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

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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.

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 (IncRNA)-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 significant 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 significant 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 significant difference.

Determining the expression level of the significantly up- and downregulated DEMs. A total of 5 significantly upregulated DEMs (inhibin bA, collagen type VIII a1, secreted frizzle related protein 4, secreted phosphoprotein 1 and thrombospondin 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 significant difference.

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 IncRNASNP (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 significant 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.
Results

Identification of DEMs and DEMis. A total of 158 DEMs (5 of the 163 genes were identified as unrecognized genes) were identified based on the cut‑off criteria using FunRich, 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.

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, SULT1F, CST1, COL8A1, SFRP4, SPP1, COL1A1, ADAMTS2, WISP1, CTHRC1, COL12A1, ASPN, CRISPLD1, THY1, COL1A2, FN1, BGN, RARRES1, CAP2, MFAP2, PDPN, PRKX1, TIMP1, SPARC, COL6A3, COL4A1, THBS1, NRP2, PDLIM7, LY6E, SPOCK1, PI15, CEMIP, CXCL8 |
| Downregulated | GKN2, GKN1, ATP4A, ATP4B, GIF, LIPF, KCNJ16, SOSTDC1 KCNE2, CWH43, ESRRG, PGC, SLC28A2, PSAP1L, KRT20, VSIG1, LTFC, CXCL17, AKR1B10, LOC643201, GSTA1, ADH7, ADH1C, GC, CAPN9, MAL, SLC26A9, HRASLS2, MFSD4A, MUC5AC, FBP2, ALDH3A1, ADGRG2, VSTM2A, LINCO00982, CAPN13, KIAA1324, CA9, TPCN2, PIK3C2G, RDH12, SLC26A7, SSTR1, VSIG2, GATA6-AS1, HPGD, UPK1B, KCNJ15, SULT1C2, LINCO0675, BTN3L, AXDN1, LINCO00992, KAZALD1, TMED6, UGT2B15, SCN1B, HAPLN1, AKR1C1, LYPD6B, FCGBP, ADTRP, IGH, CA2, RFX6, ACER2, CYP2C9, PCAT18, PK1B, SH3RF2, HHP1, HEPCAM2, AADAC, CYP2C18, RAB27B, MGAM, SPINK7, CNTN3, LINCO1133, BCAS1, SULT1B1, CAPN8, SMIM6, AMDPC1, JCHAIN, PBCL, ATP13A4, RNAE1, PLLP, B4GALT3, STYK1, CYPT2C19, SMIM24, LRRC66, RASSF6, ADAM28, FA2H, GATA5, SCIN, SGK2, TPH1, PROM2, APOBEC1, ACKR4, ADHA1, 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.

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 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
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
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
used to analyze the process of carcinogenesis to enhance the universality and reliability of the results (4-8). In the present study, 158 shared DEGs (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 DEGs (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, lysophosphatidic 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 DEGs included ‘endodermal cell differentiation’, ‘proteinaceous extracellular’ and ‘extracellular matrix binding’ while the terms enriched by downregulated DEGs 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.
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; TGFβ1, 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-κB subunit; IRF7, interferon regulatory factor 7; VEGFA, vascular endothelial growth factor A.
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

| Term | Count | P-value | Genes |
|------|-------|---------|-------|
| bta04151:Phosphoinositide-3-kinase-protein kinase B signaling pathway | 10 | 6.79 x 10^-04 | COL4A1, SGK2, COL6A3, COL1A2, COL1A1, THBS1, COL1A1, THBS2, FN1, SPP1 |
| bta04510:Focal adhesion | 9 | 9.84 x 10^-05 | COL4A1, COL6A3, COL1A2, COL1A1, THBS1, COL1A1, THBS2, FN1, SPP1 |
| bta05146:Ameobiasis | 6 | 0.001142 | COL4A1, COL1A2, CXCL8, COL1A1, COL1A1, 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 COL4A1, COL6A3, COL1A2, COL1A1, THBS1, COL1A1, THBS2, FN1, SPP1 |
| bta04512:Extracellular matrix-receptor interaction | 9 | 1.47 x 10^-07 | COL4A1, COL6A3, COL1A2, COL1A1, THBS1, COL1A1, THBS2, FN1, SPP1 |
| bta04971:Gastric acid secretion and absorption | 7 | 1.07 x 10^-05 | KCNJ16, KCNJ15, ATP4A, ATP4B, SLC26A7, KCNE2, CA2 |
| bta04974:Protein digestion | 7 | 2.61 x 10^-05 | COL4A1, COL6A3, COL1A2, COL12A1, COL1A1, COL1A1, COL10A1 |
| bta00830:Retinol metabolism | 6 | 4.79 x 10^-05 | RDH12, CYP3A5, CYP2C18, ADH1C, ADH7 |
| bta05204:Chemical carcinogenesis | 6 | 1.29 x 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 x 10^-05 | MAPK1, IL6, RELA, VEGFA, MAPK3 |
| bta04510:Focal adhesion | 4 | 0.001531 | MAPK1, JUN, VEGFA, MAPK3 |
| bta05146:Ameobiasis | 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-xβ subunit; VEGFA, vascular endothelial growth factor A; JUN, Jun proto-oncogene; TGFB1, transforming growth factor-β1.
Figure 7. Expression levels of 10 DEMs in gastric cancer tissue from the GEPIA database. (A) 5 upregulated DEMs.
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
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 indicated that IL-6 serves an important function 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
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).
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.

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.
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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. 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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