Original Article

Identification of a potential competing endogenous RNA (ceRNA) network in gastric adenocarcinoma

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Background: Recently, a growing body of evidence has revealed the role of competing endogenous RNA (ceRNA) networks in various human cancers. However, there is still a lack of research on the systemic ceRNA network related to gastric adenocarcinoma.

Methods: The intersection of differentially expressed genes (DEGs) was obtained by mining the GSE54129, GSE13861, and GSE118916 datasets from the Gene Expression Omnibus (GEO) website. The Database for Annotation, Visualization, and Integrated Discovery (DAVID) was used for the enrichment analysis. A protein-protein interaction (PPI) network was established with the STRING online database, and hub genes were identified by Cytoscape software. The prediction of key microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) was conducted by miRNet. The prognostic analysis, expression difference, and correlation analysis of messenger RNAs (mRNAs), lncRNAs, and miRNAs were carried out using the Gene Expression Profiling Interactive Analysis (GEPIA), Kaplan-Meier plotter, and Encyclopedia of RNA Interactomes (ENCORI).

Results: We identified180 significant DEGs. Extracellular matrix (ECM) receptor interaction, focal adhesion, ECM tissue, and collagen catabolic processes were the most significant pathways in the functional enrichment analysis. Nineteen upregulated hub genes and one downregulated hub gene were found to be significantly associated with the prognosis of gastric adenocarcinoma. Of the 18 miRNAs targeting 12 key genes, only six were associated with a promising prognosis in gastric adenocarcinoma. By comprehensive differential expression and survival analysis, 40 key lncRNAs were identified. Finally, we constructed a network of 24 ceRNAs associated with gastric adenocarcinoma.

Conclusions: Potential mRNA-miRNA-lncRNA subnets were constructed, each RNA of which can be used as a prognostic biomarker for gastric adenocarcinoma.

Keywords: Differentially expressed genes (DEGs); hub gene; competing endogenous RNA (ceRNA); gastric adenocarcinoma; bioinformatics analysis

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Introduction

Gastric adenocarcinoma, as a multifactorial disease, is currently the fifth most common type of cancer and the third most common cause of cancer death globally (1). Risk factors for the condition include *Helicobacter pylori* infection, age, high salt intake, and diets low in fruit and vegetables (2). Though some causal factors of gastric adenocarcinoma have been identified recently, its etiology and pathogenesis remain obscure (3). Moreover, the lack

of differential symptoms results in a low diagnostic rate for advanced gastric adenocarcinoma (4). Therefore, more effective screening methods are required to diagnose gastric adenocarcinoma. A great deal of research has shown that non-coding RNA is closely related to the occurrence and development of cancers (5-7). Competing endogenous RNA (ceRNA) is transcripts that can be mutually regulated at the post-transcriptional level by competing for shared microRNAs (miRNAs). The ceRNA network links the function of protein-coding messenger RNA (mRNA) with non-coding RNA (8,9). Bioinformatics analysis has been widely used in basic research. Therefore, this study aimed to identify the hub genes and construct a ceRNA network for gastric adenocarcinoma using bioinformatics analyses. We present the following article in accordance with the REMARK reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1201/rc).

Methods

Microarray data

Microarray data related to gastric adenocarcinoma were retrieved according to the criteria of "Homo sapiens", "expression was analyzed through array", "gastric cancer group and control group were included" and "sample size ≥30". The datasets GSE54129 (Affymetrix GPL570 platform), GSE118916 (Affymetrix GPL15207 platform), and GSE13861 (Affymetrix GPL6884 platform) were obtained from the Gene Expression Omnibus (GEO) website (https://www.ncbi.nlm.nih.gov/geo/). The Limma software package (version: 3.40.2) of R software was used to explore the expression of mRNA. The GES54129 dataset contained 111 gastric adenocarcinoma samples and 21 noncancerous samples. GSE118916 contained 15 gastric adenocarcinoma samples and 15 noncancerous samples, and GSE13861 contained 60 gastric adenocarcinoma samples and 19 noncancerous samples. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Highlight box

Key findings

- We constructed a ceRNA network of gastric adenocarcinoma.

What is known and what is new?

- ceRNA hypothesis suggested that a large-scale regulatory network was formed in the transcriptome by crosstalk between coding RNAs and non-coding RNAs via MREs (microRNA response elements, MREs).
- Combined with the difference of prognosis and expression, screening and verification were conducted layer by layer.

What is the implication, and what should change now?

- This study was a bioinformatics analysis based on the ceRNA hypothesis, further exploration and verification of RNAs from the network are needed.

Identification of differentially expressed genes (DEGs)

A GEO2R analysis was provided directly by the GEO project. A P value <0.01, a log fold change (FC) value ≥1, or a logFC value ≤–1 were defined as the thresholds for the DEGs. The overlapping DEGs were obtained by Venn diagrams.

Construction of the protein-protein interaction (PPI) network and selection of hub genes

The PPI network of the DEGs was constructed using the STRING database (http://string-db.org/). Combined scores with interactions >0.4 were considered statistically significant. The PPI networks were visualized by Cytoscape software (version 3.7.1). CytoHubba, a plugin of Cytoscape, sorted the nodes into a network according to the network characteristics. Using the degree calculation, we defined the top 20 hub genes.

Enrichment analysis of DEGs and hub genes

We conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses on the DEGs and hub genes by using the Database for Annotation, Visualization and Integrated Discovery (DAVID) version 6.8 (https://david.ncifcrf.gov/home.jsp). A P value <0.05 was considered statistically significant.

Association of hub gene expressions with the survival of gastric adenocarcinoma patients

The expression and prognostic value of the hub genes were analyzed using the Gene Expression Profiling Interactive Analysis (GEPIA) website and the Kaplan-Meier plotter online tool, and the results were applied to the search for key genes. Then the differential expression of key genes was verified using the Oncomine database analysis (http://www.oncomine.org).

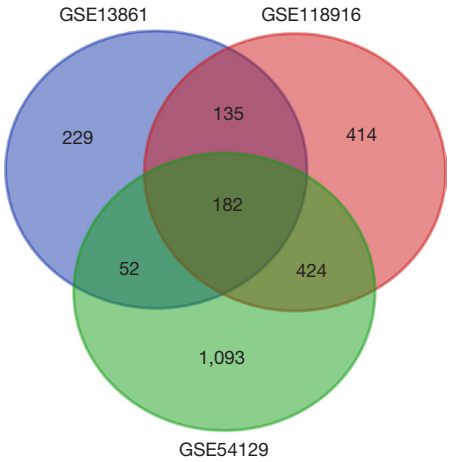


Figure 1 Identification of DEGs in the GSE118916, GSE13816, and GSE54129 datasets. DEGs, differentially expressed genes.

Construction of the miRNA subnet

The correlation between key genes and miRNA expression was analyzed by the miRNet 2.0 online database (https://www.mirnet.ca/miRNet/home.xhtml). Meanwhile, the Kaplan-Meier plotter was used to analyze the prognostic value of the predicted miRNAs on overall survival.

Prediction of upstream long noncoding RNAs (lncRNAs)

The miRNet database was used to predict the upstream lncRNAs of the miRNAs. The Kaplan-Meier plotter was used to evaluate the prognostic value of the predicted lncRNAs on overall survival. The differential expressions in gastric adenocarcinoma and normal tissues were analyzed by the Encyclopedia of RNA Interactomes (ENCORI) online dataset.

Construction of the lncRNA-miRNA-mRNA network

ENCORI 3.0 (http://starbase.sysu.edu.cn/) was used to evaluate the mRNA-lncRNA, miRNA-mRNA, and mRNA-lncRNA pairs of common expression in gastric adenocarcinoma.

Results

Identification of DEGs in gastric adenocarcinoma

We identified 1,155 genes in the GSE118916 dataset, 598 genes in the GSE13816 dataset, and 1,751 genes in the GSE54129 dataset as DEGs by standardization of the microarray results. The overlap among the three datasets is shown as a Venn diagram (*Figure 1* and *Table 1*) with 182 genes included. Two genes were excluded because of inconsistent regulation. The DEGs comprised 53 upregulated and 127 downregulated genes in gastric adenocarcinoma tissues.

The PPI network analysis.

A PPI network was constructed using 130 nodes and 390 edges to identify the key genes, as shown in *Figure 2*.

The selection of hub genes

According to the CytoHubba degree method, 20 hub genes were identified: *ATP4A*, *BGN*, *COL1A1*, *COL1A2*, *COL3A1*, *COL4A1*, *COL4A2*, *COL5A2*, *COL6A3*, *COL10A1*, *CXCL1*, *CXCL*8, *MMP3*, *PTGS*, *SERPINH1*, *SPP1*, *THBS1*, *THBS2*, *TIMP1*, and *MMP7*.

The enrichment analysis of DEGs

The DAVID website was used to perform the GO annotation and KEGG pathway enrichment analysis to ascertain the biological functions of the DEGs. Extracellular matrix (ECM) organization, digestion, collagen fibril organization, collagen catabolic process, and skeletal system development were the top five biological processes (BP) of the DEGs. The cellular composition (CC) of the DEGs mainly included extracellular space, extracellular exosome, extracellular region, ECM, and endoplasmic reticulum lumen. ECM structural constituent, oxidoreductase activity, ECM binding, platelet-derived growth factor binding, and calcium ion binding were the molecular functions (MF) of the DEGs. ECM-receptor interaction, protein digestion and absorption, focal adhesion, chemical carcinogenesis, and retinol metabolism were the main KEGG functional pathways of the DEGs (*Figure 3*).

The enrichment analysis of the hub genes

The enrichment of the GO and KEGG pathways was analyzed to explore the function of the hub genes. The most significant KEGG pathways of the hub genes were ECM-receptor interaction, focal adhesion, protein digestion and absorption, the PI3K-Akt signaling pathway, and

Table 1 DEGs detected from three datasets with 53 upregulated genes and 127 downregulated genes

DEGs	Gene
Upregulated	*CDH3, TGFBI, COL4A2, FAM83D, PLA2G2A, CEMIP, HOXB7, CPXM1, SULF2, SULF1, THBS1, CHRDL2, CLDN3, TNFRSF11B, SFRP4, THBS4,I FITM3, RARRES1, BGN, FNDC1, TMEM158, COL5A2, THBS2, COL6A3, COL1A2, MMP7, PLA2G7, OLFML2B, IGF2BP3, COMP, COL10A1, SERPINH1, COL3A1, FAP, COL4A1, SPP1, MMP3, LY6E, THY1, NID2, CTHRC1, FOXC1, CXCL1, PLAU, COL1A1, PTGS2, CXCL8, SNX10, CLDN1, TIMP1, CST1, COL8A1, CDH11*
Downregulated	*UBL3 ,POU2AF1 ,CLDN18, ANKRD22, SMIM24, ADTRP, IGFBP2, IRX3, AADAC, ADGRG2, CYP2C9, ESRRG, GUCA2B, TFF1, KCNJ16, PIGR, RNASE1, CBLIF, AMPD1, GKN2, CYP2C18, CAPN8, HPGD, PLAC8, C6ORF58, TMPRSS2, SULT2A1, C1ORF116, FBP2, CA2, BCAS1, GPT, PSCA, RDH12, TRNP1, MAL, SCIN, SULT1C2, MLPH, KRT20, CYP3A5, PIK3C2G, S100P, ITPKA, LRRC31, FOXA1, SCGB2A1, SLC28A2, DHRS7, MUCL3, CPA2, ATP4A, SSTR1, LIPF, NRG4, CTSE, ST6GALNAC1, STX19, KCNE2, CXCL17, ARL14, CWH43, ALDH1A1, FCGBP, FA2H, SCNN1B, SELENBP1, ARHGEF37, NQO1, ATP4B, NOSTRIN, LYPD6B, ANG, TCN1, REG1A, ALDH3A1, SST,T MEM171, KIAA1324, JCHAIN, ADRB2, PRSS8, AKR1C3, FAM174B, GATA5, PGC, CHGA, LTF, AKR7A3, CA9, CA4, TFF2, VILL, GPAT3, REG3A, FAM3B, MRAP2, TFCP2L1, MAOA, ADAM28, VSIG2, CAPN13, AGR2, SOSTDC1, EPN3, CYSTM1, PTGR1, TNFRSF17, GC, SPINK1, ADH7, DDX60, GPT2, TENT5C, ANXA10, KLK11, ADH1C, CAPN9, SPTSSB, AKR1B10, MFSD4A, PLAAT2,S TOX2, VSIG1, GKN1, TMED6, DNER*

DEGs, differentially expressed genes.

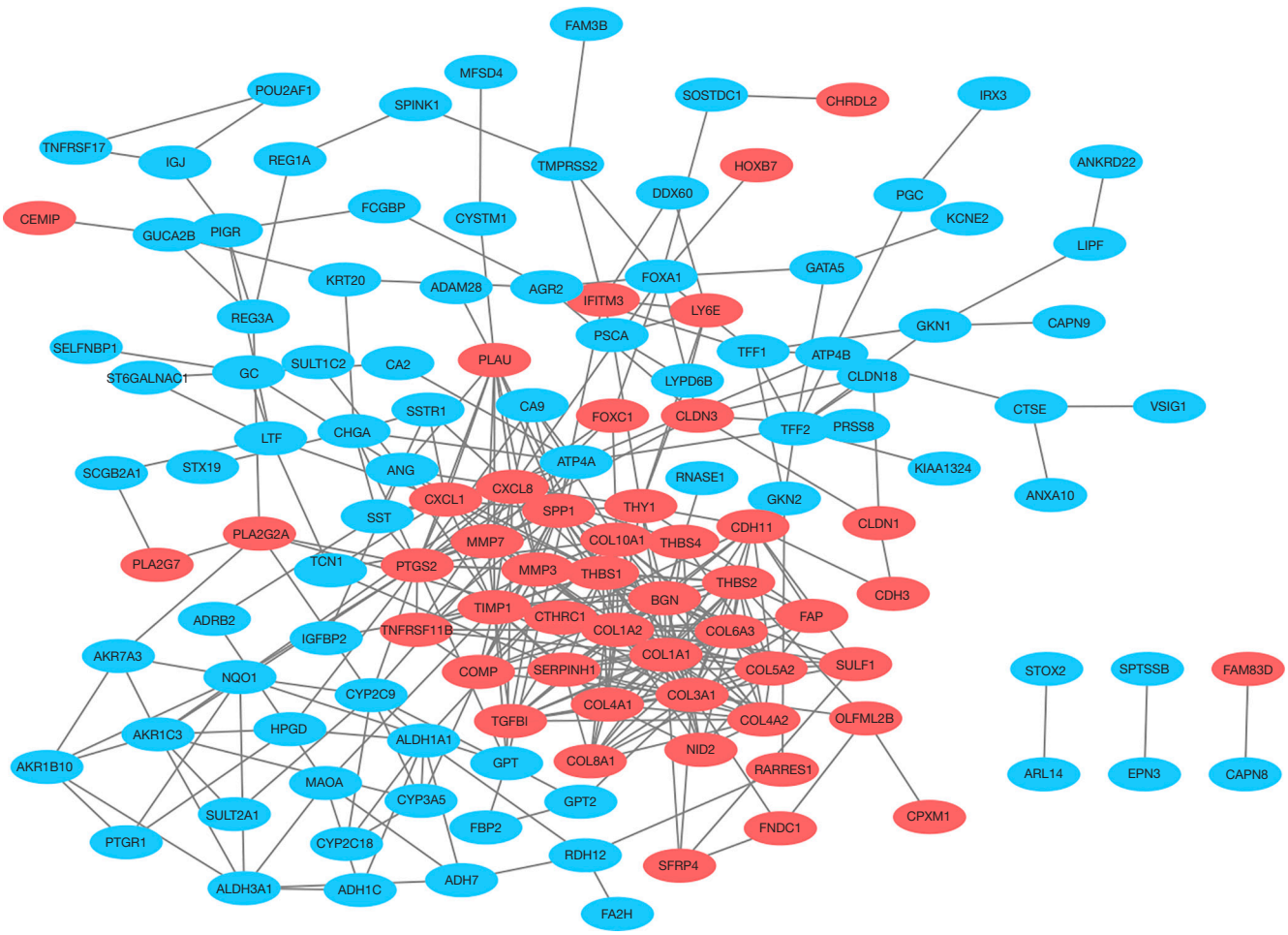
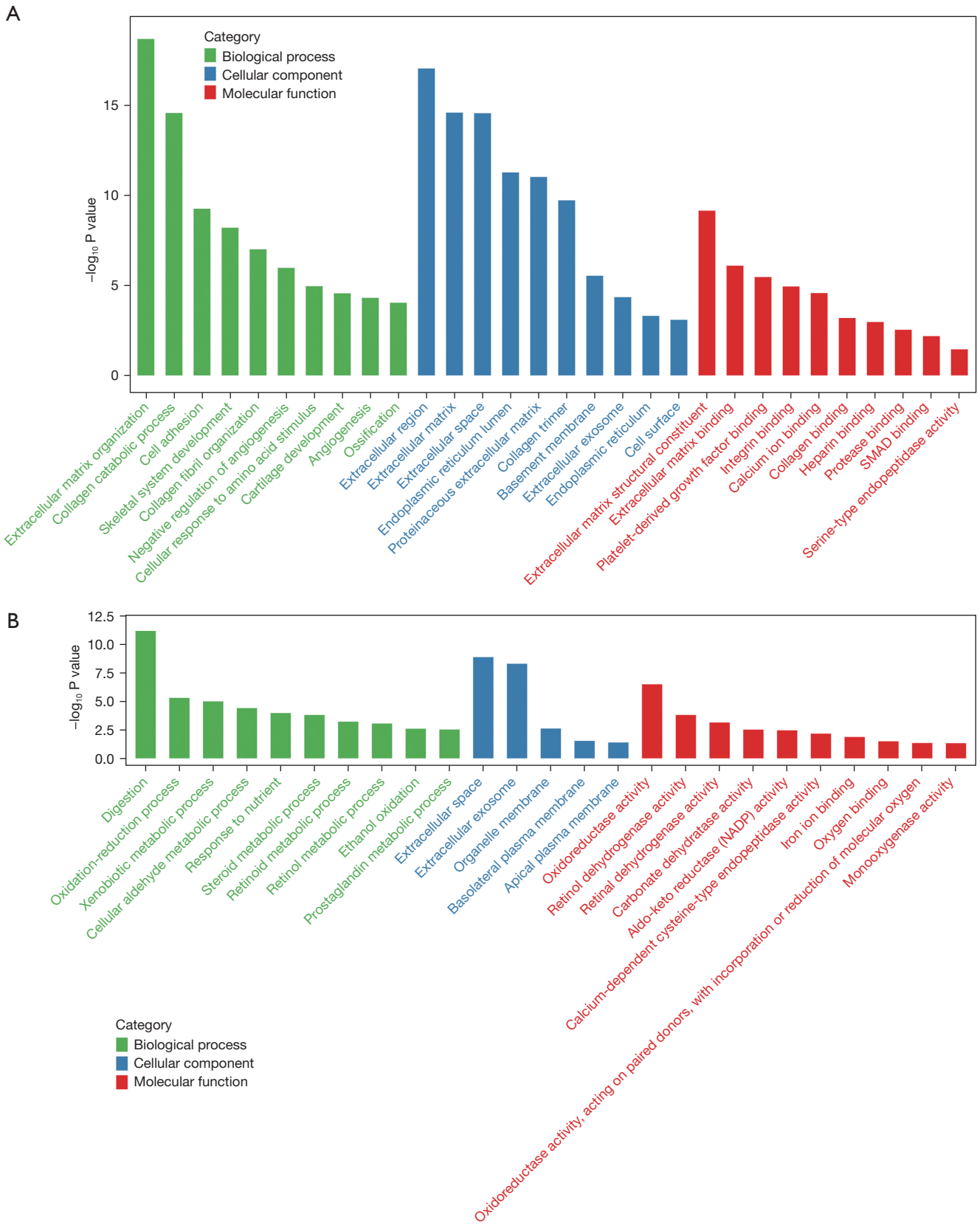


Figure 2 The PPI network with 130 nodes and 390 edges as constructed by Cytoscape. PPI, protein-protein interaction.



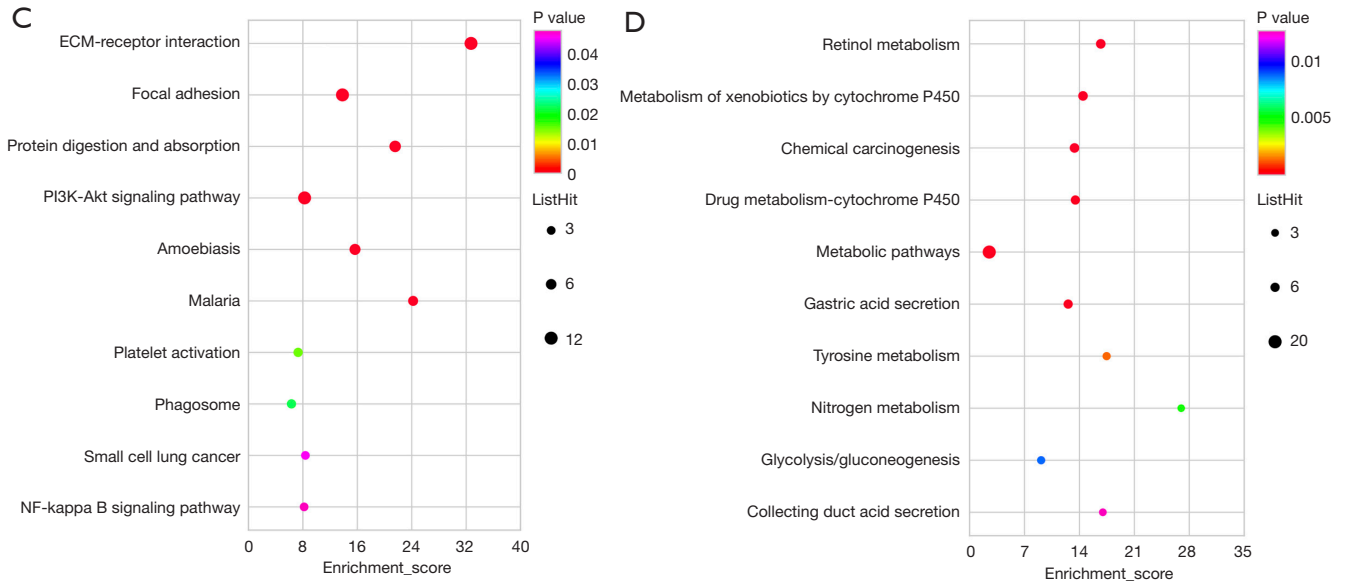


Figure 3 The enrichment analysis of DEGs and hub genes. (A) The GO annotation of the upregulated DEGs. (B) The GO annotation of the downregulated DEGs. (C) The KEGG pathways of upregulated DEGs. (D) The KEGG pathways of downregulated DEGs. GO, Gene Ontology; DEGs, differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

amoebiasis. The significant GO annotations were collagen catabolic process, endoplasmic reticulum lumen, ECM organization, extracellular region, ECM, collagen trimer, and ECM structural constituent (*Table 2*). The hub gene enrichment analysis results were visualized by ClueGo of Cytoscape (*Figure 4*).

The mRNA expression and survival analysis

Sources from the Genotype-Tissue Expression (GTEx) and The Cancer Genome Atlas (TCGA) databases were used to analyze the expression levels of the hub genes. The gastric adenocarcinoma samples possessed higher expressions of *BGN*, *COL1A1*, *COL1A2*, *COL3A1*, *COL4A1*, *COL4A2*, *COL5A2*, *COL6A3*, *COL10A1*, *CXCL1*, *CXCL8*, *MMP3*, *SERPINH1*, *SPP1*, *THBS2*, *TIMP1*, and *MMP7* compared with normal samples. Only *ATP4A* was downregulated in gastric adenocarcinoma tissues. Moreover, *ATP4A*, *COL10A1*, *BGN*, *COL1A1*, *COL1A2*, *COL4A1*, *COL4A2*, *COL5A2*, *SERPINH1*, *SPP1*, *THBS2*, *TIMP1*, and *MMP7* were related to poor overall survival in gastric adenocarcinoma, as identified by the Kaplan-Meier plotter. By comprehensive differential expression and survival analysis, we identified *COL10A1*, *BGN*, *COL1A1*, *COL1A2*, *COL4A1*, *COL4A2*, *COL6A3*, *SERPINH1*, *SPP1*, *THBS2*, *TIMP1*, and *MMP7* as key genes (*Figure 5*). The differential expression of the key genes was verified by the Oncomine database analysis (http://www.oncomine.org) (*Figure 6*).

Prediction and validation of the miRNAs

The upstream miRNAs of the key genes were predicted using miRNet 2.0. Our results showed that 18 miRNAs (*hsa-mir-29a-3p*, *hsa-mir-29b-3p*, *hsa-mir-200b-3p*, *hsa-mir-9-5p*, *hsa-mir-125a-5p*, *hsa-mir-200c-3p*, *hsa-mir-29c-3p*, *hsa-mir-429*, *hsa-mir-503-5p*, *hsa-mir-26a-5p*, *hsa-mir-10b-5p*, *hsa-mir-17-5p*, *hsa-mir-20a-5p*, *hsa-mir-22-3p*, *hsa-mir-497-5p*, *hsa-mir-224-5p*, *hsa-mir-29b*, and *hsa-mir-29c*) could potentially regulate the key genes. Based on the classical inverse relationship theory, it is assumed that upstream miRNAs may exert a positive prognostic effect. Using the Kaplan-Meier plotter tool, we found six of the 18 miRNAs (*hsa-mir-29a-3p*, *hsa-mir-200b-3p*, *hsa-mir-200c-3p*, *hsa-mir-429*, *hsa-mir-26a-5p*, *and hsa-mir-17-5p*) were associated with prognosis in gastric adenocarcinoma patients, as shown in *Figure 7*. The six miRNAs were defined as key miRNAs. The miRNA-mRNA network was obtained by Cytoscape software (*Figure 8*).

Table 2 The top 20 GO annotation and KEGG pathway hub genes

ID	Term	Count	P value	FDR	Gene
GO:0030574	Collagen catabolic process	10	8.43E-18	2.12E-15	*COL1A1, COL3A1, MMP7, COL1A2, COL4A2, COL4A1, COL5A2, MMP3, COL10A1, COL6A3*
GO:0005788	Endoplasmic reticulum lumen	11	1.12E-15	4.16E-14	*COL1A1, COL3A1, COL1A2, COL4A2, COL4A1, COL5A2, SERPINH1, COL10A1, COL6A3, PTGS2, THBS1*
GO:0030198	Extracellular matrix organization	11	3.11E-15	3.91E-13	*COL1A1, COL3A1, COL1A2, COL4A2, COL4A1, COL5A2, SPP1, BGN, COL10A1, COL6A3, THBS1*
GO:0005576	Extracellular region	17	9.58E-15	1.77E-13	*CXCL8, MMP7, MMP3, BGN, CXCL1, THBS2, THBS1, COL1A1, COL3A1, COL1A2, COL4A2, COL4A1, COL5A2, SPP1, COL6A3, COL10A1, TIMP1*
GO:0031012	Extracellular matrix	11	8.91E-14	1.10E-12	*COL1A1, COL3A1, MMP7, COL1A2, COL4A2, COL4A1, COL5A2, BGN, COL6A3, THBS2, THBS1*
hsa04512	ECM-receptor interaction	10	1.21E-13	5.47E-12	*COL1A1, COL3A1, COL1A2, COL4A2, COL4A1, COL5A2, SPP1, COL6A3, THBS2, THBS1*
GO:0005581	Collagen trimer	8	3.18E-12	2.94E-11	*COL1A1, COL3A1, COL1A2, COL5A2, SERPINH1, COL10A1, COL6A3, TIMP1*
GO:0005201	Extracellular matrix structural constituent	7	8.10E-11	4.13E-09	*COL1A1, COL3A1, COL1A2, COL4A2, COL4A1, COL5A2, BGN*
hsa04510	Focal adhesion	10	3.22E-10	7.24E-09	*COL1A1, COL3A1, COL1A2, COL4A2, COL4A1, COL5A2, SPP1, COL6A3, THBS2, THBS1*
hsa04974	Protein digestion and absorption	8	7.72E-10	1.16E-08	*COL1A1, COL3A1, COL1A2, COL4A2, COL4A1, COL5A2, COL10A1, COL6A3*
GO:0005615	Extracellular space	13	7.84E-10	5.80E-09	*CXCL8, MMP7, MMP3, CXCL1, THBS1, COL1A1, ATP4A, COL3A1, COL1A2, SERPINH1, SPP1, COL6A3, TIMP1*
GO:0005578	Proteinaceous extracellular matrix	8	5.96E-09	3.68E-08	*MMP7, COL1A2, COL5A2, MMP3, BGN, COL10A1, COL6A3, TIMP1*
hsa04151	PI3K-Akt signaling pathway	10	3.08E-08	3.47E-07	*COL1A1, COL3A1, COL1A2, COL4A2, COL4A1, COL5A2, SPP1, COL6A3, THBS2, THBS1*
GO:0030199	Collagen fibril organization	5	9.39E-08	7.86E-06	*COL1A1, COL3A1, COL1A2, COL5A2, SERPINH1*
hsa05146	Amoebiasis	7	1.25E-07	1.13E-06	*COL1A1, COL3A1, CXCL8, COL1A2, COL4A2, COL4A1, COL5A2*
GO:0048407	Platelet-derived growth factor binding	4	1.98E-07	5.06E-06	*COL1A1, COL3A1, COL1A2, COL4A1*
GO:0071230	Cellular response to amino acid stimulus	5	2.02E-07	1.27E-05	*COL1A1, COL3A1, COL1A2, COL4A1, COL5A2*
GO:0001501	Skeletal system development	5	1.49E-05	7.50E-04	*COL1A1, COL3A1, COL1A2, COL5A2, COL10A1*
GO:0022617	Extracellular matrix disassembly	4	8.19E-05	0.003428113	*MMP7, MMP3, SPP1, TIMP1*
GO:0050840	Extracellular matrix binding	3	3.84E-04	0.006525144	*SPP1, BGN, THBS1*

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

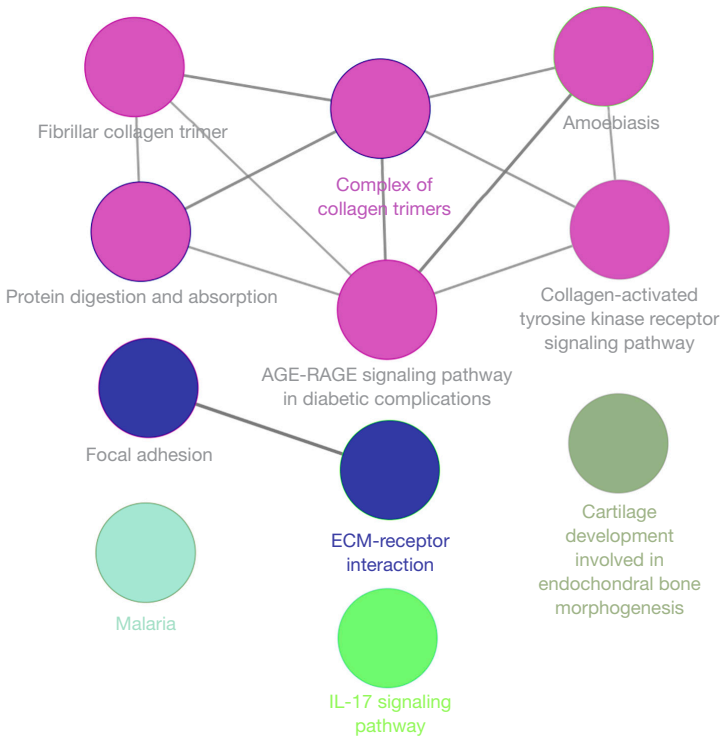
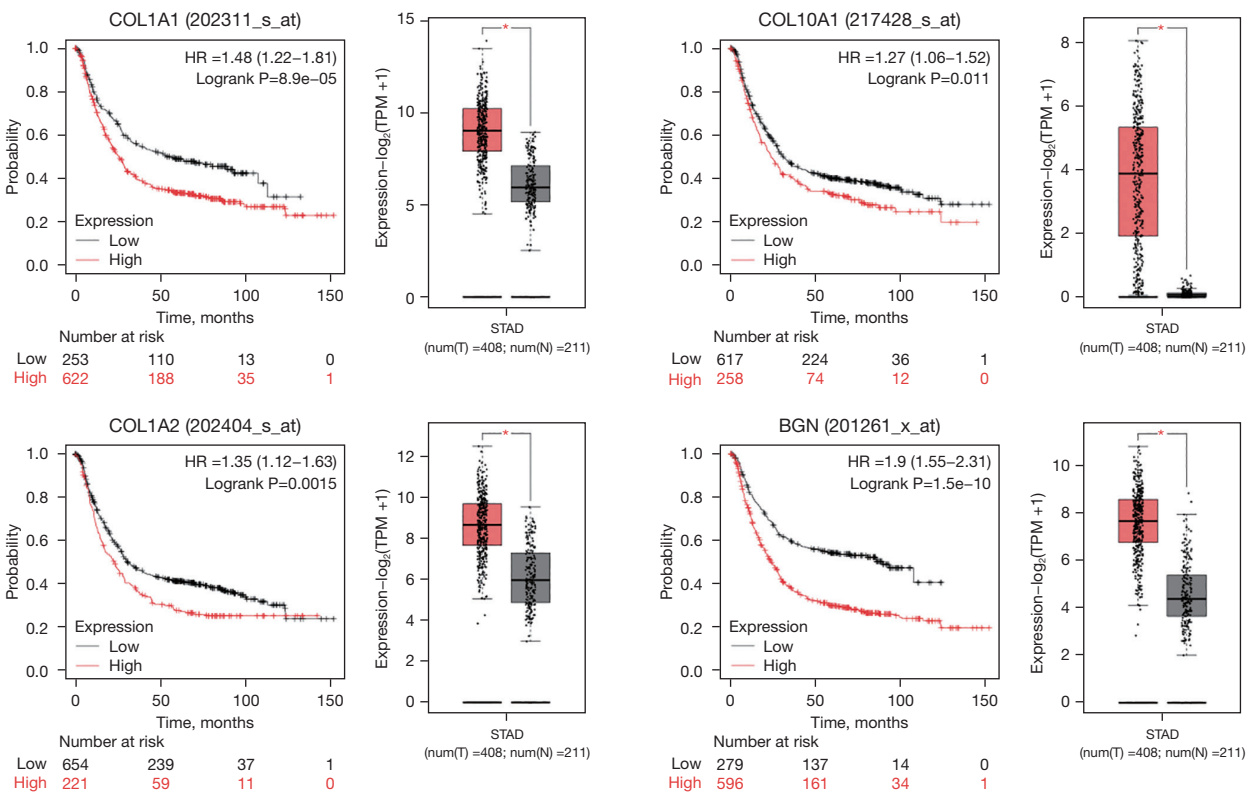


Figure 4 The GO and KEGG enrichments of the hub genes illustrated by Cluego of Cytoscape. ECM, extracellular matrix; IL, interleukin; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.



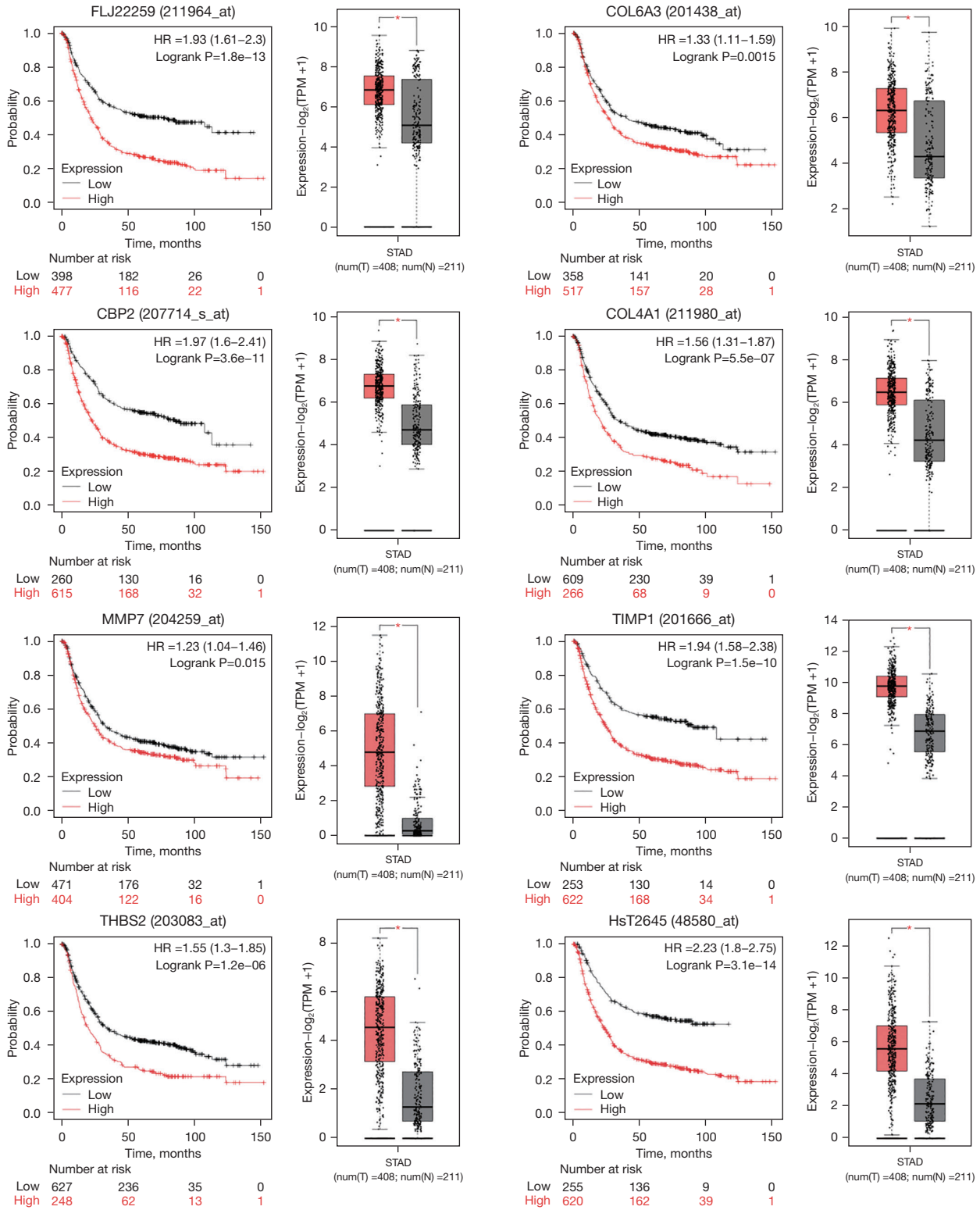


Figure 5 The expression and survival analysis of COL10A1, BGN, COL1A1, COL1A2, COL4A1, COL4A2, COL6A3, SERPINH1, SPP1, THBS2, TIMP1, and MMP7 in patients with gastric adenocarcinoma. A P value <0.05 was considered statistically significant. (FLJ22259 is the other name for COL4A2, CBP2 is the other name for SERPINH1, and HsT2645 is the other name for SPP1). *, P value <0.01. HR, hazard ratio; STAD, stomach adenocarcinomas; BGN, biglycan; TPM, transcripts per million.

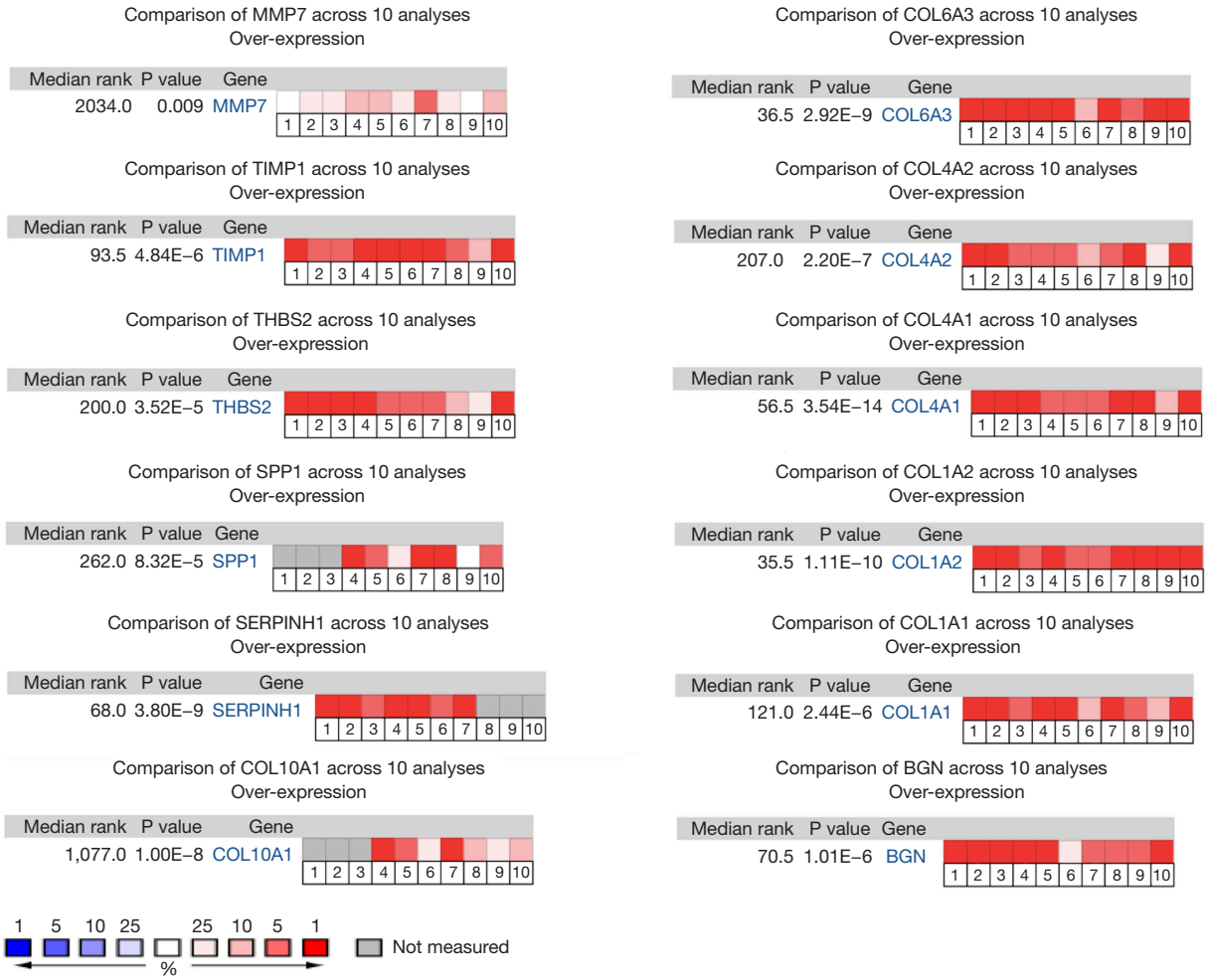


Figure 6 The differential expression of key genes verified by the Oncomine database analysis. 1. Diffuse Gastric Adenocarcinoma *vs.* Normal (10). 2. Gastric Intestinal Type Adenocarcinoma *vs.* Normal (10). 3. Gastric Mixed Adenocarcinoma *vs.* Normal (10). 4. Diffuse Gastric Adenocarcinoma *vs.* Normal (11). 5. Gastric Intestinal Type Adenocarcinoma *vs.* Normal (11). 6. Gastric Mixed Adenocarcinoma *vs.* Normal (11). 7. Gastric Cancer *vs.* Normal (12). 8. Gastric Intestinal Type Adenocarcinoma *vs.* Normal (13). 9. Gastric Mixed Adenocarcinoma *vs.* Normal (13). 10. Gastric Cancer *vs.* Normal (14). The rank for a gene is the median rank for that gene across each of the analyses. The P value for a gene is its P value for the median-ranked analysis.

Prediction of lncRNAs

The prediction of upstream lncRNAs of the six miRNAs was conducted by miRNet. Strong evidence for the potential binding of 157 lncRNAs to the six miRNAs was found. A negative correlation between lncRNAs and miRNAs was supported by the hypothesis that lncRNAs could competitively bind to miRNAs. We identified 40 lncRNAs associated with a poor prognosis for gastric adenocarcinoma patients that had a high expression in gastric adenocarcinoma tissues: *ARRDC1-AS1*, *CCDC144NL-AS1*, *DLGAP1-AS1*, *DLX6-AS1*, *GABPB1-AS1*, *GAS5*, *HCG18*, *LINC00638*, *LINC00852*, *LINC00879*, *LINC00943*, *LINC00997*, *LINC01111*, *LINC01270*, *LINC01553*, *LINC-PINT*, *MIAT*, *MIRLET7BHG*, *MMP25-AS1*, *NNT-AS1*, *PSMD6-AS2*, *PVT1*, *SNHG16*, *SNHG17*, *STK4-AS1*, *THUMPD3-AS1*, *WASIR2*, *MATN1-AS1*, *PTPRG-AS1*, *LINC00174*, *MSC-AS1*, *ZEB1-AS1*, *NUTM2B-AS1*, *KCNQ1OT1*, *HELLPAR*, *MAPKAPK5-AS1*, *LINC01303*, *RRN3P2*, *MCM3AP-AS1*, and *LINC00894*). The 40 lncRNAs were defined as key lncRNAs (*Figure 9*).

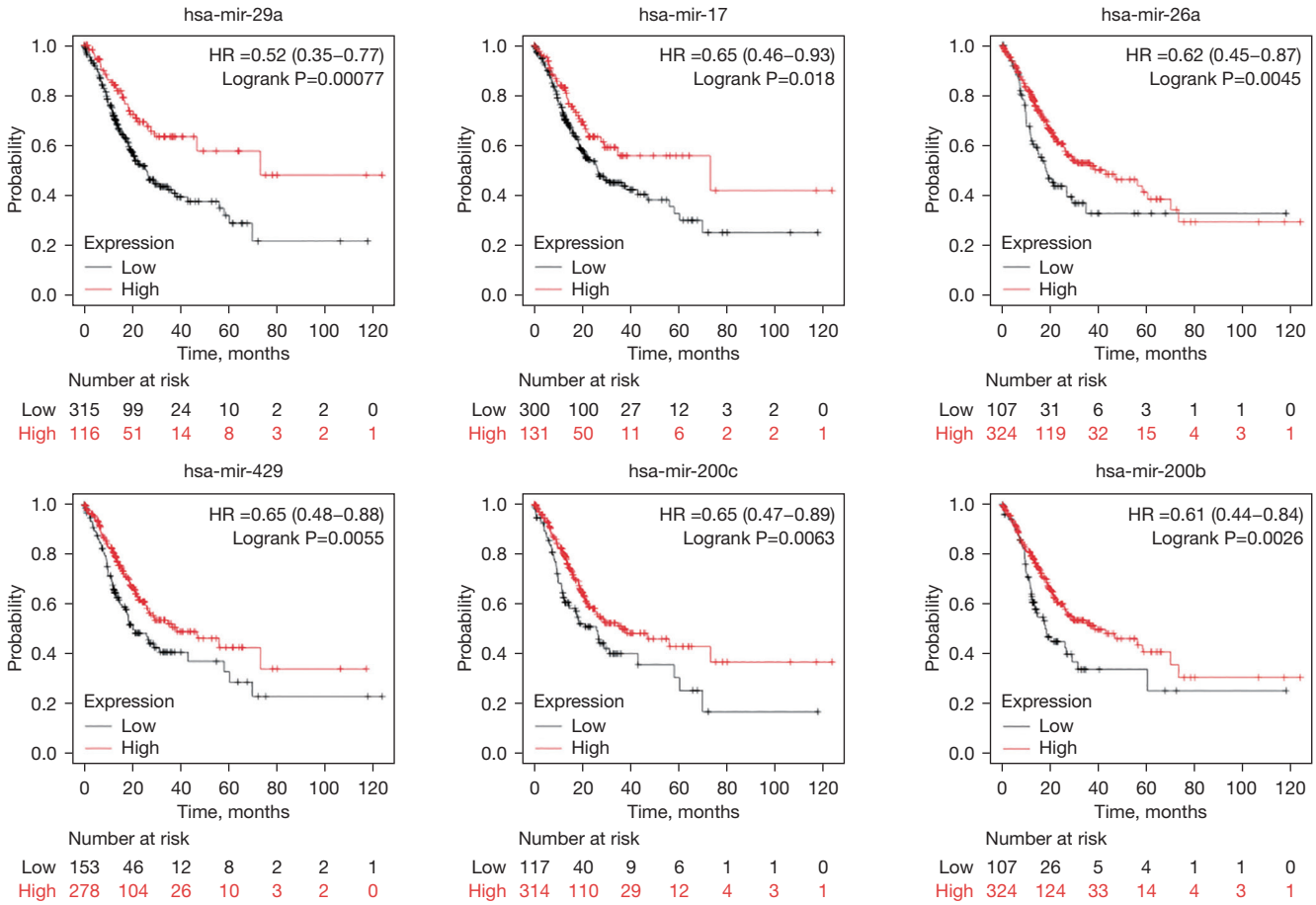


Figure 7 The survival analysis of key miRNAs (hsa-mir-29a-3p, hsa-mir-200c-3p, hsa-mir-429, hsa-mir-26a-5p, hsa-mir-17-5p and hsa-mir-200b). HR, hazard ratio; miRNA, microRNA.

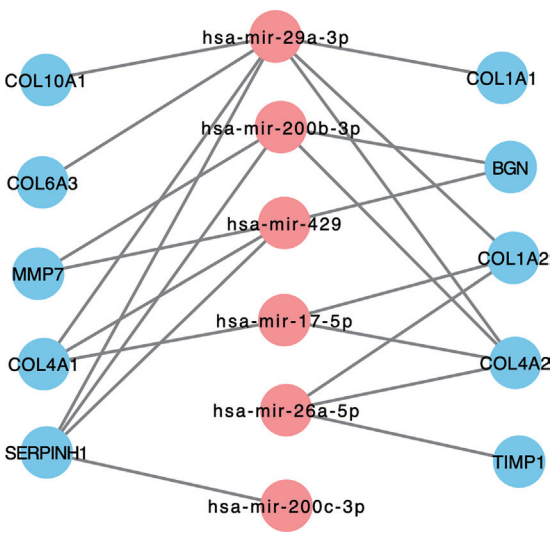


Figure 8 Construction of the miRNA-gene network using Cytoscape software. miRNA, microRNA.

Construction of the ceRNA regulatory network

Based on the previous prediction, there were 18 mRNA-miRNA pairs, 86 miRNA-lncRNA pairs, and 54 mRNA-lncRNA pairs. According to the ceRNA hypothesis, miRNAs have an opposite co-expression relationship with mRNAs and lncRNAs, whereas lncRNAs have a positive co-expression relationship with mRNAs. We assessed the correlation between all RNA interaction pairs using the ENCORI database, and found that 13 out of 18 mRNA-miRNA pairs, 18 out of 86 miRNA-lncRNA pairs, and 18 out of 54 mRNA-lncRNA pairs were consistent with the ceRNA rule (*Table 3*). Finally, 7 mRNAs (BGN, COL1A1, COL4A1, COL4A2, COL6A3, SERPINH1, MMP7) , 3 miRNAs (hsa-mir-29a-3p, hsa-mir-200b-3p, hsa-mir-429) and 5 lncRNAs (MSC-AS1, ZEB1-AS1, LINC01303, RRN3P2, CCDC144NL-AS1) constructed 24 mRNA-miRNA-lncRNA triple regulatory ceRNA network as a

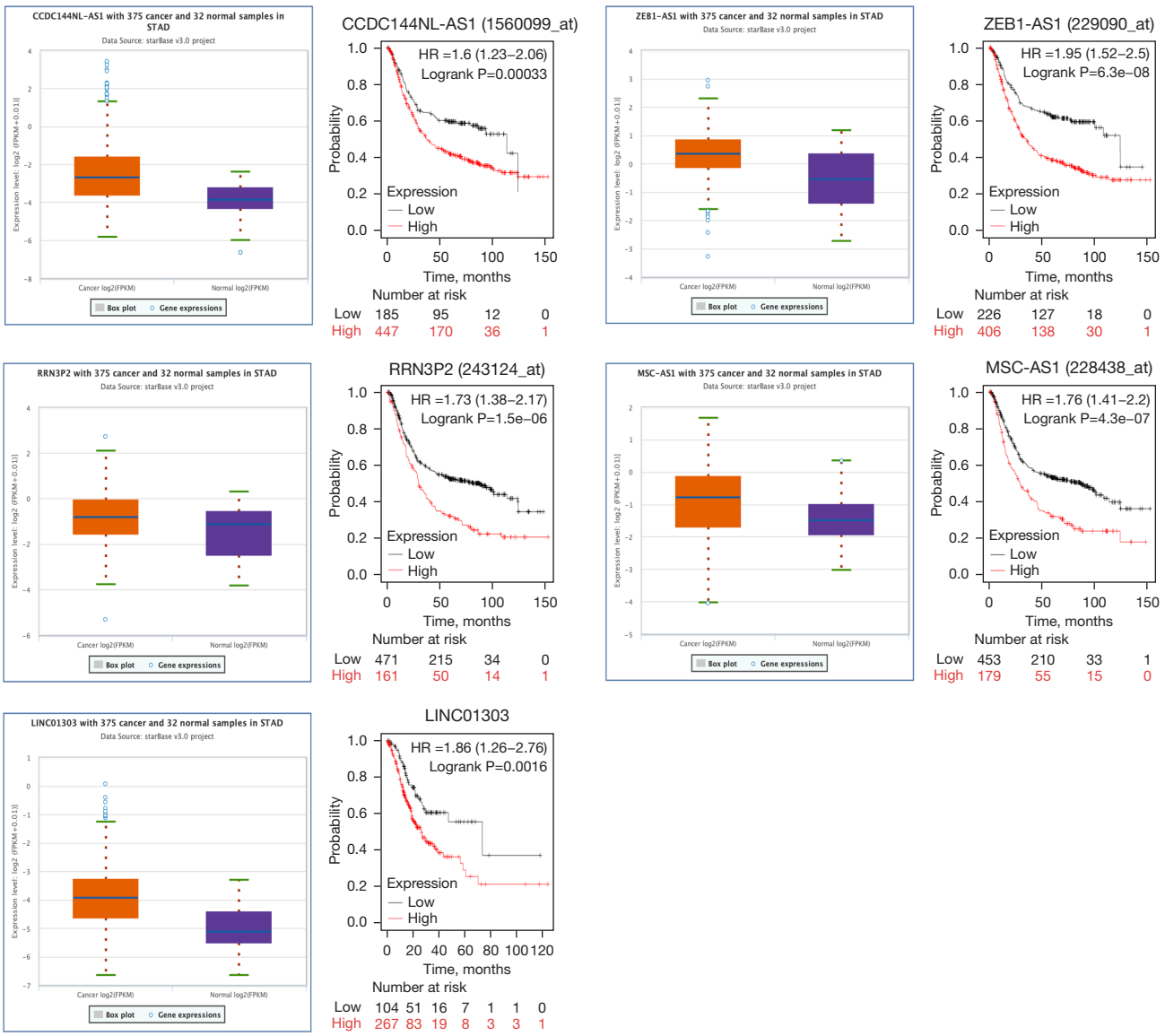


Figure 9 The differential expression and survival analysis of upstream lncRNAs (CCDC144NL-AS1, ZEB1-AS1, RRN3P2, MSC-AS1, and LINC01303). HR, hazard ratio; lncRNAs, long noncoding RNAs.

potential regulatory network for gastric carcinoma (*Table 4*). Cytoscape was used to visualize the ceRNet (*Figure 10*).

Discussion

Gastric adenocarcinoma has a high incidence and poor prognosis. Its mechanism, suggested by some studies as a latent correlation between the disease and ncRNAs (15) or miRNAs (16), requires further in-depth exploration. Additionally, the effects of ceRNAs in the context of genetic regulatory networks are still poorly understood. Identification and analysis of ceRNA network may reveal the potential pathogenesis of gastric adenocarcinoma at the molecule level and help facilitate gastric adenocarcinoma diagnosis and treatment.

In our study, the intersection of DEGs was taken from three GEO datasets (GSE54129, GSE118916, and GSE13861) for analysis, with a total of 180 DEGs identified. Results of the KEGG pathway enrichment analysis demonstrated that ECM-receptor interaction, protein

Table 3 The correlations between mRNAs, miRNAs, and lncRNAs according to the ENCORI database

mRNA	miRNA	lncRNA	R	P
MMP7	hsa-mir-429		–0.125	1.56E-02
BGN	hsa-mir-429		–0.344	9.18E-12
COL4A1	hsa-mir-429		–0.277	5.78E-08
COL1A1	hsa-mir-29a-3p		–0.159	2.04E-03
COL6A3	hsa-mir-29a-3p		–0.239	3.17E-06
COL1A2	hsa-mir-26a-5p		–0.128	1.38E-02
SERPINH1	hsa-mir-200b-3p		–0.118	2.23E-02
COL4A2	hsa-mir-200b-3p		–0.410	1.62E-16
MMP7	hsa-mir-200b-3p		–0.107	3.91E-02
BGN	hsa-mir-200b-3p		–0.366	3.16E-13
COL4A2	hsa-mir-17-5p		–0.349	4.55E-12
COL1A2	hsa-mir-17-5p		–0.292	9.41E-09
COL4A1	hsa-mir-17-5p		–0.192	1.91E-04
	hsa-mir-29a-3p	CCDC144NL-AS1	–0.253	7.47E-07
	hsa-mir-29a-3p	HCG18	–0.274	8.25E-08
	hsa-mir-29a-3p	LINC00638	–0.173	7.87E-04
	hsa-mir-17-5p	MIRLET7BHG	–0.112	3.08E-02
	hsa-mir-17-5p	PSMD6-AS2	–0.128	1.32E-02
	hsa-mir-200b-3p	MSC-AS1	–0.428	5.46E-18
	hsa-mir-200c-3p	MSC-AS1	–0.494	2.73E-24
	hsa-mir-429	MSC-AS1	–0.432	2.39E-18
	hsa-mir-200b-3p	ZEB1-AS1	–0.205	6.87E-05
	hsa-mir-200c-3p	ZEB1-AS1	–0.223	1.40E-05
	hsa-mir-429	ZEB1-AS1	–0.237	3.92E-06
	hsa-mir-29a-3p	KCNQ1OT1	–0.157	2.39E-03
	hsa-mir-200b-3p	LINC01303	–0.257	5.25E-07
	hsa-mir-200c-3p	LINC01303	–0.227	9.89E-06
	hsa-mir-429	LINC01303	–0.184	3.55E-04
	hsa-mir-200b-3p	RRN3P2	–0.219	2.12E-05
	hsa-mir-200c-3p	RRN3P2	–0.297	5.44E-09
	hsa-mir-429	RRN3P2	–0.303	2.44E-09
BGN		MSC-AS1	0.741	2.05E-66
BGN		ZEB1-AS1	0.252	7.67E-07
BGN		LINC01303	0.323	1.54E-10
BGN		RRN3P2	0.197	1.23E-04

Table 3 (*continued*)

Table 3 (*continued*)

mRNA	miRNA	lncRNA	R	P
COL1A1		CCDC144NL-AS1	0.281	3.29E-08
COL4A1		MSC-AS1	0.483	2.54E-23
COL4A1		LINC01303	0.273	7.63E-08
COL4A1		RRN3P2	0.181	4.39E-04
COL4A2		LINC00997	0.139	7.19E-03
COL4A2		MSC-AS1	0.546	1.50E-30
COL4A2		LINC01303	0.242	2.11E-06
COL4A2		RRN3P2	0.225	1.05E-05
COL6A3		CCDC144NL-AS1	0.277	4.92E-08
MMP7		MSC-AS1	0.152	3.09E-03
MMP7		LINC01303	0.165	1.36E-03
MMP7		RRN3P2	0.16	1.92E-03
SERPINH1		MSC-AS1	0.295	6.01E-09
SERPINH1		LINC01303	0.159	2.02E-03

mRNAs, messenger RNA; miRNA, microRNA; lncRNAs, long noncoding RNAs.

Table 4 Components of ceRNAs

LncRNA	miRNA	mRNA
CCDC144NL-AS1	hsa-mir-29a-3p	COL1A1
CCDC144NL-AS1	hsa-mir-29a-3p	COL6A3
MSC-AS1	hsa-mir-429	BGN
LINC01303	hsa-mir-429	BGN
RRN3P2	hsa-mir-429	BGN
ZEB1-AS1	hsa-mir-429	BGN
LINC01303	hsa-mir-429	MMP7
MSC-AS1	hsa-mir-429	MMP7
RRN3P2	hsa-mir-429	MMP7
LINC01303	hsa-mir-429	COL4A1
MSC-AS1	hsa-mir-429	COL4A1
RRN3P2	hsa-mir-429	COL4A1
MSC-AS1	hsa-mir-200b-3p	BGN
ZEB1-AS1	hsa-mir-200b-3p	BGN
LINC01303	hsa-mir-200b-3p	BGN
RRN3P2	hsa-mir-200b-3p	BGN

Table 4 (*continued*)

LncRNA	miRNA	mRNA
LINC01303	hsa-mir-200b-3p	MMP7
MSC-AS1	hsa-mir-200b-3p	MMP7
RRN3P2	hsa-mir-200b-3p	MMP7
LINC01303	hsa-mir-200b-3p	COL4A2
MSC-AS1	hsa-mir-200b-3p	COL4A2
RRN3P2	hsa-mir-200b-3p	COL4A2
LINC01303	hsa-mir-200b-3p	SERPINH1
MSC-AS1	hsa-mir-200b-3p	SERPINH1

ceRNAs, competing endogenous RNAs; lncRNAs, long noncoding RNAs; miRNA, microRNA; mRNAs, messenger RNA.

digestion, focal adhesion, and absorption were the most significant factors. Moreover, the GO enrichment analysis identified ECM organization, endoplasmic reticulum lumen, collagen catabolic process, and extracellular region as the most significant factors. In addition, we identified the top 20 hub genes by constructing a PPI, including 19 upregulated genes and one downregulated gene.

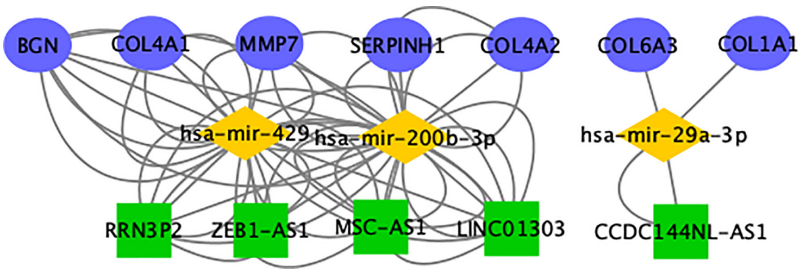


Figure 10 ceRNA networks by Cytoscape. ceRNA, competing endogenous RNA.

Interestingly, similar results for hub genes were obtained by the GO and KEGG enrichments, which indicated a close association between gastric adenocarcinoma and adhesion and ECM-related molecular mechanisms (17). Notably, the disruption of intercellular adhesion and the degradation of ECM have been found to promote the overactivated invasiveness and metastases of cancer cells (18).

Recently, a growing body of research has demonstrated the complex networks between miRNAs and lncRNAs. Based on the ceRNA hypothesis (9), lncRNAs act as important post-transcriptional regulators of downstream gene expressions through miRNA mediation and take part in the pathological processes of cancer. For instance, Wang *et al.* reported that *miR-1290* promotes the proliferation and invasion of chordoma (19), Liu *et al.* found that the *GATA3-AS1/miR-30b-5p/Tex10* axis regulates tumorigenesis of pancreatic cancer (20), Zhou *et al.* identified that *SNHG4/miR-590-3p/CDK1* modulates the colorectal cancer cell cycle and cell proliferation (21), and Chen *et al.* reported that *HIF1A-AS2/miR-30a-5p/SOX4* accelerates the malignant phenotypes of renal carcinoma (22). Furthermore, ceRNA networks were also reported to play an important role in gastric adenocarcinoma. Yang *et al.* found that *LINC01133/miR-106a-3p/APC* exerts an impact on gastric adenocarcinoma migration (23), Zhang *et al.* demonstrated that lncRNA *MT1JP* competitively binds to *miR-92a-3p* and functions as a ceRNA to regulate *FBXW7* in gastric adenocarcinoma (24), and Chen *et al.* identified that the *LINC01234/miR-204-5p/CBFB* axis is crucial in the tumorigenesis of gastric adenocarcinoma (25).

Encouragingly, we constructed 24 mRNA-miRNA-lncRNA triple regulatory networks by stepwise reverse prediction from mRNA to lncRNA. Different from other prediction construction networks, we adjusted layer by layer and verified layer by layer based on ceRNA hypothesis and combined with prognostic and expression differences. Each RNA in the network is of essential prognostic value in gastric adenocarcinoma. Some studies proved that *miRNA-429* contributes to the development of multiple cancers. For example, Wang *et al.* found that *has-miR-429* inhibits proliferation through the NF-κB pathway and suppresses cell migration-mediated epithelial-mesenchymal transition (EMT) in esophageal squamous cell carcinoma (26). It has also been confirmed that the overexpression of *miRNA-429* inhibits proliferation and invasion in glioblastoma (27). Regarding reports on miRNA-29a-3p, Liao *et al.* stated that *BLACAT1/miR-29a-3p/DVL3* promotes prostate cancer cell proliferation, migration, and invasion (28); moreover, *miR-29a-3p* is considered to be associated with gastric cancer (29). In addition, it has been proved that the *NEAT1/miR-200b-3p/SMAD2* axis promotes melanoma progression (30), and *miR-200b-3p* regulates angiogenesis in hepatocellular carcinoma (31).

In addition, biglycan (*BGN*) is an important component of the ECM. Some studies have shown that solid tumors with a poor prognosis are often accompanied by upregulation of BGN (32-34). A great deal of research has shown that the ECM is a key regulator of cell and tissue function. Collagens are major components of the ECM. It has been reported that collagens impact the proliferation, invasion, initiation, metastasis, and therapy response in tumors (35). *TGFBI*, *MMP7*, and *SERPINH1* have been found to be involved in tumor progression (36-38).

Of note, previous studies have confirmed the key roles of *CCDC144NL-AS1*, *ZEB1-AS1*, *RRN3P2*, *MSC-AS1*, and *LINC01303* in cancer. For instance, He *et al.* (39) found that *CCDC144NL-AS1* promoted the oncogenicity of osteosarcoma, Ma *et al.* (40) reported that downregulation of *ZEB1-AS1* repressed cell proliferation, migration, and invasion in prostate cancer by mediating PI3K/AKT/mTOR signaling, Hu *et al.* (41) demonstrated that *MSC-AS1* activated the Wnt/β-catenin signaling pathway to modulate cell proliferation and migration in renal clear cell carcinoma, and Cao *et al.* (42) found that the *LINC01303/*

miR-101-3p/EZH2 axis promoted gastric cancer progression. Perhaps by modifying and adjusting these RNAs, it is possible to help in the treatment of tumors.

Inevitably, the current study had some limitations. First, our conclusions were mainly based on the ceRNA hypothesis, which requires future testing and verification. Second, the omission of significant potential information in the prediction of lncRNAs by combining the prognosis results with the hypothesis of ceRNA might have led to inaccurate results. Third, the analysis of the relationship between clinical characteristics and genes was absent.

Conclusions

In conclusion, the hub genes were associated with gastric adenocarcinoma, and a ceRNA network containing 24 mRNA-miRNA-lncRNAs was constructed based on the ceRNA hypothesis. Each component of the ceRNA network had a significant prognostic value in gastric adenocarcinoma and provides more evidence for future research on tumor markers and therapeutic targets in gastric adenocarcinoma.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1201/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-1201/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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