Construction of a circRNA-miRNA-mRNA network based on differentially co-expressed circular RNA in gastric cancer tissue and plasma by bioinformatics analysis

Yu Gong
The Affiliated Changzhou No.2 People's Hospital of NanJing Medical University https://orcid.org/0000-0002-9047-4351
Xiaoyang Qi
The Affiliated Changzhou No.2 People's Hospital of NanJing Medical University
Jinjin Fu
The Affiliated Changzhou No.2 People's Hospital of NanJing Medical University
Jun Qian
The Affiliated Changzhou No.2 People's Hospital of NanJing Medical University
Yuwen Jiao
The Affiliated Changzhou No.2 People's Hospital of NanJing Medical University
Haojun Yang
The Affiliated Changzhou No.2 People's Hospital of NanJing Medical University
Liming Tang (drtanglm@163.com)
The Affiliated Changzhou No.2 People's Hospital of NanJing Medical University https://orcid.org/0000-0002-2867-5435

Primary research

Keywords: circRNA, microRNA sponge, gastric cancer, GEO

Posted Date: April 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-22009/v1

Version of Record: A version of this preprint was published at World Journal of Surgical Oncology on February 14th, 2022.
See the published version at https://doi.org/10.1186/s12957-022-02503-7.

Abstract

Background: Increasing evidence implicates circular RNAs (circRNAs) have been involved in human cancer progression. However, the mechanism remains unclear. In this study, we identified novel circRNAs related to gastric cancer and constructed a circRNA-miRNA-mRNA network.

Methods: Microarray dataset GSE83521 and GSE93541 were obtained from Gene Expression Omnibus (GEO). Then, we used computational biology to select differentially co-expressed circRNAs in GC tissue and plasma and detected the expression of selected circRNAs in gastric cell lines by quantitative real-time polymerase chain reaction (qRT-PCR). We also chose the candidate miRNAs and their target genes for circRNAs through online tools. Combining the predictions of miRNAs and target mRNAs, a competing endogenous RNA regulatory network was established. Functional and pathway enrichment analyses were performed, and interactions between proteins were predicted by using String and Cytoscape. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed to elucidate the possible functions of these differentially expressed circRNAs.

Results: The regulatory network constructed using the microarray datasets (GSE83521 and GSE93541) contained three differentially co-expressed circRNAs (DECs). A circRNA-miRNA-mRNA network was constructed based on 3 circRNAs, 43 miRNAs and 119 mRNAs. GO and KEGG analysis showed that regulation of apoptotic signaling pathway and PI3K–Akt signaling pathway were highest degrees of enrichment respectively. We established a protein-protein interaction (PPI) network consisting of 165 nodes and 170 edges and identified hub genes by MCODE plugin in Cytoscape. Furthermore, a core circRNA-miRNA-mRNA network was constructed base on hub genes. Hsa_circ_0001013 was finally determined to play an important role in the pathogenesis of GC according to the core circRNA-miRNA-mRNA network.

Conclusions: We propose a new circRNA-miRNA-mRNA network associated with the pathogenesis of GC. The network may become a new molecular biomarker and be used to develop potential therapeutic strategies for gastric cancer.

1 Background

Gastric cancer is one of the most widely malignant tumors. Every year there are nearly 1 million new cases of gastric cancer around the world, making it the third leading cause of cancer-related deaths and prompting the World Health Organization to declare it a public health problem[1]. The pathogenesis of gastric cancer is multifactorial and multi-steps, which is unclear at present. It is widely believed that Helicobacter pylori is one of the main pathogenic factors of gastric cancer[2, 3]. Almost 90% of new cases of non-cardiac gastric cancer are related to Helicobacter pylori. Although the technique for the detection and treatment of gastric cancer has been dramatically improved, the prognosis is still very poor[4]. The 5-year survival rate of patients with advanced gastric cancer is about 18% - 29%[5]. Therefore, early diagnosis and treatment are very critical to improve the curative effect and reduce mortality.

In 1976, a new type of 3'-5' head-to-tail covalently closed RNA called Circular RNAs (circRNAs) were identified[6, 7]. However, in subsequent decades, circular RNAs were thought to be the product of mis-splicing[8]. In recent years, it is recognized that circRNAs are normal co-products of numerous eukaryotic protein-coding genes[9]. It also has been the hotspot of research in the field of life science and medicine and has been identified as a critical regulator for a variety of diseases, including various malignant tumors[10-12]. CircRNAs can regulate variable splicing or the expression of its host genes by inhibiting transcriptional initiation sites, and can even be translated into proteins or peptides. But the role of competitive endogenous RNA (ceRNA) sponge miRNA is considered to be one of the main functions of circRNA in various cancers. CircRNA is also regarded to be a potential biomarker for cancers due to their better stability than linear RNA[13]. There are many differentially expressed circRNAs associated with gastric cancer. For example, Wei et al. found that circHIPK3 promotes cell proliferation and migration of gastric cancer by sponging miR-107 and regulating BDNF expression[14, 15]. He et al. confirmed that circular RNA circ_0006282 contributes to the progression of gastric cancer by

sponging miR-155 to upregulate the expression of FBXO22[16]. Pan et al. reported that circUBA1 promotes gastric cancer proliferation and metastasis by acting as a competitive endogenous RNA through sponging miR-375 and regulating TEAD4[17]. Additionally, circRNAs have also been proposed as a diagnostic or prognostic biomarker. Wang et al. demonstrated that hsa_circ_0005654 might serve as a new and promising diagnostic biomarker for screening early gastric cancer. The AUC, sensitivity and specificity of hsa_circ_0005654 are significantly higher than those of present gastric cancer associated-biomarkers[18]. Although related studies have sprung up, the network structure of circRNA regulating gastric cancer remains unclear.

In our study, the aim was to identify differentially co-expressed circRNAs in tissues and plasma of patients with gastric cancer. The expression profiles of circRNAs were obtained from Gene Expression Omnibus (GEO). The bioinformatic data were analyzed and differentially expressed circRNAs (DECs) were screened. Next, the potential miRNAs sponged by DECs and their target genes were performed by bioinformatic analysis. Moreover, the core circRNA-miRNA-mRNA regulatory network was constructed. Gene enrichment analyses of the candidate miRNAs or mRNAs were performed with the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases by R software, which resulted in the prediction of the signaling pathways involved in GC. A flow chart of the methods used in this study is provided in Figure 1. The results of this study may help to clarify the potential mechanism of the pathogenesis of gastric cancer, provide new biomarkers for gastric cancer and facilitate future research in GC treatment and diagnosis.

2 Methods

2.1 Microarray analysis of gene expression

Two circRNA expression profiles for human samples derived from patients with gastric cancer were obtained from the GEO (www.ncbi.nlm.nih.gov/geo/). We chose the GSE83521 and GSE93541 circRNA expression profiles, both of which were completed on the Agilent-069978 Arraystar Human CircRNA microarray V1 GPL19978 platform. The GSE83521 dataset contained six gastric cancer tissues and six normal mucosa tissues, and the GSE93541 dataset included three plasma samples of gastric cancer patients and three healthy controls.

2.2 Identification of DECs

Differential expression of the circRNAs in the two datasets was analyzed by using the GEO2R online analysis tool. The absolute value of log fold change > 1.5 and p value <0.05 were used as cut-off criteria. The significantly differentially expressed circRNAs in the two datasets were screened and the co-expressed circRNAs were detected by Venn analysis. The basic structural features of the differentially expressed circRNAs were obtained from the Cancer-Specific CircRNA Database (http://gb.whu.edu.cn/CSCD/).

2.3 Prediction of circRNA-miRNA and miRNA-mRNA interactions

Online tools circBank (http://www.circbank.cn/) and Circular RNA Interactome (https://circinteractome.nia.nih.gov/index.html) were used to predict the possible interactions between circRNAs and miRNAs. The co-predicted miRNAs by circBank and circinteractome were selected for candidate miRNAs. mirTarBase (http.//mirtarba se.mbc.nctu.edu.tw/php/index.php) was used to obtain experimentally strongly supported target genes of these miRNAs. Candidate genes were selected using the following criteria: verified by the Reporter assay as well as Western blot or qPCR experiments.

2.4 GO and KEEG functional enrichment analysis

FunRich software (version 3.1.1) was used to conduct GO analysis for candidate miRNAs. GO annotation and KEGG pathway analyses were conducted with the R (version 3.6) package (http://www.bioconductor.org/) clusterProfiler to

explore the potential biological roles of candidate genes. The analysis results were visualized with the ggplot2 package of the R software. Both *p* value and q value <0.05 were considered significant for GO annotation while *p* value <0.05 and q value <1 were considered significant for KEGG pathway analysis.

2.5 Construction of protein-protein interaction (PPI) and circRNA-miRNA-mRNA network

Candidate target genes of the candidate miRNAs were put into the Search Tool for the Retrieval of Interacting Genes database (STRING, https.//string-db.org/), and an interaction network chart with a combined score > 0.4 was saved and exported. Then the PPI network was visualized using Cytoscape software (version 3.6.1; http.//cytoscape.org/) . The MCODE plugin of Cytoscape software was used to identify hub genes among candidate targets. The circRNA–miRNA–mRNA network was also visualized by Cytoscape software.

2.6 Cell culture

The gastric cancer cell lines SGC-7901 and human gastric mucosal epithelial cell line GES-1 were purchased from the Cell Resource Center, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science. All of the cell lines were maintained under the recommended culture conditions and incubated at 37℃ in a humidified environment with 5% CO_2.

2.7 RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from the cell lines with TRIzol reagent (Invitrogen, CA, USA) following the manufacturer's instruction. The concentration and purity of the total RNA samples were assessed using the NanoDrop spectrophotometer (Thermo, Wilmington, DE, USA). Total RNAs were reversely transcribe using HiScript Q RT SuperMix for qPCR with gDNA wiper (Vazyme Biotech, Nanjing, China), and qPCR assays were performed in triplicate using AceQ qPCR SYBR Green Master Mix kit (Vazyme Biotech, Nanjing, China) on 7500 real time PCR system (ABI). The divergent primers used for detecting circRNAs were synthesized from Shanghai Generay Biotech (Shanghai, China), and β-actin was used as an internal control. The following primer pairs were used for qPCR: β-actin forward, 5'-AGAAAATCTGGCACCACACC-3' and reverse, 5'-CAGAGGCGTACAGGGATAGC-3'; hsa_circ_0001013 forward, 5'- GTCAAAGGAAGCAAAAGAAAGTCT-3' and reverse, 5'- GATCGCACCTCTACACTCCA-3'; hsa_circ_0007376 forward, 5'-ATCGACTCCATGGCCAACTC-3' and reverse, 5'-AAGCCCCGGAGAACAGC-3'; hsa_circ_0043947 forward, 5'-CAATTGTGGTTGTGCAGCC-3' and reverse, 5'-ACACAAACTCAGCATCATGGA-3'. The expression of circRNAs was normalized to that of internal control β-actin by using the $2^{-\Delta\Delta C}$ method[19].

2.8 Statistical analysis

All computations were carried out using the GraphPad Prism 8 (GraphPad Software, CA, USA). Data were expressed as mean ± SEM. Student's t-test was conducted to compare the differences of circRNA expression between GES-1 and SGC-7901 cells. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Identification of DECs

Two circRNA expression profiles GSE83521 and GSE93541 were obtained from GEO, and GEO2R method was applied to analysis DECs. The GSE83521 dataset derived from gastric cancer tissues and GSE93541 dataset derived from plasmas. Differential circRNAs co-expressed in tissues and plasma of gastric cancer patients are our target circRNAs. We found that 53 circRNAs were identified to be differentially expressed in GSE83521, including 39 up-regulated and 14 down-regulated circRNAs; while 267 differentially expressed circRNAs were identified in GSE93541, including 138 up-regulated and 129 down-regulated circRNAs. Among them, 3 up-regulated and 0 down- regulated circRNAs were observed in both circRNA

expression profiles. A Venn diagram of the results is shown in Figure 2A and B. The up-regulated circRNAs that overlapped in the two datasets (hsa_circ_0001013, hsa_circ_0007376, hsa_circ_0043947) were selected for further analysis. Details of the overlapped up-regulated circRNAs are listed in Table 1, and the basic structural features of the three selected circRNAs are shown in Figure 2C.

3.2 Expression of circRNAs in datasets and cell lines

As shown in Figure 3A and B, the expression patterns of the three selected circRNAs were upregulated in both tissues and plasmas according to the datasets. We also detected the expression of selected circRNAs in gastric cancer cell line SGC-7901 and human gastric epithelial cell line GES-1 by qRT-PCR. The results showed that all three selected circRNAs had higher expression levels in SGC-7901 than in GES-1 as shown in Figure 3C.

3.3 Prediction of circRNA-miRNA and their function analysis

An increasing number of evidence demonstrate that circRNAs might function as competing endogenous RNAs (ceRNAs) that operate by competitively binding common microRNAs (miRNAs) and increase the expression of the target genes of these miRNAs. Target miRNAs of the three selected circRNAs were predicted by two online tools circBank and circInteractome. A total of 43 consensus miRNAs from both prediction tools were identified and DECs potentially bind to these miRNAs were presented in Table 2. Results showed that one specific circRNA might bind to more miRNAs, while different circRNAs could interact with one specific miRNA. Rich Fun software was used to GO analysis for the 43 miRNAs. The top five enrichment items were shown respectively in Figure 4: 'Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism' , 'Regulation of cell growth', 'Cell cycle', 'Regulation of enzyme activity' and 'Cell-cell adhesion' for biological progress (BP), 'Cytoplasm and Nucleus', 'Nucleus', 'Lysosome', 'Actin cytoskeleton' and 'Endosome' for cellular component (CC), and 'Transcription factor activity', 'Receptor signaling complex scaffold activity', 'Translation regulator activity', 'Protein binding' and 'RNA binding' for molecular function(MF). All of them indicated that circRNAs might impact on GC progression by modulating various miRNAs.

3.4 Construction of the ceRNA network

We identified 119 experimentally strongly supported target genes of 43 miRNAs by mirTarBase on line tool (Table 2). Then we used 3 circRNAs, 43 miRNAs and 119 mRNAs in Cytoscape 3.6.1 to construct a circRNA-miRNA-mRNA visualization network (Figure 5).

3.5 Functional and pathway enrichment analysis and PPI network

GO analysis indicated that the 119 mRNAs were mainly enriched in 'regulation of apoptotic signaling pathway', 'autophagy', and 'process utilizing autophagic mechanism' (BPs); 'glutamatergic synapse', 'nuclear chromatin' and 'external side of plasma membrane' (CCs); and 'DNA-binding transcription activator activity, RNA polymerase□-specific' (MFs) (Figure 6A). KEGG pathway analysis revealed strong enrichment in the 'PI3K-Akt signaling pathway' (Figure 6B). After obtaining the target genes of candidate miRNAs, we created a PPI network composed of 165 nodes and 170 edges (Figure 7A). Following the identification of the vital functions of hub genes in the network, 18 hub genes (CCND2, STAT3, TP53, MCL1, MYC, FOXO1, FOXO3, BCL2L11, PTEN, MTOR, CDH1, CASP3, IL6, GSK3B, CDKN1A, MAPK1, SMAD4, CDC42) were identified in GC using the MCODE plugin, MCODE_Score = 13.76. These hub genes were predicted target genes for hsa-miR-197-3p, hsa-miR-451a, hsa-miR-136-5p, hsa-miR-337-3p, hsa-miR-654-3p, hsa-miR-182-5p, hsa-miR-1228-3p, hsa-miR-942-5p, hsa-miR-488-3p and hsa-miR-876-3p, and all these 10 miRNAs were predicted miRNAs for hsa_circ_0001013. So a core circRNA–miRNA–mRNA network based on hub genes was displayed in Figure 7B.

4 Discussion

Emerging evidence indicates that circRNAs are frequently aberrant in various cancers and may serve as a vital role in cancer progression. Moreover, the better stability of circRNAs compared with that of linear RNAs in the serum, makes circRNAs vital biomarkers for cancer diagnosis and prognosis. However, the mechanism of circRNA in cancer progression has not been clearly elucidated. Current evidence demonstrates that circRNAs can target miRNAs, often referred to as “miRNA sponges”, to reduce the level of miRNAs and release their targeting inhibition to mRNAs. These studies have shown that the circRNA-miRNA-mRNA axis can play a role as a wide range of gene expression regulatory network, and can be used as a biomarker for cancer diagnosis and prognosis. Gastric cancer is one of the most common malignant tumors of digestive tract. At present, radical resection is the main treatment for gastric cancer, but the prognosis of the patients is still not satisfactory. Previous studies have confirmed that circRNAs have been involved in tumorigenesis and progression of gastric cancer. Liu et al. found that circ-PVT1 contributes to paclitaxel resistance of gastric cancer cells through the regulation of ZEB1 expression by sponging miR-124-3p[20]. Xie et al. showed that the down-regulated expression of hsa_circ_0074362 in gastric cancer is related to lymph node metastasis and has diagnostic value for gastric cancer[21]. The expression of hsa_circ_0000190 in gastric cancer tissue and serum is down-regulated, suggesting that it may be a more potential biomarker of gastric cancer than the common tumor markers CEA and CA19-9[22]. Liu et al. attempted to construct the regulatory network of circRNA-miRNA-mRNA in gastric cancer. Their study focuses on three down-regulation circRNAs (hsa_circ_0001190, hsa_circ_0036287 and hsa_circ_0048607) in gastric cancer tissues and plasma, and successfully establishes the circRNA-miRNA-hub gene network through bioinformatics analysis[23]. However, biomarkers with relatively low abundance are less sensitive to detection than those with high abundance. Therefore, based on previous studies, we attempted to find highly expressed circRNAs in gastric cancer tissues and plasma, and further to improve the circRNA-miRNA-mRNA regulatory network , to provide theoretical basis for the study of gastric cancer. In our study, we screened the circRNA expression profiles in the GSE89143 and GSE93541 GEO datasets for gastric cancer tissue and plasma to identify differentially expressed circRNAs, with the significance threshold set as $P<0.05$ and $|log2FC|>1.5$. Three upregulated circRNAs were selected for further analysis, namely hsa_circ_0001013, hsa_circ_0007376, and hsa_circ_0043947. They have not been reported until now.

Currently, it is generally believed that circRNAs have miRNA Response Elements (MREs) and can interact with miRNA through "sponge" action. CiRS-7 is the first circRNA to be reported to perform as a ceRNA[24] and circHECTD1 has been shown to act as a ceRNA to promote gastric cancer proliferation by sponging miR-1256[25]. We also screened 43 miRNAs through bioinformatics that may interact with the three selected circRNAs, and the GO analysis showed that these 43 miRNAs were involved in regulation of nucleobase, regulation of cell growth, etc. These biological processes are also very active in the development and progression of tumors. We further predicted the downstream target genes of these 43 miRNAs by online tool and a total of 119 target mRNAs were selected. Next, we analyzed these target genes by using GO and KEEG Pathway analysis to gain an understanding of the function of the target genes. The GO analysis showed that the target genes were mainly participated in regulation of apoptotic signaling pathway for BP, glutamatergic synapse for CC and DNA−binding transcription activator activity, RNA polymerase II−specific for MF. The KEEG Pathway analysis indicated that the most enrichment item was PI3K−Akt signaling pathway, which is one of the most frequently activated downstream signal transduction pathways in human cancer. The PI3K/Akt signaling pathway serves an important role in regulating cell proliferation, growth and apoptosis. Peng et al. reported that hsa_circ_0010882 promotes the progression of gastric cancer via regulation of the PI3K/Akt/mTOR signaling pathway[26]. We established a protein-protein interaction (PPI) network consisting of 165 nodes and 170 edges and identified 18 hub genes by MCODE plugin in Cytoscape. The 18 hub genes have been reported to be associated with gastric cancer, which are CCND2[27]、STAT3[28], TP53[29], MCL1[30], MYC[31], FOXO1[32], FOXO3[33], BCL2L11[34], GSK3B[35], CDKN1A[36], CDH1[37], PTEN[38], MTOR[39], MAPK1[40], CASP3, CDC42[41], SMAD4[42] and IL6[43]. All of them were predicted target genes for hsa-miR-197-3p, hsa-miR-451a, hsa-miR-136-5p, hsa-miR-337-3p, hsa-miR-654-3p, hsa-miR-182-5p, hsa-miR-1228-3p, hsa-miR-942-5p, hsa-miR-488-3p and hsa-miR-876-3p, and all these 10 miRNAs were predicted miRNAs for hsa_circ_0001013. Therefore, a core circRNA-miRNA-mRNA regulatory network was constructed based on 1 circRNA, 10 miRNAs and 18 hub genes which called gastric cancer-related genes. Finally, hsa_circ_0001013 was determined to play a key role in the pathogenesis of GC. Although the exact

mechanisms of circRNAs in gastric cancer are not clear, our results provide insights into the underlying mechanisms of gastric cancer pathogenesis. The results of this study are based solely on bioinformatics models. This is a pilot study and further studies are needed to verify the biological role of these circRNAs in gastric cancer.

5 Conclusions

We obtained circRNA expression profiles in gastric cancer tissue and plasma from the GEO. Three up-regulated circRNAs in gastric cancer tissue and plasma were identified as potential regulators. A core circRNA-miRNA-mRNA network was constructed by using bioinformatics methods. We found that hsa_circ_0001013 may play a role of ceRNA and function as a critical role in carcinogenesis-related pathways. These findings provide a new pathway for mechanism studies and offer potential biomarkers for GC. Further studies are needed to examine the role of regulatory modules in GC carcinogenesis.

Declarations

Acknowledgments

We thank the reviewers for their constructive comments.

Author contributions

GY and QXY collected related data from GEO; identified differently expressed circRNAs; collected and analyzed information about miRNAs and mRNAs; conducted validation of qRT-PCR; constructed PPI network; conducted GO and KEGG analyses; writed the main manuscript. YHJ and TLM designed the whole experiment. FJJ and QJ helped to sort out data from datasets. JYW checked all of the data used in manuscript. All authors read and approved final manuscript.

Funding

This work was supported by the Natural Science Foundation of Jiangsu Province, China (BK20181155); the Young Scientists Foundation of Changzhou No.2 People's Hospital(2018K010).

Availability of data and materials

We declare that the data and materials in this study are provided free of charge to scientists for non-commercial purposes.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consent to publication.

Competing interests

All authors declare that there is no conflict of interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries**. *CA Cancer J Clin* 2018, **68**(6):394-424.

2. Pormohammad A, Ghotaslou R, Leylabadlo HE, Nasiri MJ, Dabiri H, Hashemi A: **Risk of gastric cancer in association with Helicobacter pylori different virulence factors: A systematic review and meta-analysis**. *Microb Pathog* 2018, **118**:214-219.
3. Sugano K: **Effect of Helicobacter pylori eradication on the incidence of gastric cancer: a systematic review and meta-analysis**. *Gastric Cancer* 2019, **22**(3):435-445.
4. Eusebi LH, Telese A, Marasco G, Bazzoli F, Zagari RM: **Gastric cancer prevention strategies: A global perspective**. *J Gastroenterol Hepatol* 2020.
5. Banks M, Graham D, Jansen M, Gotoda T, Coda S, di Pietro M, Uedo N, Bhandari P, Pritchard DM, Kuipers EJ *et al*: **British Society of Gastroenterology guidelines on the diagnosis and management of patients at risk of gastric adenocarcinoma**. *Gut* 2019, **68**(9):1545-1575.
6. Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK: **Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures**. *Proc Natl Acad Sci U S A* 1976, **73**(11):3852-3856.
7. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M *et al*: **Circular RNAs are a large class of animal RNAs with regulatory potency**. *Nature* 2013, **495**(7441):333-338.
8. Salzman J: **Circular RNA Expression: Its Potential Regulation and Function**. *Trends Genet* 2016, **32**(5):309-316.
9. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J: **The biogenesis, biology and characterization of circular RNAs**. *Nat Rev Genet* 2019.
10. Cui C, Yang J, Li X, Liu D, Fu L, Wang X: **Functions and mechanisms of circular RNAs in cancer radiotherapy and chemotherapy resistance**. *Mol Cancer* 2020, **19**(1):58.
11. Li J, Sun D, Pu W, Wang J, Peng Y: **Circular RNAs in Cancer: Biogenesis, Function, and Clinical Significance**. *Trends Cancer* 2020, **6**(4):319-336.
12. Tang Q, Hann SS: **Biological Roles and Mechanisms of Circular RNA in Human Cancers**. *Onco Targets Ther* 2020, **13**:2067-2092.
13. Chaichian S, Shafabakhsh R, Mirhashemi SM, Moazzami B, Asemi Z: **Circular RNAs: A novel biomarker for cervical cancer**. *J Cell Physiol* 2020, **235**(2):718-724.
14. Wei J, Xu H, Wei W, Wang Z, Zhang Q, De W, Shu Y: **circHIPK3 Promotes Cell Proliferation and Migration of Gastric Cancer by Sponging miR-107 and Regulating BDNF Expression**. *Onco Targets Ther* 2020, **13**:1613-1624.
15. Jahani S, Nazeri E, Majidzadeh AK, Jahani M, Esmaeili R: **Circular RNA; a new biomarker for breast cancer: A systematic review**. *J Cell Physiol* 2020.
16. He Y, Wang Y, Liu L, Liu S, Liang L, Chen Y, Zhu Z: **Circular RNA circ_0006282 Contributes to the Progression of Gastric Cancer by Sponging miR-155 to Upregulate the Expression of FBXO22**. *Onco Targets Ther* 2020, **13**:1001-1010.
17. Pan H, Pan J, Chen P, Gao J, Guo D, Yang Z, Ji L, Lv H, Guo Y, Xu D: **WITHDRAWN: Circular RNA circUBA1 promotes gastric cancer proliferation and metastasis by acting as a competitive endogenous RNA through sponging miR-375 and regulating TEAD4**. *Cancer Lett* 2020.
18. Wang Y, Xu S, Chen Y, Zheng X, Li T, Guo J: **Identification of hsa_circ_0005654 as a new early biomarker of gastric cancer**. *Cancer Biomark* 2019, **26**(4):403-410.
19. Livak KJ, Schmittgen TD: **Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method**. *Methods* 2001, **25**(4):402-408.
20. Liu YY, Zhang LY, Du WZ: **Circular RNA circ-PVT1 contributes to paclitaxel resistance of gastric cancer cells through the regulation of ZEB1 expression by sponging miR-124-3p**. *Biosci Rep* 2019, **39**(12).
21. Xie Y, Shao Y, Sun W, Ye G, Zhang X, Xiao B, Guo J: **Downregulated expression of hsa_circ_0074362 in gastric cancer and its potential diagnostic values**. *Biomark Med* 2018, **12**(1):11-20.

22. Chen S, Li T, Zhao Q, Xiao B, Guo J: **Using circular RNA hsa_circ_0000190 as a new biomarker in the diagnosis of gastric cancer**. *Clin Chim Acta* 2017, **466**:167-171.
23. Liu J, Li Z, Teng W, Ye X: **Identification of downregulated circRNAs from tissue and plasma of patients with gastric cancer and construction of a circRNA-miRNA-mRNA network**. *J Cell Biochem* 2020.
24. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J: **Natural RNA circles function as efficient microRNA sponges**. *Nature* 2013, **495**(7441):384-388.
25. Cai J, Chen Z, Wang J, Wang J, Chen X, Liang L, Huang M, Zhang Z, Zuo X: **circHECTD1 facilitates glutaminolysis to promote gastric cancer progression by targeting miR-1256 and activating beta-catenin/c-Myc signaling**. *Cell Death Dis* 2019, **10**(8):576.
26. Peng YK, Pu K, Su HX, Zhang J, Zheng Y, Ji R, Guo QH, Wang YP, Guan QL, Zhou YN: **Circular RNA hsa_circ_0010882 promotes the progression of gastric cancer via regulation of the PI3K/Akt/mTOR signaling pathway**. *Eur Rev Med Pharmacol Sci* 2020, **24**(3):1142-1151.
27. Shi H, Han J, Yue S, Zhang T, Zhu W, Zhang D: **Prognostic significance of combined microRNA-206 and CyclinD2 in gastric cancer patients after curative surgery: A retrospective cohort study**. *Biomed Pharmacother* 2015, **71**:210-215.
28. Huang Z, Cai Y, Yang C, Chen Z, Sun H, Xu Y, Chen W, Xu D, Tian W, Wang H: **Knockdown of RNF6 inhibits gastric cancer cell growth by suppressing STAT3 signaling**. *Onco Targets Ther* 2018, **11**:6579-6587.
29. Ha TMT, Le TNU, Nguyen VN, Tran VH: **Association of TP53 gene codon 72 polymorphism with Helicobacter pylori-positive non-cardia gastric cancer in Vietnam**. *J Infect Dev Ctries* 2019, **13**(11):984-991.
30. Han Y, Wu N, Jiang M, Chu Y, Wang Z, Liu H, Cao J, Liu H, Xu B, Xie X: **Long non-coding RNA MYOSLID functions as a competing endogenous RNA to regulate MCL-1 expression by sponging miR-29c-3p in gastric cancer**. *Cell Prolif* 2019, **52**(6):e12678.
31. Yang DD, Chen ZH, Yu K, Lu JH, Wu QN, Wang Y, Ju HQ, Xu RH, Liu ZX, Zeng ZL: **METTL3 Promotes the Progression of Gastric Cancer via Targeting the MYC Pathway**. *Front Oncol* 2020, **10**:115.
32. Xie C, Guo Y, Lou S: **LncRNA ANCR Promotes Invasion and Migration of Gastric Cancer by Regulating FoxO1 Expression to Inhibit Macrophage M1 Polarization**. *Dig Dis Sci* 2020.
33. He X, Zou K: **MiRNA-96-5p contributed to the proliferation of gastric cancer cells by targeting FOXO3**. *J Biochem* 2020, **167**(1):101-108.
34. Zhang H, Duan J, Qu Y, Deng T, Liu R, Zhang L, Bai M, Li J, Ning T, Ge S *et al*: **Onco-miR-24 regulates cell growth and apoptosis by targeting BCL2L11 in gastric cancer**. *Protein Cell* 2016, **7**(2):141-151.
35. Lin JX, Xie XS, Weng XF, Qiu SL, Xie JW, Wang JB, Lu J, Chen QY, Cao LL, Lin M *et al*: **Overexpression of IC53d promotes the proliferation of gastric cancer cells by activating the AKT/GSK3beta/cyclin D1 signaling pathway**. *Oncol Rep* 2019, **41**(5):2739-2752.
36. Ma JX, Yang YL, He XY, Pan XM, Wang Z, Qian YW: **Long noncoding RNA MNX1-AS1 overexpression promotes the invasion and metastasis of gastric cancer through repressing CDKN1A**. *Eur Rev Med Pharmacol Sci* 2019, **23**(11):4756-4762.
37. Shenoy S: **CDH1 (E-Cadherin) Mutation and Gastric Cancer: Genetics, Molecular Mechanisms and Guidelines for Management**. *Cancer Manag Res* 2019, **11**:10477-10486.
38. He JQ, Zhang SR, Li DF, Tang JY, Wang YQ, He X, Li YM, Wu H, Zhou M, Jiao J *et al*: **Experimental Study on the Effect of a Weifufang on Human Gastric Adenocarcinoma Cell Line BGC-823 Xenografts and PTEN Gene Expression in Nude Mice**. *Cancer Biother Radiopharm* 2020.
39. Xiang L, Wang W, Zhou Z, Lv M, Tao L, Ni T, Deng J, Masatara S, Liu Y, Zhou Y: **COX-2 promotes metastasis and predicts prognosis on gastric cancer via regulating mTOR**. *Biomark Med* 2020.

40. Diao L, Wang S, Sun Z: **Long noncoding RNA GAPLINC promotes gastric cancer cell proliferation by acting as a molecular sponge of miR-378 to modulate MAPK1 expression**. *Onco Targets Ther* 2018, **11**:2797-2804.
41. Hirano T, Shinsato Y, Tanabe K, Higa N, Kamil M, Kawahara K, Yamamoto M, Minami K, Shimokawa M, Arigami T *et al*: **FARP1 boosts CDC42 activity from integrin alphavbeta5 signaling and correlates with poor prognosis of advanced gastric cancer**. *Oncogenesis* 2020, **9**(2):13.
42. Yang T, Huang T, Zhang D, Wang M, Wu B, Shang Y, Sattar S, Ding L, Liu Y, Jiang H *et al*: **TGF-beta receptor inhibitor LY2109761 enhances the radiosensitivity of gastric cancer by inactivating the TGF-beta/SMAD4 signaling pathway**. *Aging (Albany NY)* 2019, **11**(20):8892-8910.
43. Wu X, Tao P, Zhou Q, Li J, Yu Z, Wang X, Li J, Li C, Yan M, Zhu Z *et al*: **IL-6 secreted by cancer-associated fibroblasts promotes epithelial-mesenchymal transition and metastasis of gastric cancer via JAK2/STAT3 signaling pathway**. *Oncotarget* 2017, **8**(13):20741-20750.

Tables

Table 1 Features of 3 selected circRNAs

CircRNA ID	GSE83521		GSE93541		Chromosome location	Gene symbol	Accession number
	P-Value	Log2FC	*P*-Value	Log2FC			
hsa_circ_0001013	0.0003	1.9539	0.0023	1.7488	Chr2:61339656-61345251+	KIAA1841	NM_001129993
hsa_circ_0007376	0.0000	1.9983	0.0199	3.2983	Chr19:4101016-4101278-	MAP2K2	NM_030662
hsa_circ_0043947	0.0289	1.8146	0.0000	3.1526	Chr17:41199659-41215968-	BRCA1	NM_007300

Table 2 selected circRNAs, miRNAs interact with circRNAs and their target genes.

	miRNA	mRNA
hsa_circ_0001013	hsa-miR-1197, hsa-miR-1225-3p, hsa-miR-1243, hsa-miR-1250-5p, hsa-miR-1261, hsa-miR-1294, hsa-miR-1304-5p, hsa-miR-146b-3p, hsa-miR-1827, hsa-miR-323a-3p, hsa-miR-450b-3p, hsa-miR-548g-3p, hsa-miR-548m, hsa-miR-556-3p, hsa-miR-562, hsa-miR-576-5p, hsa-miR-624-3p, hsa-miR-924	None
	hsa-miR-1228-3p	CSNK2A2, TP53
	hsa-miR-1256	TRIM68
	hsa-miR-1283	ATF4
	hsa-miR-136-5p	MTDH, PPP2R2A, RASAL2, IL6
	hsa-miR-182-5p	CDKN1A, FOXO3, FOXO1, RARG, MITF, ADCY6, CLOCK, TSC22D3, FGF9, NTM, CYLD, BCL2, CCND2, PDCD4, RECK, FLOT1, PTEN, GSK38, ZFAND4, BDNF, SATB2, CHL1, CADM1, TP53INP1, TCEAL7, FBXW7, LRRC4, ULBP2, PDK4, TRIM8, TIAM1, UQCRFS1
	hsa-miR-197-3p	FOXO3, TUSC2, NSUN5, CD82, BMF, PMAIP1, MTHFD1, FOXJ2, MAPK1
	hsa-miR-330-5p	MUC1, ITGA5, PDE4B
	hsa-miR-337-3p	RAP1A, STAT3, CSNK2A1, MZF1
	hsa-miR-451a	MIF, CAB39, ABCB1, MYC, RAB14, CPNE3, RAB5A, DCBLD2, IL6R, ADAM10, TSC1, MAPK1, CDKN2D, MAP3K1, IL6
	hsa-miR-487a-3p	ABCG2, SPRED2, PIK3R1
	hsa-miR-488-3p	SLC39A8, PAX6, BCL2L11
	hsa-miR-510-5p	SPDEF, PRDX1,
	hsa-miR-513a-3p	GSTP1, LHCGR
	hsa-miR-548c-3p	ITGAV, TWIST1
	hsa-miR-570-3p	CD274
	hsa-miR-654-3p	CDKN1A
	hsa-miR-665	CD274, CNR2
	hsa-miR-876-3p	MCL1
	hsa-miR-942-5p	CDKN1A, IFI27, SFRP4, GSK3B, TLE1, NFKBIA
hsa_circ_0007376	hsa-miR-571	None
	hsa-miR-224-5p	KLK10, CXCR4, CDC42, API5, EYA4, EDNRA, DIO1, SMAD4, PEBP1, TCEAL1, PHLPP1, HOXD10, PTX3, MBD2, TPD52, TRIB1, CDH1, APLN, CASP7, CASP3, MTOR, PHLPP2, RASSF8
hsa_circ_0043947	hsa-miR-1257	None
	hsa-miR-140-3p	NRIP1, CD38, ATP6AP2, ITGA6, MARCKSL1, COL4A1, ATP8A1
	hsa-miR-151a-3p	TWIST1, IL12RB2
	hsa-miR-660-5p	TFCP2

Figures

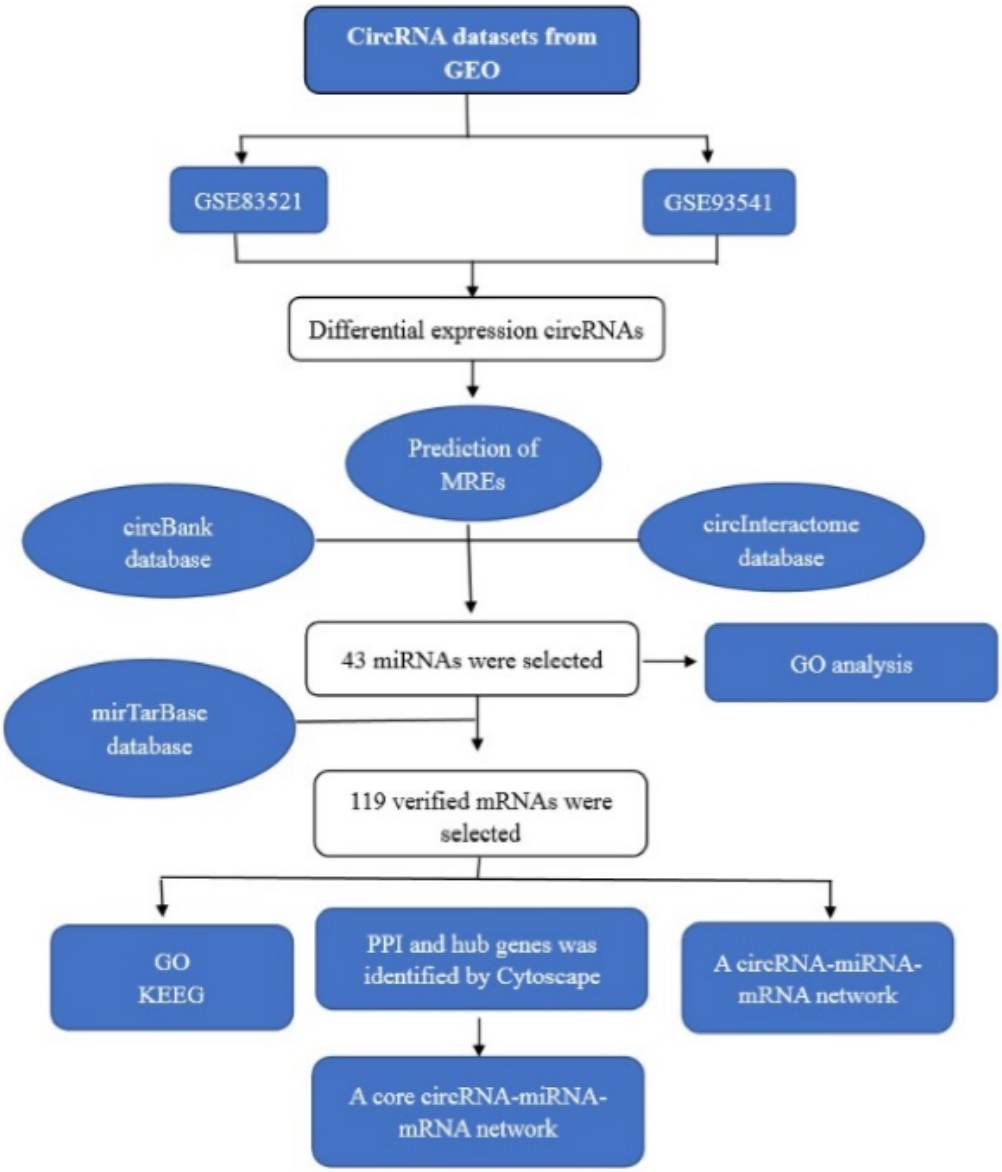

Figure 1

Flow chart of the methods used in the present study. GO, Gene Ontology; circRNA, circular RNA; KEGG, Kyoto Encyclopedia of Genes and Genome; mRNA, messenger RNA; PPI, protein-protein interaction.

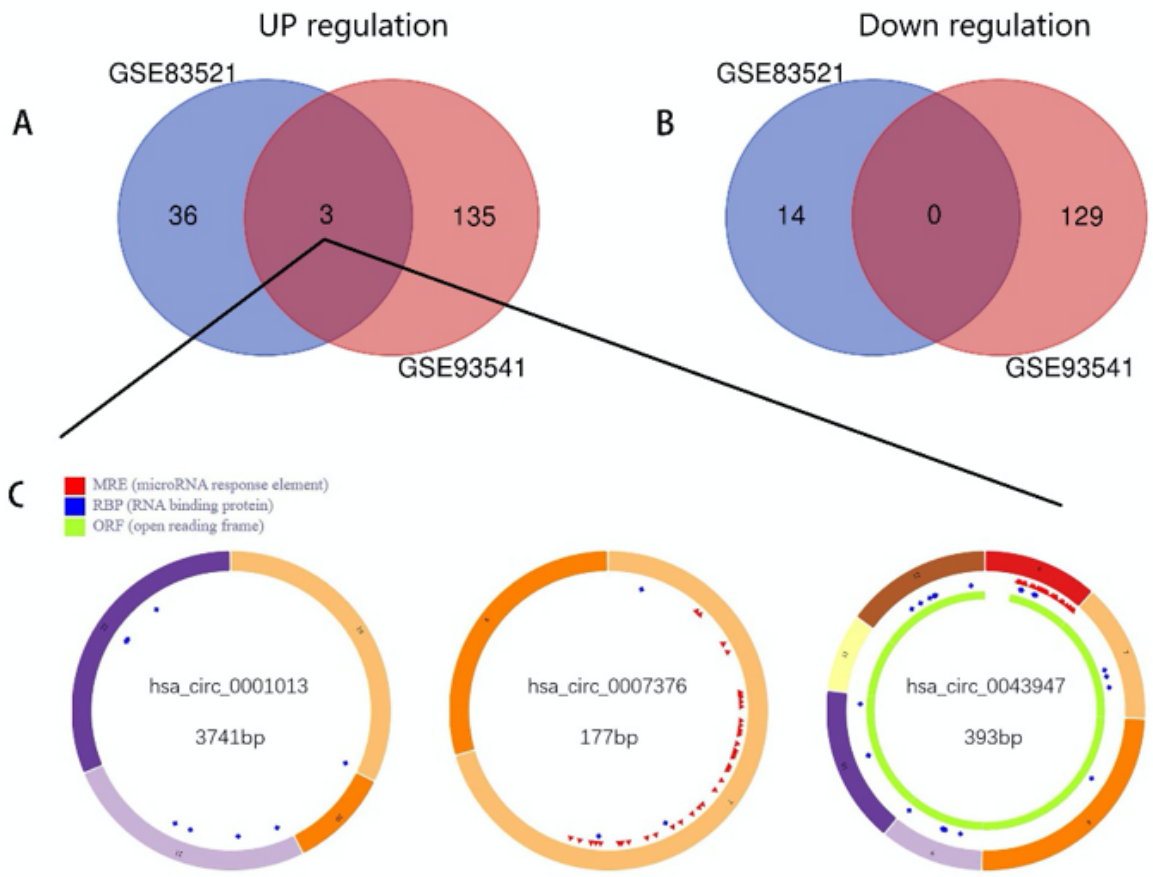

Figure 2

Differentially co-expressed circRNAs in gastric cancer tissues and plasma. (A-B) Venn diagram used to select the three overlapping differentially expressed circRNAs detected by analysis of the GSE89143 and GSE93541 datasets. (C) The essential characteristics and basic structural patterns of DECs were analyzed by the cancer-specific circRNA database; MRE, miRNA response element; RBP, RNA binding protein; ORF, open reading frame.

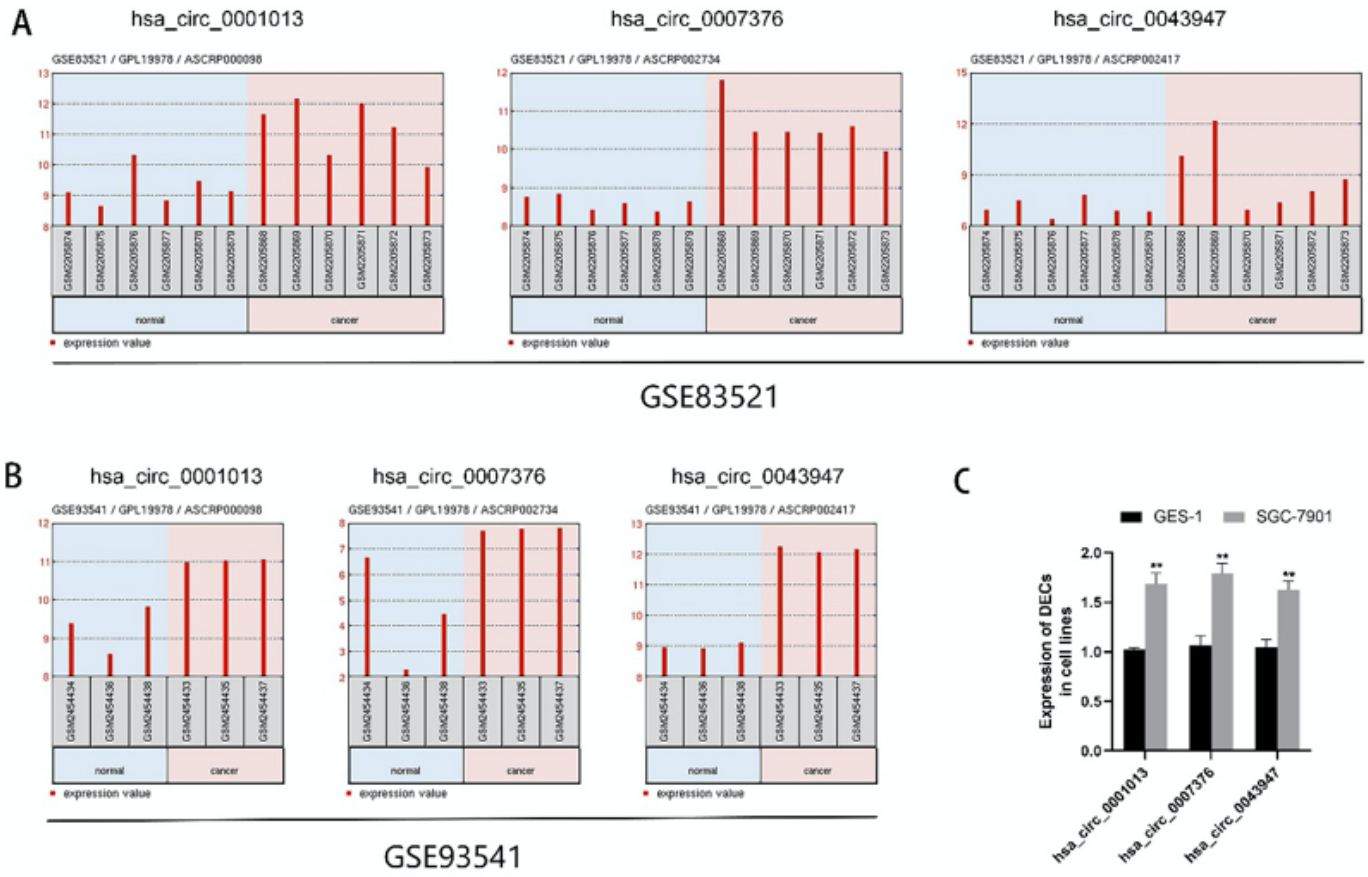

Figure 3

Expression of DECs in gastric cancer samples and cell lines. (A) The expression of three selected circRNAs in six gastric cancer tissues and six normal mucosa tissues. (B) The expression of three selected circRNAs in three gastric cancer plasmas and three healthy controls. (C) The relative expression of three selected circRNAs in gastric cancer cell line SGC-7901 and human gastric epithelial cell line GES-1. **□0.05.

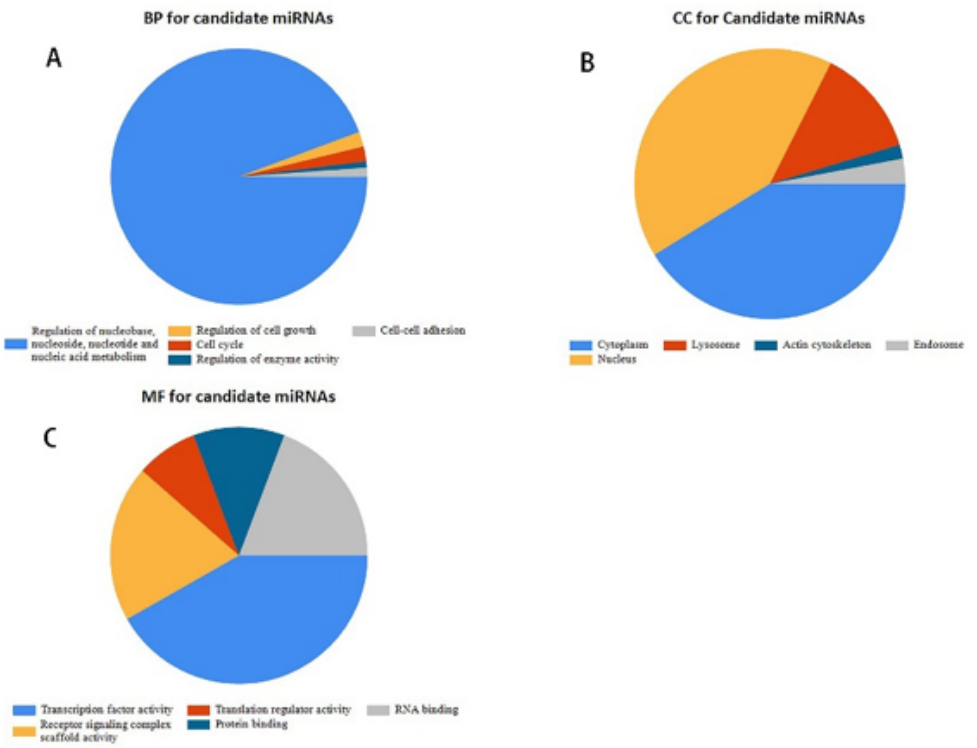

Figure 4

GO analysis for 43 miRNAs by RichFun software. (A-C) Top five enrichment items for BP, CC and MF respectively. BP, biological progress; CC, cellular component; MF, molecular function.

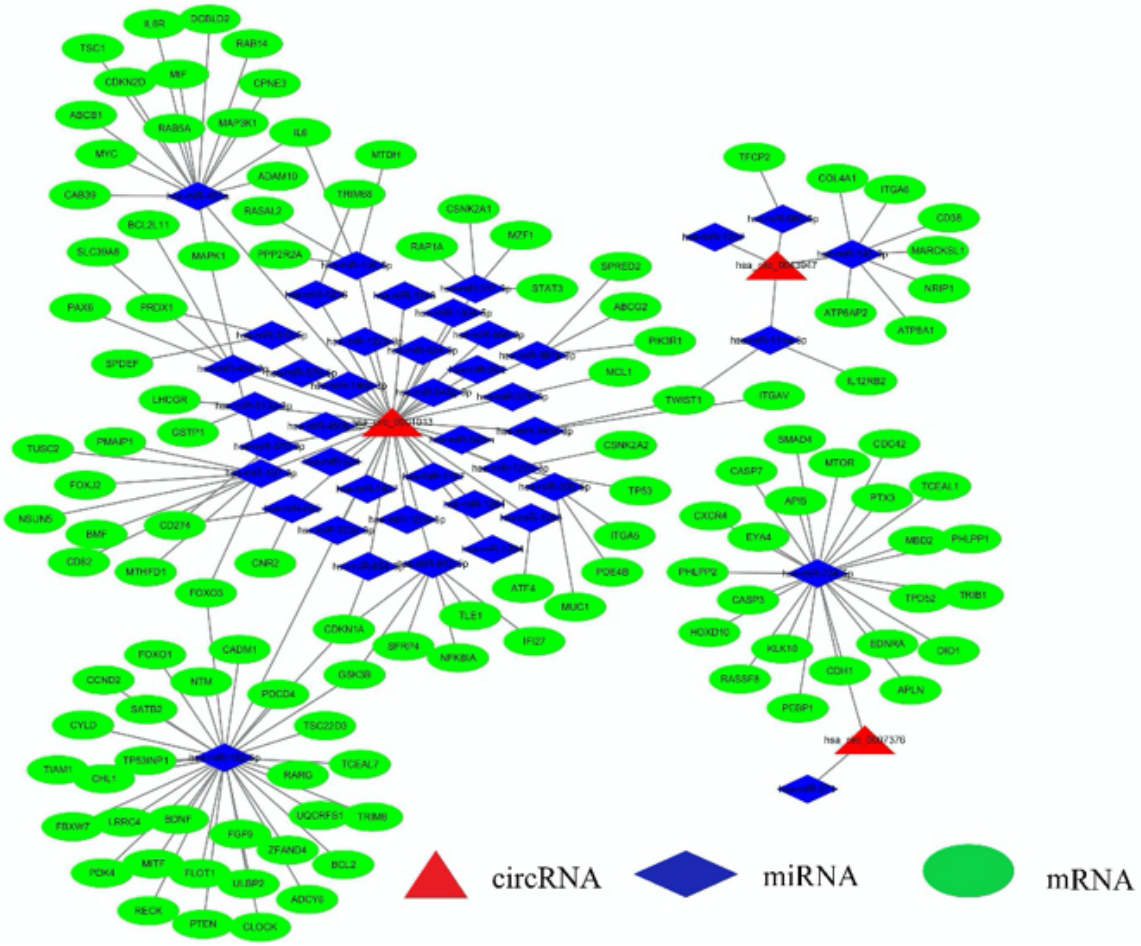

Figure 5

The circRNA-miRNA-mRNA network was constructed based on 3 circRNAs, 43 miRNAs, and 119 mRNAs.

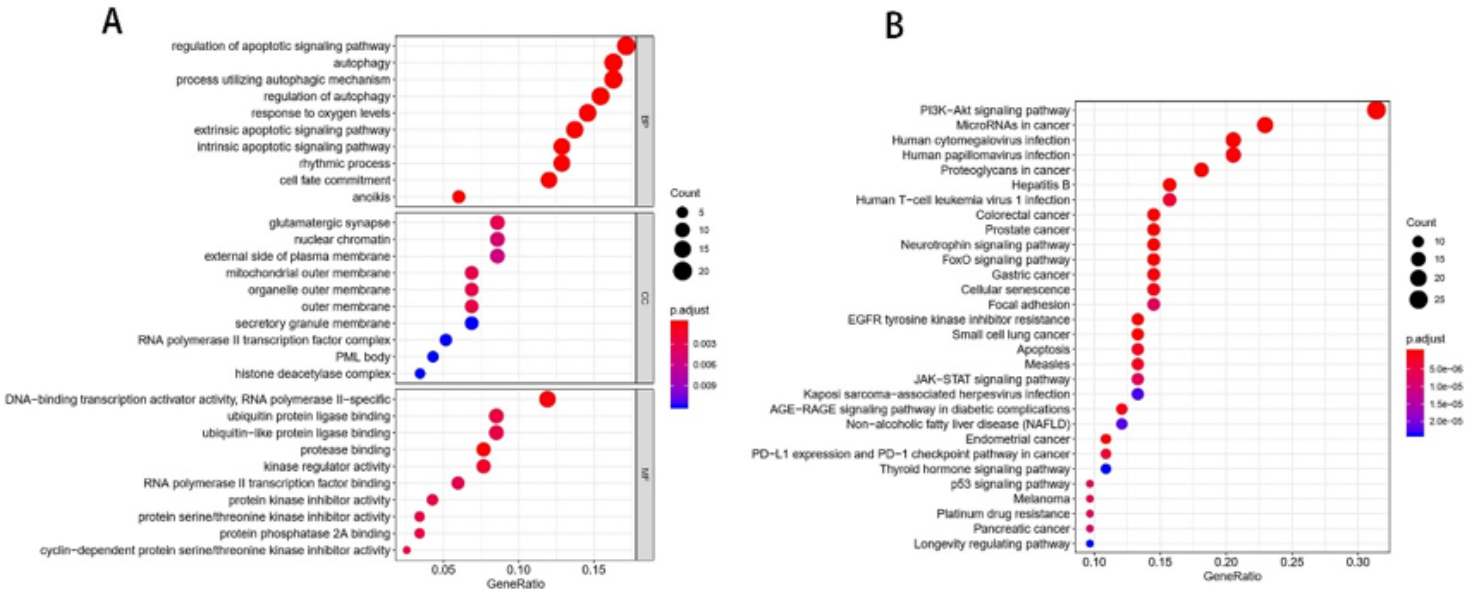

Figure 6

GO and KEEG pathway analysis for 119 mRNAs. (A) Top 10 enriched gene ontology (GO) terms. (B) Top 30 significant KEGG pathways. KEEG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function.

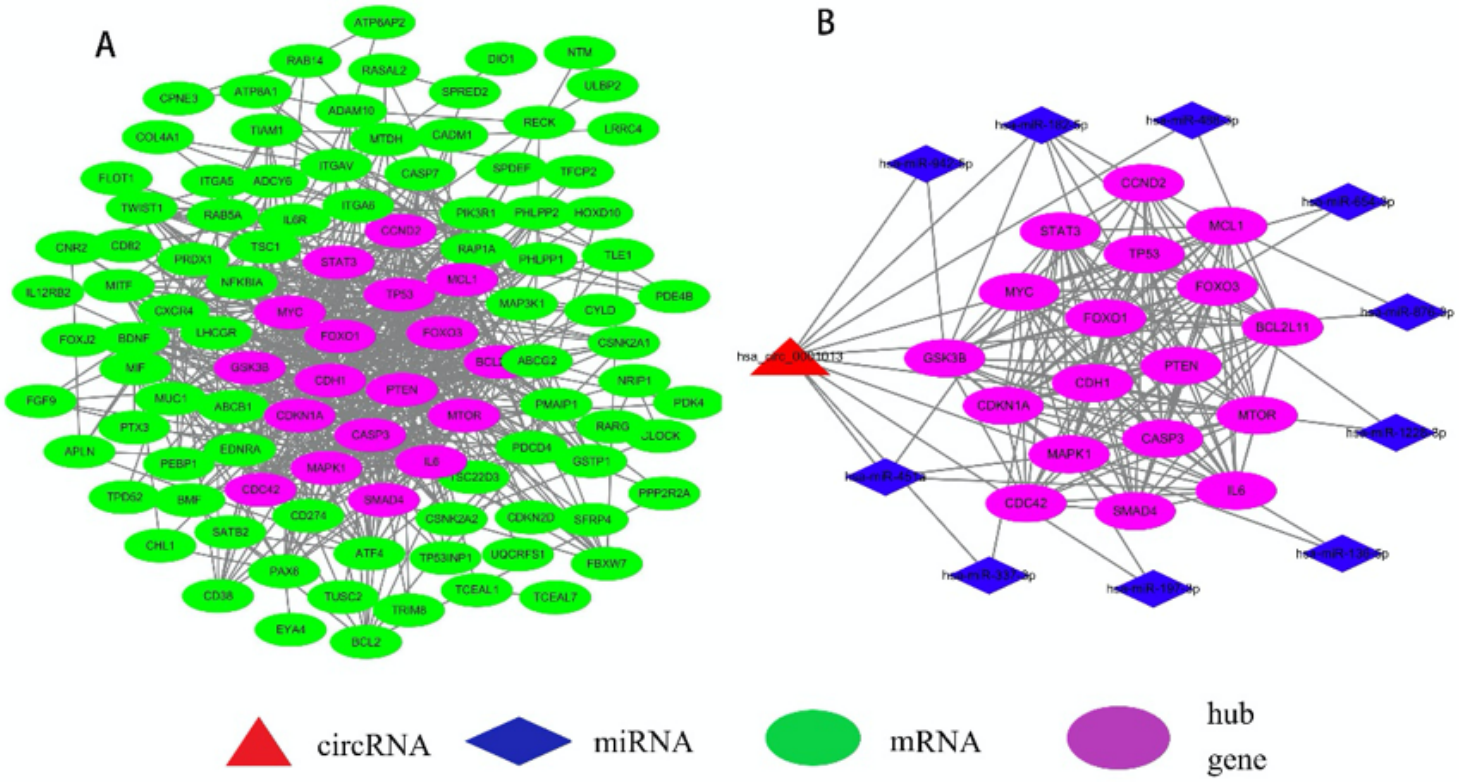

Figure 7

PPI network and core circRNA-miRNA-mRNA network. (A) PPI network composed of 165 nodes and 170 edges, and hub genes identified from the PPI network by Cytoscape. (B) The core circRNA-miRNA-mRNA network based on 1circRNA, 10 miRNAs, and the 18 hub genes. circRNA, circular RNA; miRNA, microRNA; mRNA, messenger RNA; PPI, protein-protein interaction