Analysis of serum CircRNA in related to gastric cancer

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Abstract

Gastric cancer is one of the most common malignant tumors of the digestive tract and one of the leading causes of death in patients with malignant tumors worldwide. In recent years, with the deepening of circRNA research, more and more evidence indicates that circRNA plays an important role in the occurrence and development of human malignant tumors. This study firstly conducted a retrospective analysis of the case data of gastric cancer patients who were treated at the Wuwei Cancer Hospital between 2015 and 2017. Subsequently, 18 cases of primary gastric cancer patients and 9 healthy people in Wuwei, Gansu Province were used as controls. The high-throughput microarray technology was used to screen the circRNA expression profiles of healthy and gastric cancer patients, and the expression was expressed by bioinformatics methods. Differential circRNA was used for gene ontology (GO) and KEGG pathway analysis, using its enrichment to predict the relevant biological functions of the differentially expressed circRNA and its involved pathways, and predicting miRNAs interacting with differentially expressed circRNAs, and constructing circRNA-miRNA interaction network. Q-PCR, gene organization microarray and bioinformatics techniques were used to validate candidate differential circRNAs and their linear parental genes and regulatable miRNAs. The results showed that there were 137 circRNAs with significant expression differences (including up-regulation of 67 and down-regulation of 70) in gastric cancer patients, and their differential expression may be related to the occurrence and development of gastric cancer; by GO, KEGG enrichment analysis and Regulating miRNA predictive analysis, the gastric cancer-related GO classification, KEGG Pathway and circRNA-miRNA network were preliminarily obtained, suggesting that differential circRNA may participate in gastric cancer-associated GO classification and KEGG pathway by regulating the expression of parental genes and miRNAs to influence the occurrence and development of gastric cancer. Finally, by using has verified the has_circ_0000437 and its parental genes and regulatable miRNAs, it was found that has_circ_0000437 is highly expressed in gastric cancer patients, and has a certain diagnostic value for the clinical diagnosis of gastric cancer. It may regulate its linear parental gene and The expression of miRNAs affects the development, metastasis and prognosis of clinical gastric cancer.

Background

Gastric cancer is a malignant tumor that originates from the mucosal epithelium and is one of the most common malignant tumors of the digestive system. In recent years, the incidence of gastric cancer has decreased worldwide, but according to the latest data from the Global Cancer Observatory (GCO), its incidence rate ranks fifth in the world, which is one of the leading causes of death in patients with malignant tumors in the world [1] New cases and deaths are concentrated in East Asia such as China, Japan, and Korea [2]. Wuwei is located in the Hexi Corridor area of China. It is a high-risk area of gastric cancer in China, and its morbidity and mortality are higher than the national average [3]. Therefore, studying the mechanism of the occurrence and development of gastric cancer is particularly important for the prevention and control of gastric cancer in this region.Surgery as the treatment of choice for patients with gastric cancer can greatly improve the patient's survival rate within 5 years. However, due to

the lack of obvious clinical symptoms of early gastric cancer and specific tumor markers, most patients are diagnosed as having advanced. Therefore, gastric cancer has always maintained a high mortality rate. Therefore, the search for new tumor markers and biological targets with strong specificity is necessary for the early diagnosis and intervention of gastric cancer.

CircRNA was first discovered and proposed by Sanger and colleagues in RNA viruses in 1976 [4]. Unlike linear RNA, circRNA does not have a 5'-end cap and a 3'-end poly(A) structure, which is at the 5' end. A closed loop structure that is not easily degraded is formed in the form of a covalent bond with the end of the 3' end [5]. This closed loop structure allows circRNA to have multiple biological functions such as regulation of expression of related miRNAs by the mechanism of miRNA sponge [6], regulation of linear RNA transcription and protein expression [7]. The current research results show that circRNA can be used as a miRNA sponge to participate in the development of various diseases (atherosclerosis, neurological disorders) [8]. In addition, circRNA is closely related to tumor development, invasion and metastasis, and drug resistance. Therefore, circRNA has the potential to become a novel molecular marker for tumor diagnosis and a therapeutic intervention target.

In this study, the differences in circRNA expression profiles between healthy and gastric cancer patients were analyzed. The differences in serum circRNA expression profiles between healthy and gastric cancer patients in Gansu Province were analyzed to determine the differential expression of circRNA in healthy and gastric cancer patients. The mechanism of action in gastric cancer provides a scientific basis.

Methods

1.1 Ethics Statement

The study protocol was approved by the Medical Ethics Committee of Wuwei Cancer Hospital of Gansu Province (Wuwei Academy of Medical Sciences). Each patient included in the study was required to sign the patient's informed consent before the study began. All methods were in accordance with the guidelines and related Regulations are enforced.

1.2 Patients and samples

All collected tissue samples and peripheral blood samples were obtained from primary gastric cancer patients diagnosed and operated at Wuwei Cancer Hospital from January to February 2018, 2016. The healthy blood samples from the control group were from the Wuwei Cancer Hospital during this time period. A healthy population undergoing a medical examination.

1.3 Sample total RNA extraction and quality control

Total RNA in the samples was extracted with Trizol (Invitrogen, Gaithersburg, MD, USA). Total RNA was further purified and quantified using the NucleoSpin® RNA clean-up kit (MACHEREY-NAGEL, Germany). RNA integrity was detected by agarose gel electrophoresis.

1.4 CircRNA and mRNA microarrays

CircRNA expression profiles of human species were detected using the BioBio Human CircRNA Array v2, 4x180K chip, and the resulting circRNA target sequences were derived from Circbase, Deepbase, Rybak-Wolf2015; using Agilent SurePrint G3 Human Gene Expression 8x60K v2 Microarray The Kit chip detects the mRNA expression profile of human species.

1.5 Data Analysis

The chip was scanned using an Agilent chip scanner (G2565CA) to obtain a hybrid image. The hybrid image was analyzed and extracted using Agilent Feature Extraction (v10.7) software, and then circRNA and mRNA were analyzed using GeneSpring software V13.0 (Agilent). Data summary, standardization and quality control of array data. To select differentially expressed genes, we used a threshold of ≥ 2 and ≤ -2 fold changes and a Penjamini-Hochberg corrected p-value of 0.05. The data was subjected to Log2 transformation using the Adjust Data function of CLUSTER 3.0 software and the median gene was centered and then further analyzed using average linkage hierarchical clustering (Eisen et al., 1998). Finally, we used Java Treeview (Stanford University School of Medicine, Stanford, California, USA) for tree visualization.

1.6 Gene Ontology (GO) and KEGG Path Analysis of Differential CircRNA

The GeneOntology database provides professional terminology to define the properties of gene products. GO analysis can be used to annotate gene products that are meaningful in a variety of organisms. It can be divided into three regions, biological processes (BP), molecular functions. (MF), cellular component (CC). This study used KOBAS software to annotate linear parental genes that differentially express circRNA, where -log10 (p value) is an enrichment score indicating the significance of GO term enrichment in genes that produce differentially expressed circRNA.

KEGG is used to obtain a cluster of related pathways for genes of interest in a variety of organisms, and the information contained therein covers the intermolecular interactions and related reaction networks of genes involved in the differential expression of circRNA. This study used KOBAS software to interpret pathways for circRNA-derived genes that produce differential expression.

1.7 Construction of CircRNA-miRNA Interaction Network

CircRNA can act as a miRNA sponge, targeting miRNA binding and indirectly regulating mRNA translation. In this study, based on miRanda-3.3 software, combined with entropy values below 20, select the corresponding circRNA-miRNA to construct a circRNA-miRNA network, select differentially expressed circRNA for miRNA target miRNA prediction, obtain a list of miRNAs, and use The open source bioinformatics software Cytoscape performs network diagram rendering.

1.8 Validation of candidate differential circRNA by q-PCR

Ten patients with gastric cancer and healthy people were selected. Total RNA was extracted from paired serum using TRIzol reagent (Thermo Fisher Scientific, USA), different primers for candidate circRNA validation were designed, and RNRNA was performed on each group of RNA samples by RT-qPCR. Verification. The sequence of the candidate circRNA was derived from the "circBase" database, and the relative expression of circRNA was analyzed by comparative Ct method.

1.9 Statistical analysis

Two sets of continuous data were analyzed using an independent sample t test. The receiver operating characteristic curve (ROC) and its area under the curve (AUC) were used to evaluate the clinical diagnostic performance of gastric cancer-specific circRNA. Data statistical analysis was performed using SPSS (version 21.0, IBM, USA) software, and data visualization graphics were performed using OriginPro2018 software. P values <0.05 had expression differences, and P values <0.01 had significant expression differences.

Results

2.1 Overview of differential expression of serum circRNA in normal population and gastric cancer patients

High-throughput chip technology is one of the effective ways to study the biological function of circRNA. In this study, serum samples from 18 patients with gastric cancer were used, and serum samples from 9 normal subjects were tested for human circRNA gene chip and gastric cancer. Patients were compared with normal population and gastric cancer patients for changes in circRNA expression profiles before and after treatment. The results showed that by comparing the results of gene chip screening, we found that there were 120427 circRNAs with different expression differences between gastric cancer patients and normal population, including 137 circRNAs with differential expression ≥1.5 and p value <0.05. Among them, there were 67 circRNAs with up-regulated differential expression and 70 circRNAs with differential expression (Fig. 1A). Volcanic maps and scatter plots show the overall changes of circRNA in gastric cancer patients and normal population (Fig. 1B-C). Based on the above results, we selected TOP10 differential circRNA, up-regulated by 5 and down-regulated by 5 (Table.1), among which has_circ_0000437 has the largest difference fold and the lowest P value in the above-mentioned circRNA with obvious expression difference, compared with healthy people. Has_circ_0000437 is significantly down-regulated in the serum of patients with gastric cancer.

2.2 Gene ontology analysis of differential expression circRNA

GO enrichment analysis links the linear parental genes of differential genes with three GO classifications of biological processes (BPs), molecular functions (MFs) and cellular components (CCs), and performs related functional analysis and prediction of differential genes. In one method, P-Value is used to reflect the degree of enrichment in a disease based on the correlation classification of differentially expressed genes. The smaller the P-Value, the more significant the enrichment of the GO classification in the

disease. The significance of the disease is also greater. By performing GO analysis on 115 linear parental genes of the above 137 differential circRNAs, we obtained the biological functions, molecular functions and cellular components related to the development of gastric cancer, and selected the top 30 GO classifications according to P-Value .

2.2.1 Analytical analysis of BPs differentially expressing circRNA

Based on the overall GO analysis results, we selected the top 30 biological process nodes with the most significant enrichment and analyzed the target genes(Fig. 2A). The results showed that there were a total of 26 circRNAs with differential expression in the first 30 biological processes that were significantly enriched, and two different circRNAs with differential expression involved T cell tolerance-induced positive regulation and tolerance induction. 19 biological processes including positive regulation, T cell immune tolerance induction, immature B cell differentiation, immune tolerance, and lymphoid progenitor differentiation, respectively. Three different circRNAs with differential expression are involved in regulating cell responses to heat. There are four different expressions of circRNA in the lungs, including the response of cells to heat, the positive regulation of type I interferons, the response to heat, the processing of antigens and antigen peptides by MHC I, In response to DNA damage in endogenous apoptotic signaling pathways, five different circRNAs with differential expression are involved in the regulation of type I interferon production, type I interferon production, and lung development (Fig. 2B). Among them, the main factors related to the development of gastric cancer are positive regulation of T cell tolerance induction, positive regulation of tolerance induction, induction of T cell immune tolerance, immature B cell differentiation, immune tolerance, and positive regulation of type I interferon. The expression of antigen and antigen peptide by MHC I, the response of endogenous apoptosis signaling pathway to DNA damage, and the production of type I interferon.

2.2.2 MFs annotation analysis of differential expression circRNA

Based on the results of the GO analysis, we selected the top 30 molecular functional nodes that were significantly enriched and analyzed the target genes that were annotated (Fig. 3A). The results showed that there were 59 different circRNAs with differential expression involving the first 30 molecular functions that were significantly enriched. Among them, 27 different circRNAs with differential expression involved small molecule binding, 26 different circRNAs with differential expression involved nucleotide binding, nucleotide phosphate binding, and 24 different circRNAs with differential expression involved in carbon water Compound derivative binding, 20 different circRNAs with differential expression involved ATP binding, adenine ribonucleotide binding, adenine nucleotide binding, and 12 different circRNAs with differential expression involved protein complex binding, 10 Different circRNAs with differential expression are involved in protein kinase activity, 9 different circRNAs with differential expression are involved in serine/threonine protein kinase activity, and 8 different circRNAs with differential expression are involved in ATPase activity, 5 different The circRNA with differential expression is involved in thiol-nucleotide exchange factor activity, and two different circRNAs with differential expression are involved in dna-dependent protein kinase activity, TBP-class protein binding, nitric oxide synthase binding, and 1

difference. The circRNA with differential expression involves N6-threonylcarbonyladenosine methylthiotransferase activity , 5'-3' exonuclease activity, macrophage colony-stimulating factor receptor activity, type II transforming growth factor beta receptor activity, mechanically gated ion channel activity, transcription termination site DNA binding, etc. 15 molecules Function (Fig. 3B). Among them, there are nucleotide binding, nucleotide phosphate binding, ATPase activity, macrophage colony-stimulating factor receptor activity, 5'-3' exonuclease activity, protein kinase activity, etc. related to the development of gastric cancer. .

2.2.3 CCs annotation analysis of differential expression circRNA

Based on the results of the GO analysis, we selected the top 30 cell component nodes with the highest degree of enrichment and analyzed the target genes that were annotated(Fig. 4A). The results showed that there were 47 different circRNAs with different expression differences involving the first 30 cell components that were significantly enriched. Among them, 41 different circRNAs with differential expression involved macromolecular complexes, 37 involved protein complexes, 11 involved projection neurons, 10 involved transferase complexes, 7 involved synaptic moieties, and 6 involved After the touch, the four involved ribonucleoprotein particles, cytoplasmic ribonucleoprotein particles, post-synaptic dense regions, polarized growth sites, growth cones, three involved ER to Golgi transport vesicle membrane, ER to Golgi transport Vesicle, transport vesicle membrane, histone acetyltransferase complex, dendritic spine, two involved COPII vesicle coat, DNA repair complex, and one involved nuclear RNA-directed RNA polymerase complex, RNA-directed RNA 12 different cellular components such as polymerase complex, nuclear proteasome complex, MCM8-MCM9 complex, Cul7-RING ubiquitin ligase complex, transforming growth factor beta receptor homodimer complex (Fig. 4B). Among them, macromolecular complexes, protein complexes, Cul7-RING ubiquitin ligase complexes, and DNA repair complexes are involved in the development of gastric cancer.

2.3 KEGG analysis of differential expression circRNA

KEGG Pathway is usually not used in the analysis of disease-related pathways. Based on KOBAS software, we performed KEGG Pathway enrichment analysis on the linear parental genes of differential circRNAs, and obtained the KEGG pathway related to the development of gastric cancer, and ranked according to P-Value. The first 30 KEGG pathways (Fig. 5A). The results showed that a total of 24 circRNAs with differential expression involved the above 30 pathways. Among them, 5 different circRNAs with differential expression involved ubiquitin-mediated proteolysis, and 4 involved 8 different types of Fox0 signaling pathway, influenza A, cell cycle, misregulation of transcription in tumor, MAPK signaling pathway, etc. Pathway, three involved long-term potential difference, adhesive connection, tuberculosis, herpes simplex virus infection, cAMP signaling pathway, two involved glycosaminoglycan degradation, TGF-β signaling pathway, gastric acid secretion, apoptosis, NF-KB There are 9 different pathways, such as signal pathway, and 1 different pathways involving non-homologous end joining, degradation of other polysaccharides, and pentose and glucuronate interconversion (Fig. 5B). Among them, ubiquitin-mediated protein hydrolysis, cell cycle, apoptosis, misregulation of transcription in tumors, adhesion junction,

MAPK signaling pathway, gastric acid secretion, and NF-KB signaling pathway are involved in the development of gastric cancer.

2.4 CircRNA-miRNA network analysis of differentially expressed circRNA

As a molecular sponge of miRNA, circRNA has a large number of sites that bind to miRNAs, and competitively binds miRNA molecules through binding sites, thereby exerting its miRNA molecular regulation. It is predicted that the establishment of a circRNA-miRNA regulatory network can more directly and effectively analyze the possible regulatory mechanisms between circRNA and miRNA. Based on the expression of circRNA microarray, the circRNA-miRNA co-expression network of circRNA with differential expression in gastric cancer patients and healthy people was constructed by miRanda-3.3 and Cytoscape software, combined with the entropy value of 20 or less. The results obtained were plotted for the circRNA-miRNA co-expression network map (Fig. 6). The results showed that in the gastric cancer patients and healthy people, the constructed regulatory network contained 9 differentially expressed circRNAs and 142 miRNAs; among 142 miRNAs, 20 miRNAs such as Hsa-miR-4433a-3p were available. One or more circRNAs with differential expression simultaneously establish a potentially important linkage, while in the nine differentially expressed circRNAs, Hsa_circ_0133089 and Has_circ_0070634 can establish potentially important linkages with the most miRNAs.

2.5 qPCR verification analysis of differential candidate circRNA

Due to its special ring structure and high stability and high conservatism in the body, circRNA has the potential to become a novel tumor molecular marker. Recent studies have shown that circRNA can exert its role in related biological processes or biological pathways by regulating its linear parental genes by homeopathic [9-11]. Therefore, we analyzed the linear parental genes of 10 gastric cancer-associated differential circRNAs. The results showed that the seven linear parental genes were involved in the process of tumor development, tumor cell cycle, apoptosis, migration and infiltration. Correlation, CORO1C (also known as Coronin1c) has been reported to be involved in the migration and invasion of gastric cancer cells [12-13], playing an important role in the development and metastasis of clinical gastric cancer. Therefore, we further screened the three differential circRNAs involved in CORO1C, and finally verified the RT-qPCR in each group of serum samples with hsa_circ_0000437 with the largest difference. The results showed that compared with healthy people, the expression level of hsa_circ_0000437 in serum samples of gastric cancer patients was significantly down-regulated, and the difference was statistically significant ($P \leq 0.05$) (Fig. 7). The verification results were consistent with the results of circRNA chips. Then we performed a ROC curve analysis on hsa_circ_0000437. The results showed that the AUC of hsa_circ_0000437 was 0.92, the sensitivity was 90%, and the specificity was 40%, which has certain diagnostic value for the clinical diagnosis of gastric cancer (Fig. 8).

2.6 hsa_circ_0000437 Linear Parental Gene and Related miRNA Validation Analysis

The main biological function of CircRNA is to play a miRNA molecular sponge function as a competitive endogenous RNA and to regulate the expression of the parental gene. In recent years, studies have shown

that circRNA can not only interact with RNA-binding proteins to affect the expression of parental mRNA, but also achieve a balance between linear and RNA based on competitive complementary pairing between introns during the formation process. mRNA expression, and even protein translation, is based on the above biological functions. Therefore, we performed a gene chip analysis of the linear parental gene mRNA of hsa_circ_0000437 and further predicted the relevant miRNAs.

Based on the results of tissue microarray analysis, we found that hsa_circ_0000437 linear parental gene mRNA CORO1C was highly expressed in gastric cancer tissues compared with adjacent tissues of gastric cancer patients, and the difference fold was as high as 3.81, consistent with hsa_circ_0000437 in gastric cancer. Low expression trends in patients. At the same time, based on the miRNACancerMAP database, we further analyzed 35 miRNAs that may establish important linkages with hsa_circ_0000437. Through the Pan-cancer module in the miRNACancerMAP database, we performed Pancancer microRNA-gene-pathway network prediction analysis on 35 miRNAs. Twelve miRNAs closely related to tumorigenesis and development, 10 significantly enriched signaling pathways related to tumorigenesis and development, and more than 400 miRNA target genes represented by MAPK11 were obtained (Fig. 9). Among them, 12 miRNAs are 7 miRNAs of the let-7 family (hsa-let-7a-5p; hsa-let-7b-5p; hsa-let-7c-5p; hsa-let-7e-5p; hsa-Let-7f-5p;hsa-let-7g-5p;hsa-let-7i-5p) and hsa-miR-1266-5p, hsa-miR-502-5p, hsa-miR-542-5p, hsa-miR-642a-5p, hsa-miR-23b-5p; 10 signaling pathways are MAPK signaling pathway, Axon guidance, Focal adhesion, Cell cycle, Protein digestion and absorption, Pyrimidine metabolism, Neurotrophin signaling pathway, p53 signaling pathway, Regulation of Actin cytoskeleton, ErbB signaling pathway (Table.2).

Discussion

Gastric cancer is one of the most common malignant tumors of the digestive tract. However, due to its lack of specific tumor markers and obvious clinical symptoms, most patients miss the best period of treatment, which makes stomach cancer the most deadly. One of the solid tumors. In the past few decades, researchers have sought more new discoveries and insights into the molecular mechanisms of gastric cancer, but most of the research has focused on protein-coding genes, miRNAs or long non-coding RNAs. , lcnRNA) [14-16]. CircRNA is a non-coding RNA (ncRNA) that is widely present in organisms and closely related to many diseases [17-20]. It can regulate miRNA sponge and affect the expression of related parental genes [16]. In recent years, more and more studies have shown that circRNA plays an important role in the development of tumors. Xiao Bin et al [17] analyzed the difference in circRNA expression profiles between Luminal subtype breast cancer cells and normal breast cells, and found that the expression of circRNA in normal breast cells and Luminal subtype breast cancer cells is quite different, and the up- or down-regulated circRNA may be Become a new diagnostic target for Luminal subtype breast cancer. Bai Ning et al [21] verified the difference in circRNAB expression profiles between hepatocellular carcinoma cells and normal liver tissue cells, and found that circFBLIM1 can utilize miRNA sponge to competitively bind mir-346 and regulate the expression of FBLIM1 in liver cancer. Play a regulatory role. In this study, we tested serum circRNA in healthy and gastric cancer patients. By comparing the circRNA expression profiles of healthy and gastric cancer patients, we found that there

were significant differences in circRNA expression profiles between gastric cancer patients and healthy people. GO and KEGG pathway enrichment analysis was performed on linear parental genes with differential expression of circRNA. The circRNA-miRNA interaction of differential circRNA was predicted, and the differential circRNA hsa_circ_0000437 was verified by q-PCR.

First, through the detection of high-throughput microarray, we found 137 circRNAs with significant expression differences in serum circRNA expression profiles of gastric cancer patients and healthy people, of which 67 were up-regulated and 70 were down-regulated. Previous studies have shown that there are 440 circRNAs with significant expression differences in normal gastric mucosa and gastric cancer tissues, of which 176 are significantly up-regulated in gastric cancer tissues, and 267 are significantly down-regulated [22]; circ_000026 As a potential target for the diagnosis of gastric cancer, it has been confirmed to be significantly down-regulated in gastric cancer tissues [23]; in addition, studies have shown that circPVRL may promote the development of gastric cancer by encoding related proteins and competitive binding of miRNA [24]. According to the mining and analysis of related literatures at home and abroad, it can be concluded that this study first reported the differential expression of circRNA in the serum of healthy people and gastric cancer patients in Wuwei, China.

Subsequently, based on the detection results, GO analysis was performed on the linear parental genes differentially expressing circRNA to explore the potential molecular functions, cellular components and biological processes involved in differential expression of circRNA. The results show that circRNAs differentially expressed in molecular function and biological processes are mainly involved in positive regulation of T cell tolerance induction, positive regulation of tolerance induction, induction of T cell immune tolerance, differentiation of immature B cells, Immune tolerance, nucleotide binding, macrophage colony-stimulating factor receptor activity and other molecular functions and biological processes related to immunity and gene regulation; while in cellular components, the differential expression of circRNA is mainly related to Macromolecular complexes, protein complexes, etc. Previous studies have shown that as an important part of the body's immune system, T cell activation is critical for the body's anti-tumor immune response [25], but as tumors continue to develop, tumor cells evolve different anti-tumor immune mechanisms, And use T cell immune tolerance characteristics to weaken the anti-tumor effect of T cells [26-27]; while B can inhibit the body's anti-tumor immune response by secreting tumorigenic factors or inhibiting effector cells [28]. Therefore, the above research combined with this study shows that circRNA differentially expressed in the serum of gastric cancer patients and healthy people may affect the occurrence and development of gastric cancer by participating in the biological processes related to immune system and exerting immune-related molecular functions. Changes in protein complexes are important promoters of cellular functions in the body, including chaperome [29-31], a rich family of proteins. The study found that epichaperome, which is tightly integrated by chaperome units, can serve as a network to enhance cell survival. In the more general detection of gastric cancer, liver cancer, and lung cancer, epichaperome is found in approximately 60-70% of tumors. High expression status and significant impact on diagnosis provides a new target for drug intervention in related tumors [32]. Combined with the above studies, it is shown that the differentially expressed circRNA in this study may

have an effect on the occurrence and development of gastric cancer in the protein complex, and the specific mechanism needs further research.

We also performed a KEGG pathway analysis of linear parental genes that differentially express circRNA based on high-throughput microarray results. The results showed that the differentially expressed circRNA involved in tumor-related signaling pathways mainly include ubiquitin-mediated proteolysis, NF-kB, TGFβ and the like. Protein ubiquitination plays an important role in eukaryotes. The ubiquitin-mediated protease system is the main mechanism of protein degradation in eukaryotic cytoplasm and nucleus, and is the key to maintaining the body's protein homeostasis. A growing number of studies have found [33] that tumor cells are more dependent on the homeostasis of the body's proteins, including the ubiquitin protease system, than normal cells. NF-KB is an inducible transcription factor ubiquitous in the body. Its family is considered to be the central medium in the body's inflammatory response and plays an important role in innate immunity and adaptive immunity. In malignant tumors, activation of NF-KB is very common, and it exerts its tumor-promoting effect by activating survival genes in tumor cells and pro-inflammatory genes in tumor microenvironments [34]. However, some studies have found that the ubiquitin system may be involved in the activation of NF-KB in the body [35]. TGF-β is dysregulated in many diseases including tumors. Studies have shown that TGF-β in advanced cancer, tumor cells accumulate mutations in the TGF-β cascade signal or selectively destroy anti-tumor The reaction was to escape the growth inhibition of TGF-β [36]. Combined with the above studies, it is shown that the differentially expressed circRNA in this study may play an important role in the above signaling pathway, and affect the occurrence and development of gastric cancer through the above signaling pathway.

The CircRNA-miRNA network is a method widely recognized by researchers in the field of circRNA research to study the differential expression of circRNA and related miRNA interactions. Therefore, based on the high-throughput microarray results, this study predicted the circRNA-miRNA interaction network with differential expression by miRanda-3.3 software and the entropy of the binding site below 20, and using bioinformatics methods. In this study, we predicted a total of 142 miRNAs that are importantly linked to circRNA, and can establish important connections with nine circRNAs with distinct expression differences, such as Hsa_circ_0133089 and Has_circ_0070634. Among the 142 miRNAs, 20 miRNAs such as Hsa-miR-4433a-3p and Hsa-miR-6766-5p can simultaneously connect with two or more circRNAs with different expression. Studies have shown that in liver cancer circHIPK3 can act as a molecular sponge of miRNA, bind 9 miRNAs, and there are 18 potential binding sites, including tumor suppressor mir-124, thereby inhibiting its activity [37]. Circ-ITCH can act as a tumor-associated miRNA molecule with sponge-binding regulation of miR-7, mir-17, mir-214 in esophageal squamous cell carcinoma, miR-7, miR-20a in colorectal cancer, and miR-7, mir Expression of -214 in lung cancer [38-40]. Combined with the above studies, it was shown that in this study, skRNAs with different expressions such as Hsa_circ_0133089 could establish potential linkages with targeted miRNAs such as Hsa-miR-4433a-3p and affect the occurrence and development of gastric cancer.

Recent studies have shown that circRNA can exert its corresponding biological functions by regulating its linear parental genes and affecting the expression of related mRNAs. Therefore, based on the difference in serum circRNA expression profiles between gastric cancer patients and healthy people, we selected the top 10 circRNAs. By further analysis of the six parental genes of 10 differential circRNAs, we found that 7 tumors occurred during the development of tumors. The parental gene is mainly related to the cycle, apoptosis, migration and infiltration of tumor cells, and CORO1C has been reported to be involved in the development of gastric cancer [12-13].

Therefore, we compared the three differentially expressed circRNAs involved in CORO1C in 10 differential circRNAs, and selected the has_circ_0000437 with the largest difference to verify qRT-PCR. The results showed that has_circ_0000437 was present in the serum of patients with gastric cancer. Low expression trend, and the results of the chip detection; ROC curve analysis of the expression level of has_circ_0000437, we found that the AUC of has_circ_0000437 is 0.92, its sensitivity is 90%, the specificity is 40%, which indicates that has_circ_0000437 in the clinical diagnosis of gastric cancer Has a certain diagnostic value.

Based on the research results of circRNA, its linear biological parental genes can be regulated by homeopathic effects, affecting the expression of related mRNAs. At the same time, since the linear parental gene CORO1C of has_circ_0000437 is closely related to the invasion and migration of gastric cancer cells, we have used tissue microarray technology to verify the expression levels of gastric cancer tissues and adjacent normal tissues in gastric cancer patients. The results showed that the expression level of CORO1C in gastric cancer tissues was higher than that in adjacent tissues, which was consistent with the results of the competitive complementary pairing between introns and the balance between linear RNAs in the formation of circRNA, suggesting that has_circ_0000437 may pass Homeopathic regulation of its linear parental gene CORO1C and its mRNA expression affects the development, staging, metastasis and prognosis of clinical gastric cancer.

Based on the function of miRNA molecular sponge of circRNA, we performed Pancancer microRNA-gene-pathway network prediction analysis on 35 miRNAs that may compete with has_circ_0000437. We reached 12 with hsa-miR-1266-5p. The occurrence and development of tumors are closely related to miRNAs, more than 400 miRNA target genes, and 10 biological pathways closely related to tumors represented by MAPK signaling pathway, p53 signaling pathway and ErbB signaling pathway. This suggests that has_circ_0000437 may affect the occurrence and development of gastric cancer by regulating the above 12 tumor-associated miRNAs and their target genes, and participating in signaling pathways such as MAPK signaling pathway.

Conclusions

Due to its special structure and biological function, circRNA has become a new star in the field of gastric cancer research. However, previous studies have mostly used tissue samples from patients with gastric cancer, which is difficult to compare and analyze with healthy people. And for the first time this research

from the perspective of human serum circRNA expression profiles of gastric cancer patients and healthy people in Wuwei, Gansu Province. The results showed that 137 circRNAs in gastric cancer patients were different from healthy people. Among them, the discovery of has_circ_0000437, although it has been reported in liver cancer and other diseases,but its the first time in gastric cancer, and its differential expression may be related to the occurrence and development of gastric cancer. The GO and KEGG pathway enrichment analysis was performed on the linear parental genes differentially expressing circRNA, and the relevant regulatable miRNAs were predicted. T cell immune tolerance induction, ubiquitin-mediated proteolysis, NF-kB and other signaling pathways were obtained. A number of GO classifications and KEGG pathways and circRNA-miRNA co-expression networks related to the development of gastric cancer suggest that differential circRNA may affect the occurrence and development of gastric cancer by regulating the expression of parental genes and miRNAs involved in gastric cancer-related GO classification and KEGG pathway.Subsequently, through RT-qPCR of has_circ_0000437 and its parent genes and controllable miRNA, it was found that has_circ_0000437 may have a certain tumor suppressive effect, and its expression level in gastric cancer patients is significantly lower than that in healthy people, which has certain clinical diagnosis of gastric cancer. Diagnostic value, it may affect the development, metastasis and prognosis of clinical gastric cancer by regulating the expression of miRNAs such as its linear parent genes CORO1C and hsa-miR-1266-5p, but its specific molecular mechanism remains to be further studied.

In summary, the results of this study may enrich the circRNA database related to gastric cancer, provide a new perspective for the study of circRNA in gastric cancer, and lay a certain foundation for future related studies.

Abbreviations

GC:gastric cancer; circRNA: circular RNA; miRNA: micro RNA; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; ROC: receiver operating characteristic curve; AUC: Area Under Curve; GCO:Global Cancer Observatory; BPs:biological processes; MFs:molecular functions; CCs:cellular components

Declarations

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Author contribution

GC conducted data collection, analysis and writing of manuscripts. PN, LL, FW, XW, CL, XX conducted a collection of clinical samples. YL, YS, and HG designed the research and provided financial support, and Dr. JH provided guidance and assistance in the research of the experiment and the writing of the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

The study protocol was approved by the Medical Ethics Committee of Wuwei Cancer Hospital of Gansu Province (Wuwei Academy of Medical Sciences). Each patient included in the study was required to sign the patient's informed consent before the study began. All methods were in accordance with the guidelines and related Regulations are enforced.

Conflict of interest

All authors in this article have no conflicts of interest.

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Tables

Due to technical limitations, tables are only available as a download in the supplemental files section

Figures

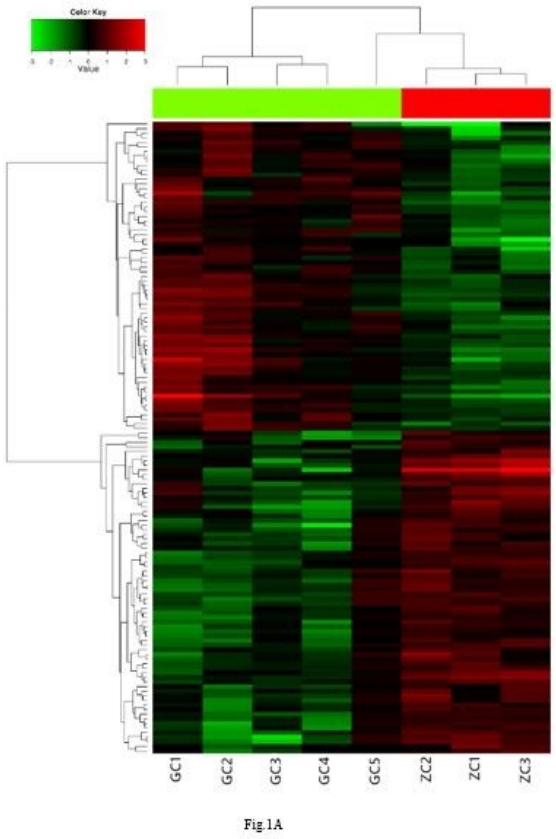

Fig.1A

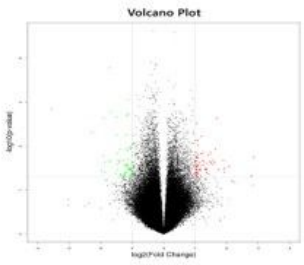

Fig.1B

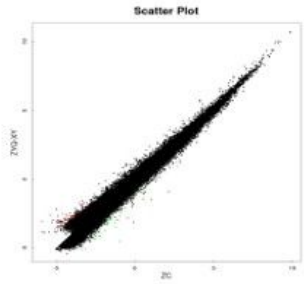

Figure 1

Hierarchical clustering maps, volcano maps, and scatter plots show circRNAs in the differential table of gastric cancer patients and healthy people. (A) Hierarchical clustering map, the number is used for the analysis of micro-column determination samples, ZC is healthy population, XY, ZYQ are gastric cancer patients. (B) The volcano map shows the differential expression of circRNA in the serum of healthy and gastric cancer patients. The green and red parts represent the down-regulation of the fold-over greater than 2.0 and the up-regulation of circRNA expression ($p<0.05$). (C) A scatter plot showing differentially expressed circRNA in healthy and gastric cancer patients. The green and red fractions represent down-regulation greater than 2.0 and up-regulation of circRNA expression, respectively ($p<0.05$).

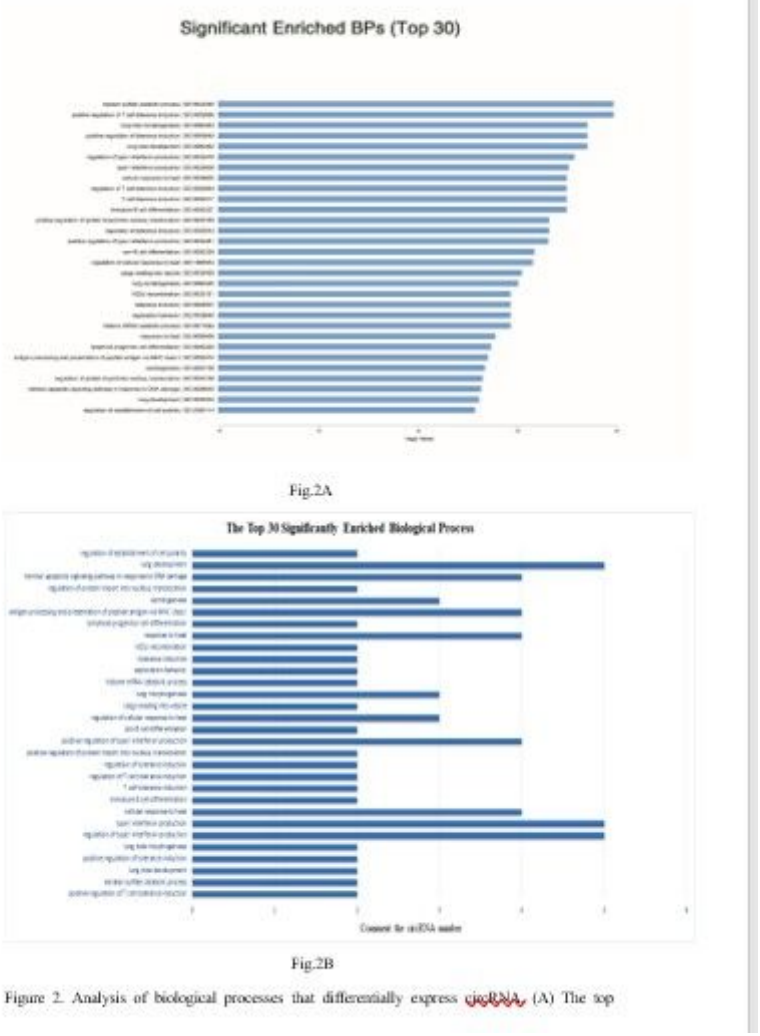

Fig.2A

Fig.2B

Figure 2. Analysis of biological processes that differentially express circRNA. (A) The top

Figure 2

Analysis of biological processes that differentially express circRNA. (A) The top 30 biological processes of enrichment; (B) The top 30 biological processes of enrichment and the number of enriched circRNAs.

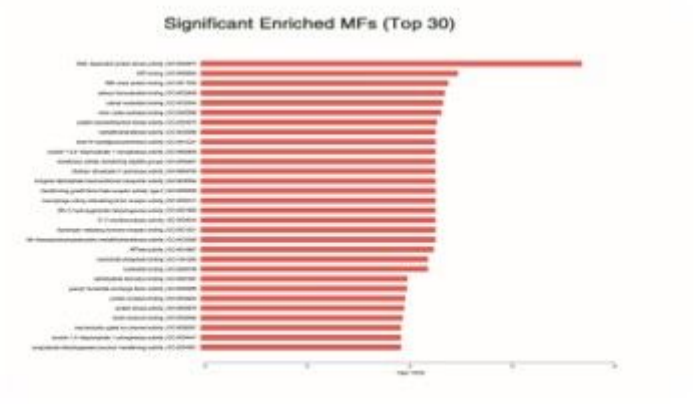

Fig.3A

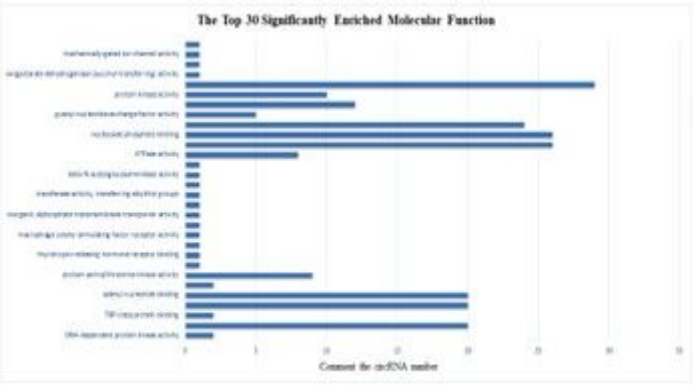

Fig.3B

Figure 3

Molecular functional analysis of differentially expressed circRNA. (A) The top 30 molecular functions of enrichment; (B) The top 30 molecular functions of enrichment and the number of circRNAs enriched.

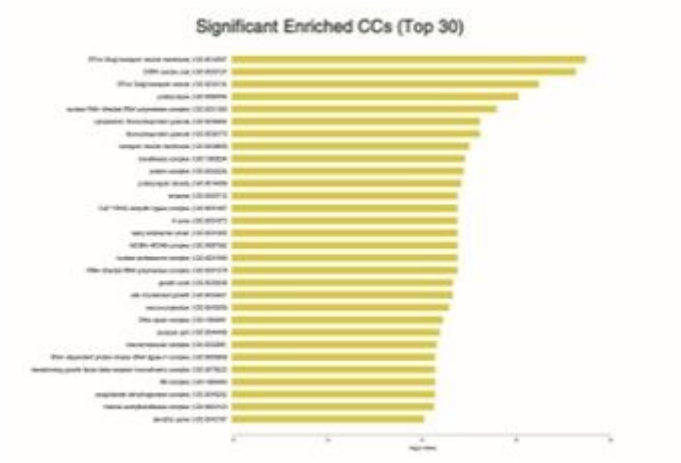

Fig.4A

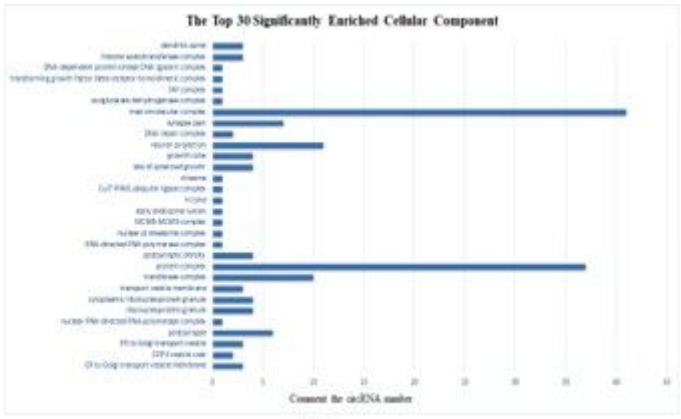

Fig.4B

Figure 4

Cell component analysis of differentially expressed circRNA. (A) The top 30 cell components of enrichment; (B) The top 30 cell components of enrichment and their number of enriched circRNAs.

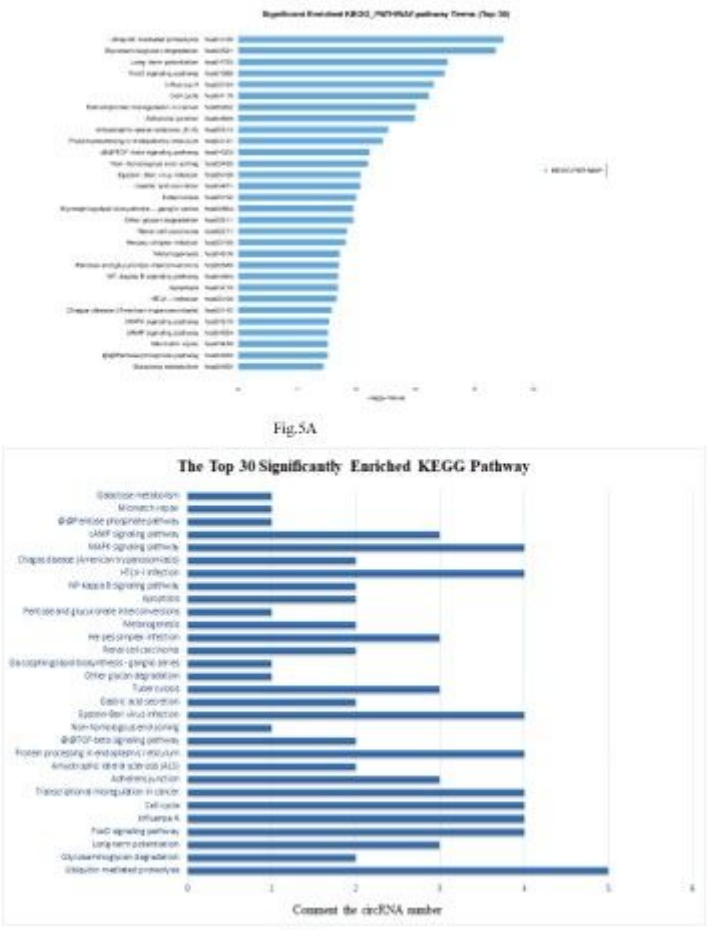

Figure 5

Analysis of KEGG pathways differentially expressing circRNA. (A) The top 30 KEGG pathways for enrichment; (B) The top 30 KEGG pathways and the number of annotated genes in enrichment.

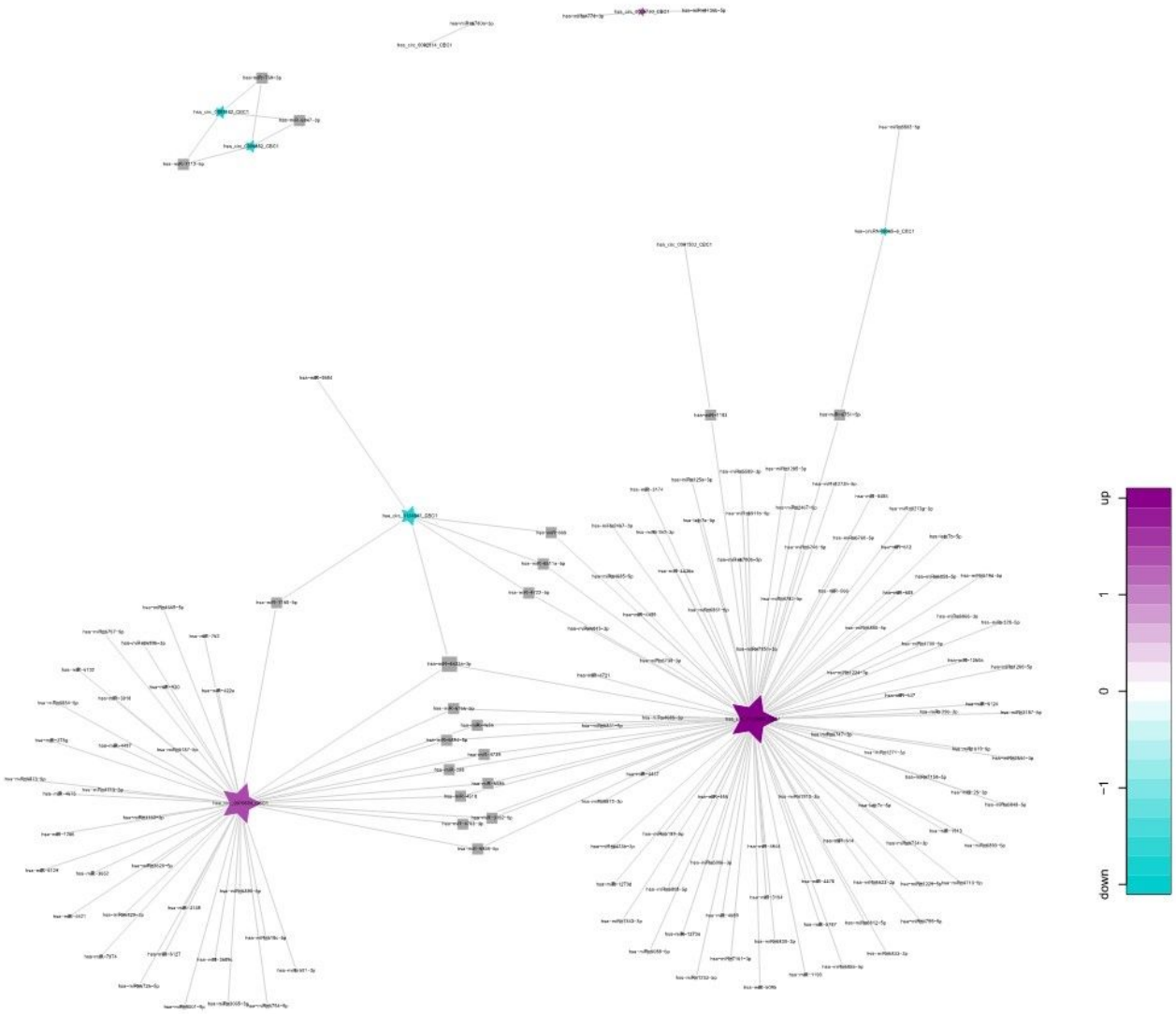

Figure 6

circRNA-miRNA network map. Based on the relationship between circRNA and target miRNA, combined with the miRanda software score, the top 9 differentially expressed circRNAs were selected to establish a circRNA-miRNA network. In the figure, the five-pointed star represents circRNA, the square represents the target miRNA, green represents down-regulation, and purple represents up-regulation. The size of the dots indicates how many circRNA-miRNA junction sites, and the larger the dots, the more points are connected. Among them, Hsa_circ_0133089 and Has_circ_0070634 predicted the largest number of bound miRNAs.

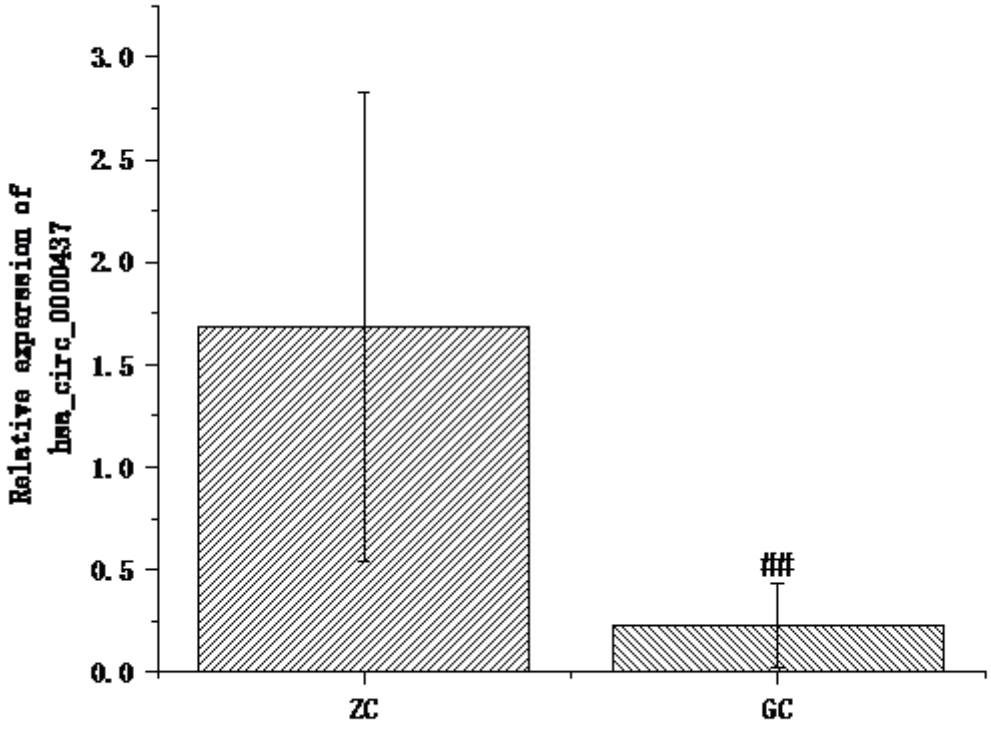

Figure 7

Hsa_circ_0000437 expression in serum samples from healthy and gastric cancer patients In this result, the number of samples of gastric cancer patients and healthy people was 10 . “#” means P<0.05 compared with healthy people, “##” means P<0.01 compared with healthy people.

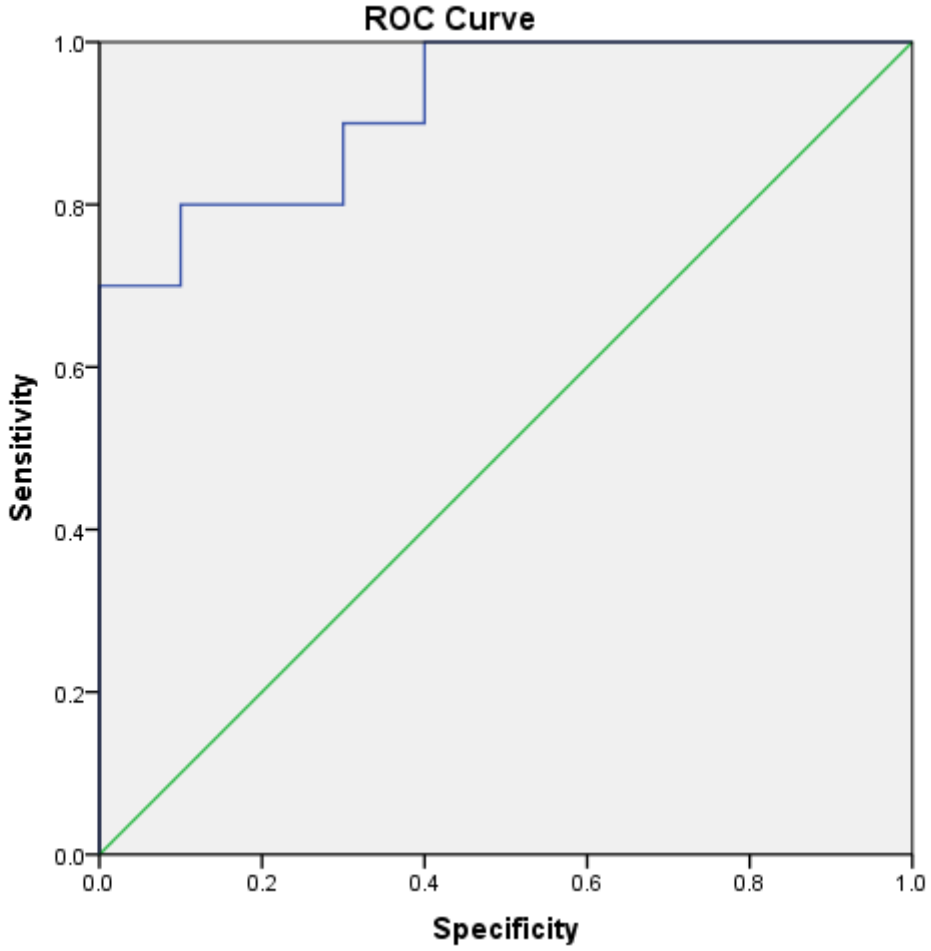

Figure 8

The ROC Curve of hsa_circ_0000437

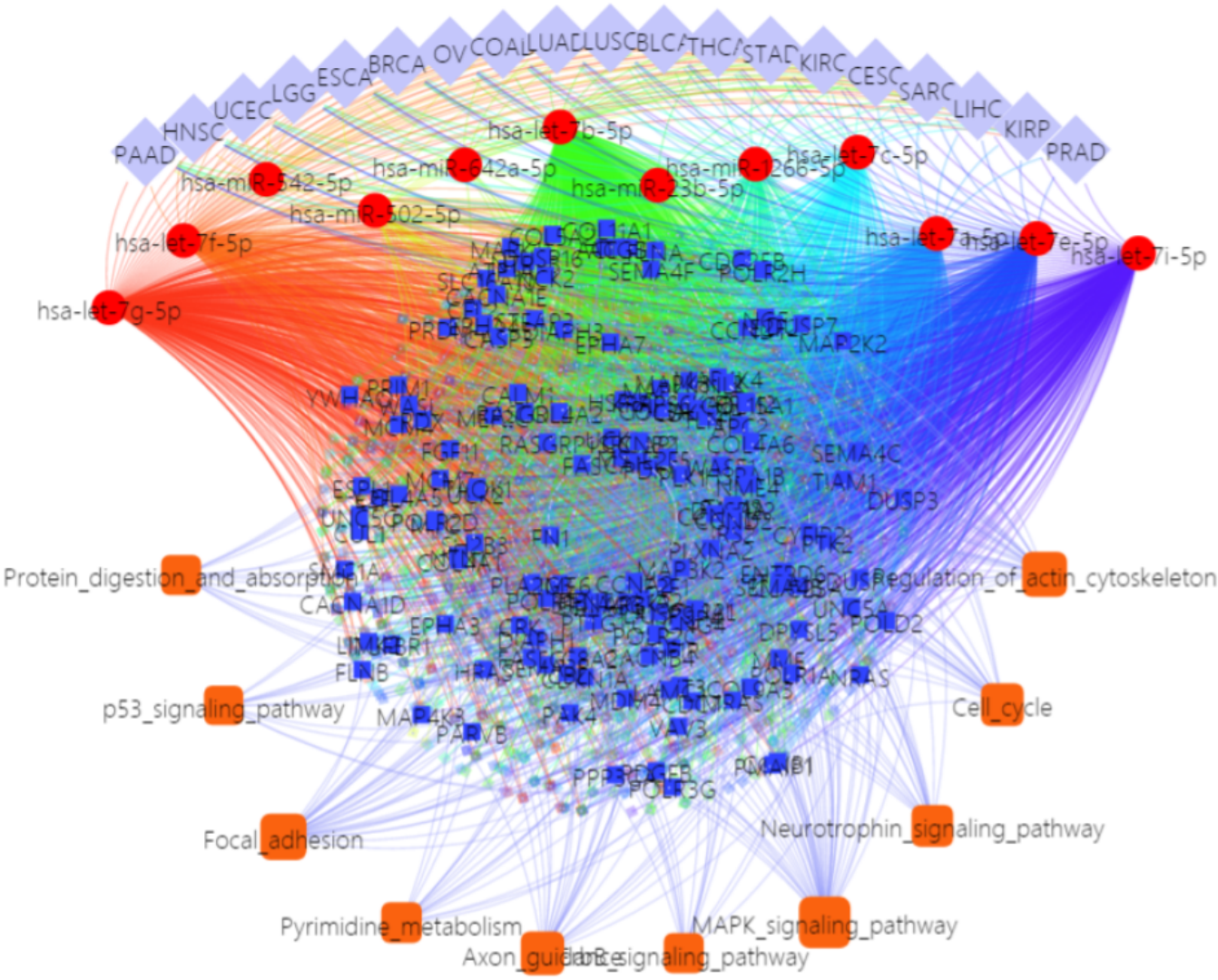

Figure 9

Pancancer microRNA-gene-pathway network. The Pan-cancer module in the miRNA CancerMAP database predicted and analyzed 35 miRNA target genes and tumor-associated pathways to construct a Pancancer microRNA-gene-pathway network. The red dots in the figure represent tumor-associated miRNAs, the blue squares represent target miRNA target genes, and the yellow squares represent tumor-associated Pathway.

Supplementary Files

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