Identification of the lncRNA-miRNA-mRNA network associated with gastric cancer via integrated bioinformatics analysis

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Received December 26, 2018; Accepted July 26, 2019

DOI: 10.3892/ol.2019.10922

Abstract. The aim of the present study was to investigate the long non-coding RNA (lncRNA)-microRNA (miRNA)-mRNA regulatory network in gastric cancer (GC) using bioinformatics analysis. Two mRNA gene expression profiles, GSE79973 and GSE54129, and two miRNA expression profiles, GSE93415 and GSE78091, were downloaded from the Gene Expression Omnibus database. The differentially expressed mRNAs (DEMs) and the differentially expressed miRNAs (DEMis) were merged separately. Gene ontology and pathway enrichment analysis were conducted using the Database for Annotation, Visualization and Integrated Discovery. A protein-protein interaction (PPI) network was then constructed and the 10 top hub genes in the network were analyzed using the Search Tool for the Retrieval of Interacting Genes. The lncRNA-miRNA-mRNA networks were visualized using Cytoscape software. As a result, 158 shared DEMs (40 upregulated and 118 downregulated) were identified from two mRNA datasets. A total of 30 upregulated miRNAs and 1 downregulated miRNA functioned as DEMis. The PPI network consisted of 129 nodes and 572 interactions. The 10 top hub genes were selected by degree using Cytohubba, including Jun proto-oncogene, mitogen-activated protein kinase (MAPK)3, transforming growth factor-β1, Fos proto-oncogene, AP-1 transcription factor subunit, interleukin (IL)-8, MAPK1, RELA proto-oncogene nuclear factor-κB subunit, interferon regulatory factor 7, ubiquitin like modifier and vascular endothelial growth factor A. In the lncRNA-miRNA-mRNA network, a total of 1,215 regulatory associations were constructed using Cytoscape. In conclusion, the present study provides a novel perspective of the molecular mechanisms underlying GC by identifying the lncRNA-miRNA-mRNA regulatory network via bioinformatics analysis.

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Abbreviations: BP, biological processes; CC, cellular component; DAVID, Database for Annotation, Visualization and Integrated Discovery; DEM, differentially expressed mRNA; DEMi, differentially expressed miRNA; GC, gastric cancer; GEO, Gene Expression Omnibus; GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; lncRNA, long non-coding RNA; MF, molecular function; miRNA, microRNA; OS, overall survival; PPI, protein-protein interaction network; STRING, Search Tool for the Retrieval of Interacting Genes

Key words: gastric cancer, long non-coding RNA, microRNA, bioinformatics analysis, gene expression omnibus, protein-protein interaction network

Introduction

Gastric cancer (GC) is one of the most common malignant diseases globally (1). Although substantial advances have been made in the diagnosis and therapy of this disease, the prognosis of GC remains poor and the 5-year survival rate is still comparatively low (2,3). Therefore, it is essential to investigate the molecular mechanism including potential biomarkers and therapeutic targets of GC.

In previous years, a large number of microarrays and bioinformatics methods have been conducted to investigate the molecular mechanism underlying cancer progression including diagnosis, treatment and prognosis (4-6). For example, bioinformatics analysis has been used to elucidate the potential key candidate genes and pathways in colorectal cancer from four cohort profile datasets (7). In addition, target genes and the prognostic value in non-small cell lung cancer have been discovered previously via bioinformatics analysis (8). Similarly, bioinformatics analysis has been performed to identify long non-coding RNA (lnc-RNA)-microRNA (miRNA/miR)-mRNA networks via the combination of lncRNA, miRNA and mRNA expression profiles based on competitive endogenous RNA in rheumatoid arthritis (9). In conclusion, it is necessary to perform further investigation of the molecular mechanism underlying GC using integrated bioinformatics analysis.

In the present study, differentially expressed mRNAs (DEMs) and differentially expressed miRNAs (DEMis) were

screened out from the Gene Expression Omnibus (GEO) database. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of DEMs were also performed. The lncRNA-miRNA-mRNA network was subsequently established, which may provide additional information on the molecular mechanism of GC.

Materials and methods

Gene expression profiles. Two human mRNA expression profiles, GSE79973 and GSE54129, were acquired from the GEO database (ncbi.nlm.nih.gov/geo/) (10), which included 121 GC and 31 normal samples. Two human miRNA expression profiles, GSE93415 and GSE78091, were also downloaded from the GEO database, which included 23 GC and 23 normal samples. FunRich version 3.1.3 was used to draw the venn diagram (11).

Identification of DEMs and DEMis. Two mRNA and two miRNA databases were analyzed using the GEO2R web tool comparing samples in the GC and control groups (12). In order to select the DEMs, an adjusted (adj.) P-value of ≤0.05 and |log2 fold change (FC)| ≥2 were selected as the cut-off values for the two mRNA databases. For DEMis, adj.P-value ≤0.05 and |log2FC| ≥1 were regarded as the cut-off criteria values.

Function analysis of DEMs. GO function and KEGG pathway enrichment analysis of the up- and downregulated genes and 10 hub genes were analyzed using the online Database for Annotation, Visualization and Integrated Discovery (DAVID; david.abcc.ncifcrf.gov/). P<0.05 was considered to indicate a statistically signicant difference.

Protein-protein interaction (PPI) network construction and sub-network identification. Search Tool for the Retrieval of Interacting Genes (STRING; string-db.org/) was used to analyze the PPI network of DEMs (13). The PPIs of DEMs were selected using a combined score of >0.9. Cytoscape version 3.5.1 (cytoscape.org/) software was utilized to construct the PPI network (14). Cytohubba in Cytoscape software was employed to identify the 10 top hub genes by degree. The sub-network in the PPI network was then visualized by MCODE with a cut-off criterion of k-score=2 (15). P<0.05 was considered to indicate a statistically signicant difference.

Survival analysis of the 10 top hub genes. The publicly available database Kaplan-Meier Plotter (www.kmplot.com) was used to identify the prognostic effect of the 10 top hub genes (16). P<0.05 was considered to indicate a statistically signicant difference.

Determining the expression level of the significantly up- and downregulated DEMs. A total of 5 significantly upregulated DEMs (inhibin bA, collagen type VIII α1, secreted frizzle related protein 4, secreted phosphoprotein 1 and thormbospondin 2) and 5 significantly downregulated DEMs [Gastrokine 2 (GKN2), GKN1, gastric intrinsic factor, Mucin like 3 and Keratin 20 (KRT20)] were selected for further analysis. The Gene Expression Profiling Interactive Analysis (GEPIA; gepia.cancer-pku.cn/) database was used to determine the expression of 10 DEMs in GC tissues (17). In order to provide more sufficient evidence to support the results, the present study investigated the data in the Cancer Cell Line Encyclopedia (CCLE) portals (broadinstitute.org/ccle/about) database, which supplied information on the expression of 10 DEMs in GC cell lines (18). P<0.05 was considered to indicate a statistically signicant difference.

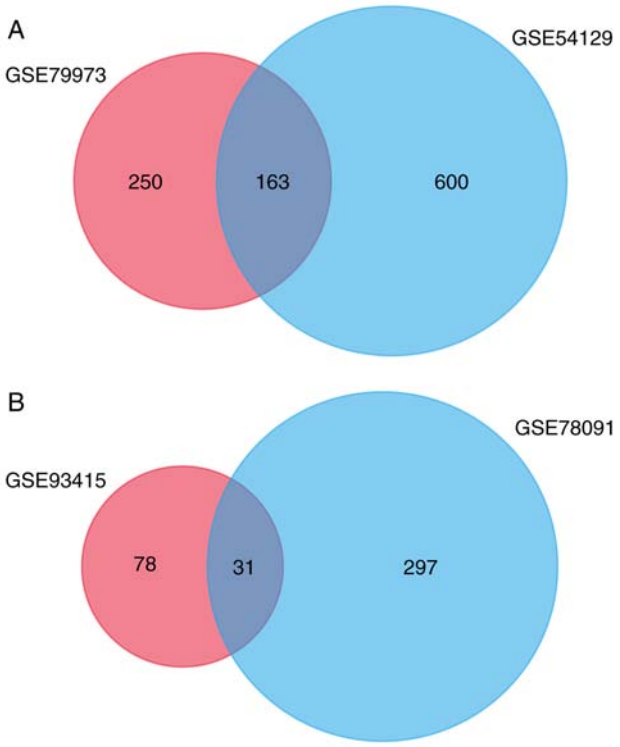

Figure 1. Identification of DEMs and DEMis. (A) A Venn diagram of DEMs in GSE79973 and GSE54129 produced using FunRich version 3.1.3. (B) A Venn diagram of DEMis in GSE93415 and GSE78091. DEM, differentially expressed mRNA; DEMis, differentially expressed microRNA.

Reconstruction of the lncRNA-miRNA-mRNA networks. The miRNA-targeted genes were screened out using the miRWalk database (mirwalk.umm.uni-heidelberg.de/) with a score of >0.9 (19,20). The targeted genes and DEMs mentioned above were merged to identify the number of miRNA-regulated target gene pairs. The prediction of lncRNA-miRNA interactions was based on the analysis of the lncRNASNP (www.lncRNAblog.com) database (21). The networks between miRNA-mRNA and lncRNA-miRNA were visualized using Cytoscape version 3.5.1. P<0.05 was considered to indicate a statistically signicant difference.

Statistical analysis. All statistical analysis was performed using corresponding databases. Data was presented as the mean ± standard deviation. A paired Student's t-test or one-way analysis of variance (ANOVA) or two-way ANOVA were used to evaluate the degree of differential expression. A log-rank test was applied to analyse the association between expression and prognosis. Post-hoc tests (least significant difference and Tukey's tests) were performed following ANOVA. P<0.05 was considered to indicate a statistically significant difference.

Table I. 158 DEMs were obtained from two datasets, including 40 upregulated DEMs and 118 downregulated DEMs. 31 DEMis were obtained between gastric cancer samples and control samples, including 30 upregulated miRNAs and 1 downregulated miRNA.

DEMs	Gene name
Upregulated	COL11A1, INHBA, IGF2BP3, COL10A1, FNDC1, FAP, THBS2, SULF1, CST1, COL8A1, SFRP4, SPP1, COL1A1, ADAMTS2, WISP1, CTHRC1, COL12A1, ASPN, CRISPLD1, THY1, COL1A2, FN1, BGN, RARRES1, CAP2, MFAP2, PDPN, PRRX1, TIMP1, SPARC, COL6A3, COL4A1, THBS1, NRP2, PDLIM7, LY6E, SPOCK1, PI15, CEMIP, CXCL8
Downregulated	GKN2, GKN1, ATP4A, ATP4B, GIF, LIPF, KCNJ16, DPCR1, SOSTDC1 KCNE2, CWH43, ESRRG, PGC, SLC28A2, PSAPL1, KRT20, VSIG1, LTF, CXCL17, AKR1B10, LOC643201, GSTA1, ADH7, ADH1C, GC, CAPN9, MAL, SLC26A9, HRASLS2, MFSD4A, MUC5AC, FBP2, ALDH3A1, ADGRG2, VSTM2A, LINC00982, CAPN13, KIAA1324, CA9, TPCN2, PIK3C2G, RDH12, SLC26A7, SSTR1, VSIG2, GATA6-AS1, HPGD, UPK1B, KCNJ15, SULT1C2, LINC00675, BTNL8, AXDND1, LINC00992, KAZALD1, TMED6, UGT2B15, SCNN1B, HAPLN1, AKR1C1, LYPD6B, FCGBP, ADTRP, IGH, CA2, RFX6, ACER2, CYP2C9, PCAT18, PKIB, SH3RF2, HHIP, HEPACAM2, AADAC, CYP2C18, RAB27B, MGAM, SPINK7, CNTN3, LINC01133, BCAS1, SULT1B1, CAPN8, SMIM6, AMPD1, JCHAIN, PBLD, ATP13A4, RNASE1, PLLP, B4GALNT3, STYK1, CYP2C19, SMIM24, LRRC66, RASSF6, ADAM28, FA2H, GATA5, SCIN, SGK2, TPH1, PROM2, APOBEC1, ACKR4, ADH1A, AKR7A3, OASL, SMPD3, XK, KLHDC7A, STX19, CYP3A5, STS, VILL, ANG, S100P, DDX60
DEMis	**miRNA name**
Upregulated	hsa-let-7i-3p, hsa-miR-100-5p, hsa-miR-106b-5p, hsa-miR-10a-5p, hsa-miR-151a-5p, hsa-miR-15a-5p, hsa-miR-195-5p, hsa-miR-199a-3p, hsa-miR-199a-5p, hsa-miR-199b-5p, hsa-miR-19a-3p, hsa-miR-20a-5p, hsa-miR-214-3p, hsa-miR-214-5p, hsa-miR-218-5p, hsa-miR-223-3p, hsa-miR-301a-3p, hsa-miR-331-3p, hsa-miR-335-5p, hsa-miR-342-3p, hsa-miR-377-3p, hsa-miR-4262, hsa-miR-4291, hsa-miR-4317, hsa-miR-454-3p, hsa-miR-455-3p, hsa-miR-4791, hsa-miR-93-5p, hsa-miR-99a-5p, hsa-miR-99b-5p
Downregulated	hsa-miR-375

DEM, differentially expressed mRNA; DEMis, differentially expressed microRNA; miRNA/miR, microRNA.

Results

Identification of DEMs and DEMis. A total of 158 DEMs (5 of the 163 genes were identified as unreconized genes) were identified based on the cut-off criteria using FunRich, including 40 upregulated DEMs and 118 downregulated DEMs (Fig. 1A). Furthermore, 31 DEMis were obtained between GC and control samples. A total of 30 upregulated miRNAs and 1 downregulated miRNA were identified as DEMis (Fig. 1B). All DEMs and DEMis are presented in Table I.

Functional analysis of DEMs. According to GO functional enrichment analysis for up- and downregulated DEMs, the significantly enriched biological process (BP), cellular component (CC) and molecular function (MF) terms were selected (Fig. 2). The values in the x-axes represent the quantification of -log10 (P-value). The GO terms enriched by upregulated DEMs were mainly associated with 'endodermal cell differentiation', 'proteinaceous extracellular' and 'extracellular matrix binding' while the GO terms enriched by downregulated DEMs were mainly associated with 'regulation of cell proliferation', 'extracellular exosome' and 'iron ion binding' (Table II). Following KEGG pathway enrichment analysis, upregulated differentially expressed genes (DEGs) were revealed to be mainly involved in 'ECM-receptor interaction', 'focal adhesion' and 'phosphoinositide-3-kinase (PI3K)-protein kinase B (Akt) signaling pathway'. Downregulated DEGs were associated with 'gastric acid secretion', 'retinol metabolism' and 'chemical carcinogenesis' (Table III).

PPI network construction and sub-network identification. The PPI network consisted of 129 nodes and 572 interactions (Fig. 3). Following the use of Cytohubba in Cytoscape software, 10 top hub genes Jun proto-oncogene (JUN), mitogen activated protein kinase (MAPK)3, transforming growth factor-β1, Fos proto-oncogene, AP-1 transcription factor subunit (FOS), interleukin (IL)-8, MAPK1, RELA proto-oncogene nuclear factor κβ (NF-κB) subunit (RELA), interferon regulatory factor 7 (IRF7), ubiquitin like modifier (ISG15) and vascular endothelial growth factor A (VEGFA) were evaluated by degree in the PPI network (Fig. 4). The sub-network in the PPI network was then visualized using MCODE with a cut-off criterion of k-score=2. The sub-network was obtained from the PPI network with 14 nodes and 86 interactions (Fig. 5).

Survival analysis of the 10 top hub genes. The present study used the Kaplan-Meier Plotter database to evaluate the prognostic effect of the 10 top hub genes by overall survival (OS; Fig. 6). The Kaplan-Meier curves indicated that a higher expression of MAPK3, TGFB1, RELA, IRF7, ISG15 and

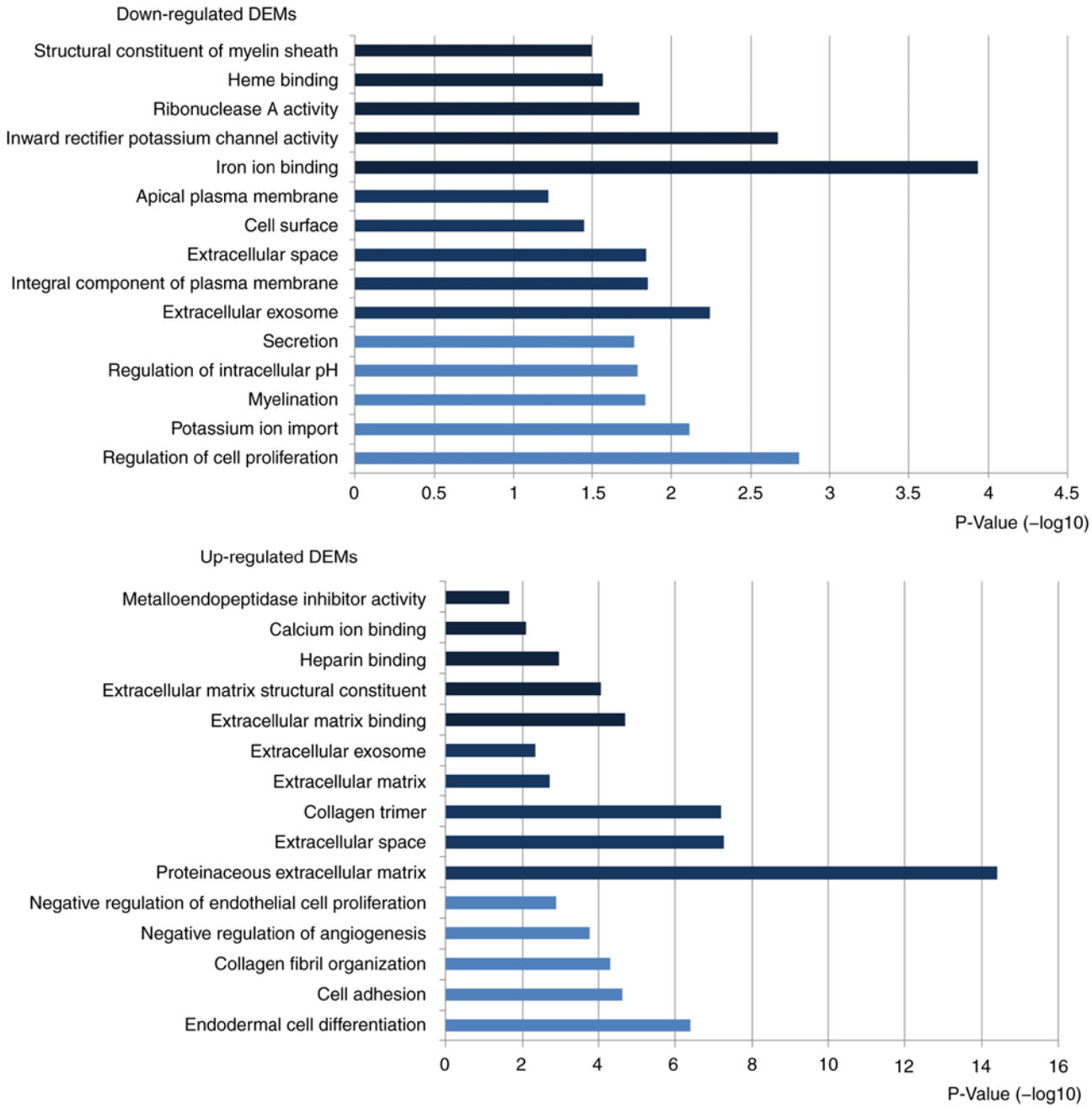

Figure 2. Top 5 enriched Gene Ontology terms of upregulated and downregulated DEMs. [Black represents biological processes, dark blue indicates cellular component, and light blue denotes molecular function; -Log10 (P-value) represents the value of x-axes]. P<0.05. DEM, differentially expressed mRNA.

VEGFA was significantly associated with poor survival in GC. On the other hand, a lower expression of JUN, FOS, IL8 and MAPK1 was significantly associated with poor survival in GC.

Functional analysis of 10 top hub genes and DEMs. In order to elucidate the specific signaling pathways that the 158 DEMs and 10 top hub genes were involved in, the DAVID database was used for further research. The results revealed that the 158 DEMs were involved in 17 KEGG pathways (Table IV). Among these pathways, 4 KEGG pathways (bta04151: PI3K-Akt signaling pathway, bta04510: Focal adhesion, bta05146: Amoebiasis and bta05144: Malaria) were identified to be significantly associated with the 10 top hub genes (Table V).

Expression levels of significantly up- and downregulated DEMs. The GEPIA database was used to reveal the expression levels of 10 DEMs in GC tissues. The results revealed that 9 DEMs presented the same trend that was previously noted and only 1 DEM (KRT20) was contrary to the aforementioned trend (Fig. 7). The CCLE database was used to obtain information on the expression levels of 10 DEMs in GC cell lines. The present study then selected 5 different GC cell lines (HGC27, HS746T, MKN1, NUGC3 and RERFGC1B) to analyze the expression levels of the 10

Table II. Top 5 enriched GO terms of upregulated and downregulated differentially expressed mRNAs.

Upregulated	Term	Count	P-value
BP			
GO:0035987	Endodermal cell differentiation	5	4.00×10^{-07}
GO:0007155	Cell adhesion	6	2.44×10^{-05}
GO:0030199	Collagen fibril organization	4	5.04×10^{-05}
GO:0016525	Negative regulation of angiogenesis	4	1.66×10^{-04}
GO:0001937	Negative regulation of endothelial cell proliferation	3	0.001248824
CC			
GO:0005578	Proteinaceous extracellular matrix	13	4.06×10^{-15}
GO:0005615	Extracellular space	14	5.60×10^{-08}
GO:0005581	Collagen trimer	6	6.88×10^{-08}
GO:0031012	Extracellular matrix	4	0.002041798
GO:0070062	Extracellular exosome	13	0.004832505
MF			
GO:0050840	Extracellular matrix binding	4	2.15×10^{-05}
GO:0005201	Extracellular matrix structural constituent	4	9.22×10^{-05}
GO:0008201	Heparin binding	4	0.001122327
GO:0005509	Calcium ion binding	6	0.008415225
GO:0008191	Metalloendopeptidase inhibitor activity	2	0.02384997
Downregulated			
BP			
GO:0042127	Regulation of cell proliferation	6	0.001561961
GO:0010107	Potassium ion import	3	0.007733402
GO:0042552	Myelination	3	0.014660727
GO:0051453	Regulation of intracellular pH	3	0.01645907
GO:0046903	Secretion	2	0.017262077
CC			
GO:0070062	Extracellular exosome	22	0.00577329
GO:0005887	Integral component of plasma membrane	11	0.014280261
GO:0005615	Extracellular space	12	0.014702585
GO:0009986	Cell surface	6	0.036068461
GO:0016324	Apical plasma membrane	4	0.06075832
MF			
GO:0005506	Iron ion binding	7	1.17×10^{-04}
GO:0005242	Inward rectifier potassium channel activity	3	0.002175164
GO:0004522	Ribonuclease A activity	2	0.016178947
GO:0020037	Heme binding	4	0.027324696
GO:0019911	Structural constituent of myelin sheath	2	0.032099763

GO, Gene Ontology; BP, biological processes; CC, cellular component; MF, molecular function.

DEMs (Fig. 8). For the upregulated DEMs, the expression level was partially different from the aforementioned results. All downregulated DEMs exhibited the same trend observed previously in the present study.

Association analysis of lncRNAs, miRNAs and mRNAs. The network of miRNAs and their targets was composed of 85 nodes and 145 interactions, determined using Cytoscape software (Fig. 9). In the lncRNA-miRNA-mRNA network, a total of 1,215 regulatory associations were screened out (Fig. 10). Pink circles indicated lncRNAs, hexagons indicated miRNAs, and diamonds represented mRNAs. With regards to the colors, red nodes indicated upregulated mRNAs and miRNAs, and green nodes represented downregulated mRNAs and miRNAs.

Discussion

The identification of the underlying molecular mechanism of GC is necessary to detect therapeutic targets in the malignant transformation process for management strategies. To date, microarrays and bioinformatics methods have been

Table III. Top 5 enriched Kyoto Encyclopedia of Genes and Genomes pathway analysis of upregulated and downregulated differentially expressed mRNAs.

ID	Term	Count	P-value
Upregulated			
cfa04512	Extracellular matrix-receptor interaction	9	$3.17x10^{-12}$
cfa04510	Focal adhesion	9	$3.53x10^{-09}$
cfa04974	Protein digestion and absorption	7	$1.38x10^{-08}$
cfa04151	Phosphoinositide-3-kinase-protein kinase B signaling pathway	9	$1.62x10^{-07}$
cfa05146	Amoebiasis	6	$2.25x10^{-06}$
ID	Term	Count	P-value
Downregulated			
bta04971	Gastric acid secretion	7	$1.29x10^{-06}$
bta00830	Retinol metabolism	6	$8.34x10^{-06}$
bta05204	Chemical carcinogenesis	6	$2.30x10^{-05}$
bta01100	Metabolic pathways	16	$5.74x10^{-04}$
bta00140	Steroid hormone biosynthesis	4	0.002819276

used to analyze the process of carcinogenesis to enhance the universality and reliability of the results (4-8). In the present study, 158 shared DEMs (40 upregulated and 118 downregulated) were obtained from the investigation of the GSE79973 and GSE54129 datasets. One previous study used GSE79973 to identify 14 significantly downregulated genes in GC, including KRT20, cytochrome P450 family 3 subfamily A member 5, RAB27B member RAS oncogene family and sulfotransferase family 1C member 2 (22). The four genes were additionally downregulated in the present study. Another previous study using GSE54129 identified 1829 DEMs including 838 upregulated genes and 991 downregulated genes (23). The Affy and limma packages in R software were used to select the DEMs in previous studies. In the present study, 158 shared DEMs (40 upregulated and 118 downregulated) were selected. The GEO2R web tool was used in the present study to identify DEMs. Although the data was from the same database, unequal results were obtained due to the different processing methods and filtering conditions utilized. For the analysis of 10 hub nodes, the previous study using the GSE54129 dataset identified 10 top hub nodes [tumor protein p53, C-X-C motif chemokine ligand (CXCL)8, tetraspanin 4, lysophospatidic acid receptor 2, adenylate cyclase 3, phosphoinositide-3-kinase regulatory subunit 1, neuromedin U, CXCL1, FOS and sphingosine-1-phosphate receptor 1] (23). FOS was also one of 10 hub genes identified in the present study. Meanwhile, the present study performed GO function and KEGG pathway enrichment analyses with 158 DEGs. The significant GO terms enriched by upregulated DEMs included 'endodermal cell differentiation', 'proteinaceous extracellular' and 'extracellular matrix binding' while the terms enriched by downregulated DEMs were mainly associated with 'regulation of cell proliferation', 'extracellular exosome' and 'iron ion binding'. The results of KEGG pathway enrichment revealed that upregulated DEGs were mainly involved in 'ECM-receptor interaction', 'focal adhesion' and 'PI3K-Akt signaling pathway', and downregulated DEGs were associated with 'gastric acid secretion', 'retinol metabolism' and 'chemical carcinogenesis'. Among the enriched pathways, the 'PI3K/Akt signaling pathway' has an essential biological function in the development of proliferation, apoptosis and invasion in various types of human cancer, including GC (24-26). Furthermore, the PI3K/Akt signaling pathway is commonly activated in advanced GC and serves an important function in resistance to chemotherapy in GC (27). LY294002 has been identified as a PI3K inhibitor and has been confirmed to suppress cell proliferation and enhance apoptosis by downregulating VEGF, matrix metalloproteinase (MMP)-2 and MMP-9 in GC (28).

In the constructed PPI network, there were 129 nodes and 572 interactions. The top 10 hub genes (JUN, MAPK3, TGFB1, FOS, IL8, MAPK1, RELA, IRF7, ISG15 and VEGFA) were evaluated by degree following Cytohubba analysis. Then, the DAVID database was used to elucidate the specific signaling pathways that the 158 DEMs and 10 top hub genes were involved in. The results revealed that 158 DEMs were involved in 17 KEGG pathways (Table IV). Amongst these pathways, 4 KEGG pathways (bta04151: PI3K-Akt signaling pathway, bta04510: Focal adhesion, bta05146: Amoebiasis and bta05144: Malaria) were also associated with the 10 hub genes (Table V). Among four common KEGG pathways, PI3K-Akt signaling pathway is closely associated with the function of miRNAs. miR-15a-5p (an upregulated DEMi) regulated granulosa cell proliferation by activating the PI3K-Akt-mechanistic target of rapamycin kinase (mTOR) signaling pathway (29). miR-4262 (an upregulated DEMi) regulates chondrocyte viability, apoptosis, autophagy by targeting sirtuin 1 and activating the PI3K/Akt/mTOR signaling pathway in rats with osteoarthritis (30). miR-375 (a downregulated DEMi) functions as a tumor suppressor in osteosarcoma and colorectal cancer by targeting phosphatidylinositol-4,5-bisphosphate

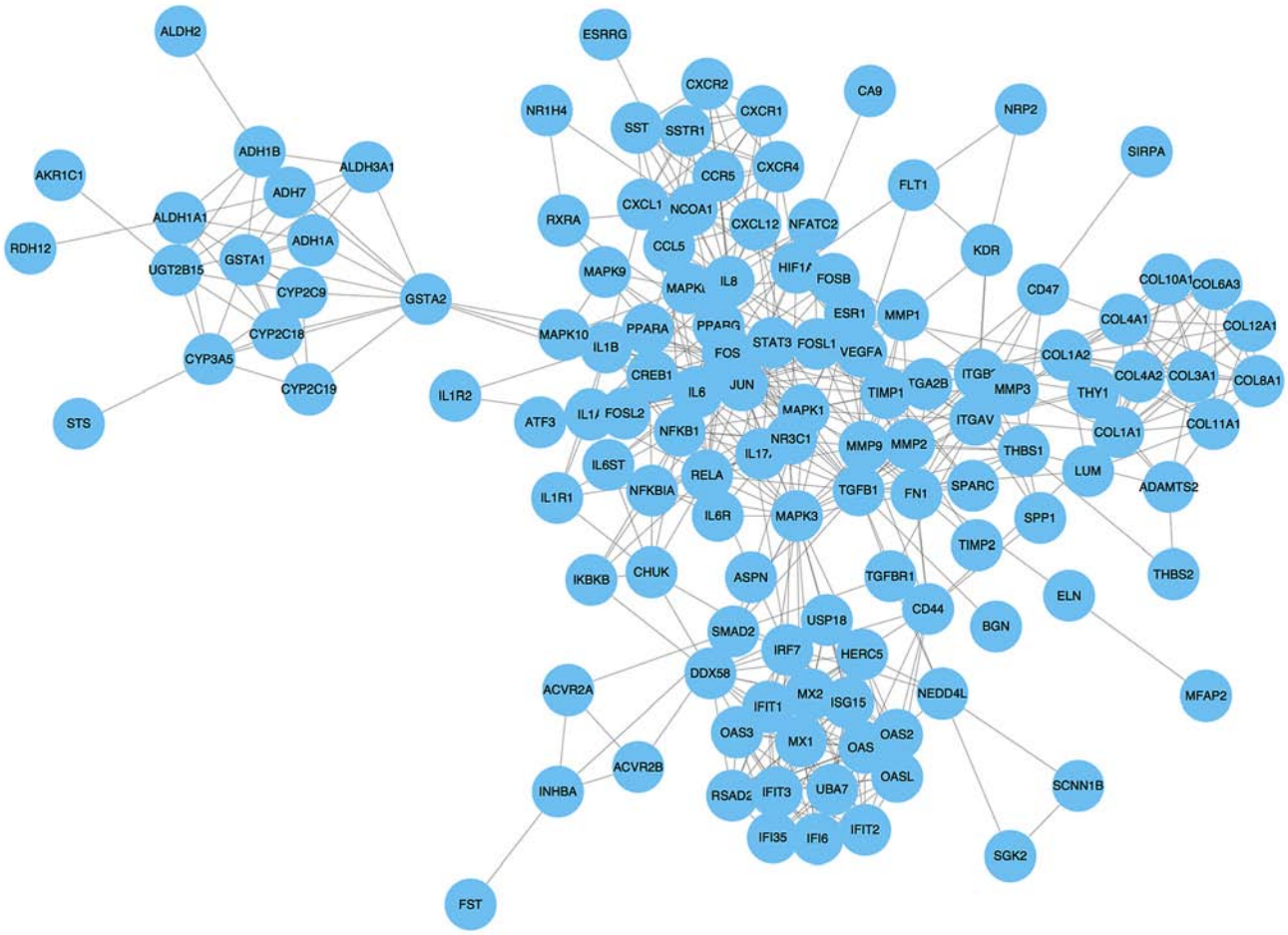

Figure 3. Construction of a protein-protein interaction network of differentially expressed mRNAs. P<0.05.

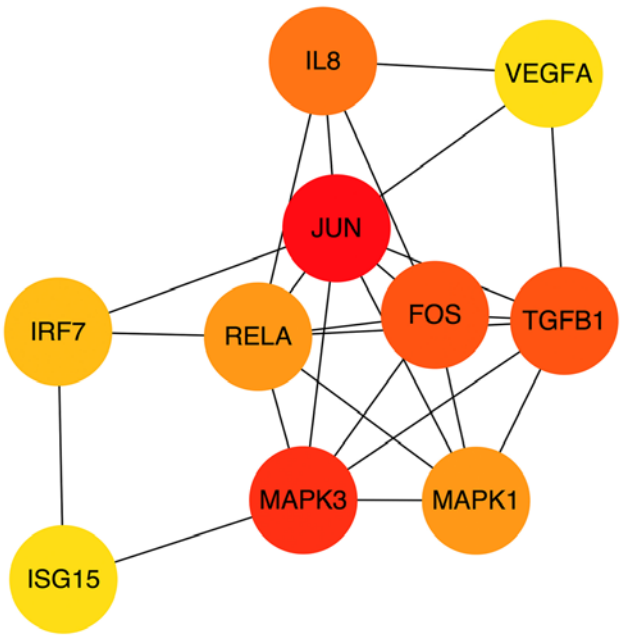

Figure 4. Top 10 hub genes by degree in the protein-protein interaction network using the Cytohubba in Cytoscape software. P<0.05. JUN, Jun proto-oncogene; TGFB1, transforming growth factor-β1; FOS, Fos proto-oncogene, AP-1 transcription factor subunit; IL-8, interleukin 8; MAPK, mitogen-activated protein kinase; RELA, RELA proto-oncogene NF-ein kinase; IRF7, interferon regulatory factor 7; VEGFA, vascular endothelial growth factor A; ISG15, ISG15 ubiquitin like modifier.

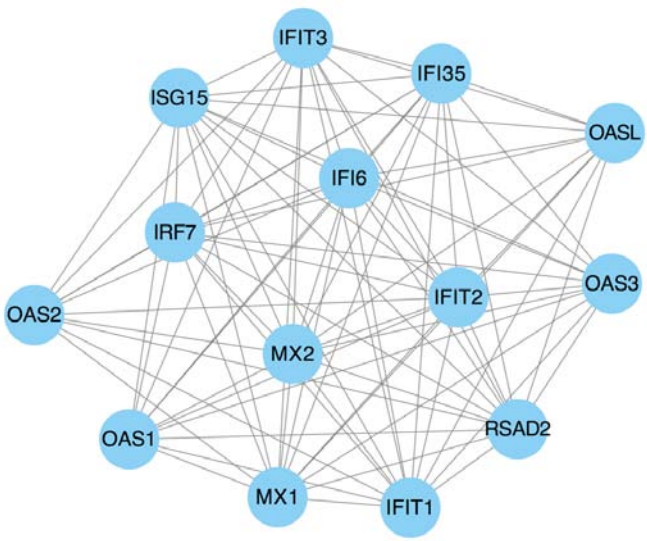

Figure 5. Identification of a sub-network using MCODE in Cytoscape software. P<0.05.

3-kinase catalytic subunit α (31,32), which indicates that miR-375 may be associated with the PI3K-Akt signaling pathway. Furthermore, survival analysis of the top 10 hub genes was performed using the publicly available database Kaplan-Meier Plotter. The Kaplan-Meier curves indicated

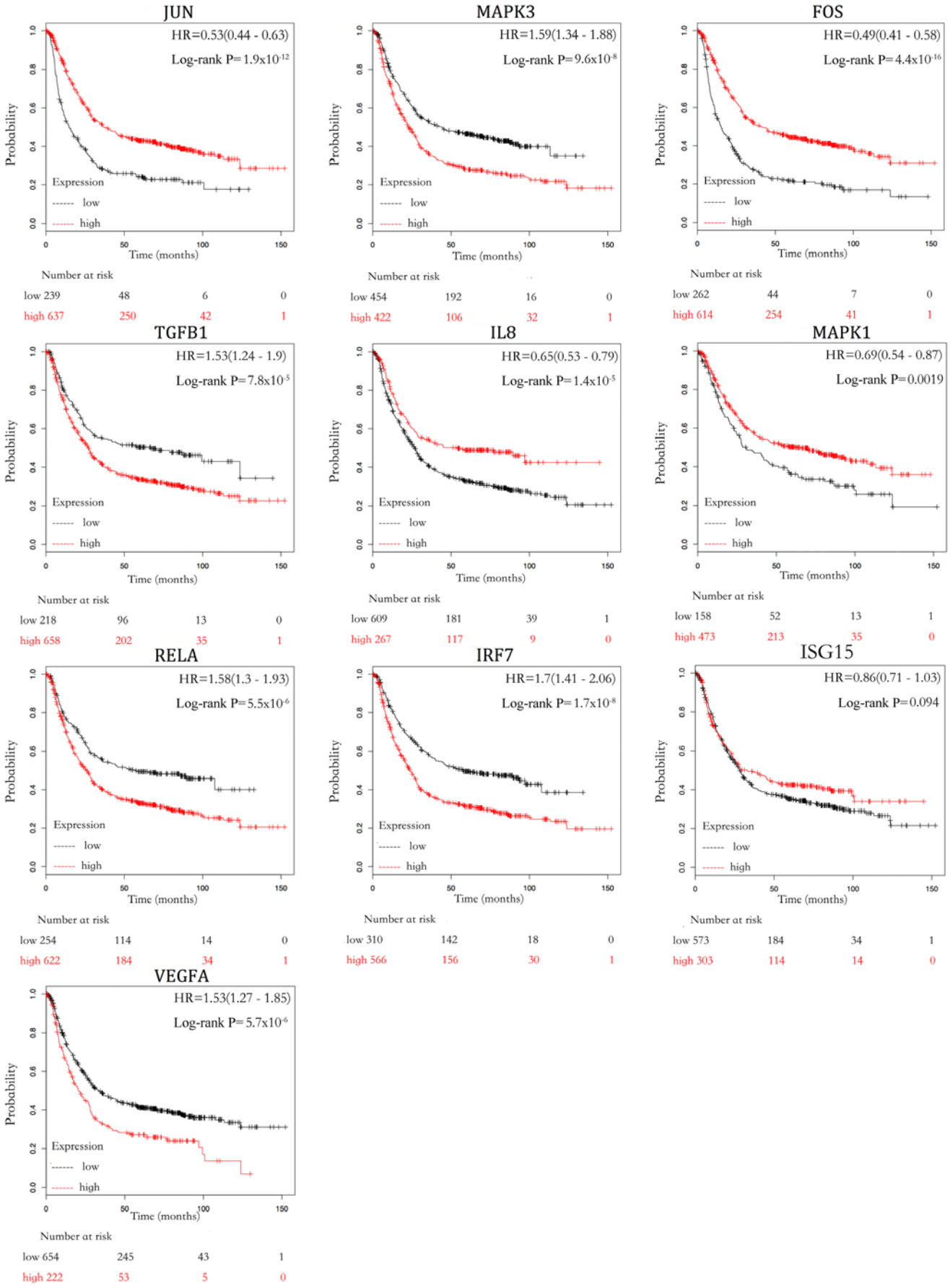

Figure 6. Survival analysis of 10 top hub genes. The survival data for patients with gastric cancer were obtained from Kaplan-Meier Plotter database. P<0.05. HR, hazard ratio; JUN, Jun proto-oncogene; TGFB1, transforming growth factor-β1; FOS, Fos proto-oncogene, AP-1 transcription factor subunit; IL-8, interleukin 8; ubiquitin like modifier (ISG15); MAPK, mitogen-activated protein kinase; RELA, RELA proto-oncogene NF-κβ subunit; IRF7, interferon regulatory factor 7; VEGFA, vascular endothelial growth factor A.

Table IV. Enriched Kyoto Encyclopedia of Genes and Genomes pathway analysis of 158 differentially expressed mRNAs.

Term	Count	P-value	Genes
bta04151:Phosphoinositide-3-kinase-protein kinase B signaling pathway	10	6.79×10^{-04}	COL4A1, SGK2, COL6A3, COL1A2, COL1A1, THBS1, COL11A1, THBS2, FN1, SPP1
bta04510:Focal adhesion	9	9.84×10^{-05}	COL4A1, COL6A3, COL1A2, COL1A1, THBS1, COL11A1, THBS2, FN1, SPP1
bta05146:Amoebiasis	6	0.001142	COL4A1, COL1A2, CXCL8, COL1A1, COL11A1, FN1
bta05144:Malaria	3	0.054453	CXCL8, THBS1, THBS2
bta01100:Metabolic pathways	16	0.024433	CYP3A5, PIK3C2G, CYP2C18, ACER2, ADH1C, ADH7, FBP2, AMPD1, ALDH3A1, RDH12, AKR1B10, MGAM, TPH1, SMPD3, LIPF
bta04512:Extracellular matrix-receptor interaction	9	1.47×10^{-07}	COL4A1, COL6A3, COL1A2, COL1A1, THBS1, COL11A1, THBS2, FN1, SPP1
bta04971:Gastric acid secretion	7	1.07×10^{-05}	KCNJ16, KCNJ15, ATP4A, ATP4B, SLC26A7, KCNE2, CA2
bta04974:Protein digestion and absorption	7	2.61×10^{-05}	COL4A1, COL6A3, COL1A2, COL12A1, COL1A1, COL11A1, COL10A1
bta00830:Retinol metabolism	6	4.79×10^{-05}	RDH12, CYP3A5, CYP2C18, ADH1C, ADH7
bta05204:Chemical carcinogenesis	6	1.29×10^{-04}	CYP3A5, CYP2C18, ADH1C, ADH7, ALDH3A1
bta00140:Steroid hormone biosynthesis	4	0.007616	CYP3A5, STS, CYP2C18
bta00010:Glycolysis/Gluconeogenesis	4	0.010031	ADH1C, ADH7, FBP2, ALDH3A1
bta04966:Collecting duct acid secretion	3	0.015591	ATP4A, ATP4B, CA2
bta00591:Linoleic acid metabolism	3	0.028276	CYP3A5, CYP2C18
bta00350:Tyrosine metabolism	3	0.032672	ADH1C, ADH7, ALDH3A1
bta00982:Drug metabolism-cytochrome P450	3	0.063878	ADH1C, ADH7, ALDH3A1
bta00980:Metabolism of xenobiotics by cytochrome P450	3	0.065826	ADH1C, ADH7, ALDH3A1

Table V. Four enriched Kyoto Encyclopedia of Genes and Genomes pathway analysis of 10 hub genes that are identical to the results of 158 differentially expressed mRNAs.

Term	Count	P-value	Genes
bta04151:Phosphoinositide-3-kinase-protein kinase B signaling pathway	6	2.16×10^{-05}	MAPK1, IL6, RELA, VEGFA, MAPK3
bta04510:Focal adhesion	4	0.001531	MAPK1, JUN, VEGFA, MAPK3
bta05146:Amoebiasis	3	0.007335	IL6, RELA, TGFB1
bta05144:Malaria	2	0.061465	IL6, TGFB1

MAPK, mitogen-activated protein kinase; IL-6, interleukin 6; RELA, RELA proto-oncogene NF-κβ subunit; VEGFA, vascular endothelial growth factor A; JUN, Jun proto-oncogene; TGFB1, transforming growth factor-β1.

that the higher expression of MAPK3, TGFB1, RELA, IRF7 and VEGFA were associated with poor survival in GC. By contrast, a lower expression of JUN, FOS, IL8, ISG15 and MAPK1 were associated with poor survival in GC. Similar to the present study, a previous study applied bioinformatics analysis to identify the key genes in NCI-N87 GC cells exposed to quercetin. A PPI network was constructed, and hub genes were identified according to degree level, including FOS (degree=12) and JUN (degree=11) (33). One genome-wide search was performed to identify the genes epigenetically silenced by CpG methylation in GC. Three GC cell lines (SNU-1, SNU-601 and SNU-719) treated with 5Aza-Dc were analyzed to identify 143 associated genes by microarrays. Among the associated genes, IRF7 exhibited

Figure 7. Expression levels of 10 DEMs in gastric cancer tissue from the GEPIA database. (A) 5 upregulated DEMs.

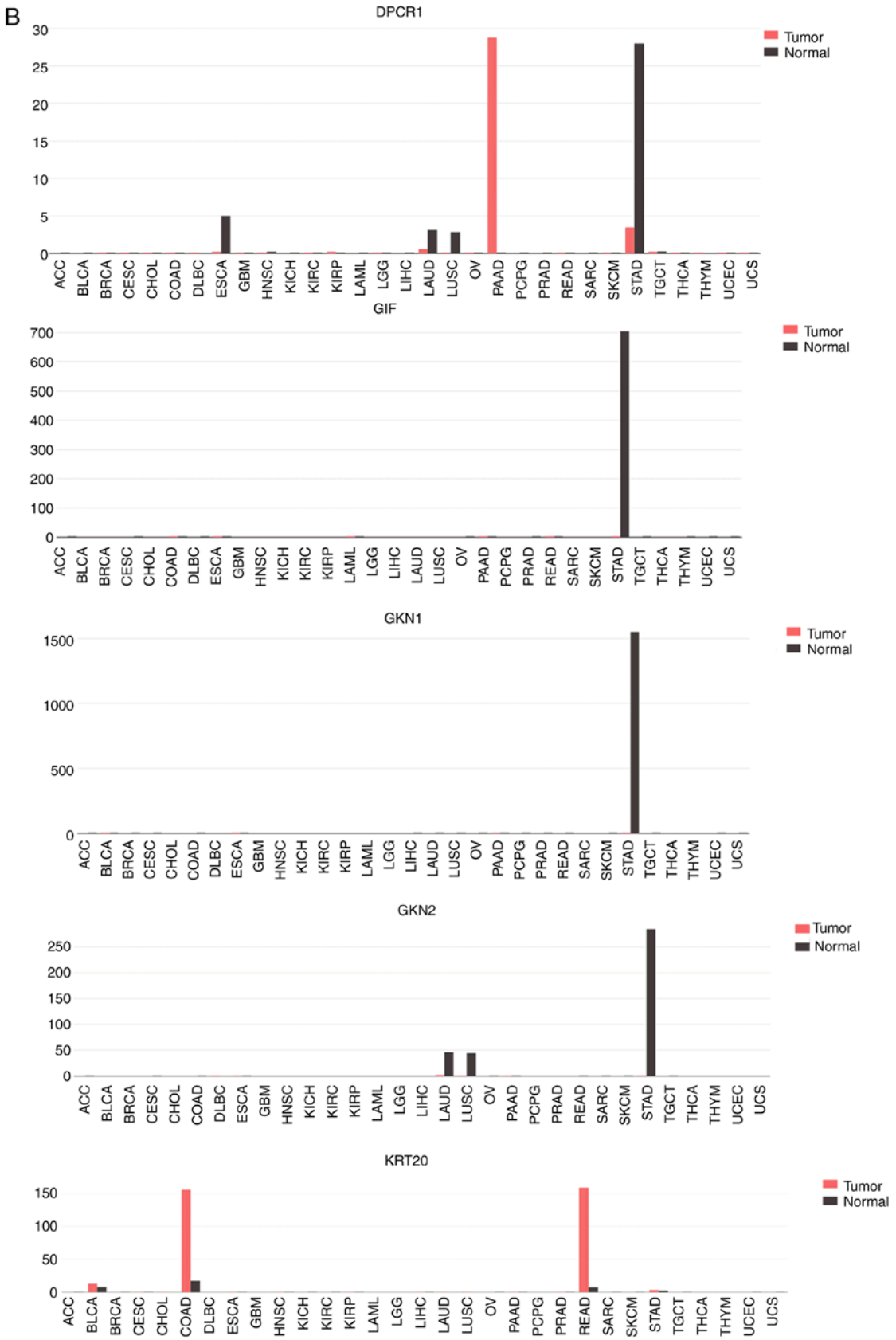

Figure 7. Continued. Expression levels of 10 DEMs in gastric cancer tissue from the GEPIA database. (B) 5 downregulated DEMs. P<0.05. DEM, differentially expressed mRNA.

promoter hypermethylation in one or more gastric cancer cell lines (34). As the intracellular signal transduction pathway, the MAPK cascade serves an important function in the progression of various tumor types. MAPK1 was upregulated in GC tissues and participated in the proliferation and cell migration of GC cells. In addition, miRNAs

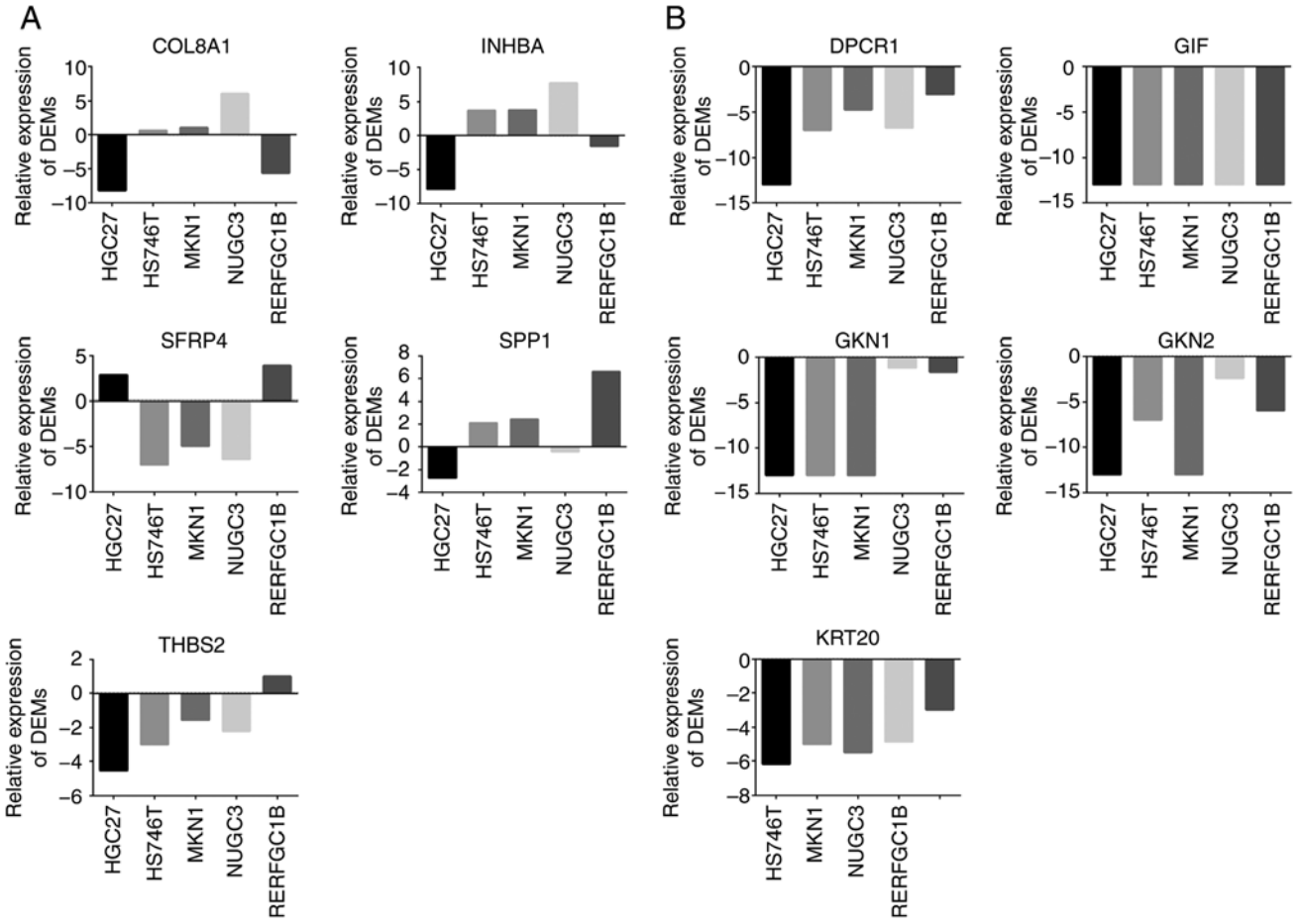

Figure 8. Expression levels of 10 DEMs in the Cancer Cell Line Encyclopedia database. (A) Expression levels of 5 upregulated DEMs in 5 GC cell lines. (B) Expression levels of 5 downregulated DEMs in 5 GC cell lines. P<0.05. DEM, differentially expressed mRNA; GC, gastric cancer.

may regulate the expression of MAPK1 and were therefore involved in the development and progression of GC (35,36). In the analysis of patients with resected GC, the results of patient prognosis analysis indicated that MAPK3/1 expression was an independent prognostic marker for patients with resected GC (37). The NF-κB signaling pathway serves an important function in the biological process of GC, including cell migration, cell invasion and cell apoptosis. As a basic component of the NF-κB signaling pathway, RELA has been reported to be activated in the progression of GC. The results of a study by Huang *et al* (38) revealed that RELA was upregulated in GC tumor tissues and GC cell lines compared with control groups. Furthermore, the upregulation of RELA was significantly correlated with poor OS in the prognosis of 876 patients with GC (P<0.001), which was consistent with the results of the present study. As an inflammatory factor, IL-6 serves an important function in the process of various types of cancer. Previous studies have revealed that IL-6 is involved in the regulation of invasion and prognosis in GC (39,40). Mechanistically, mesenchymal stem cells promote the activation of neutrophils through the IL-6-STAT3 axis to mediate GC progression (41). IL8 has been reported to be involved in the development of GC. A previous study demonstrated that IL-8 was upregulated in GC cells and enhanced cell proliferation, invasion and migration in GC (42). In addition, IL8 served as an inflammatory cytokine serving an important function in the angiogenesis of GC (43). Furthermore, a previous study also conducted a meta-analysis to investigate the potential functions of IL-6 and IL-8 polymorphisms in the development of GC. The results revealed that IL-6 rs1800796 and IL-8 rs4073 polymorphisms may serve as genetic biomarkers of GC in an Asian population (44). A number of studies have indicated that VEGFA functions as a direct target of miRNAs to participate in the tumor progression of GC (45,46). Furthermore, VEGFA may also be a predictive biomarker for antiangiogenic therapy in GC, which is consistent with the present bioinformatics results (47). A previous study also conducted analysis to reveal the correlation between polymorphisms of TGFB1 and VEGF genes and the survival of patients with GC. The results suggested that the TGFB1+915CG/CC and VEGF-634CG genotypes were associated with short-term survival in patients with GC (48).

MiRNAs serve an important function in the downregulation of the transcription of target mRNAs by binding to complementary 3'-untranslated regions of genes (49). An accumulating body of evidence has demonstrated the association between miRNAs and targets in tumor progression in various types of cancer. For example, it has been confirmed that miR-218 is a tumor suppressor in glioblastoma cells by directly targeting E2F transcription factor 2 (50). miR-155

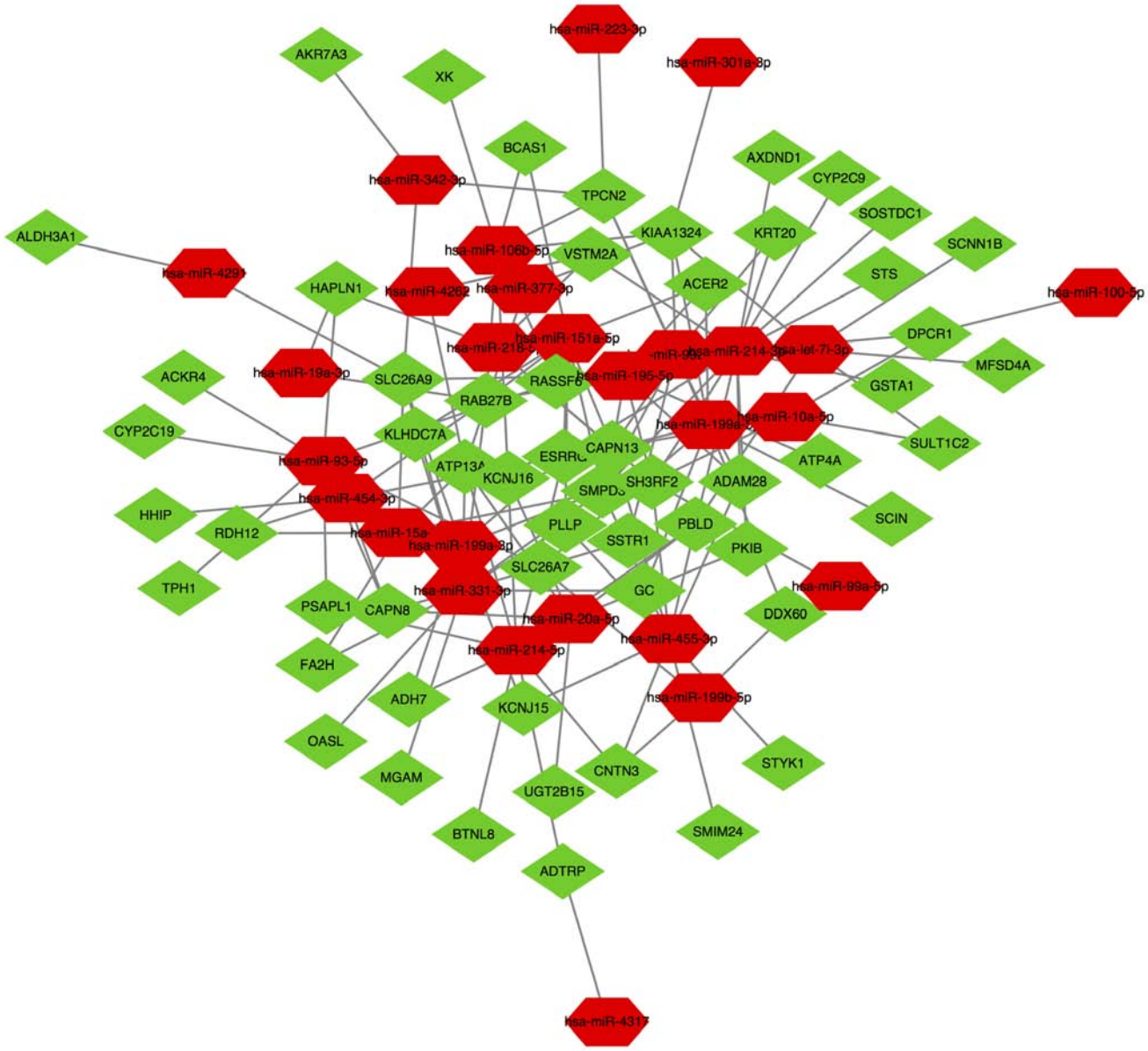

Figure 9. Network of microRNAs and targets. P<0.05.

has also been demonstrated to enhance cell growth and invasion by regulating transforming growth factor-β receptor 2 (51). In the present study, a total of 30 upregulated miRNAs and 1 downregulated miRNA were identified between the GC and control samples using systematic analysis. Then, a network of miRNAs and targets was constructed using the miRwalk database and Cytoscape software, which consisted of 124 nodes and 490 interactions. According to the results of the present analysis, miR-375 was downregulated in GC tissues when compared with the NC group. Similarly, a number of studies have confirmed the function of miR-375 as a tumor suppressor in GC. MiR-375 has been reported to be involved in cell proliferation, migration and invasion by regulating janus kinase 2 and macrophage stimulating 1 receptor (52,53). MiR-375 serves as a controller of the Hippo signaling pathway by targeting the Yes associated protein 1-TEA domain transcription factor 4-cellular communication network factor 2 axis (54). A previous study indicated that miR19a3p is upregulated in GC and promotes epithelialmesenchymal transition via the PI3K/Akt signaling pathway (55). Furthermore, miR-19a-3P has been confirmed to mediate metastasis by directly targeting MAX dimerization protein 1 in GC cells (56). Emerging evidence has indicated that miR-214-3p serves an essential oncogenic function and is correlated with distant metastasis as a novel biomarker of GC (57). In addition, miR-214-3p negatively regulates phosphatase and tensin homolog and is involved in GC cell proliferation, migration and invasion (58,59).

Previous studies have suggested that lncRNAs participate in the development of cell growth, metastasis and invasion progression in various types of cancer (60-62). LncRNAs have also been demonstrated to function as miRNA sponges that are involved in a variety of cancer types. For example, the lncRNA XIST has been demonstrated to function as a molecular sponge of miR-101 to modulate enhancer of zeste 2 polycomb repressive complex 2 subunit expression in GC (63).

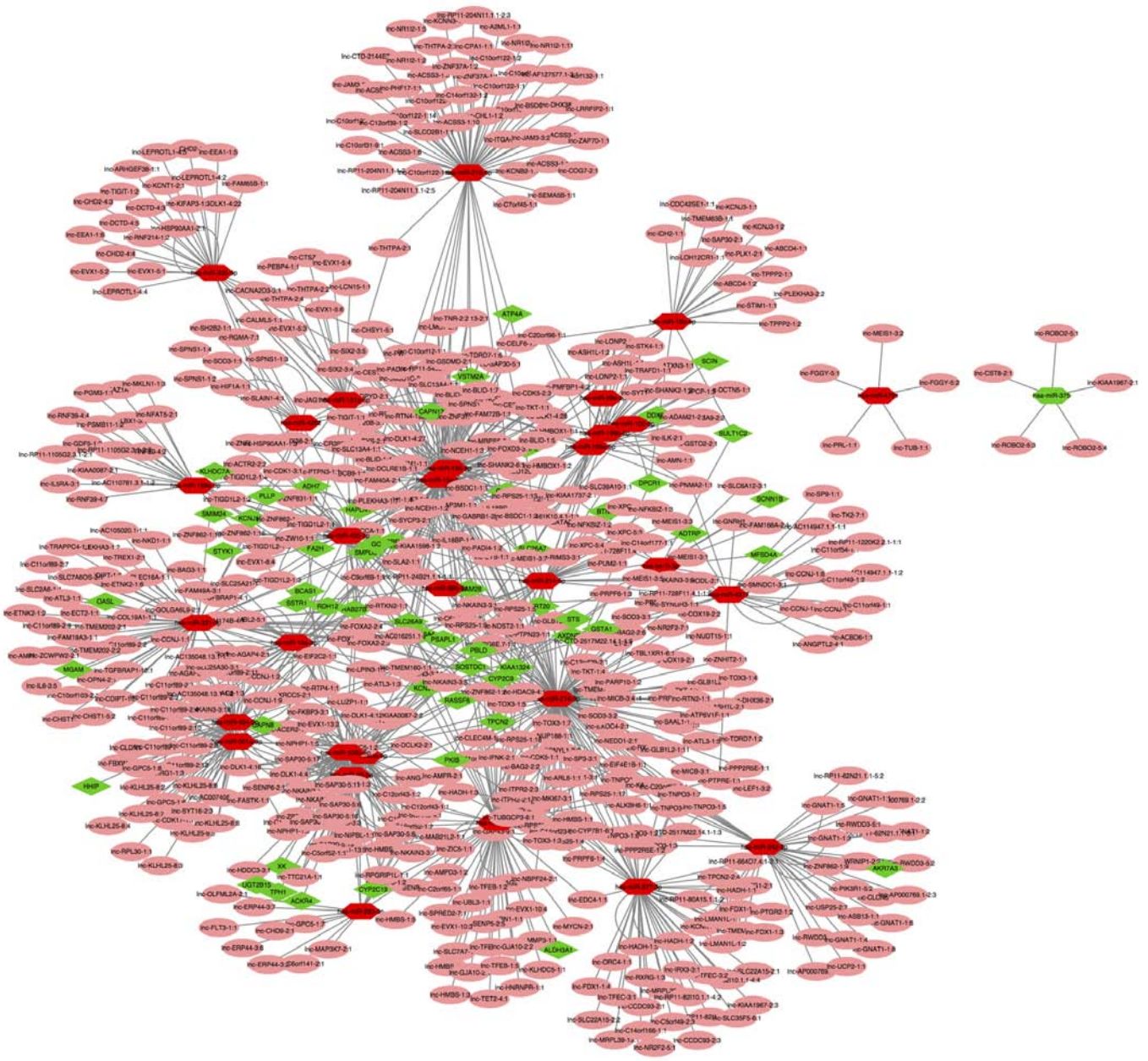

Figure 10. LncRNA-miRNA-mRNA regulatory network. Pink circles indicate lncRNAs, hexagons indicate miRNAs and diamonds represent mRNAs. Red nodes indicate upregulated mRNAs and miRNAs, and green nodes represent the downregulated mRNAs and miRNAs. P<0.05. LncRNA, long non-coding RNA; miRNA, microRNA.

LncRNA-regulator of reprogramming regulates the expression of miR-145 and ADP ribosylation factor 6 by functioning as a sponge in triple-negative breast cancer (64). Therefore, it is essential to investigate the regulatory mechanism underlying the action of lncRNAs, miRNAs and mRNAs. In the present study, the lncRNASNP database was used to perform prediction analysis of DEMis-lncRNA pairs. In addition, a lncRNA-miRNA-mRNA network was constructed using the combination of DEMis-target pairs and DEMis-lncRNA pairs, which included 1,215 regulatory associations. Altogether, these results provide a better understanding of the potential functions of lncRNA, miRNA and mRNA in GC.

However, there were a number of limitations in the present analysis. Firstly, the present study lacked experimental evidence on the expression levels and biological functions of genes. Further detection and experiments on the associated genes in larger sample sizes should be validated in future studies. Secondly, lncRNAs were only predicted using the lncRNASNP database without the investigation of expression profile databases, which are different from mRNAs and miRNAs. In order to increase the credibility, GC-associated lncRNAs require further analysis in the future.

In conclusion, the present study analyzed 158 DEMs, 31 DEMis and the top 10 hub genes in GC from multiple profile datasets by integrated bioinformatical analysis. In addition, lncRNA-miRNA-mRNA regulatory networks were established to gain a better understanding of the underlying molecular mechanism of GC. These results provide an effective foundation for further research on potential target therapy strategies in GC.

Acknowledgements

Not applicable.

Funding

The present study was supported in part by the Department of Gastrointestinal Endoscopy, First Affiliated Hospital of China Medical University (Shenyang, China).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MS designed the study. XM, YM, HZ and HJZ performed the research. XM analyzed the data and wrote the paper.

Ethics approval and consent to participate

Not applicable

Patient consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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