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Original Article

Identification of key miRNA-gene pairs in gastric cancer through integrated analysis of mRNA and miRNA microarray

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Abstract: Nowadays, the current bioinformatic methods have been increasingly applied in the field of oncological research. In this study, we expect a better understanding of the molecular mechanism of gastric cancer from the bioinformatic methods. By systematically addressing the differential expression of microRNAs (miRNAs) and mRNAs between gastric cancer specimens and normal gastric specimens with the application of bioinformatics tools, A total of 206 DEGs and 38 DEMs were identified. The Gene Ontology (GO) analysis of Annotation, Visualization and Integrated Discovery (DAVID) database revealed that the differentially expressed genes (DEGs) were significantly enriched in biological process, molecular function and cellular component, while Kyoto Encyclopedia of Genes and Genomes (KEGG) database showed DEGs were significantly enriched in 8 signal pathways. The miRNA-gene regulatory network was constructed based on 385 miRNA-gene (DEM-DEG) pairs, consisting of 35 miRNAs and 107 target genes. In the regulatory network, the top 5 up-regulated genes were Transmembrane Protease, Serine 11B (TMPRSS11B), regulator of G protein signaling 1 (RGS1), cysteine rich angiogenic inducer 61 (CYR61), inhibin subunit beta A (INHBA), syntrophin gamma 1 (SNTG1), and the top 5 down-regulated genes were tumor necrosis factor receptor superfamily, member 19 (TNFRSF19), pleckstrin homology domain containing B2 (PLEKHB2), Tax1 binding protein 3 (TAX1BP3), presenilin enhancer, gamma-secretase subunit (PSENEN), NME/NM23 nucleoside diphosphate kinase 3 (NME3). Based on the gastric cancer patient database from Kaplan-Meier Plotter tools, we found that 8 of 10 genes with most significant changes in the miRNA-gene regulatory network possessed a prognostic value for survival time of gastric cancer patients. Patients with higher level of RGS1, PLEKHB2, TAX1BP3 and PSENEN in gastric cancer had a longer survival time compared with the patients with lower level of these genes. On the contrary, patients with higher level of INHBA, SNTG1, TNFRSF19 and NME3 were found associated with a shorter survival time. In conclusion, our findings provided several potential targets regarding gastric cancer, which may result in a new strategy to treat gastric cancer from a system rather than a single-gene perspective.

Keywords: Gastric cancer, mRNA, miRNA, prognosis

Introduction

Gastric cancer is the fourth leading cause of cancer death worldwide, which has a high incidence in East Asia, South America, and Eastern Europe [1-4]. Although the high mortality of this malignant carcinoma has been more recognized, there still lacks effective detective tools and treatments for gastric cancer in the clinic. Currently, microarray-based approaches have been increasingly applied to identify miRNA and mRNA signatures, which promotes the development of cancer diagnosis and treatment [5-8]. We expected the genome-wide microRNAs (miRNAs) and mRNA expression profiling by microarray-based approaches providing a prospective way for gastric cancer detection and treatment.

For now, a mass of genes have been confirmed associated with the development and progression of gastric cancer, such as the mutation of phosphatase, tumor protein p53, vascular endothelial growth factor and so on [9-14]. miRNAs have gene regulatory functions, which could bind to the 3'-untranslated region of

their target mRNA, so as to inhibit translation or induce mRNA degradation. Many miRNAs could affect biological behavior of gastric cancer in vivo. For example, miRNA-454 could inhibit the cell proliferation and invasion of gastric cancer cells by targeting mitogen-activated protein kinase; miRNA-28 promotes cell proliferation and invasion of gastric cancer via the phosphatase and tensin homolog (PTEN)/phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) signaling pathway; miRNA-216a inhibits the metastasis of gastric cancer cells by targeting Janus kinase2 (JAK2)/Signal Transducer and Activator of Transcription3 (STAT3)-mediated epithelial-mesenchymal transition process [15-17]. Therefore, it is of great importance to elucidate the role of the miRNA-gene regulatory network in development and progression of gastric cancer. However, to date, there are only a few reports regarding the comprehensive regulatory network of miRNA-gene in gastric cancer.

In this study, we analyzed the profiles of mRNAs and miRNAs by comparing gastric cancer specimens with the normal gastric specimens to reveal the regulatory network between miRNAs and mRNAs. Our data may provide important information helping elucidate the molecular mechanisms of gastric cancer and contribute to a new treatment for gastric cancer from a systematic perspective.

Materials and methods

Microarray data

The miRNA expression dataset GSE78091 [18] and mRNA expression dataset GSE33651 [19] were downloaded from National Center of Biotechnology Information (NCBI) Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) database [20]. The miRNA dataset GSE78091 contained 6 specimens, including 3 gastric cancer specimens and 3 adjacent normal gastric specimens. The miRNA expression profile was detected via the miRCURY LNA microRNA Array GPL21439 platform. The mRNA dataset GSE33651 contained 52 specimens, including 40 gastric cancer specimens and 12 normal gastric specimens. The microarray data we used was detected based on GPL2895 GE Healthcare/Amersham Biosciences CodeLink Human Whole Genome Bioarray platform.

Identification of differentially expressed miRNAs (DEMs) and genes (DEGs)

GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) is an interactive online tool of GEO that permits users to compare two or more groups of samples in a GEO Series and it can analyze most GEO series with gene symbol [21]. For the miRNA dataset, DEMs between gastric cancer specimens and adjacent normal gastric specimens were identified via the GEO2R. The screening threshold was false discovery rate (FDR) corrected $P<0.01$ and $|\log2 (\text{fold-change (FC)})|>2$. For the mRNA dataset, DEGs were also identified via the GEO2R. The DEGs were identified according to the criteria of FDR corrected $P<0.01$ and $|\log2FC|>2$.

Gene ontology (GO) terms enrichment analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/) was a widely used web-based tool for genomic functional annotations [22]. In the present study, GO was performed for enrichment analysis on DEGs via DAVID [23]. A $P< 0.05$ was considered as a significant change and the pie chart was drawn for all the changes with the most statistical significance.

Pathway enrichment analysis

Target pathways for DEGs were predicted by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis via DAVID [24]. All pathways showing $P<0.05$ were considered significantly enriched between the compared groups.

Screening of DEGs associated DEMs

The DEGs associated miRNAs, as well as the miRNA-gene pairs, were identified based on the analysis from miRWalk 3.0 platform (http://mirwalk.umm.uni-heidelberg.de/) [25].

Construction of the miRNA-gene regulatory network

The overlaps between the DEMs and the DEGs associated miRNAs were selected. The miRNA-gene regulatory network was constructed based on the DEM-DEG pairs and then visualized via Cytoscape software 3.4.0 (http://www.cytoscape.org) [26].

Table 1. Characteristics of mRNA and miRNA expression profiling of gastric cancer specimens with normal gastric specimens

GEO ID	Platform	Sample (cases: control)	Country	Year	Reference
GSE78091	GPL21439 miRCURY LNA microRNA Array	3:3	China	2016	X. Xu et al. [18]
GSE33651	GPL2895GE Healthcare/Amersham Biosciences CodeLink Human Whole Genome Bioarray	40:12	South Korea	2011	J.H. Park et al. [19]

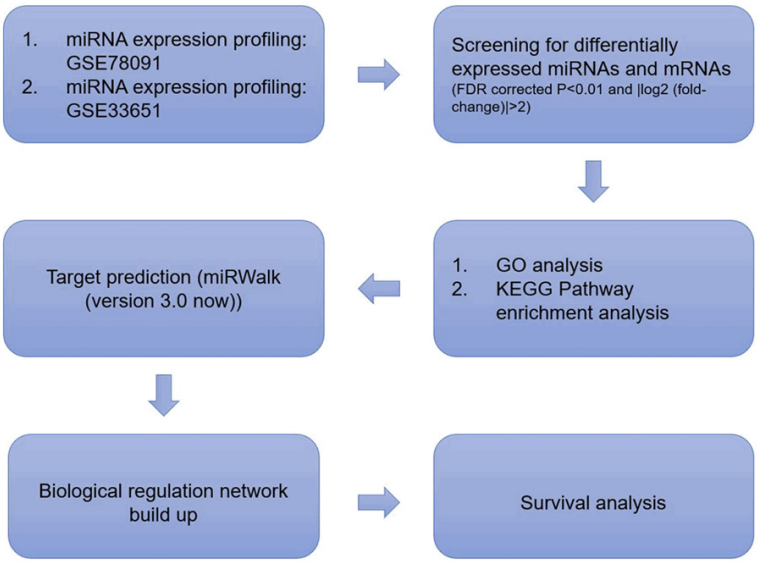

Figure 1. Workflow of the study design.

Gastric cancer data set analysis

Kaplan Meier-plotter (KM plotter, http://kmplot.com/analysis/) could assess the effect of 54,675 genes on survival using 10,461 cancer samples, including 5,143 breast, 1,816 ovarian, 2,437 lung and 1,065 gastric cancer patients with a mean follow-up of 69, 40, 49 and 33 months [27]. The mRNA data of 1,065 patients was utilized to verify our results from the GEO dataset. The DEGs, which were top 5 upregulated and top 5 downregulated in the miRNA-gene regulatory network, were selected to verify their correlation with survival time of gastric cancer patients. The hazard ratio (HR) with 95% confidence intervals and log rank *P* value were calculated. The log-rank P<0.05 was considered statistically significant.

Results

Differential expression of miRNAs and mRNAs in gastric cancer

In this study, we investigated the differentially expressed miRNA and mRNA expression profiles between gastric cancer specimens and normal gastric specimens based on GEO datasets, see **Table 1**. The study design was presented in **Figure 1**. In total, 38 miRNAs, with a threshold of P<0.01 and |log2FC|>2 were considered as significantly differentially expressed, all of which were up-regulated. The whole DEMs were listed in **Table 2**. A total of 206 mRNAs were identified as being differentially expressed between gastric cancer specimens and normal gastric specimen under the threshold of P<0.01 and |log2FC|>2, see **Figure 2**, including 155 up-regulated genes and 51 down-regulated genes, see top 20 up-regulated genes and top 20 down-regulated genes in **Table 3**.

GO terms enrichment analysis and KEGG pathway enrichment analysis of DEGs

To further gain insights into the biological roles of DEGs in gastric cancer, we performed GO terms enrichment analysis for 206 DEGs. Firstly, in the analysis of biological processes, these genes were mainly enriched for of immune response, cell adhesion and negative regulation of angiogenesis, see **Figure 3A**. Secondly, in the analysis of molecular function, these genes were enriched for heparin binding, integrin binding, extracellular matrix structural constituent, and CXCR chemokine receptor binding, see **Figure 3B**. Thirdly, in cell component analysis, these genes were mainly enriched for extracellular exosome, extracellular space, and cytosol, **Figure 3C**. We also performed KEGG pathway enrichment analysis to predict the signaling pathways mediated by these 206 DEGs, which revealed that 21 DEGs

Table 2. List of differentially regulated miRNAs in GSE78091

Up-regulated			Up-regulated		
miRNA_ID_LIST	logFC	adj. *P*. Val	miRNA_ID_LIST	logFC	adj. *P*. Val
kshv-miR-K12-3-3p	2.054444	0.00967	hsa-miR-3140-3p	2.731037	0.00774
hsa-miR-4801	2.092554	0.00755	hsa-miR-5003-5p	2.747225	0.00801
hsa-miR-222-5p	2.150165	0.00801	hsa-miR-499a-3p	2.761745	0.00582
hsa-miR-548v	2.181981	0.00774	hsa-miR-3671	2.776101	0.00842
hsa-miR-148b-5p	2.185893	0.00801	hsa-miR-4714-3p	2.786759	0.00648
hsa-miR-4517	2.235501	0.00934	hsa-miR-4715-5p	2.924227	0.0066
hsa-miR-3664-3p	2.302251	0.00801	hsa-miR-494-3p	3.04274	0.00801
hsa-miR-93-3p	2.373845	0.0076	hsa-miR-3607-5p	3.074306	0.00582
hsa-miR-4699-5p	2.467019	0.00663	hsa-miR-214-5p	3.07533	0.00755
hsa-miR-5008-5p	2.516168	0.00582	hsa-miR-548au-3p	3.179879	0.00648
hsa-miR-342-3p	2.578716	0.00728	hsa-miR-5692b/hsa-miR-5692c	3.300774	0.00582
hsa-miR-3074-3p	2.58266	0.00582	hsa-miR-4774-5p	3.421769	0.00582
hsa-miR-3663-3p	2.60645	0.00801	hsa-miR-3910	3.57701	0.00582
hsa-miR-4776-5p	2.621787	0.00582	ebv-miR-BHRF1-2-5p	3.666725	0.00582
hsa-miR-323b-3p	2.621831	0.00774	hsa-miR-5000-3p	3.810626	0.00582
hsa-miR-4691-5p	2.628232	0.00671	hsa-miR-4418	3.89057	0.00582
hsa-miR-3132	2.668607	0.00648	hsa-miR-187-5p	4.026276	0.00801
ebv-miR-BART22	2.699331	0.00801	hsa-miR-5579-3p	4.032345	0.00582
hsa-miR-34c-3p	2.721863	0.00582	hsa-miR-4474-5p	4.390643	0.00582

Note: FC: Fold change.

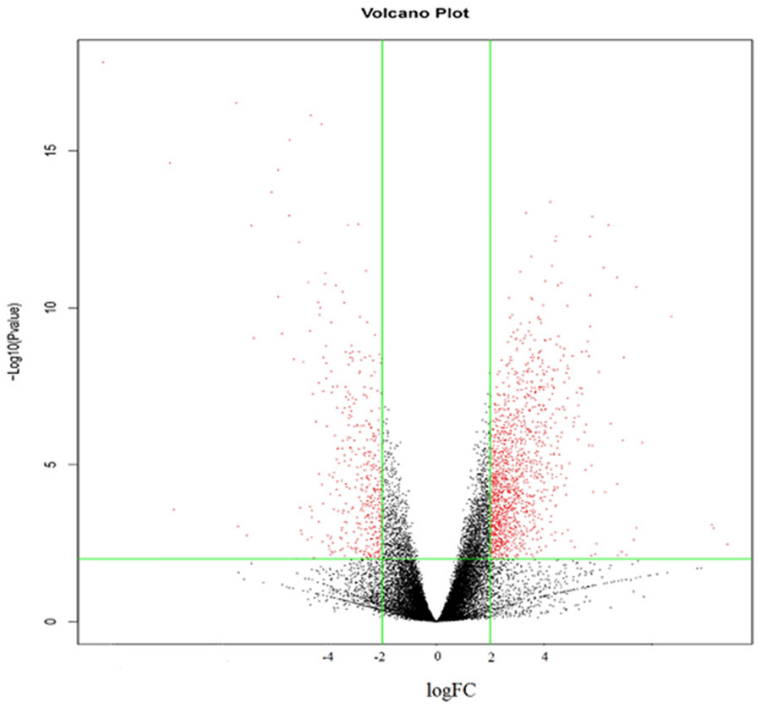

Figure 2. Differentially expressed genes (DEGs) between gastric cancer specimens and normal gastric specimen. Microarray data from GSE33651 were analyzed by GEO2R to screen the most significantly changed mRNAs between gastric cancer specimens and normal gastric specimens. A total of 206 mRNAs, for which P<0.01 and |logFC|>2 were selected and considered as the most significantly differentially expressed mRNAs.

were significantly enriched in 8 pathways. They were extracellular matrix (ECM)-receptor interaction, amoebiasis, focal adhesion, systemic lupus erythematosus, alcoholism, protein digestion and absorption, p53 signaling pathway and pertussis, see **Figure 3D** and **Table 4**.

The miRNA-Gene regulatory network in gastric cancer

Among the 206 DEGs, 159 DEGs could be found with corresponding miRNAs via miRWalk 3.0 platform. Using the DEGs to detect the miRNAs, the miRWalk platform identified 2491 miRNAs corresponding to DEGs, including 35 overlapping miRNAs with DEMs. Based on the overlapping miRNAs, a total of 385 miRNA-gene (DEM-DEG) regulatory pairs were identified, including 35 DEMs and 107

Table 3. List of top 20 up-regulated and top 20 down-regulated DEGs in GSE33651

Up-regulated			Down-regulated		
Gene.symbol	adj. *P*. Val	logFC	Gene.symbol	adj. *P*. Val	logFC
TPM2///SETDB1	1.11E-08	3.0131642	MUC5AC	1.52E-18	-6.1737005
IGFBP7///TLCD1	5.24E-12	3.0976027	HIST1H4C	2.41E-15	-4.9362316
LCN2	7.47E-05	3.126299	LINC00607	2.69E-04	-4.8650322
COL4A1	2.30E-13	3.1867801	MT1E	2.97E-17	-3.7052832
CXCL2///CXCL2	4.96E-07	3.2312427	CKM	9.30E-04	-3.6714809
IGFBP5	1.07E-11	3.3471907	TNFRSF19	1.77E-03	-3.5093672
CXCL8	4.03E-05	3.3528075	SNHG5	2.43E-13	-3.4262254
CLRN3	7.65E-03	3.3624408	HIST1H4D	9.26E-10	-3.3827768
LOC100506937	5.96E-03	3.431249	SMG1P5	2.08E-14	-3.0511987
C3///ADH1B	1.66E-06	3.4564663	MIF	4.06E-15	-2.9295263
FCGR3B///FCGR3A///FCGR3B	3.79E-09	3.4759273	PLEKHB2	4.38E-11	-2.9285128
SNTG1	7.49E-03	3.5194497	TAX1BP3	6.66E-10	-2.8569623
INHBA	2.43E-03	3.6596828	FICD	1.16E-13	-2.727911
RGS1	2.15E-11	3.7045805	PSENEN	4.49E-16	-2.7148007
CST13P	1.03E-03	3.7083948	NME3	4.33E-09	-2.6402314
CYR61	1.97E-06	3.8153501	PPP1R15B	9.98E-03	-2.5830217
RGS1	1.90E-10	4.3497434	CBX5	8.21E-13	-2.5424735
ATG16L2	8.05E-04	5.1042386	SST	2.32E-04	-2.5222501
TMPRSS11B	1.04E-03	5.1351031	CHD9	1.27E-03	-2.5074243
TMPRSS11A	3.37E-03	5.3930129	MSH4	1.60E-03	-2.4931796

Note: FC: Fold change.

DEGs, see **Table 5**, and the miRNA-gene regulatory network was constructed by the Cytoscape software, see **Figure 4**. In the constructed miRNA-gene regulatory network, 5 mRNAs [chromobox 5 (CBX5), calmodulin 1 (CALM1), potassium channel modulatory factor 1 (KCMF1), autophagy cargo receptor (NBR1) and prostaglandin I^2 synthase (PTGIS)] were found having a relative higher connectivity with miRNA and connected with 10 or more than 10 miRNAs, they were connected with 12, 11, 11, 10, 10 miRNAs respectively. Eight miRNAs, hsa-miR-5008-5p, hsa-miR-4715-5p, hsa-miR-3140-3p, and hsa-miR-3132, hsa-miR-222-5p, hsa-miR-3074-3p, hsa-miR-4776-5p, hsa-miR-93-p were found having a relative higher associations with mRNAs and connected with 20 or more than 20 RNAs, regulating 24, 24, 24, 23, 21, 20, 20, 20 mRNAs respectively.

miRNA-gene-pathway relationship in gastric cancer

Based on the constructed miRNA-gene regulatory network and the KEGG analysis for DEGs, the common RNAs between these two were screened out. The miRNA-gene-pathway relationship in gastric cancer was elucidated via the connection of common RNAs, see **Table 6**.

Verification of mRNA expression levels in an independent database containing 1,065 gastric cancer patients from Kaplan-Meier Plotter tools

It is reasonable to confirm the above findings of GEO data in other independent datasets. The top 5 up-regulated DEGs (Transmembrane Protease, Serine 11B (TMPRSS11B, logFC= 5.14), regulator of G protein signaling 1 (RGS1, logFC=4.35), cysteine rich angiogenic inducer 61 (CYR61, logFC=3.82), inhibin subunit beta A (INHBA, logFC=3.66), syntrophin gamma 1 (SNTG1, logFC=3.52)) and top 5 down-regulated DEGs (tumor necrosis factor receptor superfamily, member 19 (TNFRSF19, logFC=-3.51), pleckstrin homology domain containing B2 (PLEKHB2, logFC=-2.93), Tax1 binding protein 3 (TAX1BP3, logFC=-2.86), presenilin enhancer, gamma-secretase subunit (PSENEN, logFC=-2.71), NME/NM23 nucleoside diphos-

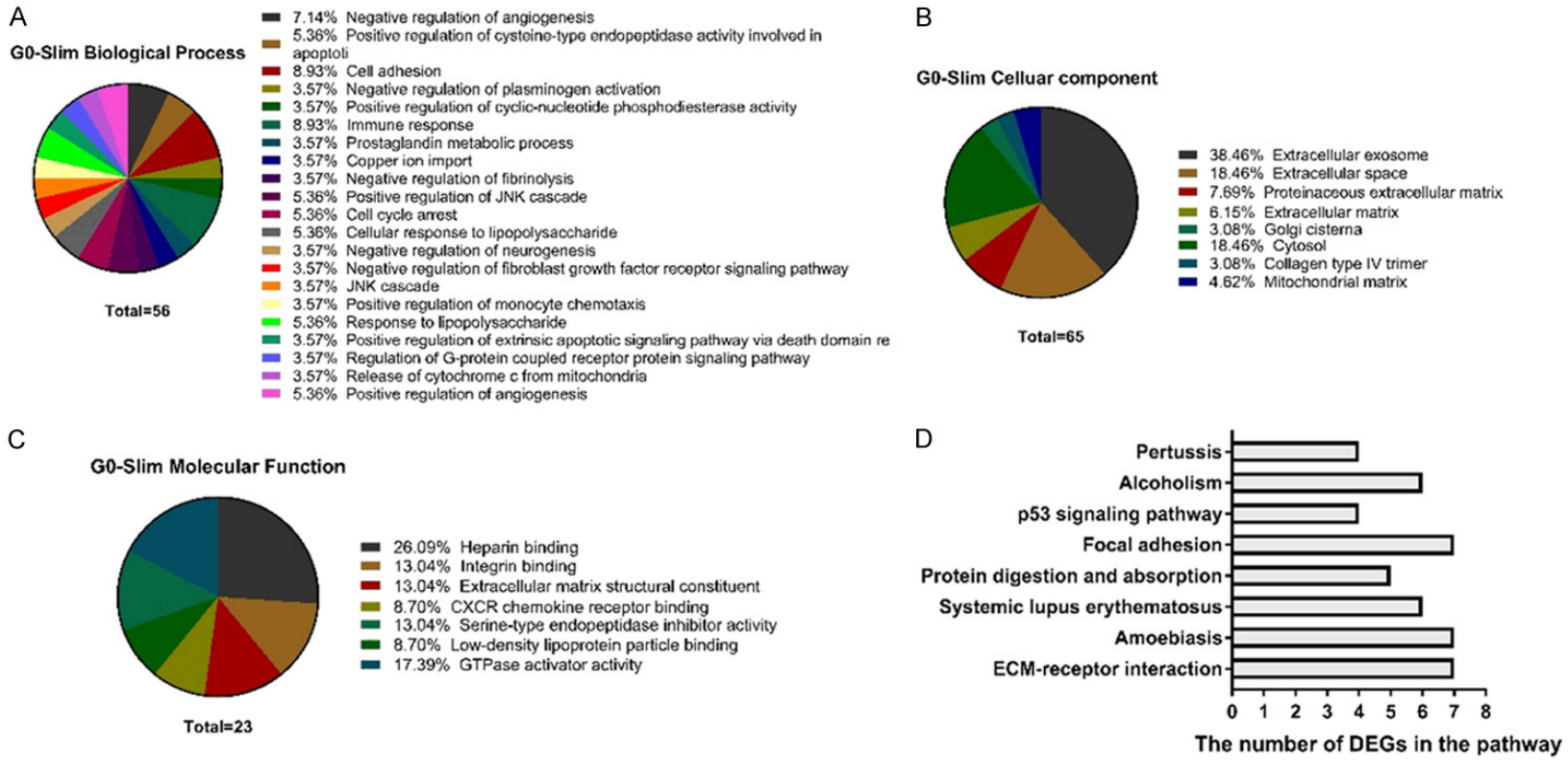

Figure 3. The significantly enriched functional annotation of DEGs. Gene Ontology (GO) enrichment analysis was performed to determine the function of the DEGs in gastric cancer. Biological processes (A), cellular components (B), and molecular functions (C) of the DEGs. (D) Bar graph of the significantly enriched signaling pathways for DEGs.

Table 4. KEGG pathway analysis of differentially expressed genes associated with gastric cancer

Term	Count	%	*P* Value	Genes	FDR
hsa04512: ECM-receptor interaction	7	0.029	2.90E-04	COL4A2, COL4A1, COL1A2, COL1A1, THBS1, COL5A1, FN1	0.346683487
hsa05146: Amoebiasis	7	0.029	8.37E-04	COL4A2, COL4A1, COL1A2, CXCL8, COL1A1, COL5A1, FN1	0.997222076
hsa05322: Systemic lupus erythematosus	6	0.025	0.013335949	HIST1H4L, HLA-DRB1, FCGR1A, HIST1H4C, HIST1H4D, HIST3H3	14.84457736
hsa04974: Protein digestion and absorption	5	0.021	0.013718549	COL4A2, COL4A1, COL1A2, COL1A1, COL5A1	15.23896471
hsa04510: Focal adhesion	7	0.029	0.021197208	COL4A2, COL4A1, COL1A2, COL1A1, THBS1, COL5A1, FN1	22.61965164
hsa04115: p53 signaling pathway	4	0.017	0.033479742	CYCS, SERPINE1, THBS1, GADD45B	33.4742564
hsa05034: Alcoholism	6	0.025	0.03864605	HIST1H4L, GNB4, HIST1H4C, HIST1H4D, HIST3H3, CALM1	37.60782894
hsa05133: Pertussis	4	0.017	0.044476951	LY96, CXCL8, SERPING1, CALM1	41.98955616

Table 5. The total 385 pairs of miRNA and gene

Gene	miRNA	Gene	miRNA
ADAMTS9	hsa-miR-3074-3p	NBR1	hsa-miR-4418
ADAMTS9	hsa-miR-5008-5p	NBR1	hsa-miR-4715-5p
ARID5B	hsa-miR-214-5p	NBR1	hsa-miR-5000-3p
ARID5B	hsa-miR-222-5p	NME3	hsa-miR-214-5p
ARID5B	hsa-miR-4714-3p	NOL7	hsa-miR-187-5p
ARID5B	hsa-miR-93-3p	NOL7	hsa-miR-222-5p
ARID5B	hsa-miR-4517	NOL7	hsa-miR-3663-3p
ARID5B	hsa-miR-4691-5p	PAIP2	hsa-miR-93-3p
ARID5B	hsa-miR-4776-5p	PAIP2	hsa-miR-4474-5p
ARID5B	hsa-miR-5008-5p	PAIP2	hsa-miR-4715-5p
ASAP2	hsa-miR-4776-5p	PCBP3	hsa-miR-5003-5p
ASAP2	hsa-miR-499a-3p	PCBP3	hsa-miR-4691-5p
ATF3	hsa-miR-148b-5p	PCBP3	hsa-miR-5008-5p
ATF3	hsa-miR-222-5p	PCM1	hsa-miR-214-5p
ATF3	hsa-miR-3140-3p	PCM1	hsa-miR-4774-5p
ATF3	hsa-miR-4418	PDCD5	hsa-miR-148b-5p
ATF3	hsa-miR-4691-5p	PDCD5	hsa-miR-3132
ATF3	hsa-miR-4715-5p	PDCD5	hsa-miR-4715-5p
ATP2A3	hsa-miR-214-5p	PDPN	hsa-miR-222-5p
ATP2A3	hsa-miR-3132	PDPN	hsa-miR-342-3p
ATP2A3	hsa-miR-3140-3p	PDPN	hsa-miR-5579-3p
ATP2A3	hsa-miR-3664-3p	PDPN	hsa-miR-4715-5p
BOD1L1	hsa-miR-3132	PDPN	hsa-miR-5008-5p
BOD1L1	hsa-miR-5008-5p	PLEKHB2	hsa-miR-222-5p
BRIX1	hsa-miR-3607-5p	PLEKHB2	hsa-miR-3074-3p
CALM1	hsa-miR-148b-5p	PLEKHB2	hsa-miR-5579-3p
CALM1	hsa-miR-222-5p	PLEKHB2	hsa-miR-4418
CALM1	hsa-miR-3074-3p	PLEKHB2	hsa-miR-4715-5p
CALM1	hsa-miR-323b-3p	PLEKHB2	hsa-miR-494-3p
CALM1	hsa-miR-34c-3p	PLEKHB2	hsa-miR-548v
CALM1	hsa-miR-3671	POLR2E	hsa-miR-3074-3p
CALM1	hsa-miR-93-3p	POLR2E	hsa-miR-4714-3p
CALM1	hsa-miR-4691-5p	POLR2E	hsa-miR-5003-5p
CALM1	hsa-miR-4776-5p	POLR2E	hsa-miR-4418
CALM1	hsa-miR-494-3p	POLR2E	hsa-miR-499a-3p
CALM1	hsa-miR-5008-5p	POLR2E	hsa-miR-5008-5p
CBX5	hsa-miR-222-5p	PPP1R15B	hsa-miR-187-5p
CBX5	hsa-miR-3074-3p	PPP1R15B	hsa-miR-342-3p
CBX5	hsa-miR-3132	PPP1R15B	hsa-miR-4801
CBX5	hsa-miR-3140-3p	PPP1R15B	hsa-miR-4418
CBX5	hsa-miR-5003-5p	PPP1R15B	hsa-miR-5000-3p
CBX5	hsa-miR-548au-3p	PPP1R7	hsa-miR-187-5p
CBX5	hsa-miR-4418	PPP1R7	hsa-miR-34c-3p
CBX5	hsa-miR-4715-5p	PPP1R7	hsa-miR-3664-3p
CBX5	hsa-miR-4774-5p	PPP1R7	hsa-miR-4714-3p
CBX5	hsa-miR-4776-5p	PPP1R7	hsa-miR-548au-3p
CBX5	hsa-miR-499a-3p	PPP1R7	hsa-miR-4774-5p

CBX5	hsa-miR-5008-5p	PSENEN	hsa-miR-3140-3p
CHD9	hsa-miR-5579-3p	PSMD14	hsa-miR-3132
CHD9	hsa-miR-4776-5p	PTGIS	hsa-miR-187-5p
CLRN3	hsa-miR-3140-3p	PTGIS	hsa-miR-222-5p
CLRN3	hsa-miR-34c-3p	PTGIS	hsa-miR-3132
CLRN3	hsa-miR-4714-3p	PTGIS	hsa-miR-3140-3p
COL16A1	hsa-miR-5003-5p	PTGIS	hsa-miR-3663-3p
COL16A1	hsa-miR-4715-5p	PTGIS	hsa-miR-3664-3p
COL4A1	hsa-miR-548v	PTGIS	hsa-miR-4714-3p
COL4A2	hsa-miR-4691-5p	PTGIS	hsa-miR-93-3p
COL4A2	hsa-miR-4715-5p	PTGIS	hsa-miR-494-3p
COL5A1	hsa-miR-4715-5p	PTGIS	hsa-miR-5000-3p
CTGF	hsa-miR-93-3p	RAB31	hsa-miR-3074-3p
CXCL10	hsa-miR-3140-3p	RAB31	hsa-miR-3132
CXCL10	hsa-miR-3663-3p	RAB31	hsa-miR-342-3p
CXCL10	hsa-miR-93-3p	RAB31	hsa-miR-4418
CXCL10	hsa-miR-4776-5p	RAB31	hsa-miR-4715-5p
CXCL10	hsa-miR-5008-5p	RACK1	hsa-miR-148b-5p
CXCL8	hsa-miR-34c-3p	RGS1	hsa-miR-3663-3p
CYCS	hsa-miR-214-5p	RGS1	hsa-miR-4714-3p
CYCS	hsa-miR-222-5p	S100A10	hsa-miR-3140-3p
CYCS	hsa-miR-3074-3p	SERPINE1	hsa-miR-214-5p
CYCS	hsa-miR-34c-3p	SERPINE1	hsa-miR-222-5p
CYCS	hsa-miR-3664-3p	SERPINF1	hsa-miR-5003-5p
CYCS	hsa-miR-93-3p	SERPING1	hsa-miR-3132
CYCS	hsa-miR-4715-5p	SERPING1	hsa-miR-4715-5p
CYCS	hsa-miR-5008-5p	SIK1	hsa-miR-3074-3p
CYCS	hsa-miR-548v	SIK1	hsa-miR-3132
CYR61	hsa-miR-3140-3p	SIK1	hsa-miR-548au-3p
CYR61	hsa-miR-34c-3p	SIK1	hsa-miR-4776-5p
CYR61	hsa-miR-548au-3p	SIK1	hsa-miR-5008-5p
CYR61	hsa-miR-4691-5p	SLC31A1	hsa-miR-214-5p
CYR61	hsa-miR-548v	SLC31A1	hsa-miR-3074-3p
DNAJA1	hsa-miR-3664-3p	SLC31A1	hsa-miR-3132
DNAJA1	hsa-miR-5579-3p	SLC31A1	hsa-miR-323b-3p
DUSP5	hsa-miR-187-5p	SLC31A1	hsa-miR-3671
DUSP5	hsa-miR-3140-3p	SLC31A1	hsa-miR-548au-3p
DUSP5	hsa-miR-323b-3p	SLC31A1	hsa-miR-4691-5p
ETS2	hsa-miR-34c-3p	SLC31A1	hsa-miR-4699-5p
ETS2	hsa-miR-4714-3p	SLC31A1	hsa-miR-5008-5p
ETS2	hsa-miR-5008-5p	SNTG1	hsa-miR-187-5p
FAM171B	hsa-miR-499a-3p	SNTG1	hsa-miR-3140-3p
FAP	hsa-miR-5579-3p	SNTG1	hsa-miR-3663-3p
FBXO32	hsa-miR-214-5p	SNTG1	hsa-miR-93-3p
FBXO32	hsa-miR-3074-3p	SNTG1	hsa-miR-4517
FBXO32	hsa-miR-3140-3p	SOD2	hsa-miR-187-5p
FBXO32	hsa-miR-548au-3p	SOD2	hsa-miR-214-5p
FBXO32	hsa-miR-93-3p	SOD2	hsa-miR-3074-3p
FBXO32	hsa-miR-4418	SOD2	hsa-miR-3607-5p

FBXO32	hsa-miR-4517	SOD2	hsa-miR-3663-3p
FBXO32	hsa-miR-5000-3p	SOD2	hsa-miR-4714-3p
FCGR1A	hsa-miR-3132	SOD2	hsa-miR-4418
FCGR1A	hsa-miR-5003-5p	SOD2	hsa-miR-4776-5p
FTSJ3	hsa-miR-3140-3p	SOD2	hsa-miR-5008-5p
FTSJ3	hsa-miR-93-3p	SORL1	hsa-miR-187-5p
GABARAP	hsa-miR-4714-3p	SORL1	hsa-miR-3074-3p
GADD45B	hsa-miR-214-5p	SORL1	hsa-miR-3664-3p
GADD45B	hsa-miR-4715-5p	SORL1	hsa-miR-4714-3p
GLIPR1	hsa-miR-222-5p	SOSTDC1	hsa-miR-93-3p
GLIPR1	hsa-miR-548au-3p	SOSTDC1	hsa-miR-4776-5p
GLIPR1	hsa-miR-4474-5p	SOSTDC1	hsa-miR-5000-3p
GLIPR1	hsa-miR-4776-5p	SOX21	hsa-miR-3132
GLIPR1	hsa-miR-5008-5p	SOX21	hsa-miR-4715-5p
GNB4	hsa-miR-4714-3p	SRGAP2C	hsa-miR-222-5p
GNB4	hsa-miR-4776-5p	SRGAP2C	hsa-miR-93-3p
GNB4	hsa-miR-499a-3p	SRGAP2C	hsa-miR-4474-5p
GNB4	hsa-miR-5008-5p	SRGAP2C	hsa-miR-494-3p
GPSM1	hsa-miR-3074-3p	SRP14	hsa-miR-4776-5p
GPSM1	hsa-miR-3664-3p	STEAP4	hsa-miR-214-5p
GPSM1	hsa-miR-548au-3p	STEAP4	hsa-miR-3074-3p
GPSM1	hsa-miR-93-3p	STEAP4	hsa-miR-3140-3p
GPSM1	hsa-miR-4774-5p	STEAP4	hsa-miR-4714-3p
GUCY1A3	hsa-miR-187-5p	STEAP4	hsa-miR-548au-3p
GUCY1A3	hsa-miR-222-5p	STEAP4	hsa-miR-4691-5p
GUCY1A3	hsa-miR-3140-3p	STEAP4	hsa-miR-5000-3p
GUCY1A3	hsa-miR-4714-3p	STEAP4	hsa-miR-5008-5p
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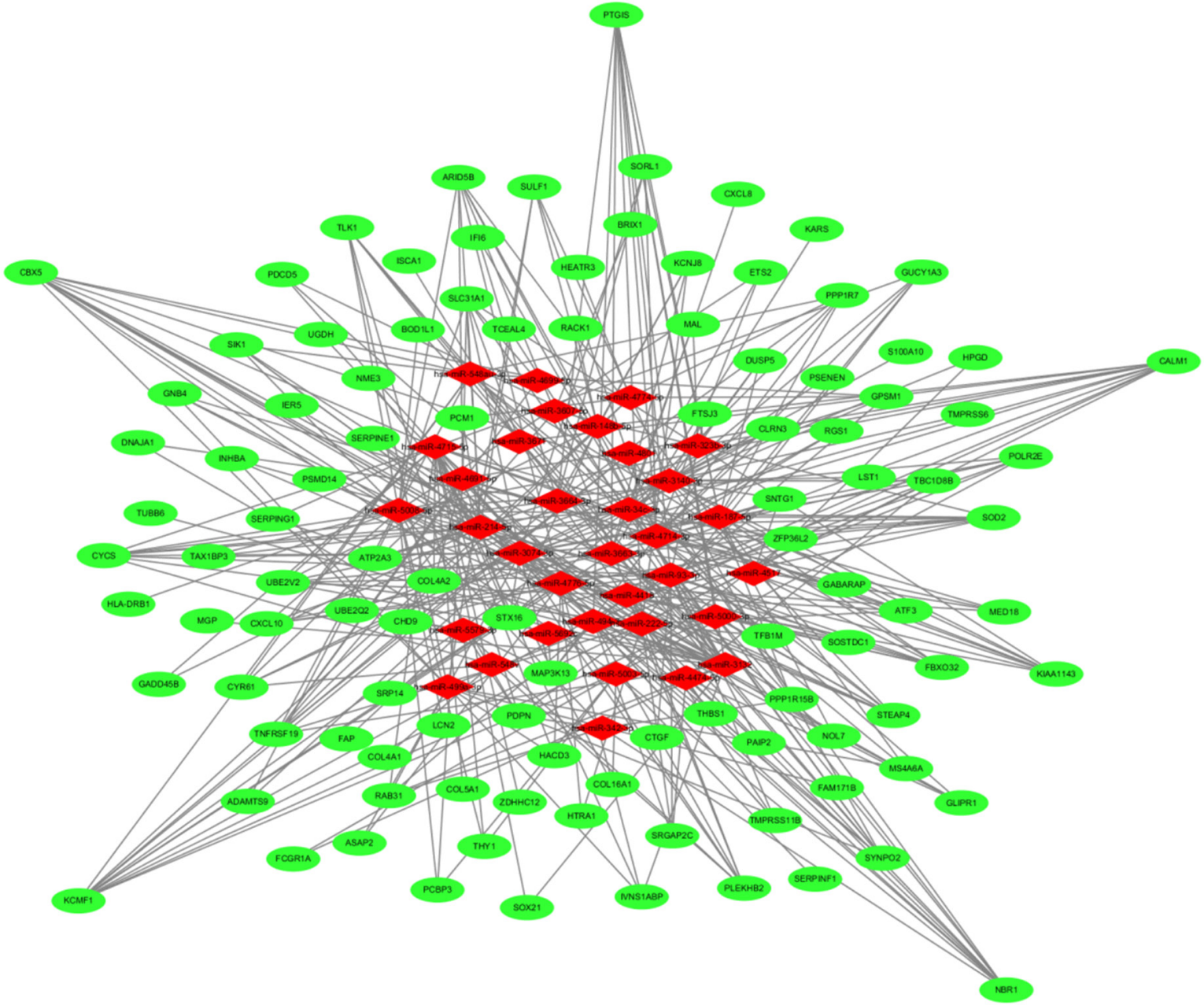
HACD3	hsa-miR-4715-5p	STX16	hsa-miR-3074-3p
HACD3	hsa-miR-4776-5p	STX16	hsa-miR-3132
HACD3	hsa-miR-499a-3p	STX16	hsa-miR-3140-3p
HEATR3	hsa-miR-4774-5p	STX16	hsa-miR-93-3p
HLA-DRB1	hsa-miR-5008-5p	STX16	hsa-miR-4691-5p
HPGD	hsa-miR-3140-3p	STX16	hsa-miR-4715-5p
HPGD	hsa-miR-4517	SULF1	hsa-miR-148b-5p
HTRA1	hsa-miR-222-5p	SULF1	hsa-miR-214-5p
HTRA1	hsa-miR-4776-5p	SULF1	hsa-miR-3140-3p
IER5	hsa-miR-3132	SULF1	hsa-miR-4714-3p
IFI6	hsa-miR-3132	SULF1	hsa-miR-499a-3p
IFI6	hsa-miR-3664-3p	SYNPO2	hsa-miR-214-5p
INHBA	hsa-miR-214-5p	SYNPO2	hsa-miR-3140-3p
INHBA	hsa-miR-3132	SYNPO2	hsa-miR-34c-3p
ISCA1	hsa-miR-3671	SYNPO2	hsa-miR-3607-5p
IVNS1ABP	hsa-miR-3132	SYNPO2	hsa-miR-3671
IVNS1ABP	hsa-miR-4715-5p	SYNPO2	hsa-miR-548au-3p
IVNS1ABP	hsa-miR-4776-5p	SYNPO2	hsa-miR-4776-5p
IVNS1ABP	hsa-miR-499a-3p	SYNPO2	hsa-miR-499a-3p
KARS	hsa-miR-3663-3p	TAX1BP3	hsa-miR-214-5p
KCMF1	hsa-miR-222-5p	TAX1BP3	hsa-miR-3074-3p

KCMF1	hsa-miR-3140-3p	TAX1BP3	hsa-miR-5008-5p
KCMF1	hsa-miR-3663-3p	TBC1D8B	hsa-miR-222-5p
KCMF1	hsa-miR-3664-3p	TBC1D8B	hsa-miR-3074-3p
KCMF1	hsa-miR-4801	TBC1D8B	hsa-miR-3140-3p
KCMF1	hsa-miR-5003-5p	TBC1D8B	hsa-miR-93-3p
KCMF1	hsa-miR-548au-3p	TBC1D8B	hsa-miR-4474-5p
KCMF1	hsa-miR-93-3p	TCEAL4	hsa-miR-3132
KCMF1	hsa-miR-4774-5p	TFB1M	hsa-miR-222-5p
KCMF1	hsa-miR-4776-5p	TFB1M	hsa-miR-4714-3p
KCMF1	hsa-miR-5000-3p	THBS1	hsa-miR-222-5p
KCNJ8	hsa-miR-148b-5p	THBS1	hsa-miR-4418
KCNJ8	hsa-miR-222-5p	THY1	hsa-miR-5003-5p
KCNJ8	hsa-miR-3663-3p	THY1	hsa-miR-5579-3p
KCNJ8	hsa-miR-3664-3p	THY1	hsa-miR-5008-5p
KIAA1143	hsa-miR-187-5p	TLK1	hsa-miR-3132
KIAA1143	hsa-miR-3140-3p	TLK1	hsa-miR-3663-3p
KIAA1143	hsa-miR-323b-3p	TLK1	hsa-miR-548au-3p
KIAA1143	hsa-miR-34c-3p	TLK1	hsa-miR-5579-3p
KIAA1143	hsa-miR-4691-5p	TLK1	hsa-miR-499a-3p
KIAA1143	hsa-miR-4715-5p	TLK1	hsa-miR-5008-5p
KIAA1143	hsa-miR-4776-5p	TMPRSS11B	hsa-miR-3671
KIAA1143	hsa-miR-5008-5p	TMPRSS11B	hsa-miR-93-3p
LCN2	hsa-miR-5008-5p	TMPRSS11B	hsa-miR-4691-5p
LST1	hsa-miR-3140-3p	TMPRSS11B	hsa-miR-4715-5p
MAL	hsa-miR-323b-3p	TMPRSS6	hsa-miR-3140-3p
MAL	hsa-miR-3663-3p	TNFRSF19	hsa-miR-222-5p
MAP3K13	hsa-miR-3074-3p	TNFRSF19	hsa-miR-3132
MAP3K13	hsa-miR-342-3p	TNFRSF19	hsa-miR-3607-5p
MAP3K13	hsa-miR-4714-3p	TNFRSF19	hsa-miR-4801
MAP3K13	hsa-miR-5579-3p	TNFRSF19	hsa-miR-548au-3p
MAP3K13	hsa-miR-5692c	TNFRSF19	hsa-miR-4691-5p
MAP3K13	hsa-miR-494-3p	TNFRSF19	hsa-miR-4699-5p
MAP3K13	hsa-miR-5008-5p	TNFRSF19	hsa-miR-4774-5p
MED18	hsa-miR-214-5p	TUBB6	hsa-miR-3132
MED18	hsa-miR-4714-3p	UBE2Q2	hsa-miR-3074-3p
MED18	hsa-miR-4715-5p	UBE2Q2	hsa-miR-5579-3p
MED18	hsa-miR-4776-5p	UBE2V2	hsa-miR-148b-5p
MGP	hsa-miR-3132	UBE2V2	hsa-miR-3132
MS4A6A	hsa-miR-187-5p	UBE2V2	hsa-miR-34c-3p
MS4A6A	hsa-miR-93-3p	UBE2V2	hsa-miR-3663-3p
MS4A6A	hsa-miR-499a-3p	UBE2V2	hsa-miR-93-3p
NBR1	hsa-miR-148b-5p	UBE2V2	hsa-miR-4418
NBR1	hsa-miR-214-5p	UBE2V2	hsa-miR-4715-5p
NBR1	hsa-miR-222-5p	UGDH	hsa-miR-4776-5p
NBR1	hsa-miR-3074-3p	ZDHHC12	hsa-miR-4715-5p
NBR1	hsa-miR-342-3p	ZFP36L2	hsa-miR-3664-3p
NBR1	hsa-miR-5003-5p	ZFP36L2	hsa-miR-5003-5p
NBR1	hsa-miR-93-3p		


PTGIS
SORL1
ARID5B
SULF1
CXCL8
BRIX1
KARS
TLK1
IFI6
ISCA1
HEATR3
KCNJ8
ETS2
GUCY1A3
CBX5
PDCD5
SLC31A1
PPP1R7
MAL
BOD1L1
TCEAL4
RACK1
UGDH
SIK1
DUSP5
S100A10
HPGD
CALM1
NME3
PSENEN
GNB4
IER5
GPSM1
PCM1
FTSJ3
TMPRSS6
CLRN3
RGS1
DNAJA1
SERPINE1
POLR2E
INHBA
LST1
TBC1D8B
PSMD14
SNTG1
TUBB6
SOD2
SERPING1
ZFP36L2
CYCS
TAX1BP3
ATP2A3
GABARAP
UBE2V2
COL4A2
HLA-DRB1
ATF3
MED18
UBE2Q2
MGP
CXCL10
CHD9
STX16
TFB1M
SOSTDC1
FBXO32
KIAA1143
GADD45B
CYR61
MAP3K13
SRP14
PPP1R15B
STEAP4
PDPN
THBS1
TNFRSF19
FAP
LCN2
CTGF
NOL7
PAIP2
COL4A1
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FAM171B
ADAMTS9
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HTRA1
TMPRSS11B
SRGAP2C
ASAP2
FCGR1A
THY1
SYNPO2
PCBP3
SERPINF1
KCMF1
SOX21
IVNS1ABP
PLEKHB2
NBR1

Figure 4. The regulatory network between DEMs and DEGs in gastric cancer specimens. The diamond nodes and oval nodes represent miRNAs and mRNA, respectively. The miRNA-gene regulatory network was constructed based on 385 miRNA-gene (DEM-DEG) pairs, consisting of 35 miRNAs and 107 target genes. Five mRNAs [CBX5, CALM1, KCMF1, NBR1 and PTGIS] were found having the highest connectivity with miRNAs, associated with 12, 11, 11, 10, 10 miRNAs, respectively.

Table 6. The miRNA-gene-pathway relationship

mRNA	miRNA	Pathway	mRNA	miRNA	Pathway
CALM1	hsa-miR-148b-5p	Alcoholism	COL5A1	hsa-miR-4715-5p	Focal adhesion
CALM1	hsa-miR-222-5p	Alcoholism	CXCL8	hsa-miR-34c-3p	Amoebiasis
CALM1	hsa-miR-3074-3p	Alcoholism	CXCL8	hsa-miR-34c-3p	Pertussis
CALM1	hsa-miR-323b-3p	Alcoholism	CYCS	hsa-miR-214-5p	p53 signaling pathway
CALM1	hsa-miR-34c-3p	Alcoholism	CYCS	hsa-miR-222-5p	p53 signaling pathway
CALM1	hsa-miR-3671	Alcoholism	CYCS	hsa-miR-3074-3p	p53 signaling pathway
CALM1	hsa-miR-93-3p	Alcoholism	CYCS	hsa-miR-34c-3p	p53 signaling pathway
CALM1	hsa-miR-4691-5p	Alcoholism	CYCS	hsa-miR-3664-3p	p53 signaling pathway
CALM1	hsa-miR-4776-5p	Alcoholism	CYCS	hsa-miR-93-3p	p53 signaling pathway
CALM1	hsa-miR-494-3p	Alcoholism	CYCS	hsa-miR-4715-5p	p53 signaling pathway
CALM1	hsa-miR-5008-5p	Alcoholism	CYCS	hsa-miR-5008-5p	p53 signaling pathway
CALM1	hsa-miR-148b-5p	Pertussis	CYCS	hsa-miR-548v	p53 signaling pathway
CALM1	hsa-miR-222-5p	Pertussis	FCGR1A	hsa-miR-3132	Systemic lupus erythematosus
CALM1	hsa-miR-3074-3p	Pertussis	FCGR1A	hsa-miR-5003-5p	Systemic lupus erythematosus
CALM1	hsa-miR-323b-3p	Pertussis	GADD45B	hsa-miR-214-5p	p53 signaling pathway
CALM1	hsa-miR-34c-3p	Pertussis	GADD45B	hsa-miR-4715-5p	p53 signaling pathway
CALM1	hsa-miR-3671	Pertussis	GNB4	hsa-miR-4714-3p	Alcoholism
CALM1	hsa-miR-93-3p	Pertussis	GNB4	hsa-miR-4776-5p	Alcoholism
CALM1	hsa-miR-4691-5p	Pertussis	GNB4	hsa-miR-499a-3p	Alcoholism
CALM1	hsa-miR-4776-5p	Pertussis	GNB4	hsa-miR-5008-5p	Alcoholism
CALM1	hsa-miR-494-3p	Pertussis	HLA-DRB1	hsa-miR-5008-5p	Systemic lupus erythematosus
CALM1	hsa-miR-5008-5p	Pertussis	SERPINE1	hsa-miR-214-5p	p53 signaling pathway
COL4A1	hsa-miR-548v	ECM-receptor interaction	SERPINE1	hsa-miR-222-5p	p53 signaling pathway
COL4A1	hsa-miR-548v	Amoebiasis	SERPINE1	hsa-miR-214-5p	Pertussis
COL4A1	hsa-miR-548v	Protein digestion and absorption	SERPINE1	hsa-miR-222-5p	Pertussis
COL4A1	hsa-miR-548v	Focal adhesion	SERPINF1	hsa-miR-5003-5p	p53 signaling pathway
COL4A2	hsa-miR-4691-5p	ECM-receptor interaction	SERPINF1	hsa-miR-5003-5p	Pertussis
COL4A2	hsa-miR-4715-5p	ECM-receptor interaction	SERPING1	hsa-miR-3132	p53 signaling pathway
COL4A2	hsa-miR-4691-5p	Amoebiasis	SERPING1	hsa-miR-4715-5p	p53 signaling pathway
COL4A2	hsa-miR-4715-5p	Amoebiasis	SERPING1	hsa-miR-3132	Pertussis
COL4A2	hsa-miR-4691-5p	Protein digestion and absorption	SERPING1	hsa-miR-4715-5p	Pertussis
COL4A2	hsa-miR-4715-5p	Protein digestion and absorption	THBS1	hsa-miR-222-5p	ECM-receptor interaction
COL4A2	hsa-miR-4691-5p	Focal adhesion	THBS1	hsa-miR-4418	ECM-receptor interaction
COL4A2	hsa-miR-4715-5p	Focal adhesion	THBS1	hsa-miR-222-5p	p53 signaling pathway
COL5A1	hsa-miR-4715-5p	ECM-receptor interaction	THBS1	hsa-miR-4418	p53 signaling pathway
COL5A1	hsa-miR-4715-5p	Amoebiasis			

phate kinase 3 (NME3, logFC=-2.64)) in the miRNA-gene regulatory network were selected and analyzed in an independent gastric cancer database from Kaplan-Meier Plotter tools. The overall survival time of patients with gastric cancer was evaluated based on the average level of these 10 gene sets in this database. Results showed that two genes, TMPRSS11B and CYR61, were found no significant predictive value for the survival of gastric cancer patients ($P>0.05$). While patients with higher level of RGS1, PLEKHB2, TAX1BP3 and PSENEN in gastric cancer had a longer survival time compared with the patients with lower

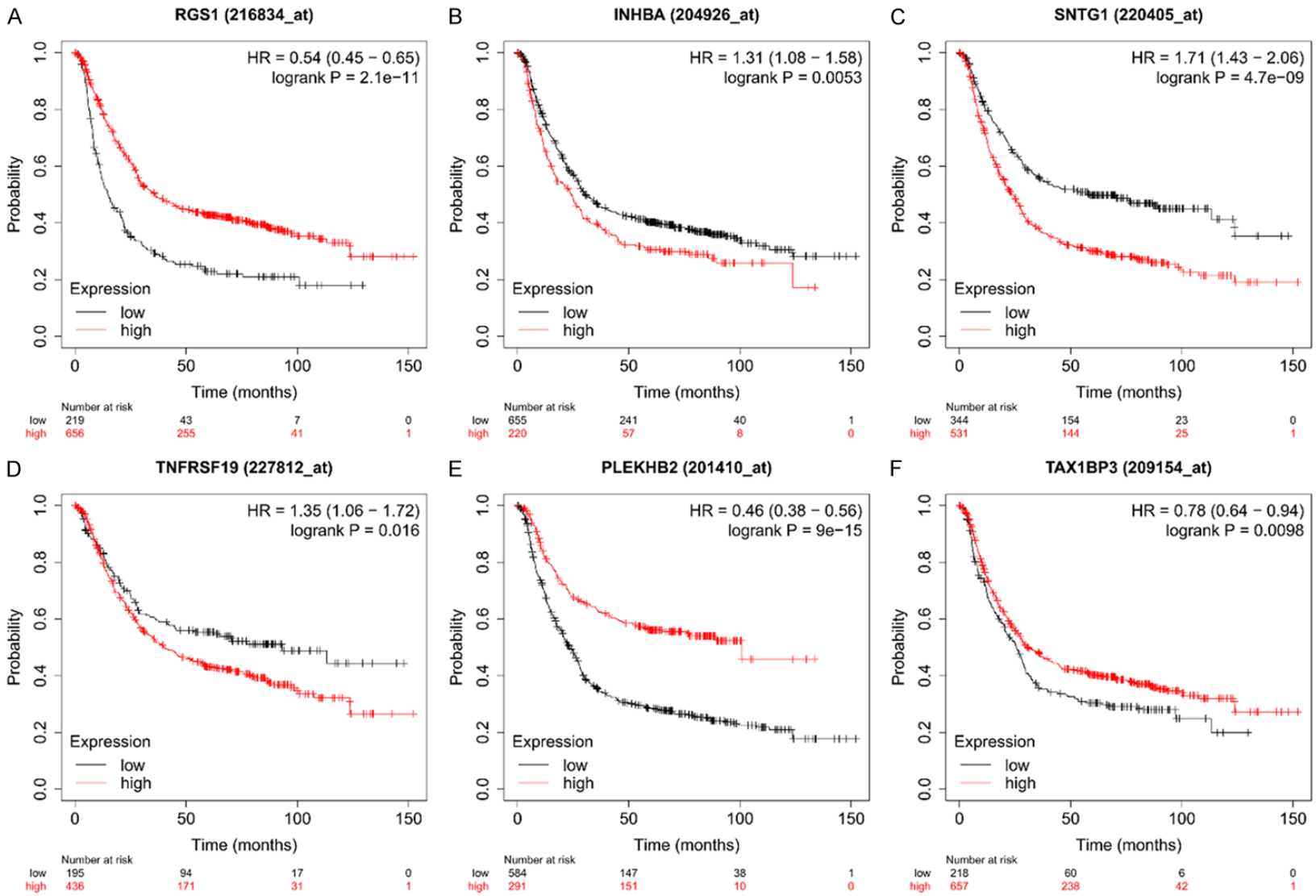
level of these genes. On the contrary, the patients with higher level of INHBA, SNTG1, TNFRSF19 and NME3 were found associated with a shorter survival time, see **Figure 5**.

Discussion

With the development of bioinformatics technology, a new era of the detection of cancer has arrived. The recognition of differentially expressed genes and miRNAs from primary gastric carcinoma specimens may provide effective diagnostic biomarkers and potential treatment target for gastric cancer. In the present study, based on a series of bioinformatics tools, a miRNA-gene regulatory network has been concluded by comparing gastric cancer specimens with normal gastric specimens, which may be critically involved in the development and progression of gastric cancer. What' more, our results revealed that these differentially expressed mRNAs play a role in a series of biological processes and signaling pathway in gastric cancer. Meanwhile, we found that 8 of 10 genes with the most significant changes in our miRNA-gene regulatory network based on GEO database, possessed an ability of survival prediction in another clinical database including 1067 gastric cancer patients.

The difference in their transcriptional responses is a key cause for the differences between gastric cancer specimens and normal gastric specimens in situ. Hence, the exploration of the differential expression of the miRNA-gene regulatory network between them is of importance with the purpose of extensive understanding of the molecular mechanism of gastric cancer. With the construction of the miRNA-gene regulatory network in our study, the relationship between DEMs and DEGs during the development and progression of gastric cancer became clearer. In our study, the miRNA-gene regulatory network was drawn based on 385 miRNA-gene correlation pairs, in which, CBX5, CALM1, KCMF1, NBR1, PTGIS were associated with at least 10 miRNAs, and defined as having higher connectivity with miRNAs. It has been reported that gene expression of CXB5 elevated in gastric tumor tissue and was down-regulated more than 2-fold by vorinostat treatment in gastric cancer cell lines, which suggested that the gene CXB5 may represent an emerging molecule to discover therapeutic agents for gastric cancer [28]. KCMF1 mRNA expression has been found up-regulated in gastric cancer cells and epithelial tumors, and previous reports have linked KCMF1 to cellular motility, invasion, and cancer progression [29]. However, the role of CALM1, NBR1, PTGIS in gastric cancer was under investigate and may be the next diagnostic and therapeutic targets for gastric cancer.

In addition, mRNA of RGS1, INHBA, SNTG1, TNFRSF19, PLEKHB2, TAX1BP3, PSENEN, NME3, which were among the 10 most significant changed mRNA in the miRNA-gene regulatory network, have been confirmed associated with the prognosis of gastric cancer patients from another independent database. Patients with higher level of RGS1, PLEKHB2, TAX1BP3 and PSENEN in gastric cancer had a longer survival time compared with the patients with lower level of these genes. On the contrary, patients with higher level of INHBA, SNTG1, TNFRSF19 and NME3 were found associated with a shorter survival time. The role RGS1, INHBA, TNFRSF19 in gastric cancer has been once investigated. RGS1, encoding a member of the regulator of G-protein signaling family, has been shown to be up-regulated in diffuse-type gastric cancer cells compared with mesenchymal stem cells and thought a critical role to response cancer cells, however, its role in survival prediction of gastric cancer patients has not been investigated [30-32]. Our study suggested higher expression of RGS1 associated longer survival time. INHBA, is a ligand belonging to the transforming growth factor-β superfamily, and its expression levels were significantly higher in gastric cancer tissue than in adjacent normal mucosa, which were related to TNM stage and venous invasion and high INHBA gene expression was correlated with significantly poorer 5-year overall survival than was low expression [31]. In our study, we also found high INHBA correlated to a poorer survival. TNFRSF19, encoding a member of the TNF-receptor superfamily, has been found more commonly in intestinal type gastric cancer compared with diffuse type gastric cancer, correlated inversely with tumor grade and nodal spread, and supposed an inverse prognosticator of poor patient outcome [32]. In our study, we confirmed that TNFRSF19 was a survival predictor. However, the role of SNTG1,


A
RGS1 (216834_at)
HR = 0.54 (0.45 − 0.65)
logrank P = 2.1e−11
B
INHBA (204926_at)
HR = 1.31 (1.08 − 1.58)
logrank P = 0.0053
C
SNTG1 (220405_at)
HR = 1.71 (1.43 − 2.06)
logrank P = 4.7e−09
D
TNFRSF19 (227812_at)
HR = 1.35 (1.06 − 1.72)
logrank P = 0.016
E
PLEKHB2 (201410_at)
HR = 0.46 (0.38 − 0.56)
logrank P = 9e−15
F
TAX1BP3 (209154_at)
HR = 0.78 (0.64 − 0.94)
logrank P = 0.0098
Probability
Time (months)
Expression
low
high
Number at risk
low 219 43 7 0
high 656 255 41 1
low 655 241 40 1
high 220 57 8 0
low 344 154 23 0
high 531 144 25 1
low 195 94 17 0
high 436 171 31 1
low 584 147 38 1
high 291 151 10 0
low 218 60 6 0
high 657 238 42 1

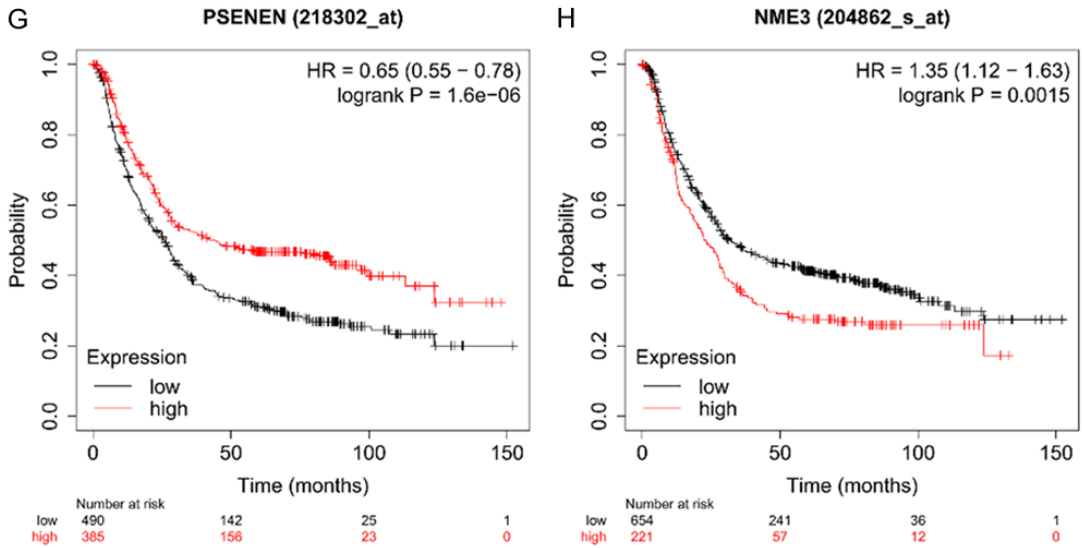


Figure 5. Prognostic value of eight genes, RGS1, PLEKHB2, TAX1BP3, PSENEN, INHBA, SNTG1, TNFRSF19 and NME3 for survival time in gastric cancer patients.

PLEKHB2, TAX1BP3, PSENEN, NME3 in gastric cancer was under investigate and may be the next diagnostic and therapeutic targets for gastric cancer.

In our study, we found the DEGs were significantly enriched in 8 signal pathways. Several pathways have been demonstrated participating in the pathological process gastric cancer, including focal adhesion, p53 signaling pathway signaling pathway, with the rest unexplored pathways may be the next potential therapeutic targets [33-38].

Nevertheless, our study was performed based on bioinformatics method and GEO database, which lacked basic experimental and clinical investigation. Further experimental and clinical studies were expected to verify our findings.

By bioinformatics method, we identified a miRNA-gene-pathway regulatory work net, which provided several potential targets regarding gastric cancer and may result in a new strategy to treat gastric cancer from a systems-rather than a single-gene perspective.

Disclosure of conflict of interest

None.

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