNA was extracted from gastric cancer tissues and from adjacent noncancerous tissues, according to Trizol protocol (Takara, Japan). Total RNA was used for circRNA microarray analysis (Agilent human circRNA). RNA labeling and hybridization were performed in accordance to the manufacturer's indications. Agilent scanner and the Feature Extraction 10.7.1.1 software (Agilent Technologies) were used to obtain the microarray raw data. Microarray results were analyzed using the GeneSpring GX 12.5 software (Agilent Technologies). Differentially expressed circRNAs were identified by using a moderated t-test and Benjamini-Hochberg correction (adjusted $P \leq 0.05$ and fold change ≥ 2). Differentially expressed genes were measured similarity with circRNAs by the Pearson correlation and were analyzed in Gene Ontology (GO) analysis, KEGG pathway analysis, and cluster analysis.

Differential expression analysis of miRNAs

The miRNA expression, clinical, and meta- and manifest data on GC were derived from The Cancer Genome Atlas (TCGA). MiRNA-sequencing data was downloaded, a Bioconductor package based on the R language, to screen differentially expressed miRNAs between GC tissue and adjacent normal tissue. Differentially expressed miRNAs with FDR values < 0.05 and $|\log_2FC| > 1$ were considered significantly.

CircRNA targeting miRNA prediction and prognosis of miRNAs

According to the results of the differential circRNA expression analysis, targeted miRNAs of hsa_circRNA_0008727 were predicted via Cancer-Specific CircRNA Database (CSCD). We used the keyword "hsa_circ_0008278" in the CSCD and obtained the miRNAs that could bind to the hsa_circ_0008278. Furthermore, we extracted the expression and survival time of miRNAs related GC via TCGA. The overall survival curves were analyzed using the R language survival package. Finally, we took the intersection among the differential expression miRNAs, the predicted targeting miRNAs and the survival-related miRNAs. According to the outcome of intersection, Kaplan-Meier survival curve were constructed to evaluate diagnostic and prognostic values.

Construction of the circRNA-miRNA regulatory network

According to the results of the above analysis. Type and network txt files were designed. The interaction ceRNA network of circRNA–miRNA was constructed using Cytoscape software 3.6.0 [11].

Human tissue samples

This research was approved by the Institutional Ethics Committee of Nanjing Medical University. All cancerous and adjacent noncancerous tissues were gathered from the Jiangsu Province Hospital (Nanjing, China), which were eventually diagnosed as GC via postoperative pathological analysis. These patients were not undergone the chemotherapy or radiotherapy before resection.

Cell culture and transfection

Normal gastric epithelial cell line GES-1 and GC cell line SGC-7901 were obtained from the Shanghai Institute of Cell Biology (Shanghai, China). Cells were cultured in 10% fetal ox-like serum PRIM-1640 (GIBCO, Rockville, USA) medium supplemented with 1% penicillin at 37 degrees C in a humidified atmosphere of 5%CO₂/95% air incubator. Cells cultured on the six-well plate were transfected. Plasmid mediated YY1 or circRNA_0008278 overexpression vector, siRNA targeting YY1 or circRNA_0008278 vector, miR-378 mimic, inhibitor and their respective controls were purchased from Genechem. Cells are transiently transfected with plasmids by utilizing Lipofectamine RNAimax (Invitrogen, Carlsbad, CA, USA). Finally, cells were used in further experiments 48h post-transfection.

RNA fluorescence in situ hybridization (FISH)

AGS cells were grown to the exponential phase and were 80–95% confluent at the time of fixation. After pre-hybridization (1 × PBS/0.5% Triton X-100), the cells were hybridized in hybridization buffer (40% formamide, 10% dextran sulfate, 1 × Denhardt's solution, 4× SSC, 10 mM DDT, 1 mg ml⁻¹ yeast transfer RNA, 1 mg ml⁻¹ sheared salmon sperm DNA) with DIG-labelled probes specific to circRNA_0008278 and miR-378 at 60 °C overnight. The expression level of circRNA_0008278 and miR-378 was evaluated by FISH. The probe signals were determined with the Fluorescent in Situ Hybridization Kit (RiboBio, Guangzhou, China) according to the manufacturer's guidelines. Alexa Fluor 555-labeled circRNA_0008278 probes and Alexa Fluor 488-labeled miR-378 probes were designed and synthesized by Life Technologies. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI). Images were acquired using a fluorescence microscope (Eclipse E600; Nikon Corporation, Tokyo, Japan).

RNA isolation, RNase R digestion and qRT-PCR

Total RNA was isolated from human tissue samples and cells by use of Trizol reagent (Life Technologies, CA, US), then we measured the concentration by NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). RNA was reverse transcribed the total RNAs by Reverse Transcription Kit (Takara, Tokyo, Japan) and then to measure the expression by qRT-PCR using the SYBR Green PCR Kit (Takara) on the ABI 7900HT (Applied Biosystems). All tests were rehashed multiple times. U6 was an internal control. Fold changes in expression were determined utilizing $2^{-\Delta Ct}$ or $2^{-\Delta\Delta Ct}$.

Protein extraction and Western blotting

Total proteins were extracted using RIPA buffer, moreover, protein concentrations generally are determined by the Bicinchoninic Acid (BCA) Protein Assay. The Western Blot was conducted as followed: first, protein was isolated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Second, we exchanged proteins to polyvinylidene difluoride membrane (PVDF) (Millipore, Billerica, USA). Third, we use 5% bovine serum albumin (BSA) to block the membrane for 2h. Fourth, antibodies YY1 and β -actin (Abcam, Cambridge, USA) were incubated at 4 °C overnight. Fifth, the membranes were washed multiple times. Sixth, the secondary antibody was incubated at room temperature for 1h. Finally, proteins were envisioned with upgraded chemiluminescence reagents (Pierce, Rockford, IL, USA).

Dual-luciferase reporter assay

Cells were plated in the 24-well plate for 24 hours. Then, we transfected with a mixture of firefly luciferase reporter, pRL-CMV Renilla luciferase reporter, and miR-378 mimic or inhibitor. Firefly and Renilla luciferase activities were estimated with the Dual-Luciferase Report Assay System after 48h incubation (Promega, Madison, WI, USA)

Cell Migration and Invasion Assay

In the upper chamber, we seeded 4×10^4 cell with serum-free medium. Cell was seeded with 600ul medium containing 10% serum in the lower chamber. After 48h, cells were colored with dying solution, and tallied under 20x amplification.

Grow cells in RPMI-1640 supplemented with 10% FBS. Gently scratch the monolayer with a 1 ml pipette tip over the focal point of the well. Scratch another straight line opposite to the main line to make a cross in each well. In the wake of scratching, delicately wash the well twice with medium to expel the withdrew cells. Renew the well with new medium. Micrographs were taken after a traditional scratch wound assay of SCG-7901 cells 48 hours after wounding.

Colony formation assay

The SCG-7901 cell was trypsinized, and we plated 1×10^3 cells in a 6-well plate, then incubated at 37 °C for 10 days. Cells were dyed with crystal violet staining solution (Beyotime, China), then tallied cell colonies.

Cell proliferation assay

1×10^4 cells per ml in RPMI-1640 medium with 10% FBS and penicillin (100 U/ml) were evenly seeded in 96-well plates. Then, mixed 10ul/well CCK-8 solution and incubated with CCK8(Beyotime, Nantong, China) solution at 37 °C for 1h. Finally, we measured the absorbance at 450 nm by use of a microplate reader (Bio-Rad, USA).

Statistical analysis

GraphPad Prism 7.00 Software (USA) and SPSS version 22.0 (SPSS, USA) were used to conduct the statistical analyses. Expression is presented as mean $\pm$ SEM of at least three independent experiments for all results. One-way analysis of variance (ANOVA) or student's *t* test was performed to determine statistical differences among two or more groups. Sensitivity, specificity, and area under the curve (AUC), including 95% confidence interval (CI), were computed with the aid of the constructed receiver-operating characteristic (ROC) curves [25] to calculate the optimum cut-off values. The survival analysis included log-rank tests and Kaplan–Meier analyses. *P* values less than 0.05 were considered statistical significance. For all figures: *, *P* < 0.05.

Results

Identification of differentially expressed circRNAs and miRNAs in GC

A total of 3 gastric cancer tissues and their matched non-cancerous tissues were collected and screened for dysregulated circRNA using human circRNA microarray. These results are showed in the volcano plot and heat map (Figure 1). A total of 32 circRNAs were differentially expressed in the GC samples compared to the adjacent noncancerous tissues, 22 of which were downregulated and 10 of which were upregulated (Supplementary table 1). A total of 198 differentially expressed miRNAs were obtained in TCGA database (Figure 2), among the differentially expressed miRNAs, 65 were downregulated and 133 were upregulated (Supplementary table 2). These differentially expressed circRNAs and miRNAs may contribute to the pathogenesis or phenotypic behavior of gastric cancer and are potential markers for the diagnosis of gastric cancer.

Searching for the targeting miRNAs and survival-related miRNAs

The results of predicted targeting miRNAs shown that hsa_circ_0008278 had 54 targeting miRNAs (Supplementary Table 3). The relationship of miRNA expression with survival is depicted in supplementary table 4. A total of 115 miRNAs were significant related with the prognosis of gastric cancer (*P* < 0.05). Among these, 44 downregulated miRNAs were negatively associated with survival in GC, and 71 upregulated miRNAs were positively related with survival. Finally, the intersection of miRNAs (different expression miRNAs, the predicted targeting miRNAs and the survival-related miRNAs) was conducted. We got 2 miRNAs (hsa_miR-378 and hsa_miR-548) by taking the intersection (Figure 3A). Furtherly, Kaplan–Meier curves of hsa_miR-378 and hsa_miR-548 were showed Figure 3B and 3C. The expression of hsa_miR-378 and hsa_miR-548 was significant associated with increased survival in GC. Over-expression of miR-378 inhibits cell migration and invasion [11]. Previous study reported that the levels of serum miR-378 was significantly decreased in GC and CRC patients [12]. However, the expression of GC tissue miR-378 was not reported until now. This underscores the need to further assess the mechanism underlying the role of miR-378 in GC.

Constructed interaction of the circRNA–miRNA–mRNA ceRNA network

The ceRNA network of circRNA–miRNA–mRNA in GC was constructed (Figure 3D). Green quadrangle indicates has_circRNA_0008278, pink ellipses indicates miRNAs, and yellow triangle indicates mRNA. YY1 was one of downstream genes.

Expression of circ_0008278 and clinical pathologic information in patients with GC

As human circRNA microarray showed, circ_0008278 was significantly up-regulated in GC. However, its role in the occurrence and development of GC remains unknown. Structure diagram of hsa_circ_0008278 was showed in Figure 3E. As shown in Table 1, the expression of circ_0008278 was not associated with age, gender, differentiation, tumor size, nerves invasion or differentiation grade in patients with GC. However, up-expression of circ_0008278 level was positively associated with TNM stage ($P=0.031$) and lymphatic metastasis ($P=0.001$). By using the ROC curve. The circ_0008278 diagnostic efficacy for GC show that the AUC of 0.738, with a specificity of 80.1% and a sensitivity of 73.2% (Figure 4I). By using the median value of circ_0008278 expression as the cut-off value, the Kaplan-Meier survival curve showed high expression of circ_0008278 in GC tissues was significantly associated with poor prognosis in GC patients ($P=0.012$, log-rank test; Figure 4J).

The expression of circRNA_0008278, miR-378, and YY1 in tissue and cells

We examined the mRNA expression of circRNA_0008278, miR-378, and YY1 in 106 pairs primary cancerous and adjacent noncancerous tissues from GC patients. We found that circRNA_0008278 and YY1 were up-regulated in GC compared with the adjacent noncancerous tissues. By the contrary, miR-378 was down-regulated in human GC tissue (Figure 4A-C). Colocalization and inverse expression levels of circRNA_0008278 and miR-30c-2-3p were observed based on FISH in human tissue (Figure 4D). Then, we found the protein expression of YY1 was also high expressed in GC tissue (Figure 4E-F). As well as cell lines, we also validated an increased expression of circRNA_0008278 and YY1 in SGC-7901 and AGS, compared with GES-1, while miR-378 was down-regulated in gastric cancer cell line and up-regulated in the typical gastric epithelial cell line (Figure 4G). Next, FISH against circRNA_0008278 and miR-378 demonstrated that they both localized within the cytoplasm in AGS cells (Figure 4H).

CircRNA_0008278 acts as a miRNA sponge for miR-378 targeted YY1 in GC cells

We searched for the targets of miR-378, the targetscan database showed the YY1 was one of the target genes. To investigate whether circRNA_0008278 could regulate YY1 expression by combining with miR-378, we analyzed their expression in gastric cancer cell line SGC-7901 and AGS.

First, we transfected with ov-circRNA_0008278, ov-control. qRT-PCR analysis showed high expression of YY1 and low expression of miR-378 (Figure 5A). The level of YY1 were reduced and miR-378 was increased, compared to si-circRNA transfected. (Figure 5B). As well as the protein of YY1, the expression was increased by ov-circRNA_0008278 transfected (Figure 5C).

Second, to determined the potential mechanism of how miR-378 contributed to gastric cancer, the expression level of YY1 was likewise inspected after transfection with ov-circRNA_00082727 plus miR-378

mimic and si-circRNA_0008727 plus miR-378 inhibitor in SGC-7901 and AGS cells indicated by qRT-PCR. YY1 was reduced in the presence of miR-378 mimic and increased in miR-378 inhibitor compared to its control (Figure 5A and B). Then, we also exam the protein level by Weston-Blot, which was in parallel with the mRNA levels (Figure 5C).

Thirdly, to further verify whether YY1 was a downstream gene of miR-378 in AGS cells, we cloned the wild-type or mutant type 3'-UTR of YY1 with the putative miR-378 binding sites into the reporter plasmid. The results revealed that the luciferase intensity driven of wild-type 3'-UTR of YY1 was attenuated by miR-378 mimic and increased by miR-378 inhibitor, while the mutant 3'-UTR of YY1 was not affected. In general, the finding revealed that YY1 targets miR-378 (Figure 5E).

Fourthly, RIP assay applied that circRNA_0008727, served as a molecular sponge for miR-637, recruited argonaute 2 (Ago2) via WB and qRT-PCR analysis (Figure 5F, G). Moreover, to evaluate the ability of miR-378 to bind circRNA_0008727, biotin-labeled miR-378 probe to pull-down targeting circRNA_0008727 in the cells. The result showed that circRNA_0008727 was significantly pulled down (Figure 5H).

Taken together, these results showed that circRNA_0008278/miR-378 could target YY1 to promote gastric cancer progress.

The effects of circRNA_0008278 on GC cell proliferation, invasion and migration by miR-378 in vitro

Further, to show the effects of circRNA_0008278 on miR-378 in vitro, we performed the cell proliferation, invasive potential, and migration by use of transfecting SGC-7901 and AGS cells with ov-circRNA_0008278, ov-circRNA_0008278+miR-378 mimic, si-circRNA_0008278 and si-circRNA_0008278+miR-378 inhibitor.

First, the CCK-8 and colony formation demonstrated that improved cell proliferation in ov-circRNA_0008278 group. Transwell assays and scratch wound assay revealed that the migration and invasive potential of SCG-7901 and AGS cells were promoted dramatically after circRNA_0008278 over-expression. However, all these results were reversed by co-transfection of ov-circRNA_0008278 and miR-378 mimic (Figure 6 A-D).

Second, we also transfected the si-circRNA_0008278 and si-circRNA_0008278+ miR-378 inhibitor. The cell proliferation, invasion and migration of SCG-7901 and AGS cells were attenuated. These inhibitory effects were rescued by co-transfection of si-circRNA_0008278 and miR-378 inhibitor. Overall, these results revealed that miR-378 is a functional component of circRNA_0008278 which contributed to enhance invasive potential and migration in SGC-7901 and AGS cells (Figure 7A-D).

CircRNA_0008278/miR-378 axis on gastric cancer cell growth in vivo.

In order to validate the effect of circRNA_0008278/miR-378 axis on gastric cancer in vivo, we subcutaneously injected the different cells into nude mice: non-transfected SGC-7901 cells, ov-circRNA_0008278 transfected cells and ov-circRNA_0008278 and miR-378 mimic transfected cells. Then,

we drew the tumor growth-curve of tumor weight and volume after euthanasia. We found that the tumor growth-curve was increased after circRNA_0008278 overexpression, and the tendency was reversed by co-transfected ov-circRNA_0008278 and miR-378 mimic (Figure 8).

Discussion

CircRNA is a newly-identified noncoding RNA, associated with carcinogenesis at both transcriptional and post-transcriptional levels[13-15]. Due to its covalently closed cyclic structure, circRNA could be stable in the tissues and serum, which have emerged as the most interesting molecules in a field of much cancer research.[16-19]. Increasing evidence has showed that circRNA took part in the development and progression of tumor. For instance, circRNA_002059 was significantly decreased in gastric cancer[20]. Upregulation of circRNA_0007534 promotes breast cancer cell multiplication [21]. Importantly, circRNAs could serve as miRNA sponge involved in cancer pathogenesis[22, 23]. Several evidences reported that circRNAs was associated with numerous diseases by acting as a miRNA sponge, such as Cartilage Degradation[24], heart failure[25], diabetes[26] and so on. Zhang et al reported upregulation of circRNA_0072995 was associated with breast cancer cell proliferation and migration by miR-378[27].

Our study found the expression of circRNA_0008278 was upgraded in the gastric cancer tissue and cells. We also identified the promoting effects in cancer by gain- and loss-of-function methods. Our study demonstrated that the proliferation, invasion and migration of gastric cancer cells was suppressed via siRNA against circRNA_0008278. Moreover, the circRNA_0008278 plasmid has the opposite functions. Take together, our results implied a new possible etiology of gastric cancer, which may contribute to gastric cancer progression.

Further, we picked up miR-378 for study. Many previous studies revealed miR-378 related with many types of cancer. MiR-378 suppresses the proliferation, migration and invasion of colon cancer cells by inhibiting SDAD1[28]. It could decrease miR-378 levels indicate high tumor invasiveness and poor prognosis in gliomas[29]. Another study in hepatocellular carcinoma reported the low expression of miR-378 was associated with a better survival[30]. In our study, the miR-378 fold-changes of qRT-PCR was agreement with the Cancer Genome Atlas (TCGA) database. Furthermore, overexpression of miR-378 suppressed gastric cancer cells proliferation, migration and invasion.

Subsequently, we continued studying the relationship between miR-378 and YY1. Indeed, miR-378 regulates several target genes, such as SuFu and Fus-1 in tumor cell[31]. Li et al found that CASP3 as a downstream gene of miR-378 to attenuated high glucose-suppressed osteogenic differentiation[32]. YY1 is a recently validated as an oncogene with the promoting effect of tumor genes. Our results confirmed that YY1 also was a downstream gene of miR-378 in gastric cancer. Besides, the expression of miR-378 was contrarily associated with YY1 in gastric cancer tissues in our study. Moreover, down-regulation of miR-378 significantly increased the mRNA and protein levels of YY1 in gastric cancer cells. Besides, the promoting tumor effects of YY1 in gastric cancer cells was weakened by miR-378 mimic. Additionally, luciferase reporter activity of wild-type 3'-UTR was declined in condition with miR-378 overexpression,

while this impact was attenuated when mutant YY1 3'-UTR was treated. Finally, the oncogenic impact of miR-378 overexpression was enhanced by transfected with pc-YY1. Above all, these results uphold the speculation that YY1 is a target gene of miR-378 in gastric cancer.

In conclusion, our study reveals that circRNA_0008278 is upregulated in GC tissues and that high circRNA_0008278 expression is associated with poor prognosis, lymph node metastasis and poor TNM stage in GC patients. Moreover, enhanced circRNA_0008278 expression promoted GC cell growth and motility in vitro and in vivo through sponging miR-378 to upregulate YY1. Consequently, circRNA_0008278 may have considerable potential as a prognostic predictor and therapeutic target for GC.

Abbreviations

GC: Gastric cancer; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; TCGA: The Cancer Genome Atlas; CSCD: CircRNA Database; RNase R: Ribonuclease R; FBS: Fetal bovine serum; CCK-8: Cell counting kit-8; DAPI: 4',6-diamidino-2-phenylindole; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; circRNA: Circular RNA; miRNA: microRNA; YY1: Yin Yang 1; qRT-PCR: Reverse transcription quantitative polymerase chain reaction; FISH: fluorescence in situ hybridization; BCA: Bicinchoninic Acid; SDS-PAGE: sodium dodecyl sulphate-polyacrylamide gel electrophoresis; PVDF: polyvinylidene difluoride membrane; WB: Western blot; SEM: Standard error of the mean; SPSS: Statistical product and service solutions; AUC: area under the curve; CI: confidence interval; ROC: receiver-operating characteristic; Ago2: recruited argonaute 2.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University and written informed consent forms were obtained from all recruited patients. The experiments concerning animals were approved by the Nanjing Medical University Ethics Committee.

Consent for publication

Not applicable.

Availability of data and materials

All data in our study will be available upon reasonable request.

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Authors' contributions

XL and HSQ designed the project. XL, HD, and YND performed the experiments. LP and YND processed the data. XL and HSQ wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J: **Cancer statistics in China, 2015.** *Ca Cancer J Clin* 2016, **66**:115-132.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: **Global cancer statistics, 2012.** *Ca A Cancer Journal for Clinicians* 2015, **65**:87-108.
3. Borie F, Millat B, Fingerhut A, Hay JM, Fagniez PL, Saxce BD: **Lymphatic involvement in early gastric cancer: prevalence and prognosis in France.** *Archives of Surgery* 2000, **135**:1218.
4. Siewert JR: **Gastric cancer: the dispute between East and West.** *Gastric Cancer* 2005, **8**:59-61.
5. Parisa K, Farhad I, Sharmila A, Freedman ND, Farin K: **Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention.** *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2014, **23**:700-713.
6. Chen LL, Yang L: **Regulation of circRNA biogenesis.** *Rna Biol* 2015, **12**:381-388.
7. Qu S, Zhong Y, Shang R, Zhang X, Song W, Kjems J, Li H: **The emerging landscape of circular RNA in life processes.** *Rna Biol* 2017, **14**:992-999.
8. Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, Sun W, Dou K, Li H: **Circular RNA: A new star of noncoding RNAs.** *Cancer Lett* 2015, **365**:141-148.
9. Xie H, Ren X, Xin S, Lan X, Lu G, Yuan L, Yang S, Zeng Z, Liao W, Ding YQ: **Emerging roles of circRNA_001569 targeting miR-145 in the proliferation and invasion of colorectal cancer.** *Oncotarget* 2016, **7**:26680-26691.
10. Ke Z, Xie F, Zheng C, Chen D: **CircHIPK3 promotes proliferation and invasion in nasopharyngeal carcinoma by abrogating miR-4288-induced ELF3 inhibition.** *J Cell Physiol* 2019, **234**:1699-1706.
11. Li B, Wang Y, Li S, He H, Tao B: **Decreased expression of miR-378 correlates with tumor invasiveness and poor prognosis of patients with glioma.** *International Journal of Clinical & Experimental Pathology* 2015, **8**:7016-7021.
12. Huang SK, Wang J, Li Y, Lin H, Huang CZ: **Clinical application value of combined detection of serum miR-378 and miR-21 in gastric cancer.** *Zhonghua zhong liu za zhi Chinese journal of oncology* 2018, **40**:441-445.

13. Julia S, Chen RE, Olsen MN, Wang PL, Brown PO: **Cell-type specific features of circular RNA expression.** *Plos Genet* 2013, **9**:e1003777.
14. Stoffelen R, Jimenez MI, Dierckxsens C: **Circular RNAs Are the Predominant Transcript Isoform from Hundreds of Human Genes in Diverse Cell Types.** *Plos One* 2012, **7**:e30733.
15. Qu S, Yang X, Li X, Wang J, Gao Y, Shang R, Sun W, Dou K, Li H: **Circular RNA: A new star of noncoding RNAs.** *Cancer Lett* 2015, **365**:141-148.
16. Yu Z, Wei L, Peng Z, Chen J, Hui Q, Xu Z, Xu W: **Circular RNAs: emerging cancer biomarkers and targets.** *Journal of Experimental & Clinical Cancer Research Cr* 2017, **36**:152.
17. Zhang HD, Jiang LH, Sun DW, Hou JC, Ji ZL: **CircRNA: a novel type of biomarker for cancer.** *Breast Cancer-Tokyo* 2018, **25**:1-7.
18. Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P, Wu M: **CircRNA: functions and properties of a novel potential biomarker for cancer.** *Mol Cancer* 2017, **16**:94.
19. Han YN, Xia SQ, Zhang YY, Zheng JH, Li W: **Circular RNAs: A novel type of biomarker and genetic tools in cancer.** *Oncotarget* 2017, **8**:64551-64563.
20. Li P, Chen S, Chen H, Mo X, Li T, Shao Y, Xiao B, Guo J: **Using circular RNA as a novel type of biomarker in the screening of gastric cancer.** *Clin Chim Acta* 2015, **444**:132-136.
21. Song L, Xiao Y: **Downregulation of hsa_circ_0007534 suppresses breast cancer cell proliferation and invasion by targeting miR-593/MUC19 signal pathway.** *Biochem Biophys Res Commun* 2018, **503**:2603-2610.
22. Zheng Q, Bao C, Guo W, Li S, Jie C, Bing C, Luo Y, Lyu D, Yan L, Shi G: **Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs.** *Nat Commun* 2016, **7**:11215.
23. Zhong Z, Lv M, Chen J: **Screening differential circular RNA expression profiles reveals the regulatory role of circTCF25-miR-103a-3p/miR-107-CDK6 pathway in bladder carcinoma.** *Sci Rep* 2016, **6**:30919.
24. Liu Q, Zhang X, Hu X, Dai L, Fu X, Zhang J, Ao Y: **Circular RNA Related to the Chondrocyte ECM Regulates MMP13 Expression by Functioning as a MiR-136 'Sponge' in Human Cartilage Degradation.** *Sci Rep* 2016, **6**:22572.
25. Wang K, Long B, Liu F, Wang JX, Liu CY, Zhao B, Zhou LY, Sun T, Wang M, Yu T: **A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223.** *Eur Heart J* 2016, **37**:2602-2611.
26. Huanyu X, Sen G, Wei L, Ping Y: **The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells.** *Sci Rep-Uk* 2015, **5**:12453.
27. Zhang HD, Jiang LH, Hou JC, Zhou SY, Zhong SL, Zhu LP, Wang DD, Yang SJ, He YJ, Mao CF, et al: **Circular RNA hsa_circ_0072995 promotes breast cancer cell migration and invasion through sponge for miR-30c-2-3p.** *Epigenomics-Uk* 2018, **10**:1229-1242.

28. Zeng M, Zhu L, Li L, Kang C: **miR-378 suppresses the proliferation, migration and invasion of colon cancer cells by inhibiting SDAD1.** *Cell Mol Biol Lett* 2017, **22**:12.
29. Li B, Wang Y, Li S, He H, Tao B: **Decreased expression of miR-378 correlates with tumor invasiveness and poor prognosis of patients with glioma.** *International Journal of Clinical & Experimental Pathology* 2015, **8**:7016-7021.
30. An J, Liu J, Liu L, Liu Y, Pan Y, Huang M, Qi F, Wen J, Xie K, Ma H: **A Genetic Variant in Primary miR-378 Is Associated with Risk and Prognosis of Hepatocellular Carcinoma in a Chinese Population.** *Plos One* 2014, **9**:e93707.
31. Lee DY, Deng Z, Wang CH, Yang BB: **MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression.** *Proc Natl Acad Sci U S A* 2007, **104**:20350-20355.
32. You L, Gu W, Chen L, Pan L, Chen J, Peng Y: **MiR-378 overexpression attenuates high glucose-suppressed osteogenic differentiation through targeting CASP3 and activating PI3K/Akt signaling pathway.** *International Journal of Clinical & Experimental Pathology* 2014, **7**:7249-7261.

Table

Table 1 Relationship between circRNA_0008278 expression and clinicopathologic factors of gastric cancer patients. The TNM Staging System is based on the tumor (T), the extent of spread to the lymph nodes (N), and the presence of metastasis (M); LVI: Lymphatic invasion* *P*< 0.05

Table 1. Pathologic features of the included patients.

	no of patients (n=106)	circRNA_0008278(low)	circRNA_0008278 (high)	<i>P</i> value
Gender				0.695
Female	42	22	20	
Male	64	31	33	
Age				0.200
≤60	47	29	18	
≥60	59	24	35	
Size				0.335
≤5cm	57	31	26	
≥5cm	49	22	27	
Lymph node status				0.031
Negative	30	20	10	
Positive	76	33	43	
Nerves invasion				0.078
Negative	61	26	35	
Positive	45	27	18	
Differentiation grade				0.441
Differentiated	56	26	30	
Undifferentiated	40	27	13	
TMN				0.001
Stage I-III	85	47	38	
Stage IV	21	6	15	

Supplementary Information

Supplementary Table 1 CircRNAs differentially expressed in gastric cancer tissues and matched noncancerous gastric tissues.

Supplementary Table 2 MiRNAs differentially expressed in gastric cancer tissues and matched noncancerous gastric tissues.

Supplementary Table 3 CircRNAs targeted miRNAs prediction by CSDC.

Supplementary Table 4. Relationship between expression of miRNAs with GC survival. HR: Hazard Rate.

Figures

Figure 1

CircRNA microarray chip was used to identify the differentially expressed circRNAs between GC and adjacent normal tissues. A) Heatmap. (B) Volcano plot

Figure 2

Heatmap of differentially expressed miRNAs between GC and adjacent normal tissues on TCGA. A) Heatmap. (B) Volcano plot a Hierarchical clustering heat map of differential expression of miRNAs. Red represents upregulated miRNAs and green represents downregulated miRNAs.

Figure 3

Identification of ceRNA network. (A) Two sharing downregulated miRNAs. (B) Relationship between hsa_miR-378 and survival. (C) Relationship between hsa_miR-548 and survival. Blue circle represents the predicted targeting miRNAs, yellow circle represnets different expression miRNAs, and green circle represents the survival-related miRNAs. Red line represents high expression, and blue line represents low expression. (D) The ceRNA network of the has_circRNA_0008278. (E) Structure diagram of circ_0008278. The red, blue, and green regions inside the circular RNA molecule respectively represent MRE (microRNA response element), RBP (RNA binding protein), ORF (open reading frame).

Figure 4

Correlation of circ_0008278, miR-378 and YY1 mRNA expression in gastric cancer tissues corresponding noncancerous tissues, and cell lines. (A-C) Relative expression of circ_0008278, miR-378 and YY1 mRNA in gastric cancer tissues compared with corresponding noncancerous tissues examined by qRT-PCR. Circ_0008278 and YY1 mRNA expression was normalized to the GAPDH expression while miR-378 expression was normalized to U6 expression. (D) RNA fluorescence in situ hybridization (FISH) for

circ_0008278 and miR-378 in human tissue. (E-F) Western blot analysis of YY1 in gastric cancer tissues compared with corresponding noncancerous tissues. (G) circ_0008278, miR-378 and YY1 mRNA expression was examined by qRT-PCR in gastric cancer cell line SGC-7901, AGS and compared with their expression in normal gastric epithelial cell line GES-1. (H) FISH for circ_0008278 and miR-378. The nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Scale bar, 5 μ m. (I) The sensitivity and specificity of circ_0008278 for GC prediction was evaluated by Receiver-operating characteristic (ROC) curve analysis. (J) Kaplan-Meier analysis of the correlation between circ_0008278 expression and overall survival (OS) with GC.

Figure 5

YY1 is a potential target of circRNA_0008278/miR-378 in SGC-7901 gastric cancer cell line. (A) circRNA_0008278, miR-378, and YY1 expression were detected by qRT-PCR in gastric cancer cells after transfection with ov-circRNA_0008278, ov-circRNA_0008278+miR-378 and ov-control in AGS and SGC-7901 (B) circRNA_0008278, miR-378, and YY1 expression were detected by qRT-PCR in gastric cancer cells after transfection with si-circRNA_0008278, si-circRNA_0008278 + miR-378 inhibitor and si-control in

AGS and SGC-7901. (C) Western blot analysis of YY1 protein expression in gastric cancer cells. si-circRNA_0008278, si-circRNA_0008278+miR-378 inhibitor, ov-circRNA_0008278, ov-circRNA_0008278+miR-378 mimic and their respective controls; An unrelated protein GAPDH was used as the control. (D) Western blot analysis of YY1 protein expression in gastric cancer cells transfected with miR-378 mimic, inhibitor and their respective controls; An unrelated protein GAPDH was used as the control. (E) Left: Diagram of putative miR-378 binding sites in the 3'-UTR of YY1. The mutant sequences YY1 3'-UTR sequences used in the luciferase reporter constructs are indicated in red. Right: Relative activities of luciferase reporters containing YY1 3'-UTR variants cotransfected with miR-378 or negative control mimics and inhibitor in AGS cells. (F-G) The assessment of the enrichment in Ago2 immunoprecipitates by WB and qRT-PCR. (H) CircRNA_0008727 were pulled down by biotin-labelled miR-378 and confirmed by qRT-PCR in AGS and SGC_7901 cells. For all quantitative results, data are presented as the mean $\pm$ SD of three independent experiments. *P < 0.05.

Figure 6

Over-expressed circRNA_0008278 promoted the cell proliferation, invasion, and migration of miR-378. (A) Cell proliferation analysis by measuring colony formation of SGC-7901 and AGS gastric cancer cells after their transfection with empty vector, ov-circRNA_0008278, and ov-circRNA_0008278+ miR-378 mimic. (B) Transwell assays were performed to determine the migration in SGC-7901 and AGS gastric cancer cells after transfection with empty vector, ov-circRNA_0008278, and ov-circRNA_0008278+ miR-378 mimic. (C) scratch wound assay was applied to examine the invasion in SGC-7901 and AGS gastric cancer cells after transfection with empty vector, ov-circRNA_0008278, and ov-circRNA_0008278+ miR-378 mimic. (D) Cell proliferation analysis using CCK-8 in SGC-7901 and AGS gastric cancer cells after their transfection

with empty vector, ov-circRNA_0008278, and ov-circRNA_0008278+ miR-378 mimic. For all quantitative results, data are presented as the mean $\pm$ SD of three independent experiments. *P < 0.05.

Figure 7

Suppressed circRNA_0008278 mediates the cell proliferation, invasion, and migration of miR-378. (A) Cell proliferation analysis by measuring colony formation of SGC-7901 and AGS gastric cancer cells after their transfection with empty vector, si-circRNA_0008278, and si-circRNA_0008278+ miR-378 inhibitor. (B) Transwell assays were performed to determine the migration in SGC-7901 and AGS gastric cancer cells after transfection with empty vector, si-circRNA_0008278, and si-circRNA_0008278+ miR-378 inhibitor. (C) scratch wound assay was applied to examine the invasion in SGC-7901 and AGS gastric cancer cells after transfection with empty vector, si-circRNA_0008278, and si-circRNA_0008278+ miR-378 inhibitor. (D) Cell proliferation analysis using CCK-8 in SGC-7901 and AGS gastric cancer cells after their transfection with empty vector, si-circRNA_0008278, and si-circRNA_0008278+ miR-378 inhibitor. For all quantitative results, data are presented as the mean $\pm$ SD of three independent experiments. *P < 0.05.

Figure 8

Overexpression of circRNA_0008278 partially increased tumor growth induced by miR-378 in nude mice. (A) SGC-7901 gastric cancer cells were used for the in vivo study. Nude mice carrying tumors from respective treatment groups were shown. (B) Tumor weight was measured after mice were euthanized. (C) Tumor volume was calculated every week. (D) Hematoxylin-eosin (HE) staining was done after mice were euthanized. Data are presented as the mean $\pm$ SD of three independences.

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