Identification of Key Genes and MicroRNAs in Gastric Cancer via miRNA-mRNA Regulatory Network

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Primary research

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Abstract

Background

Gastric cancer (GC) is a malignant tumor with high mortality. MicroRNAs (miRNAs) participate in various biological processes and disease pathogenesis by targeting messenger RNA (mRNA). The purpose of this study was to identify potential prognostic molecular markers of GC and to characterize the molecular mechanisms of GC.

Methods

A gene expression profiling dataset (GSE54129) and miRNA expression profiling dataset (GSE113486) were downloaded from the Gene Expression Omnibus (GEO) database. A miRNA-mRNA interaction network was established. Functional and pathway enrichment analyses were performed for differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMs) using FunRich, the clusterProfiler package, and DIANA-mirPath. Survival analysis of key molecular markers was performed using the online tool Kaplan-Meier Plotter and the database OncomiR. Finally, experiments were carried out to verify the expression levels and biological functions of a key gene.

Results

A total of 390 DEMs and 341 DEGs were identified. Ultimately, 45 genes and 31 miRNAs were selected to establish a miRNA-mRNA regulatory network. Four hub genes (ZFPM2, FUT9, NEUROD1 and LIPH) and six miRNAs (hsa-let-7d-5p, hsa-miR-23b-3p, hsa-miR-23a-3p, hsa-miR-133b, hsa-miR-130a-3p and hsa-miR-124-3p) were identified in the network. DEGs and DEMs were associated with ECM-receptor interactions and metabolic pathways. Two genes (ZFPM2 and LIPH) and two miRNAs (hsa-miR-23a-3p and hsa-miR-130a-3p) were observed to be related to the prognosis of GC. ZFPM2 was highly expressed in GC tissues and various GC cell lines and could promote the proliferation, invasion and migration of GC cells.

Conclusion

The expression levels of ZFPM2, LIPH, hsa-miR-23a-3p and hsa-miR-130a-3p were closely related to the prognosis of GC. ZFPM2 may serve as a potential molecular marker and therapeutic target for GC. ECM receptor interactions and metabolic abnormalities play a critical role in the GC progression.

Background

Gastric cancer (GC) is a tumor of the digestive system with the third highest mortality rate [1]. After radical gastrectomy for early gastric cancer (EGC), the 5-year survival rate can reach more than 90%, while that for advanced gastric cancer (AGC) is less than 10% [2–3]. Therefore, elucidating the mechanism of GC is essential to identify molecular biomarkers for early detection. MicroRNAs (miRNAs) are a class of small, endogenous, noncoding RNAs that bind to their target mRNA through the 3' untranslated region (3'UTR) to cause mRNA degradation or inhibit mRNA translation [4–6]. MiRNAs participate in the regulation of various biological processes and metabolic pathways, and their dysregulated expression is closely associated with the development of cancer [7–9]. MiRNA-106a is overexpressed in GC and is involved in the invasion and metastasis of GC [10–11]. MiRNA-145 can inhibit the metastasis and angiogenesis of GC cells [12]. MiR-150 and miR-130b play an important role in promoting the progression of GC, which not only increases the invasiveness of GC cells but also inhibits the apoptosis of GC cells [13–14]. The miR-143 and miR-145 can be used as biomarkers for EGC [15]. However, the regulatory relationship between miRNAs and genes is not simplistic, but forms a complex network of miRNAs and mRNAs during disease progression [16–17]. In cancer, miRNA-mRNA regulatory networks can regulate cell proliferation, invasion and migration [18–20]. Therefore, the study of miRNA-mRNA regulatory networks is crucial for understanding the developmental mechanisms of GC.

In this study, we identified DEGs and DEMs in GC through a complete bioinformatics analysis of two microarray datasets downloaded from the GEO database. Moreover, we conducted experiments to verify the expression levels and biological functions of key genes. The purpose of this study was to construct a reliable miRNA-mRNA interaction network to identify key genes and miRNAs of GC, providing potential molecular markers and therapeutic targets for the treatment of GC.

Materials And Methods

Microarray data

The gene expression profiling dataset (GSE54129) and miRNA expression profiling dataset (GSE113486) were obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo). The GSE54129 and GSE113486 dataset were based on the GPL10687 platform (Affymetrix Human Genome U133 Plus 2.0 Array, RuiJin Hospital,SJTU) (submission date: Jan 16, 2014) and the GPL21263 platform (3D-Gene Human miRNA V21_1.0.0, Toray Industries Inc.) (submission date: Apr 20, 2018), respectively. The array data of GSE54129 includes 111 gastric cancer samples and 21 normal samples. The array data of GSE113486 includes 40 gastric cancer samples and 100 normal samples.

Data processing

Background correction and standardization were performed on the downloaded original series matrix files and the annotation SOFT tables using Perl 5.26 (http://www.perl.org/). In the case of multiple probes corresponding to a single gene, the gene expression value was determined as the average of the probes. R software (version 3.6.1, https://www.r-project.org/) and the "limma" package [21] were used to identify DEGs and DEMs. Analysis was performed using the "limma" package, and adjusted *p*-value < 0.05 and | $\log_2$FC | > 2 were used as cutoff criteria for the identification of DEGs and DEMs.

Identification of upstream TFs and target genes of DEMs

FunRich [22], which provides functional explanations for a mass of genes (http://www.funrich.org/), was used to identify the ten most significant upstream TFs that regulate DEMs. The target genes of DEMs were predicted using TargetScan 7.2 (http://www.targetscan.org/), which predicts the target genes of miRNAs by finding specific sequence complements for each miRNA seed region.

Establishment of a miRNA-mRNA regulatory network

First, the target genes were combined with DEGs to select DEM-differentially expressed target gene pairs, and a miRNA-mRNA regulatory network was established. Then, we used Cytoscape software (version 3.7.0) to visualize the network. Finally, cytoHubba, a Cytoscape plugin, was used to identify the top ten key molecular markers ranked by "Degree".

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of DEMs

We used FunRich for GO enrichment analysis and DIANA-mirPath v3.0 [23] to identify the KEGG pathways that were significantly related to DEMs in the regulatory network. For this tool, we chose to use the archives in microT Web Server v5.0 to analyze validated miRNA-gene interactions [24]. The GO analysis includes biological processes, cellular components and molecular functions categories. An adjusted *p*-value < 0.05 was considered statistically significant.

GO and KEGG pathway enrichment analysis of DEGs

GO and KEGG pathway enrichment analysis was performed using the clusterProfiler package [25]. A *p*-value < 0.05 was used as a threshold for identifying significant GO terms and pathways. A circle diagram of the GO enrichment results for mRNAs in the miRNA-mRNA interaction network was plotted using the GOplot package.

Hub genes and miRNA analysis

Survival analysis of genes was performed using the online tool Kaplan-Meier Plotter (http://kmplot.com/); survival analysis of miRNAs was performed using the online database OncomiR [26] (http://www.oncomir.org/). A log-rank test *p*-value < 0.05 was

considered statistically significant. The online database Oncomine (http://www.oncomine.com) was used to evaluate the expression of key genes in GC.

Clinical samples and immunohistochemistry assay

The 56 GC specimens and 47 adjacent tissue specimens (at least 5 cm from the tumor edge) were collected. All patients with GC were diagnosed by pathology before surgery and did not undergo chemoradiotherapy. Our study was approved by the Ethical Committee of the Second Affiliated Hospital of Nanchang University and written informed consent was obtained from all patients. The samples were incubated with an anti-ZFPM2 (ab121212, Abcam, 1:100) antibody. The expression of the ZFPM2 protein was assessed according to the degree of staining.

Establishment of GC cells with stable knockdown of ZFPM2

The GES-1, AGS, MKN45, MKN28, BGC803, MGC803, BGC823 and SGC7901 cell lines were cultured in Roswell park memorial institute (RPMI) 1640 medium or Dulbecco's modified Eagle medium (DMEM) at 37 °C and 5% CO2, and the medium was replaced once every 2 to 3 days. All experiments were performed when the cells reached 75%-85% confluence and were in a logarithmic growth phase. SGC7901 and MGC803 cells were transfected with lentivirus-mediated control shRNA or shRNA targeting ZFPM2 and were designated vector and shZFPM2, respectively. The lentiviral-mediated shRNA was obtained from Hanbio Biotechnology (Shanghai, China); the following sequence fragments were inserted into the vector: control shRNA, GACGUAGCCAACCUCAAUAUU; shRNA-1, 5'-GGCUCUGUUUGCACUUUAUUU-3' and shRNA-2, 5'-GCUGCAAGUAUGAAUUUAAUU-3'. At 72 hours post infection, the cells were cultured in medium containing 5 μg/ml puromycin (Sigma, Missouri, USA) until all uninfected cells were killed by puromycin, thus obtaining stably transfected cell lines.

Western blot assay

Stably transfected cells were lysed with RIPA buffer to obtain total protein, and then the protein was transferred to a PVDF membrane after separation by gel electrophoresis. Anti-ZFPM2 (PA5-29094, Invitrogen, 1:3000) was used as the primary antibody. HRP-linked anti-rabbit IgG (ab6721, Abcam, 1:5000) and HRP-linked anti-mouse IgG (ab6709, Abcam, 1:5000) were used as the secondary antibodies. GAPDH (5174T,CST□1:1000) and actin (8457T□CST□1:1000) served as internal standards. The protein quantity was assessed by a BCA assay kit (Beyotime Biotechnology, China).

Quantitative real-time polymerase chain reaction (qRT-PCR) assay

Total RNA was isolated from the samples with TRIzol reagent (Invitrogen), and then total RNA was used to synthesize cDNA using the PrimeScript reverse transcriptase kit (TaKaRa, Shiga, Japan). qRT-PCR was performed to analyze the levels of ZFPM2 mRNA in samples. The primer sequences for amplification were as follows: ZFPM2-forward (5'-TTGCTCATCTCCGAACGTGAA-3'), ZFPM2-reverse (5'-CGCAGCTCAGATTTTCAGGC-3'), GAPDH-forward (5'-CTTTGGTATCGTGGAAGGACTC-3'), and GAPDH-reverse (5'-AGTAGAGGCAGGGATGATGT-3'). The mRNA expression was analyzed using the $2^{-\Delta\Delta CT}$ method.

Cell proliferation assay

SGC7901 and MGC803 cells in the vector/shZFPM2 groups were inoculated into 6-well plates. When the cell density reached 75%-85%, we used a pipette tip to make a 0.5 cm line in the middle of the well. The cells were then cultured with serum-free medium, and placed in a 37 °C and 5% CO2 incubator. Next, we obtained images at 0 hours, 24 hours, and 48 hours to observe the wound healing process. Additionally, when the cell density reached 75%-85%, the cells were labeled with EDU, fixed with 4% paraformaldehyde, decolorized with glycine solution, incubated with 0.3% Triton X-100 PBS, and then sequentially stained with Apollo and Hoechst. After staining, laser scanning confocal microscopy observation and imaging were performed (Hoechst 33342 is blue light). An anchorage-independent colony formation assay was performed using soft agar growth of 3×10^3 cells for 10–14 days.

Cell migration and invasion assays

Precooled Matrigel was added to the bottom of the Transwell chamber to coat it with Matrigel. It was then placed in an incubator until Matrigel solidified. SGC7901 and MGC803 cells (control group and shZFPM2 group) were resuspended and seeded into the upper chamber in serum-free medium at a density of 2×10^5/ml, and the lower chamber was filled with 20% FBS medium. After 24

hours of incubation, the non-migrated cells in the upper chamber were wiped off with a sterile cotton swab. Finally, the migratied cells were examined by crystal violet (0.1%) staining and counted under a microscope. Migration experiments did not include Matrigel.

Statistical analysis

The t test was used for comparison between the two groups. A *p*-value < 0.05 was considered statistically significant. All experiments were repeated three or more times. Data analysis was performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Identification of DEMs and DEGs

Based on the "limma" package analysis, 390 DEMs and 341 DEGs were identified in GC tissue samples compared to the normal samples group (**Supplementary Fig. 1 and** Fig. 2). The GSE113486 dataset had 124 upregulated miRNAs and 266 downregulated miRNAs (Fig. 1A). The GSE54129 dataset had 159 upregulated genes and 182 downregulated genes (Fig. 1B).

Identification of upstream transcription factors (TFs) and target genes of DEMs

FunRich was used to identify upstream TFs that regulate DEMs. The most significant ten upstream TFs were ZFP161, YY1, NKX6-1, RREB1, NFIC, MEF2A, POU2F1, SP4, SP1 and EGR1 (Fig. 2). We identified 5797 DEM-gene regulatory relationships based on TargetScan **(Supplementary Table 1).**

Establishment of the miRNA-mRNA regulatory network

There were 45 overlapping genes between the target genes of the DEMs and the DEGs, among which 17 were upregulated and 28 were downregulated. These 45 genes were regulated by 31 miRNAs, of which 20 were upregulated and 11 were downregulated. A miRNA-mRNA regulatory network was established. We then used Cytoscape software (version 3.7.0) for visualization (Fig. 3). Next, cytoHubba, a Cytoscape plugin, was used to identify ten Hub genes or miRNAs. According to the MOCDE score, the first one was ZFPM2, followed by hsa-let-7d-5p, FUT9, NEUROD1, hsa-miR-23b-3p, hsa-miR-23a-3p, hsa-miR-133b, LIPH, hsa-miR-130a-3p and hsa-miR-124-3p.

GO and KEGG pathway enrichment analysis of DEMs

The most significantly enriched GO terms were "Nucleus" (ontology: CC), "Transcription factor activity" (ontology: MF), and "Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism" (ontology: BP) (Fig. 4). The 64 KEGG pathways significantly enriched the upregulated DEMs **(Supplementary Table 2)**. The most significant was the ECM-receptor interaction, followed by Proteoglycans in cancer, Glioma, TGF-beta signaling pathway, Pathways in cancer, Focal adhesion, Mucin type O-Glycan biosynthesis, Signaling pathways regulating pluripotency of stem cells, Endocytosis, and Wnt signaling pathway (Fig. 5A). The 17 KEGG pathways significantly enriched the downregulated DEMs (Fig. 5B).

GO and KEGG pathway enrichment analysis of DEGs

The most significantly enriched GO terms were "extracellular matrix component" (ontology: CC) and "enteroendocrine cell differentiation" (ontology: BP); the significantly enriched KEGG pathways were maturity onset diabetes of the young and proximal tubule bicarbonate reclamation (**Table 1**). A circle diagram was generated using the GOplot package (Fig. 6).

Hub genes and miRNA analysis

GC patients with high expression of ZFPM2, FUT9 and NEUROD1 had a shorter survival, while GC patients with high expression of LIPH had a longer survival (Fig. 7). However, differential analysis showed that ZFPM2 was highly expressed in GC samples, while

FUT9, NEUROD1 and LIPH were expressed at low levels in GC samples. We further analyzed ZFPM2 and LIPH using the online database Oncomine. In the Forster Gastric Dataset, the expression of ZFPM2 in diffuse gastric adenocarcinoma was significantly higher than that in gastrointestinal adenocarcinoma, while the expression of LIPH in diffuse gastric adenocarcinoma was significantly lower than that in gastric adenocarcinoma [27] (Fig. 8A). In the Wang Gastric Dataset, the expression of ZFPM2 in GC was higher than that in gastric mucosa, while LIPH expression in GC was lower than that in gastric mucosa [28] (Fig. 8B). Survival analysis of key miRNAs using the online database OncomiR indicated that GC patients with upregulated hsa-miR-23b-3p expression had a shorter survival (Fig. 9A). Although the survival curve of hsa-miR-130a-3p was not statistically significant (Fig. 9B), survival outcome analysis demonstrated that it was related to GC survival (P = 0.0276).

Experimental verification

The expression of ZFPM2 protein and mRNA in tumor tissues was significantly higher than that in adjacent tissues (Fig. 10A and 10B). ZFPM2 protein and mRNA were significantly highly expressed in various GC cell lines (Fig. 10C). Interference with ZFPM2 expression decreased colony forming ability and cell proliferation activity of SGC7901 and MGC803 cells (Fig. 11A and 11B). After knocking down ZFPM2, the ability of SGC7901 and MGC803 cells to heal, migrate and invade was reduced (Fig. 12A, 12B, 12C and 12D).

Discussion

MiRNAs are key regulators of carcinogenesis and are involved in the development and progression of cancer, including GC [29]. A total of 390 DEMs were identified in this study. Then, the associated TFs were predicted, and the ten most significant TFs were identified as EGR1, SP1, SP4, POU2F1, MEF2A, NFIC, RREB1, NKX6-1, YY1 and ZFP161. EGR1 can significantly upregulate and enhance cell proliferation and promotes cell cycle progression in GC tissues [30]. Sp1 activity was significantly increased in GC specimens and was related to poor survival [31]. Related studies also showed that members of the Sp1 family regulate multiple aspects of angiogenesis [32]. Moreover, studies have found that Sp1 plays an important role in cancer migration and metastasis [33–35]. POU2F1 belongs to the POU homeodomain transcription factor family, and its overexpression was negatively correlated with the survival of GC patients [36]. Qian et al. [37] demonstrated that high expression of OCT1 was related to the activation of the ERK mitogen-activated protein kinase signaling pathway in GC tissues. MEF2A is most highly expressed in GC, and p38 can phosphorylate MEF2A via transcription of GLUT4 to promote glycolysis [38]. Moreover, MEF2A can also induce CDKN1A expression through KLF4 and has a tumor suppressor effect [39]. In the established miRNA-mRNA regulatory network, there were a total of 45 genes and 31 miRNAs. Four key genes (ZFPM2, FUT9, NEUROD1 and LIPH) and six key miRNAs (hsa-let-7d-5p, hsa-miR-23b-3p, hsa-miR-23a-3p, hsa-miR-133b, hsa-miR-130a-3p and hsa-miR-124-3p) were identified. The expression of ZFPM2 and LIPH in GC tissues was consistent with the survival curve results. ZFPM2, also known as FOG2, is located on chromosome 8q23 and belongs to the GATA gene family. ZFPM2 is expressed in the heart, brain, testis, liver, lung and skeletal muscle [40–41] and has been studied in neuroblastoma [42]. ZFPM2 can act as a repressor or activator, depending on the specific promoter and cell type [43]. ZFPM2 was related to circulating vascular endothelial growth factor levels, which may influence venous thrombosis risk by regulating circulating vascular endothelial growth factor and disrupting the thrombotic balance in the venous system [44]. The expression of ZFPM2 antisense RNA 1 (ZFPM2-AS1) in GC was higher than that in normal gastric tissues, which was related to tumor size, infiltration depth, differentiation degree and TNM staging, and promoted the occurrence of GC by stabilizing migration of inhibitory factor (MIF) to inhibit the p53 pathway [45]. GO enrichment analysis suggested that ZFPM2 was enriched during embryonic organ development. There is growing evidence that EMT is critical in the development of cancer. [46–47]. Type 3 EMT can transform primary cancer epithelial cells into invasive, metastatic mesenchymal cancer cells [48]. Type 3 EMT related to tumorigenesis can be considered an abnormal type of EMT, which normally occurs during embryonic development [49]. Our experimental results indicated that ZFPM2 was highly expressed in GC tissues and various GC cell lines and could promote the proliferation, invasion and migration of GC cells. LIPH is involved in the proliferation of lung adenocarcinoma cells, can be used as a novel indicator of lung cancer [50]. Cui et al. [51] showed that the expression of LIPH was associated with tumor size, histological grade and staging of breast cancer. Ishimine et al. [52] demonstrated that the high expression of LIPH in esophageal adenocarcinoma was positively correlated with increased patient survival, suggesting that LIPH had certain predictive value for the survival of esophageal cancer. Our analysis showed that LIPH was expressed at low levels in GC tissues. The survival curve indicated that patients with low expression of GC had a shorter survival.

Previous studies indicated that miR-23b-3p may reverse drug resistance in GC [53–54]. MiR-23b-3p targets ATG12 and HMGB2 to inhibit autophagy in multidrug-resistant GC [55]. However, decreased levels of miR-23b-3p increased autophagy in pancreatic cancer [56]. MiR-23b-3p was downregulated in colon cancer and mediated tumor metastasis by regulating FZD7 and MAP3K1 [57]. In addition, studies also found that miR-23b-3p was upregulated in GC tissues [58]. Previous studies indicated that miR-130a-3p was downregulated and inhibited cancer in many cancers [59–62]. Moreover, related studies indicated that miR-130a-3p was upregulated in GC and promoted the invasion of GC cells [63]. Rossi et al. [64] indicated that the miR-130a in diffuse GC was higher than that in intestinal type GC. In addition, some studies suggested that miR-130a was downregulated in GC and was related to the migration and metastasis of GC [65]. Wang et al. [66] also found that miR-130a-3p can inhibit cell migration and invasion by inhibiting TBL1XR1-mediated EMT in GC, suggesting that the decrease in miR-130a-3p was related to poor prognosis of GC. Wei et al. [67] showed that miR-130a was an oncogenic miRNA. Duan et al. [68] indicated that miR-130a could directly target TGFβR2 in GC and acted as an oncogene. Our analysis indicated that ECM-receptor interaction was a KEGG pathway enriched by miR-23b-3p and miR-130a-3p. Studies have demonstrated that ECM receptor-mediated signaling is a major signaling category that promotes cell survival [69]. ECM can also be involved in cell proliferation, differentiation and carcinogenesis [70]. In this study, 18 of the 20 DEMs were enriched in this pathway.

In conclusion, we identified 45 genes and 31 miRNAs and established a miRNA-mRNA regulatory network. Two genes (ZFPM2 and LIPH) and two miRNAs (hsa-miR-23a-3p and hsa-miR-130a-3p) were identified by analysis and experimental verification. These four molecular markers were closely related to the prognosis of GC. ZFPM2 may serve as a potential molecular marker and therapeutic target for GC. ECM receptor interactions and metabolic abnormalities play a critical role in the GC progression. These findings can enhance our understanding of the pathogenesis of GC and associated molecular events, contribute to timely diagnosis and treatment, and lay the foundation for future clinical research. This study also has some limitations. First, a small number of gene and miRNA expression profiling datasets was analyzed. Second, the key genes ZFPM2 and LIPH were not enriched in the KEGG enrichment analysis. Third, LIPH and key miRNA expression levels and biological functions were not experimentally verified. Therefore, further studies are necessary to analyze the underlying mechanisms and related pathways of these molecular markers.

Abbreviations

GC: gastric cancer; messenger RNA: mRNA; GEO: Gene Expression Omnibus; DEGs: differentially expressed genes; DEMs: differentially expressed miRNAs; EGC: early gastric cancer; AGC: advanced gastric cancer; GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; RPMI: Roswell park memorial institute; DMEM: Dulbecco's modified Eagle medium; qRT-PCR: Quantitative real-time polymerase chain reaction; TFs: transcription factors; CC: cellular component; BP: biological process; MF: molecular function; ZFPM2-AS1: ZFPM2 antisense RNA 1; MIF: migration of inhibitory factor

Declarations

Ethics approval and consent to participate

All procedures were performed in accordance with the guidelines of the Helsinki Declaration. The study was approved by the Ethical Committee of the Second Affiliated Hospital of Nanchang University and written informed consent was obtained from all patients.

Consent for publication

Not applicable.

Availability of data and materials

All the data in this study are available from TCGA database (https://portal.gdc.cancer.gov).

Competing interests

The authors declare no competing financial interests.

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Author contributors

CH and XJZ designed the study. CH, XJZ, and JFZ collected the data. CH, JH and FQB analyzed the data. CH and XJZ performed the experiments and statistical analysis. CH wrote the manuscript with contributions from all authors. All authors read and approved the final version of the paper.

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Table

Category	Term	Description	p.adjust	Gene	Gene counts
GO:0035883	BP	enteroendocrine cell differentiation	0.0005	NEUROD1/PAX6/INSM1/RFX6	4
GO:0031018	BP	endocrine pancreas development	0.0015	NEUROD1/PAX6/INSM1/RFX6	4
GO:0002067	BP	glandular epithelial cell differentiation	0.0015	NEUROD1/PAX6/INSM1/RFX6	4
GO:0002687	BP	positive regulation of leukocyte migration	0.0027	PLVAP/CCL21/THBS1/VEGFC/THY1	5
GO:0003309	BP	type B pancreatic cell differentiation	0.0054	PAX6/INSM1/RFX6	3
GO:0031016	BP	pancreas development	0.0054	NEUROD1/PAX6/INSM1/RFX6	4
GO:0002691	BP	regulation of cellular extravasation	0.0054	PLVAP/CCL21/THY1	3
GO:2000177	BP	regulation of neural precursor cell proliferation	0.0074	PTPRZ1/PAX6/VEGFC/INSM1	4
GO:0002685	BP	regulation of leukocyte migration	0.0095	PLVAP/CCL21/THBS1/VEGFC/THY1	5
GO:0048871	BP	multicellular organismal homeostasis	0.0118	MFAP2/CA2/ESRRG/NEUROD1/ELOVL6/AQP1/CLDN18	7
GO:0002065	BP	columnar/cuboidal epithelial cell differentiation	0.0121	NEUROD1/PAX6/INSM1/RFX6	4
GO:0035270	BP	endocrine system development	0.0192	NEUROD1/PAX6/INSM1/RFX6	4
GO:0048545	BP	response to steroid hormone	0.0195	CA2/ESRRG/PAQR8/AQP1/THBS1/SST	6
GO:2000179	BP	positive regulation of neural precursor cell proliferation	0.0195	PAX6/VEGFC/INSM1	3
GO:0090596	BP	sensory organ morphogenesis	0.0204	MFAP2/PAX6/COL8A1/THY1/PRRX1	5
GO:0002604	BP	regulation of dendritic cell antigen processing and presentation	0.0204	CCL21/THBS1	2
GO:0019755	BP	one-carbon compound transport	0.0204	CA2/AQP1	2
GO:0061351	BP	neural precursor cell proliferation	0.0204	PTPRZ1/PAX6/VEGFC/INSM1	4

GO = gene ontology, BP = biological process, CC = cellular component, KEGG = Kyoto Encyclopedia of Genes and Genomes.

Table 1 Significantly enriched GO biological process terms and KEGG pathways.

Category	Term	Description	p.adjust	Gene	Gene counts
GO:0048568	BP	embryonic organ development	0.0204	MFAP2/NEUROD1/PAX6/ZFPM2/PRRX1/RSPO3	6
GO:0048592	BP	eye morphogenesis	0.0204	MFAP2/PAX6/COL8A1/THY1	4
GO:0045123	BP	cellular extravasation	0.0204	PLVAP/CCL21/THY1	3
GO:0002468	BP	dendritic cell antigen processing and presentation	0.0204	CCL21/THBS1	2
GO:0002693	BP	positive regulation of cellular extravasation	0.0309	PLVAP/THY1	2
GO:0002690	BP	positive regulation of leukocyte chemotaxis	0.0426	CCL21/THBS1/VEGFC	3
GO:0002577	BP	regulation of antigen processing and presentation	0.0426	CCL21/THBS1	2
GO:0015669	BP	gas transport	0.0426	CA2/AQP1	2
GO:0023019	BP	signal transduction involved in regulation of gene expression	0.0426	NEUROD1/PAX6	2
GO:0002052	BP	positive regulation of neuroblast proliferation	0.0455	PAX6/VEGFC	2
GO:0001654	BP	eye development	0.0488	MFAP2/NEUROD1/PAX6/COL8A1/THY1	5
GO:0150063	BP	visual system development	0.0490	MFAP2/NEUROD1/PAX6/COL8A1/THY1	5
GO:0010810	BP	regulation of cell-substrate adhesion	0.0490	CCL21/THBS1/COL8A1/THY1	4
GO:0048880	BP	sensory system development	0.0497	MFAP2/NEUROD1/PAX6/COL8A1/THY1	5
GO:0044420	CC	extracellular matrix component	0.0234	MFAP2/PTPRZ1/COL8A1	3
hsa04950	KEGG	Maturity onset diabetes of the young	0.0017	NEUROD1/PAX6/RFX6	3
hsa04964	KEGG	Proximal tubule bicarbonate reclamation	0.0395	CA2/AQP1	2

GO = gene ontology, BP = biological process, CC = cellular component, KEGG = Kyoto Encyclopedia of Genes and Genomes.

Table 1 Significantly enriched GO biological process terms and KEGG pathways.

Supplementary Figure Legends

Supplementary Figure 1 Heat map of the significantly differentially expressed miRNAs in gastric tumor tissue samples and normal samples.

Supplementary Figure 2 Heat map of the significantly differentially expressed genes in gastric tumor tissue samples and normal samples.

Figures

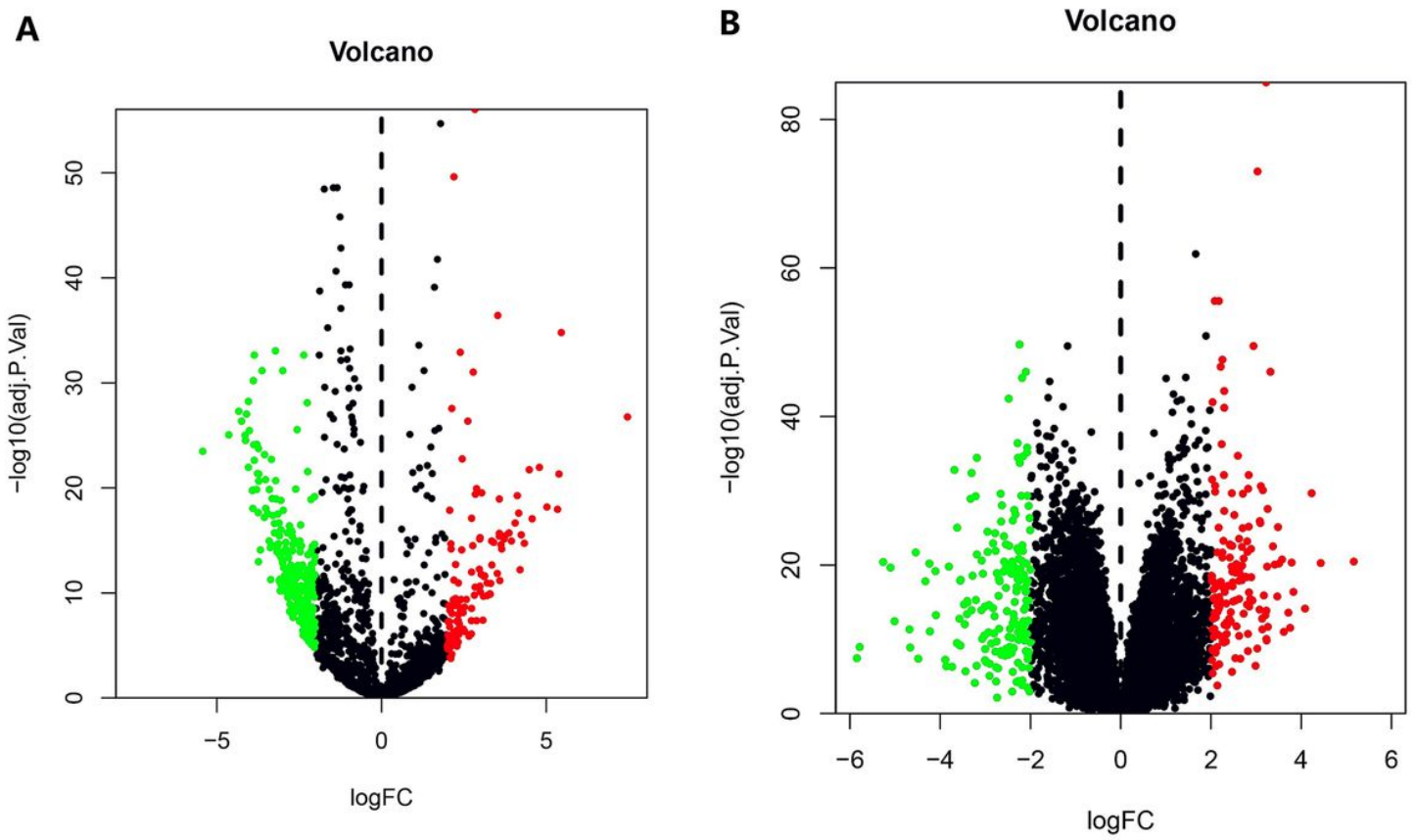

Figure 1

Volcano plots. (A) Differentially expressed miRNAs. (B) Differentially expressed genes. The green indicates downregulated, and red indicates upregulated.

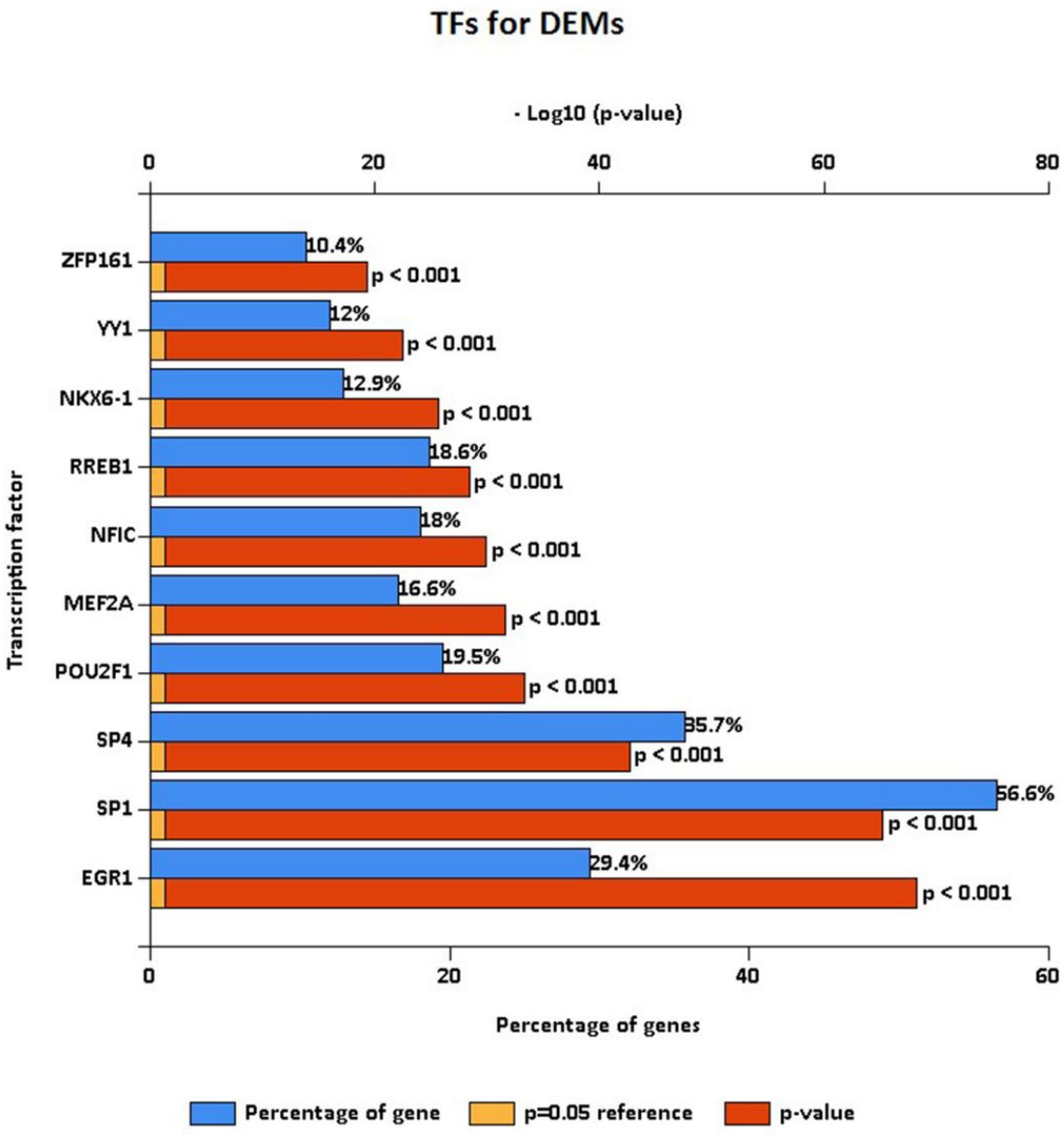

Figure 2

Upstream TFs of differentially expressed miRNAs.

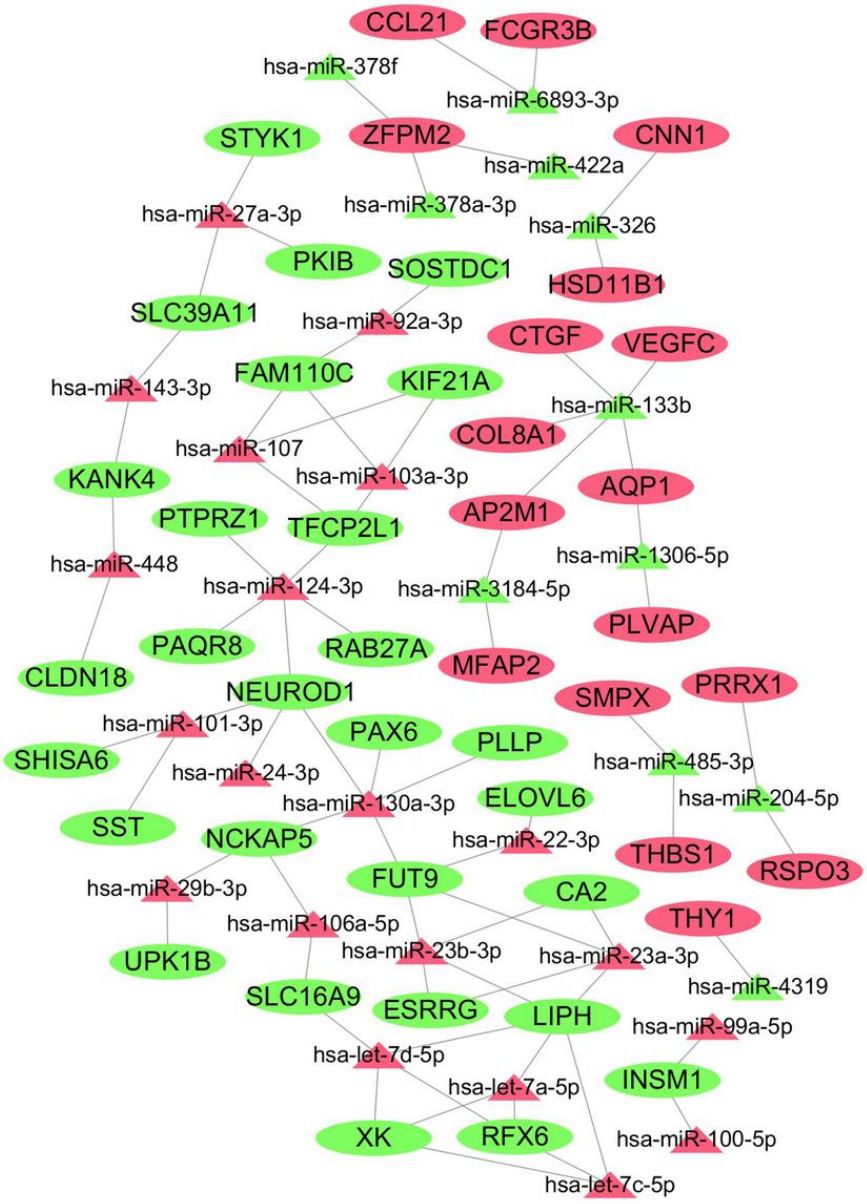

Figure 3

A miRNA-mRNA regulatory network. The green indicates downregulated, and red indicates upregulated.

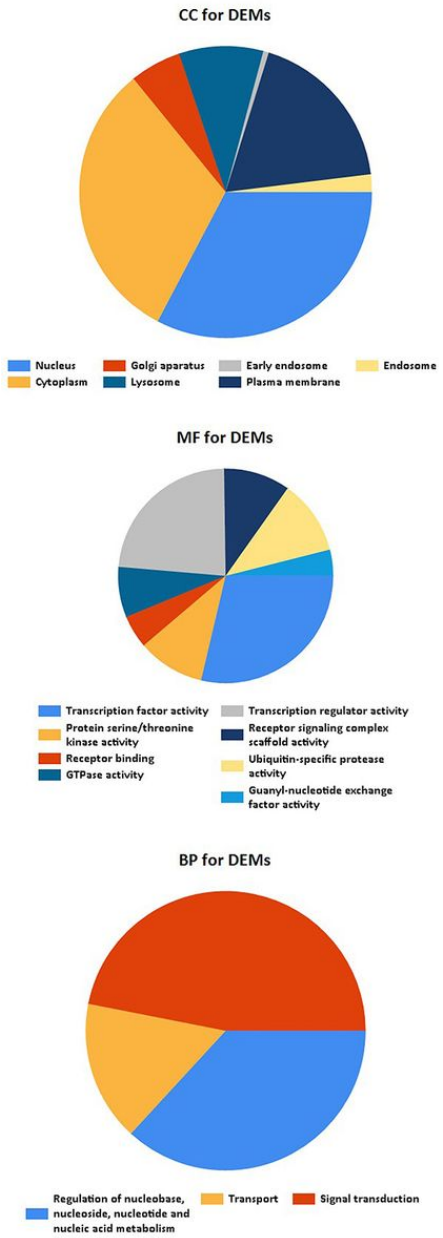

Figure 4

Significantly enriched Gene Ontology biological process terms of differentially expressed miRNAs. BP indicates biological process, CC indicates cellular component, and MF indicates molecular function.

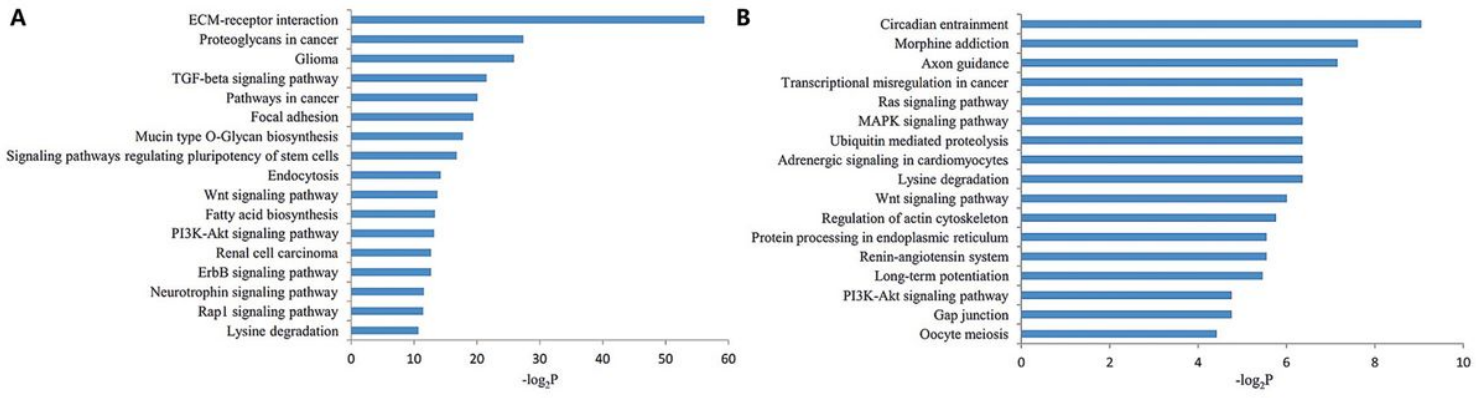

Figure 5

Significantly enriched KEGG pathways of upregulated (A) and downregulated differentially expressed miRNAs (B).

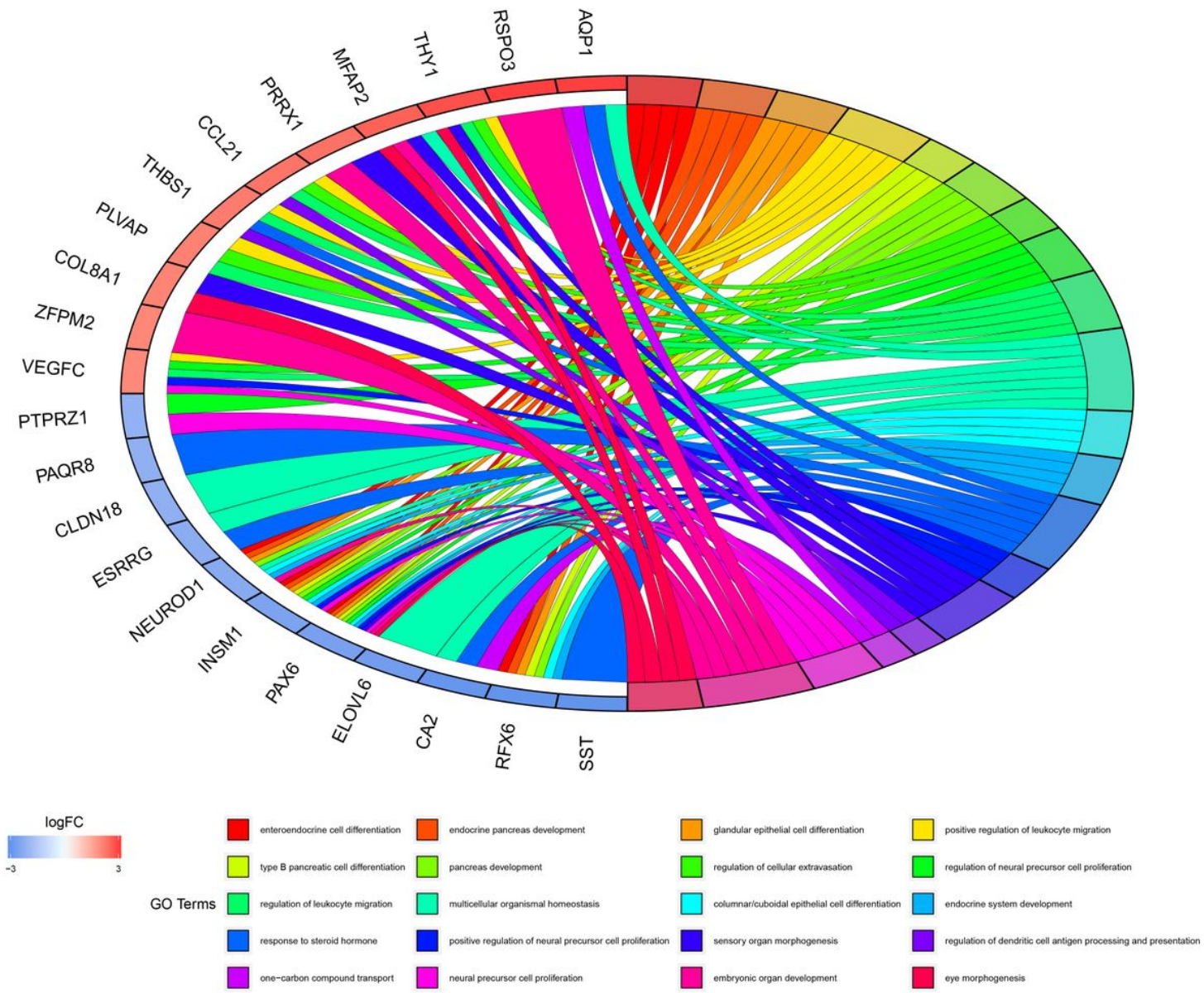

Figure 6

Circle diagram of differentially expressed genes.

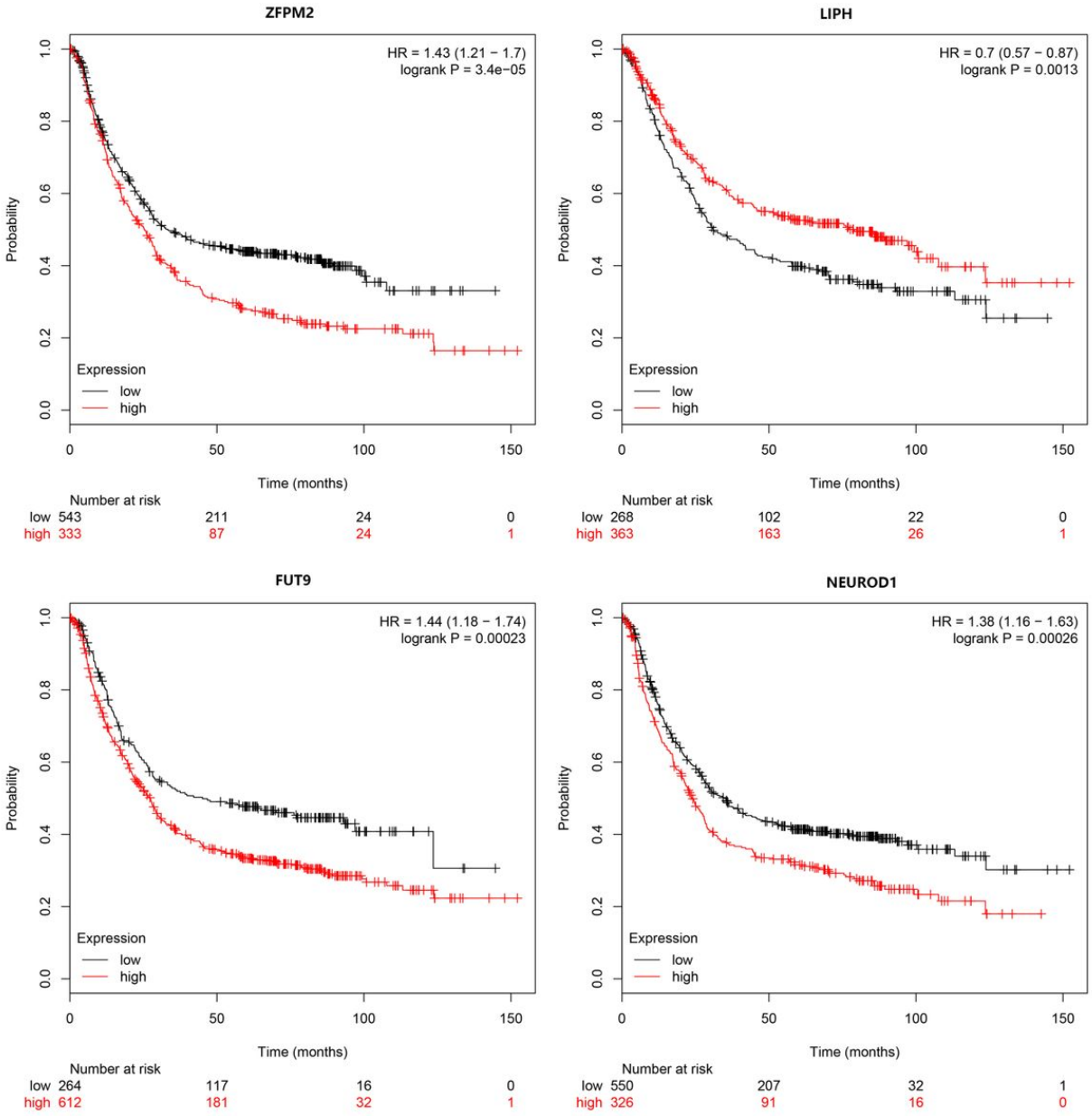

Figure 7

Survival curves of hub genes using the online tool Kaplan-Meier Plotter.

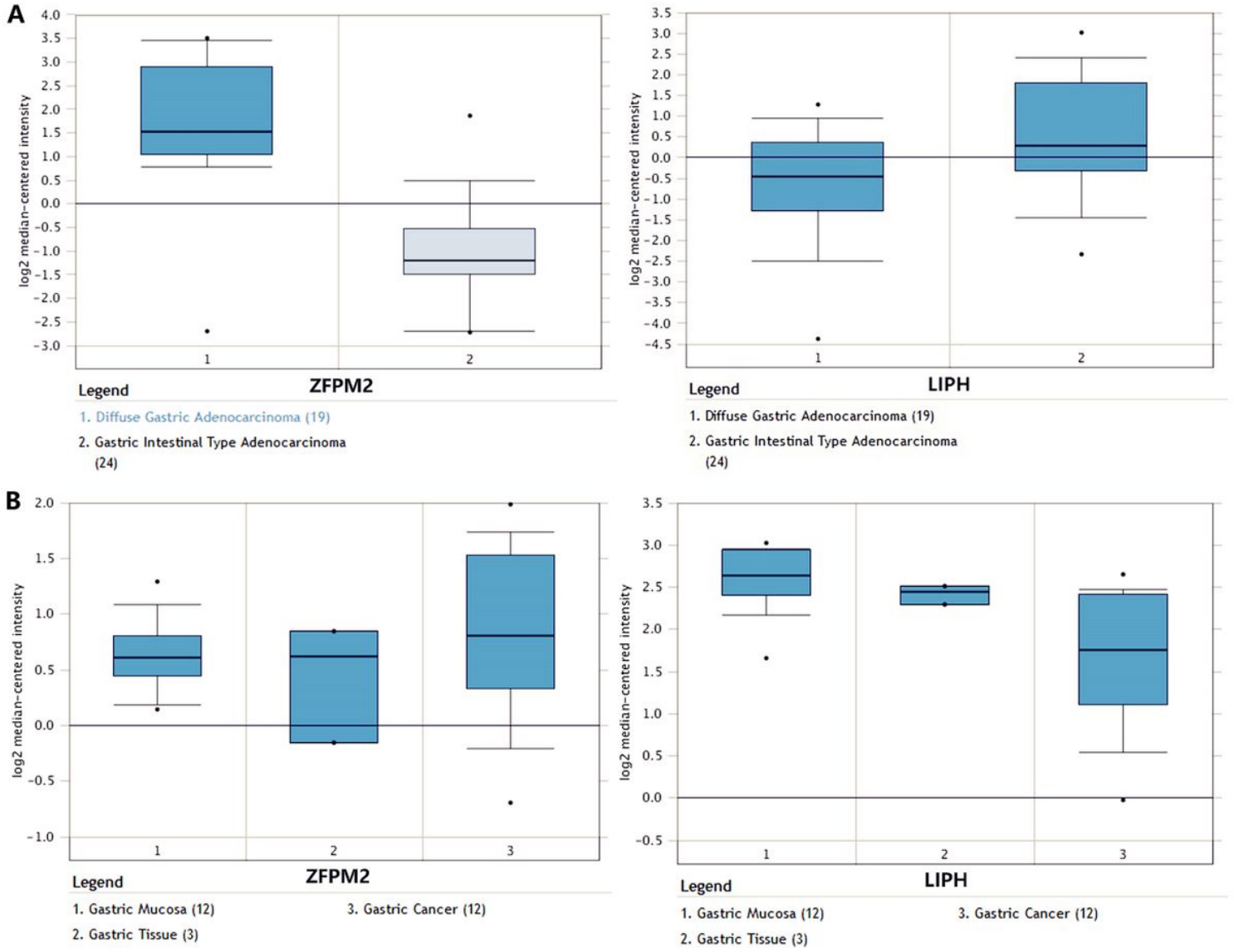

Figure 8

Expression of ZFPM2 and LIPH based on the online database Oncomine. (A) Expression of ZFPM2 and LIPH between diffuse gastric adenocarcinoma and gastric adenocarcinoma. (B) Expression of ZFPM2 and LIPH between gastric cancer and gastric mucosa samples.

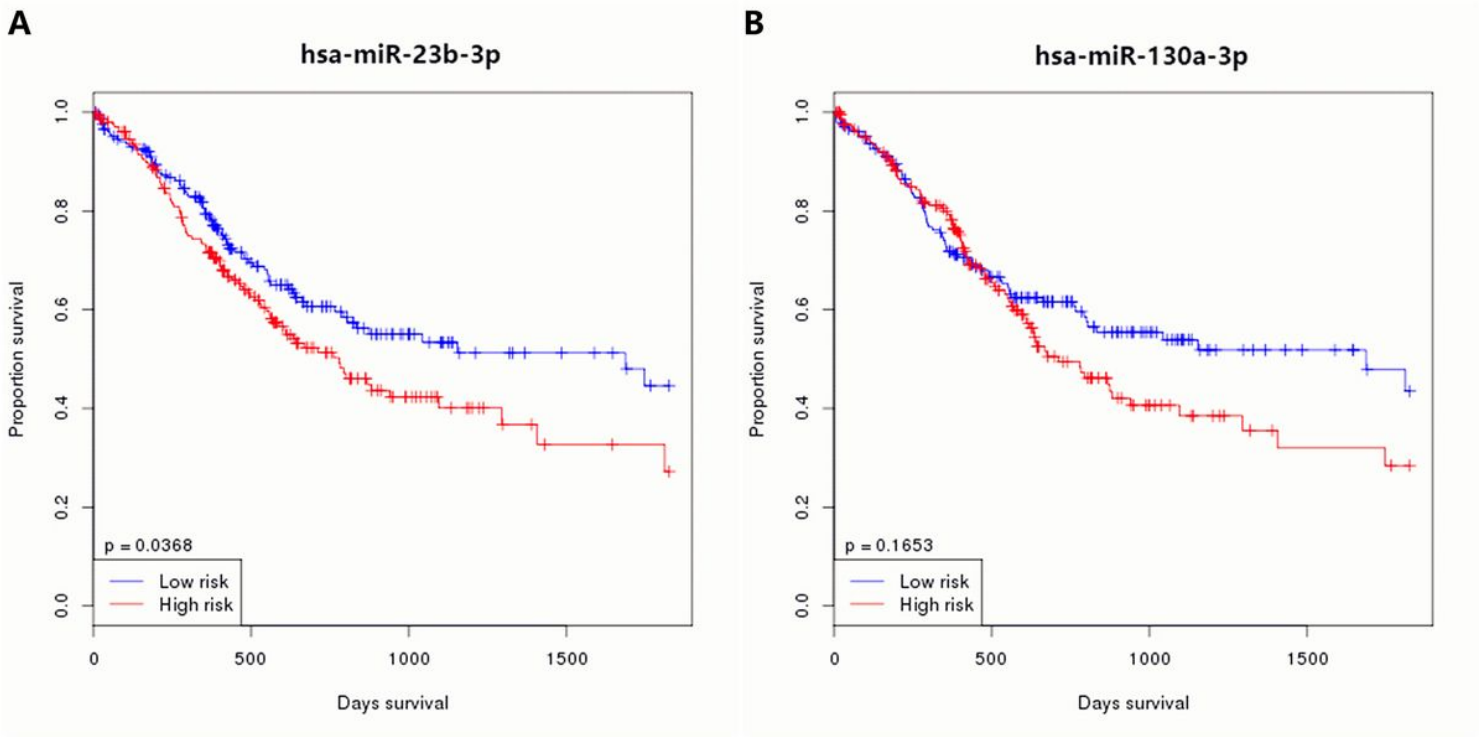

Figure 9

Survival curves of hsa-miR-23b-3p (A) and hsa-miR-130a-3p (B).

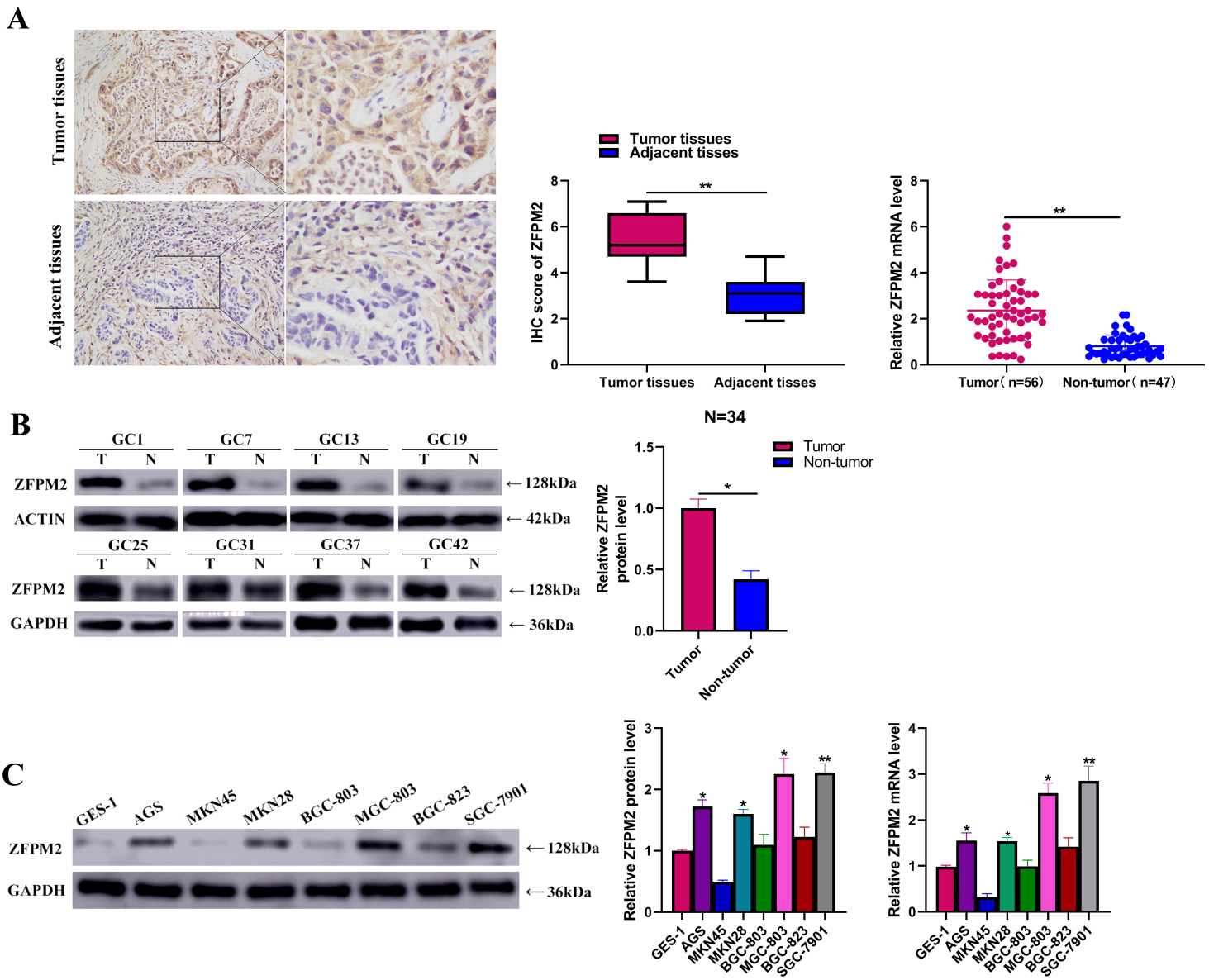

Figure 10

Immunohistochemistry, qRT-PCR and Western blot assays. A: Expression levels of ZFPM2 in tumor tissues and adjacent tissues, and expression levels of ZFPM2 mRNA in tumor and non-tumor tissues. B: Expression levels of ZFPM2 protein in tumors and non-tumors. C: Expression levels of ZFPM2 mRNA and protein in various gastric cell lines. *P < 0.05, **P < 0.01.

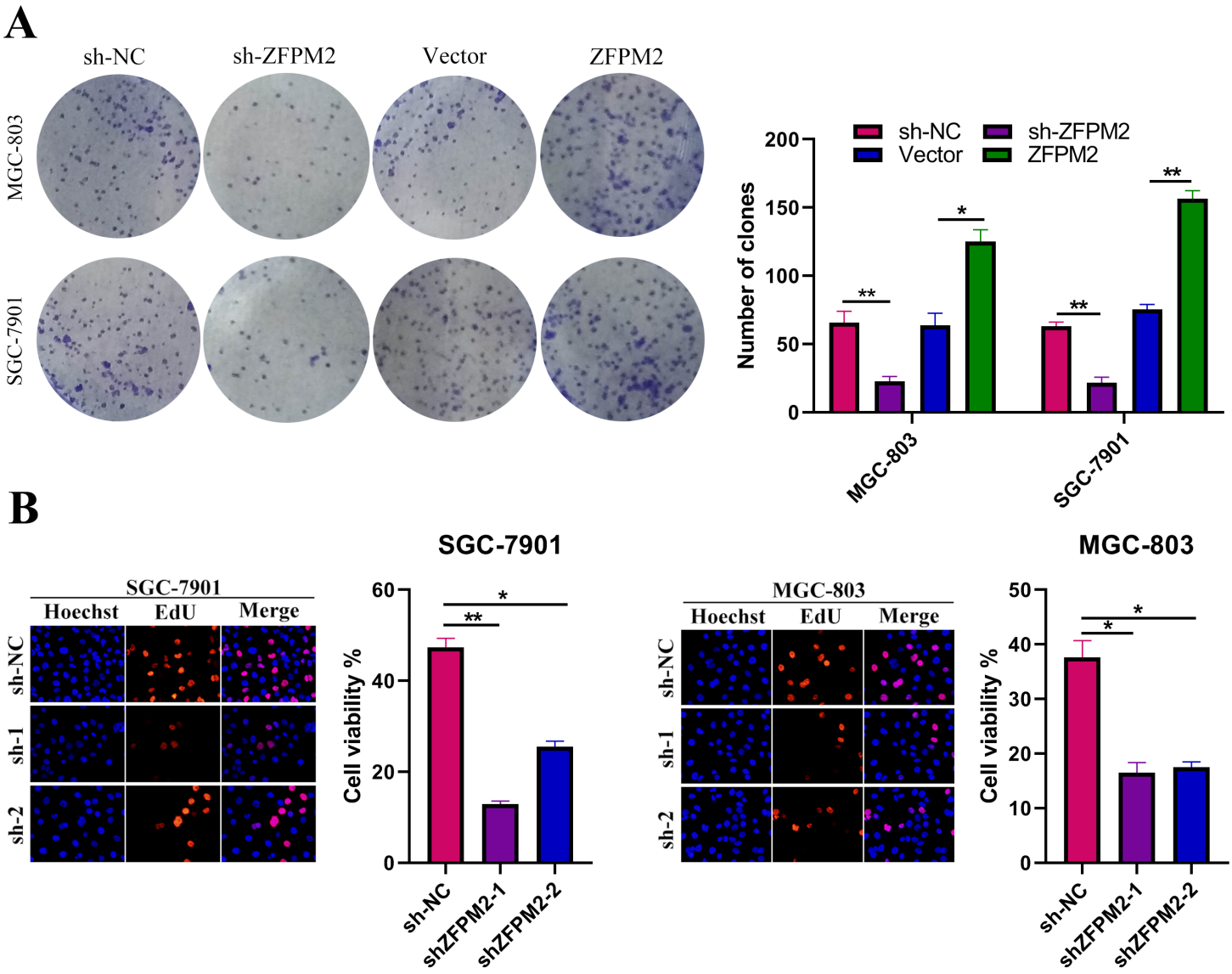

Figure 11

Cell proliferation experiments. A: The number of colonies of SGC7901 and MGC803 cells after different treatments. B: Cell viability of SGC7901 and MGC803 cells transfected with sh-NC, shZFPM2-1 and shZFPM2-2. *P < 0.05, **P < 0.01.

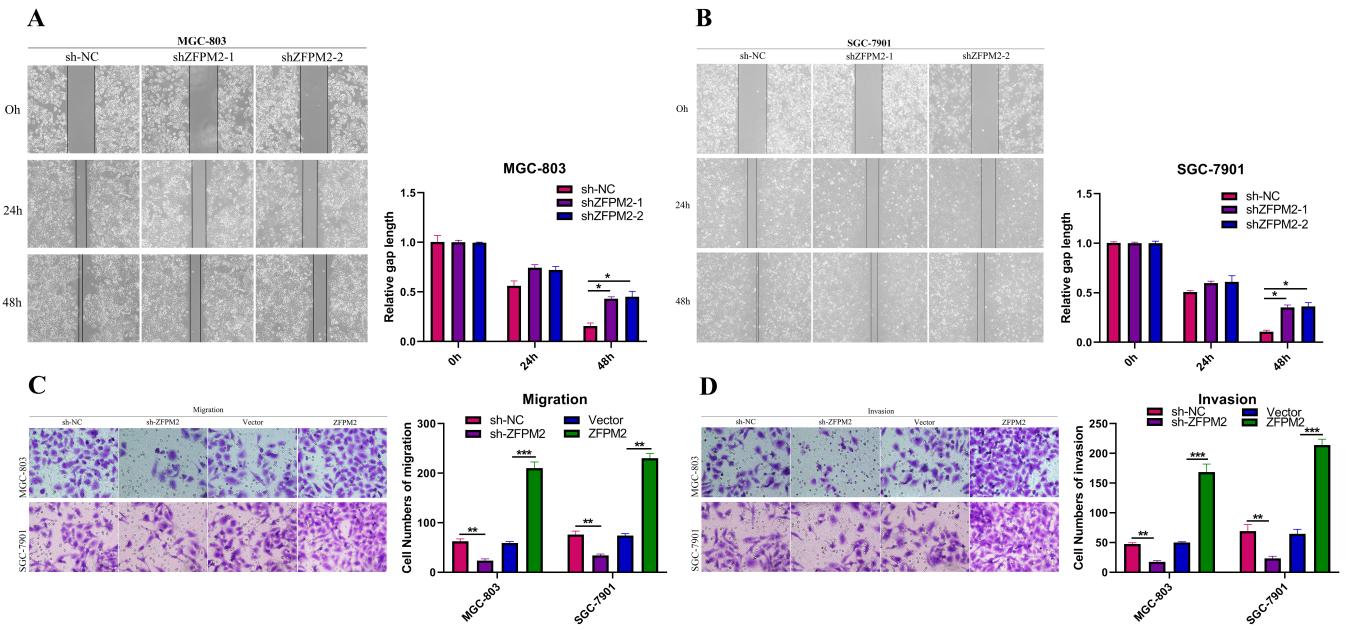

Figure 12

Cell scratch, migration, and invasion experiments. A: Relative gap length in MGC803 cells transfected with sh-NC, shZFPM2-1 and shZFPM2-2. B: Relative gap length in SGC7901 cells with transfected sh-NC, shZFPM2-1 and shZFPM2-2. C: Numbers of migrated of SGC7901 and MGC803 cells after different treatments. D: Numbers of invaded of SGC7901 and MGC803 cells after different treatments. *P < 0.05, **P < 0.01 ***P < 0.001.

Supplementary Files

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- SupplementaryFigure2.jpg