Int J Clin Exp Med 2019;12(5):4600-4613
www.ijcem.com /ISSN:1940-5901/IJCEM0087405

Original Article

Long noncoding RNA MT1JP suppresses gastric cancer cell proliferation and migration in TGF-β induced EMT

Chenglou Zhu[2*], Tianxiang Liu[1*], Xiaoxiong Wen[3], Jichun Ma[4], Yaoqi Li[3], Jian Yang[1], Mingxu Da[1]

[1]Department of Surgical Oncology, Gansu Provincial Hospital, Lanzhou, China; [2]College of Clinical Medicine, Gansu University of Chinese Medicine, Lanzhou, China; [3]College of Clinical Medicine, Ningxia Medical University, Yinchuan, Ningxia, China; [4]Department of General Surgery Intensive Care Unit, Lanzhou University Second Hospital, Lanzhou, China. [*]Equal contributors.

Received October 21, 2018; Accepted March 9, 2019; Epub May 15, 2019; Published May 30, 2019

Abstract: Background: The early diagnosis rate of gastric cancer (GC) is low. Further research on the mechanisms of gastric cancer occurrence and development and finding biomarkers for the invasion and metastasis of gastric cancer are of great significance for the prevention and treatment of gastric cancer. Objective: To screen the differential expressions of long chain noncoding RNA (LncRNA) related to gastric cancer and to explore new targets for the diagnosis and treatment of gastric cancer. Methods: The microarray data associated with gastric cancer were downloaded in Gene Expression Omnibus (GEO), the expression difference of long noncoding RNA was analyzed with R software, and gene annotation and clustering was performed in the DAVID database. The differences in gene expression were enriched in the signaling pathways and in the protein interaction network. GES-1, BGC-823 cells were cultured with 5 ng/ml TGF-β, with the aim of stimulating the cells and evaluating the changes in cell migration, proliferation, and invasiveness after establishing an epithelial-mesenchymal transition (EMT) cell model. E-cadherin, N-cadherin, and vimentin expression levels were detected by Western-blot, and LncRNA MT1JP was investigated by performing a qRT-PCR. The relationship between the epithelial-mesenchymal markers and the expression level of LncRNA MT1JP was analyzed using Spearman's test. Results: The microarray GSE103236 involving 10 normal tissues and 9 gastric cancer tissues was analyzed. Based on the data from the microchip, 65 differentially expressed genes including 40 up-regulated LncRNAs and 25 down-regulated LncRNAs were screened. The results of the gene enrichment analysis showed that most of the differentially expressed genes were enriched in the cell matrix and adhesion molecules ($P<0.01$). The cell proliferation, migration, and invasion abilities of GES-1, BGC-823 induced by TGF-β were significantly enhanced ($P<0.01$). In the TGF-β induced group, the expression levels of LncRNA MT1JP and E-cadherin were significantly decreased in the BGC-823 cell line. However, the expression levels of N-cadherin and vimentin were increased in the TGF-β induced EMT group ($P<0.01$). Conclusions: The expression of gastric cancer related LncRNA is different. Most LncRNAs may be associated with the process of gastric cancer invasion and metastasis. The low expression level of LncRNA MT1JP can promote the EMT process of gastric cancer cells. It may be a major target for the invasion and metastasis of gastric cancer. Additional exploration of its related mechanisms is expected, which could become a new target for the diagnosis and treatment of gastric cancer.

Keywords: Gastric cancer, long noncoding RNA, epithelial-mesenchymal transition, TGF-β

Introduction

Gastric cancer (GC) is one of the most common malignancies worldwide [1]. Surgery is regarded as the only treatment strategy that may be effective, but due to the lack of specific symptoms in early stages of gastric cancer, most patients are diagnosed in the advanced stages and lose the chance for a complete cure [2]. Although radiochemotherapy and targeted therapy have a high rate of successfully treating advanced gastric cancer, the ability to achieve early diagnosis is still the key to improving the prognosis of gastric cancer patients [3]. The occurrence of tumor invasion and metastasis is the main reason for gastric cancer recurrence, and it also affects the effectiveness of treatment to a large extent [4]. Therefore, the in-depth study of the mechanisms of the occurrence and development of gastric cancer is expected to improve the early diagnosis rate and the anti-tumor efficacy.

Long noncoding RNA (LncRNA) is a member of the noncoding RNA family. Its length is usually larger than 200nt, less than 100 kb. Studies have confirmed that LncRNA not only participates in the regulation of the biological behaviors of normal cells, such as differentiation and growth, but it also plays a significant role in normal life activities. It also plays a crucial role in the development and progression of tumors, invasion, and metastasis [5]. The expression of long-chain noncoding RNA in tumor tissues and normal tissues has certain differences [6]. Some LncRNAs are similar to oncogenes and are highly expressed in tumor tissues [7, 8] such as MALAT1, HOTAIR, etc., while some LncRNAs are functionally similar to tumor suppressor genes and show a low expression in tumor tissues [9, 10] such as MEG3, LET, etc. Due to the heterogeneity among tumors, some LncRNAs have different expression levels in different tumors. For example, LncRNA BANCR is highly expressed in melanoma and low expressed in lung cancer tissues [11]. However, the relevant mechanisms of the LncRNA and EMT processes in gastric cancer are still unknown, but it has been determined that some long-chain noncoding RNAs do participate in the gastric cancer EMT process [12].

At present, more studies have started to focus on the function of long-chain noncoding RNAs in the development of tumors. LncRNAs were previously considered to be biological "noise" or "junk" and have been re-recognized by researchers [13]. However, compared with miRNA research, the understanding of the biological behavior of LncRNA is still at an exploratory stage. However, the invasion and metastasis of malignant tumors and the EMT process are the result of multi-linkages of multiple molecules involved in cells, and the relevant mechanisms are complex and unclear [14]. Whether long-chain non-coding RNA is regulated by EMT during gastric cancer or whether it is affected by the expression of other genes, and how to determine which specific genes and molecules interact with each other are still unknown. Although existing studies provide ideas for understanding the function of LncRNA in the EMT of gastric cancer, with the continuous advancement of biological technology, the emergence of more effective techniques and tools may be helpful for the further study of LncRNA, gastric cancer EMT, invasion, and metastasis. This study intends to explore the role and possible regulatory mechanisms of long-chain non-coding RNA MT1JP in the EMT of gastric cancer by using bioinformatics technology and the functional verification of cell experiments to provide new ideas for the early diagnosis of gastric cancer and the prognosis of gastric cancer.

Materials and methods

Chip data source

A computer search GEO database (https://www.ncbi.nlm.nih.gov/gds/) was utilized to search for gastric cancer-related chip data for long-chain noncoding RNA expression levels. The search terms were: long nocoding RNA, long noncoding RNA, lncRNA, gastric cancer, stomach cancer, gastric neoplasm, and finally data analysis based on GSE 103236 chip original data and platform information (GPL4133, published on August 30, 2017). GSE 103236 contains 19 samples, including 10 normal tissues of gastric mucosa and 9 tissues of gastric cancer.

Differential gene screening

The original data of the GSE103236 chip we obtained is a TXT format file, which we imported into the GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) online system for chip quality evaluation. The R software was used to retrieve the "limma" package for differential gene screening. Differential gene screening conditions were set to: the expression multiple was more than 2.5 times and the statistical p value was ≤ 0.03. Gene expression status was distinguished by the positive/negative value of the log fold change (logFC), where positive values represent up-regulated expression and negative values represent down-regulated expression.

Gene GO analysis and signal pathway enrichment analysis

The differential genes obtained from the screening were genetically annotated and clustered on the DAVID online website (https://david.ncifcrf.gov/home.jsp). The GO analysis was based on three levels: biological Processes (BP), molecular functions (MF), and cell components (CC). A volcano figure was generated and heat maps were used to describe the expression levels and classifications of the differential

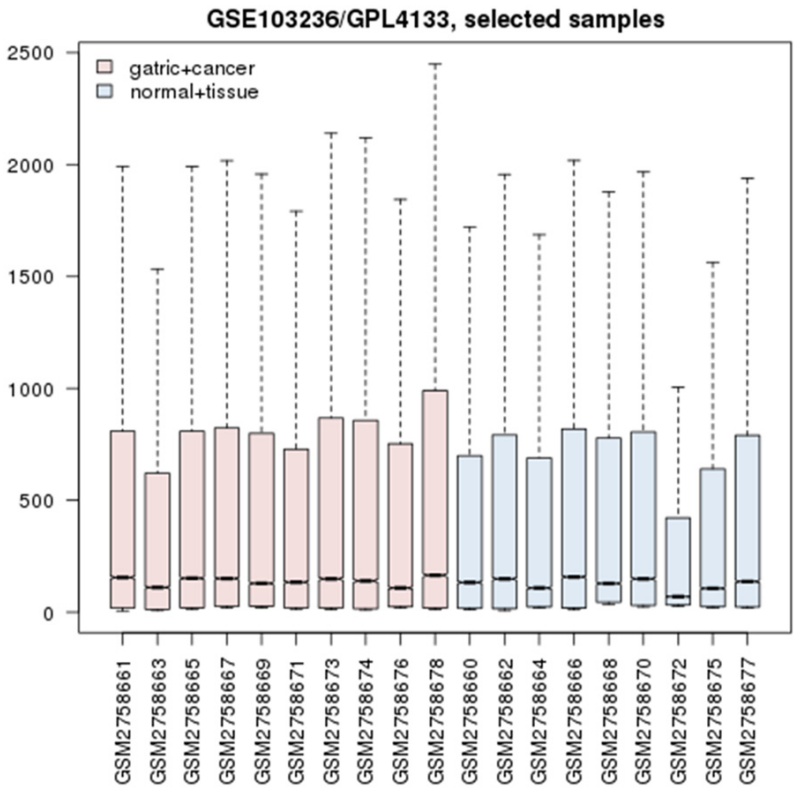

Figure 1. GSE103236 chip quality evaluation.

genes. Differential gene names were imported into DAVID's third-party website for the analysis of the gene signaling pathways (KEGG).

Differential gene protein interaction analysis

Differential gene names were imported into the STRING online tool (https://string-db.org/) for a protein-protein interaction analysis (PPI), and the protein interaction maps were generated based on the differences in gene enrichment.

Cell culture

Normal gastric mucosal epithelial cell GES-1 and gastric cancer cell line BGC823 (low differentiation) were purchased from the Chinese Academy of Sciences Cell Bank (Shanghai, China). These cells were cultivated in DMEM supplemented with 10% fetal calf serum. The culture conditions were: 37°C, 5% CO_2, saturated humidity.

MTT proliferation assay

One hundred microliters of cell suspension with a concentration of 1.5×10^5 cells/ml was inoculated into a 96-well plate, 20 μl MTT solution was added for 2 h at a concentration of 5 mg/L, and then DMSO 200 μl was added for 10 minutes, and the plate reader was set at a wavelength of 490 nm. The absorbance (A) value of each well was measured, and the cell survival rate of each group was calculated.

Invasion assay

The transwell chamber was placed in a 24-well plate. After that, 500 μL of DMEM medium containing 10% fetal bovine serum was added to the lower layer of the transwell chamber. After 24 to 48 hours of incubation, the transwell chamber was removed, fixed with paraformaldehyde, and stained with crystal violet fuel to dry at room temperature for 2 hours. The number of cells passing through the membrane in the field of 5 high-power microscopes was counted, and the number of cells permeating the membrane in each high-powered microscope field was finally determined. The number of cells passing through the membrane was used to evaluate the gastric epithelial cells and the invasive ability of the tumor cells.

Wound healing assay

GES-1 and BGC-823 cells with a cell density of 5×10^4 cells/ml were seeded in 6-well plates, and each well was scratched 24 hours after the disinfection of the ruler. After the scratches were completed, they were rinsed with prepared PBS. FBS-containing DMEM was added to each well and placed in the incubator for further incubation.

RNA extraction and qRT-PCR analysis

Total RNA was extracted from the cells using Trizol (Takara, Dalian, China). Reverse transcription of the total RNA from the sample of cDNA was performed using the PrimeScript RT rea-

Table 1. Differentially expressed genes

Gene name	logFC	t	*P*. value	B	Expression type
TREM2	3.5184	10.6028	5.24E-10	12.0823	Up
COL10A1	6.0841	9.5351	3.50E-09	10.5260	Up
KIAA1199	4.9871	8.0656	6.03E-08	8.1018	Up
CTHRC1	3.3432	7.8500	9.38E-08	7.7172	Up
SALL4	4.2045	7.8450	9.48E-08	7.7081	Up
HOXC9	3.9205	7.2229	3.51E-07	6.5553	Up
TNFRSF11B	2.5253	6.6368	1.26E-06	5.4122	Up
INHBA	4.0764	6.4684	1.84E-06	5.0740	Up
SDS	3.2009	6.4331	1.99E-06	5.0026	Up
COL1A1	2.8228	6.2091	3.30E-06	4.5448	Up
SPHK1	2.6137	6.0681	4.56E-06	4.2530	Up
KRT80	3.8673	5.9807	5.57E-06	4.0708	Up
F12	2.7550	5.9347	6.20E-06	3.9743	Up
ETV4	2.9119	5.7937	8.59E-06	3.6773	Up
TNFRSF12A	2.6818	5.6512	1.20E-05	3.3745	Up
ONECUT2	4.3326	5.6146	1.30E-05	3.2963	Up
CLDN1	3.6588	5.5285	1.60E-05	3.1117	Up
IL11	3.5593	5.4353	1.99E-05	2.9111	Up
FAP	3.4693	5.3149	2.64E-05	2.6504	Up
FLJ39632	3.2109	5.2468	3.11E-05	2.5024	Up
MMP11	3.3208	5.2390	3.16E-05	2.4854	Up
SLC4A11	2.9594	5.1976	3.49E-05	2.3951	Up
CLDN4	3.6157	5.0372	5.12E-05	2.0441	Up
KRT17	4.4471	5.0117	5.44E-05	1.9879	Up
APOC1	3.2446	4.9928	5.69E-05	1.9465	Up
CKMT2	-3.8748	-8.2137	4.47E-08	8.3617	Down
FIGF	-4.2692	-7.7033	1.27E-07	7.4510	Down
LIFR	-2.5271	-6.6761	1.16E-06	5.4906	Down
APOBEC2	-3.8317	-6.5342	1.59E-06	5.2068	Down
SCARA5	-4.2081	-6.2020	3.36E-06	4.5302	Down
PLCXD3	-2.6867	-6.0749	4.49E-06	4.2671	Down
GPER	-3.1642	-5.9834	5.54E-06	4.0764	Down
MYOC	-5.4140	-5.8472	7.59E-06	3.7903	Down
MAL	-3.6087	-5.8202	8.08E-06	3.7333	Down
C16orf89	-3.3517	-5.7533	9.43E-06	3.5918	Down
RERGL	-2.5583	-5.6865	1.10E-05	3.4498	Down
PLP1	-2.9515	-5.3954	2.18E-05	2.8249	Down
MT1E	-2.6774	-5.2734	2.92E-05	2.5603	Down
PI16	-3.5027	-5.1692	3.73E-05	2.3331	Down
DPT	-3.1734	-5.1167	4.23E-05	2.2183	Down
LGI1	-2.5387	-5.1133	4.27E-05	2.2109	Down
CCKBR	-3.6775	-4.8688	7.66E-05	1.6731	Down
TTR	-3.5670	-4.8460	8.09E-05	1.6228	Down
CKB	-2.5323	-4.6818	0.000120133	1.2593	Down
LYVE1	-2.6857	-4.6392	0.000133139	1.1647	Down
NPY	-2.6488	-4.6200	0.000139448	1.1221	Down
ESRRG	-3.4429	-4.4822	0.000194527	0.8156	Down
DRD5	-3.4612	-4.4540	0.000208265	0.7528	Down
PPP1R1A	-3.3649	-4.4045	0.00023473	0.6426	Down
MT1JP	-2.5498	-4.3580	0.000262683	0.5391	Down

gent kit along with a gDNA eraser kit (Takara, Dalian, China). Using the cDNA obtained by reverse transcription as a template, gene amplification was performed using a Light Cycler® Nano (Roche) fluorescence quantitative PCR machine, and relative quantification was performed using the SYBR® Select Master Mix. Using the housekeeping gene β-actin as an internal reference, the expression level of LncRNA MT1P was quantified using the ΔCt value.

Western blotting analysis

The cells were lysed with a RIPA lysate containing a phosphoprotein cocktail inhibitor. The cells were completely lysed and centrifuged under the following conditions: 4°C 12000 rpm 20 min. The protein samples were separated with 10% SDS-polyacrylamide, electrophoresed on a gel and transferred to a 0.22 μm PVDF membrane. After the membrane was completed, it was blocked with 5% nonfat milk. We incubated the primary antibody for 1 hour at room temperature and placed it in a 4°C freezer overnight. We incubated at room temperature for another 1 hour and wash it extensively. The membrane was subsequently placed in a secondary antibody and incubated for 2 hours at room temperature.

Statistical analysis

SPSS 17.0 software was used for the statistical analysis, and GraphPad Prism 6 software was used for the statistical chart making. The data were expressed as the mean ± SD, and the measurement data included a *t* test, the two classified variables used a chi-squared test, and the correlation between them was analyzed using Spearman's correlation analysis. The test level was 0.05, and the difference was considered statistically significant when $P<0.05$.

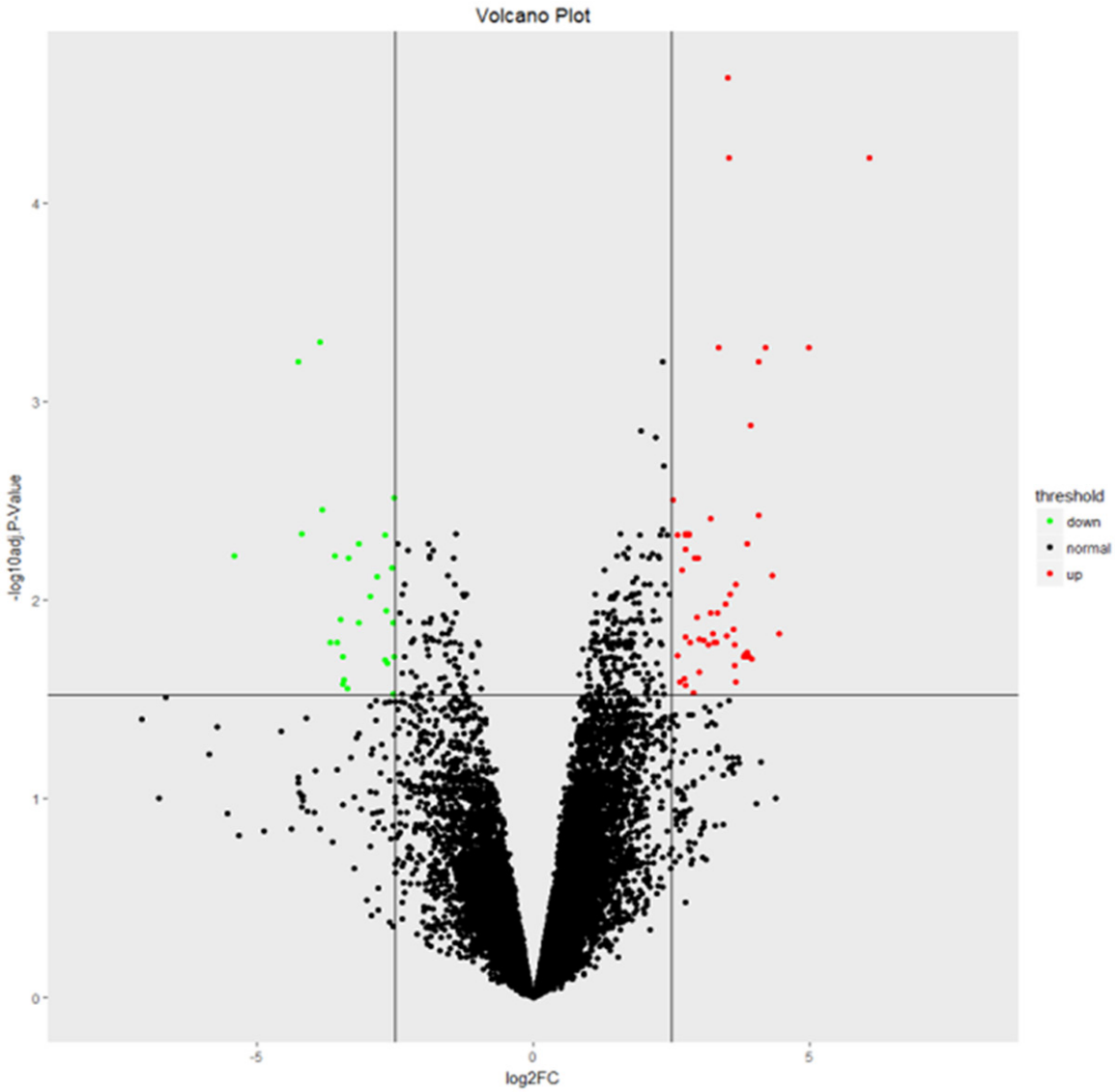

Figure 2. Volcano map of differentially expressed genes.

Results

Chip quality evaluation

The GSE103236 chip included 10 normal gastric mucosal tissues and 9 gastric cancer tissues. The quality evaluation results showed that the gastric cancer tissues and the normal gastric mucosa tissues did not show significant bias in the process of total RNA extraction and chip analysis, and there was no significant difference in the mean levels between the two groups (**Figure 1**).

Differential genetic screening

According to the expression multiples of 2.5 times and the statistical P≤0.03, the microarray differentially expressed genes were screened. Finally, 65 differential genes were screened, of which 40 were up-regulated and 25 were down-regulated. The up-regulation and down-regulation of differentially expressed genes were sorted by expression ratio and p-value, and the up-regulated expression of the top 25 were: TREM2, COL10A1, KIAA1199, CTHRC1, SALL4, HOXC9, TNFRSF11B, INHBA, SDS, COL1A1, SPHK1, KRT80, F12, ETV4, TNFRSF12A, ONECUT2, CLDN1, IL11, FAP, FLJ39632, MMP11, SLC4A11, CLDN4, KRT17, APOC1; the down-regulated expression of the top 25 were: CKMT2, FIGF, LIFR, APOBEC2, SCARA5, PLCXD3, GPER, MYOC, MAL, C16-orf89, RERGL, PLP1, MT1E, PI16, DPT, LGI1, CCKBR, TTR, CKB, LYVE1, NPY, ESRRG, DRD5,

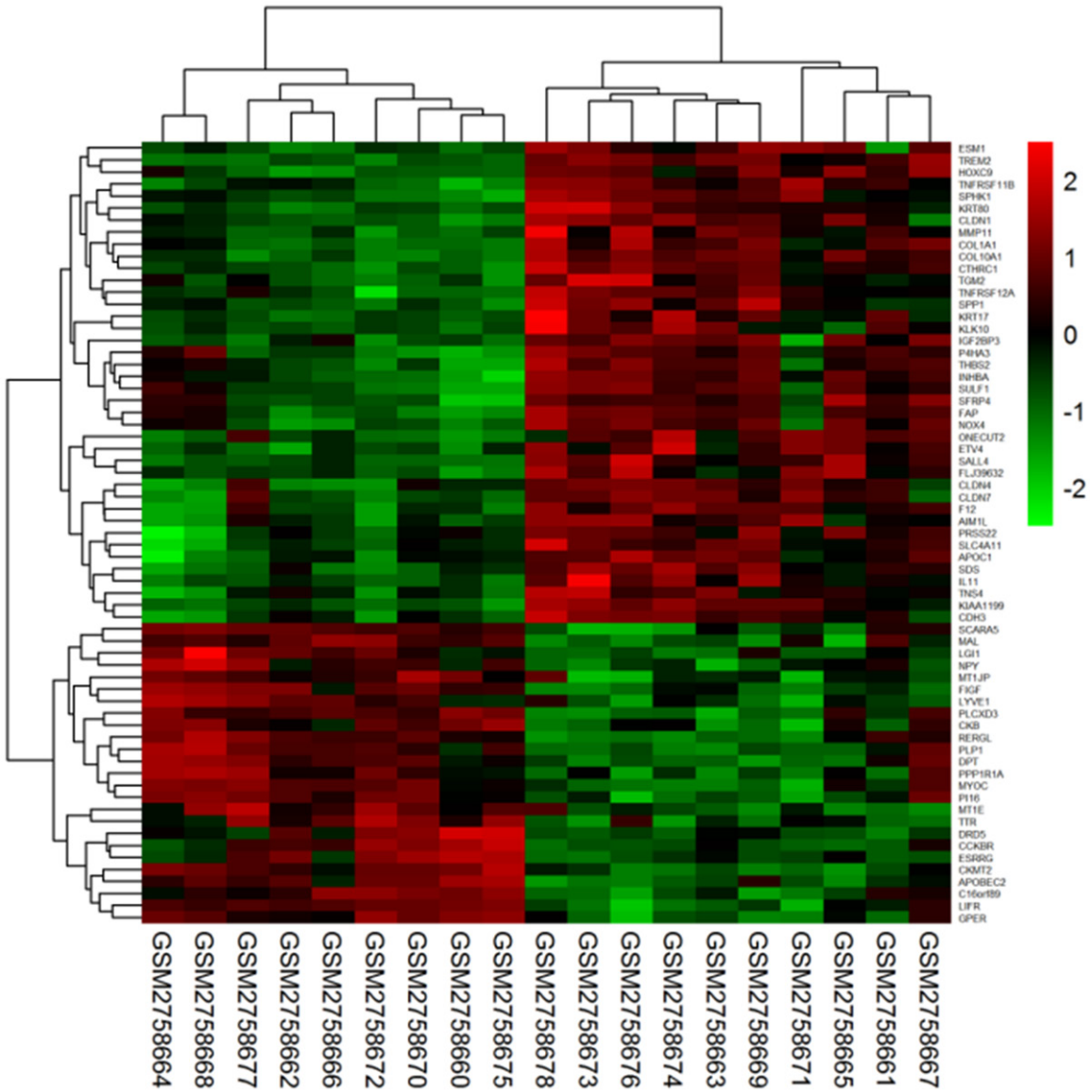

Figure 3. Cluster analysis diagram of differentially expressed genes.

PPP1R1A, MT1JP (**Table 1**). The differentially expressed gene volcano map is shown in **Figure 2**, and the cluster analysis is shown in **Figure 3**.

Gene GO analysis results

According to the different levels of gene expression regulation, the up-regulation and down-regulation genes were separately analyzed using a GO analysis. The results showed that the up-regulated genes were involved in the positive regulation of signal transduction and cell proliferation regulation at the BP level, and they were involved in the extracellular space substance regulation at the CC level. And the cell junctions regulated the serine endopeptidase activity and structural molecule activity at the MF level; the downregulated genes participated in the ion balance and neural system development at the BP level, and participated in the extracellular space substance regulation at the CC and MF levels in the regulation of receptor activity. The gene GO analysis in **Table 2** shows that regardless of the up-regulation or down-regulation of the expression genes, they are all involved in the regulation of extracellular space substance expression at the CC level. The occurrence and development of gastric cancer involves the epithelial-mesenchymal transition process. It is associated with the

Table 2. Differential gene GO analysis

Expression	Category	Term	Count	*P* Value	Genes
Up-regulation	GO_BP	GO: 0009967~positive regulation of signal transduction	12	4.61E-06	NOX4, TNFRSF11B, TNFRSF12A, CDH3, COL1A1, ESM1, INHBA, IL11, SFRP4, SPHK1, SULF1, TREM2
	GO_BP	GO: 0042127~regulation of cell proliferation	12	6.10E-06	ETV4, NOX4, TNFRSF11B, CDH3, CTHRC1, ESM1, FAP, INHBA, IL11, SFRP4, SPHK1, SULF1
	GO_CC	GO: 0005615~extracellular space	10	3.88E-04	TNFRSF11B, APOC1, F12, CTHRC1, COL1A1, FAP, INHBA, SFRP4, SPP1, SULF1
	GO_CC	GO: 0030054~cell junction	7	0.0084	CDH3, CLDN1, CLDN4, CLDN7, FAP, TNS4, TGM2
	GO_MF	GO: 0004252~serine-type endopeptidase activity	4	0.0045	F12, FAP, KLK10, PRSS22
	GO_MF	GO: 0005198~structural molecule activity	6	0.0064	CLDN1, CLDN4, CLDN7, COL1A1, KRT17, KRT80
Down-regulation	GO_BP	GO: 0050801~ion homeostasis	6	4.99E-04	CCKBR, CKB, MT1E, MYOC, SCARA5
	GO_BP	GO: 0042552~myelination	3	0.0050	MAL, MYOC, PLP1
	GO_BP	GO: 0007399~nervous system development	7	0.0168	CKB, CKB, LGI1, MA, MYOC, NPY, PLP1
	GO_CC	GO: 0005615~extracellular space	7	0.0010	CKB, DPT, LGI1, MYOC, NPY, TTR
	GO_CC	GO: 0044421~extracellular region part	10	0.0072	CKB, DPT, LGI1, LIFR, LYVE1, MYOC, NPY, PI16, TTR
	GO_MF	GO: 0004872~receptor activity	6	0.0436	CCKBR, DRD5, ESRRG, LIFR, LYVE1, SCARA5

Table 3. KEGG analysis results

Category	Term	Genes	Count
KEGG_PATHWAY	cfa04514: Cell adhesion molecules (CAMs)	CDH3, CLDN1, CLDN4, CLDN7	4
KEGG_PATHWAY	cfa04512: ECM-receptor interaction	COL1A1, SPP1, THBS2	3
KEGG_PATHWAY	cfa04670: Leukocyte transendothelial migration	CLDN1, CLDN4, CLDN7	3
KEGG_PATHWAY	cfa00330: Arginine and proline metabolism	CKB, CKMT2	2

differential expression of epithelial phenotypic markers, such as the CDH3 gene.

Key gene pathway enrichment analysis

The key signal pathways involved in the differential gene KEGG enrichment analysis are: ① cell adhesion molecules (CAMs), of which the related genes are CDH3, CLDN1, CLDN4, and CLDN7; ② extracellular matrix (ECM) receptor interaction, and the related genes there are COL1A1, SPP1, and THBS2; ③ leukocytes by endothelial migration, and the related genes are CLDN1, CLDN4, and CLDN7; ④ arginine and proline metabolism, and the related genes are: CKB and CKMT2. The key pathway enrichment results of the KEGG differentially expressed genes are shown in **Table 3**.

Protein interaction network analysis

The differentially expressed genes were imported into the STRING online website for PPI analysis. The results of the PPI analysis of 40 up-regulated genes showed that they focused on the formation of three major gene accumulation regions: ① Aggregation areas with SMAD2, BMP7, SOX2, LEFTY2, ACVR2A, ACVR2B, BMP2, NANOG, and POU5F1 as the core; ② With COL10A1, COL3A1, COL5A2, P4HA3, LUM, COL6A3, COL1A2, and COL1A1 as the core of the aggregation; ③ CYBB, NCF4, NOX1, NCF1, CYBA, NCF2, and NOX4 as the core of the aggregation area. Twenty-five down-regulated PPIs were analyzed to form two aggregation areas: ① Aggregation areas with NPS, GCG, GAST, CCK, NPY, POMC, DRD5, CRH, CCKAR, CCKBR genes as the core; ② With FLT4, FIGF, VEGFA, and NRP1, the LYVE1, VEGFC, KDR, and CDH5 genes are the core aggregation regions (**Figures 4** and **5**).

TGF-β promotes the proliferation of GES-1 and BGC-823 cells

The GES-1 and BGC-823 cells were induced with TGF-β at a final concentration of 5 ng/ml, and the OD values of each well at 0 h, 12 h, 24 h, 48 h, and 72 h after TGF-β induction were measured using an MTT assay. The survival rate of cells was calculated as shown in **Figure 6A** and **6B**, suggesting that the proliferation of the GES-1 and BGC-823 cells was significantly increased, and the growth rate was increased

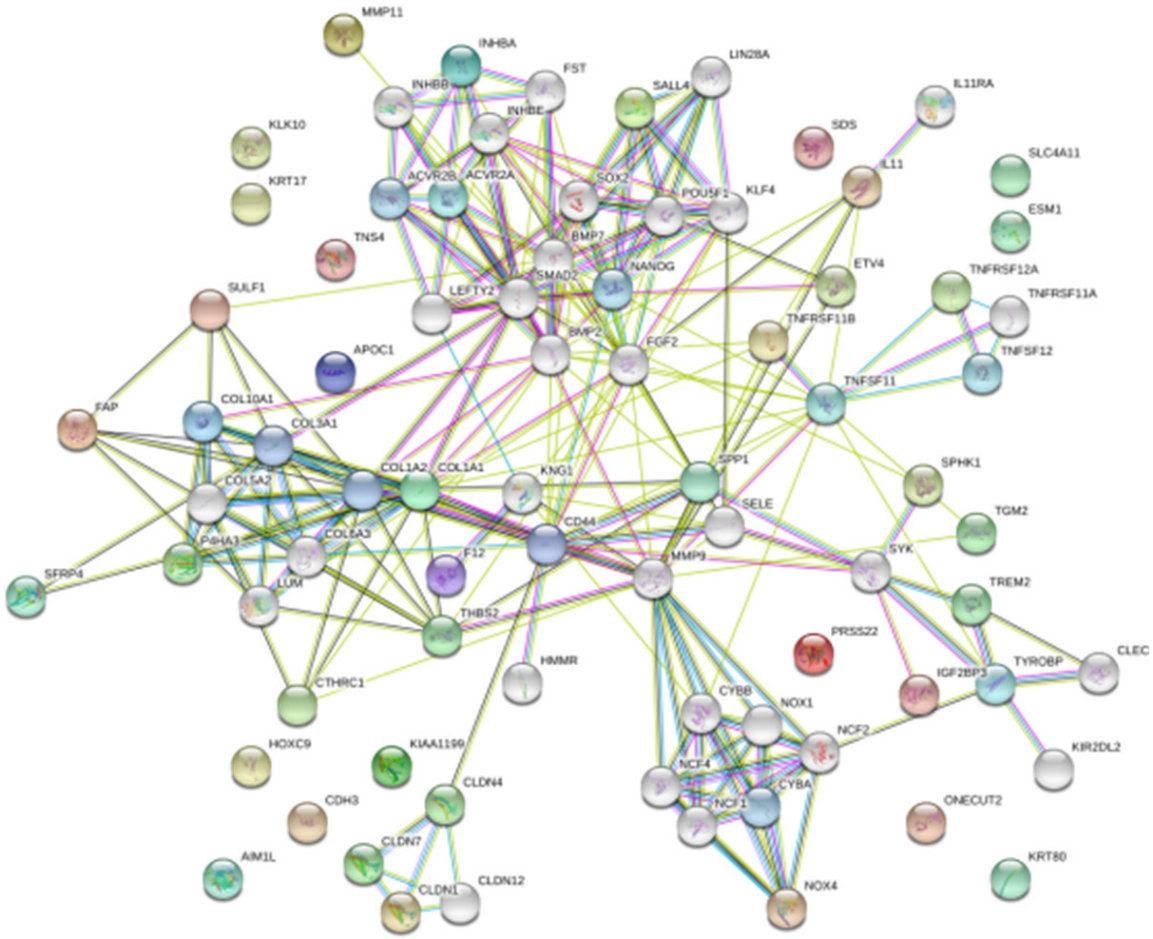

Figure 4. PPI network diagram of up-regulated genes.

after the TGF-β treatment, which was significant at 48 h and 72 h (P<0.01).

TGF-β enhances the migration of BGC-823 Cells

At 12 h, 24 h, 48 h and 72 h after scratching, the number of cells that migrated to the scratches and the cell morphology were observed. As shown in **Figure 7A**, no obvious changes were observed in the cells at the scratches of the BGC-823 cells at 12 h. At 48 h, the scratches on the cells gradually became narrower, and the distance between the scratches gradually decreased, and the cells broke the scratch line toward the scratches. Growing: In the TGF-β-induced cell group, the cells grew faster toward the scratch and the trend was more pronounced. The trend of cell scratches is shown in **Figure 7B**. There was a statistically significant difference between the groups at 48 h and 72 h (P<0.05).

TGF-β increases the invasive ability of BGC-823 cells

A transwell assay was used to evaluate the invasion and metastasis ability of the cells. TGF-β induced the cells 24 h after inoculation in the transwell chamber, and we incubated them for 48 hours, then we fixed, stained, and counted the number of cells passing through the chamber, and continuously counted the number of cells in the 5 high-power fields, and obtained the average The value is the average number of cells passing through the cell. **Figure 8** shows that the invasion and metastasis of the GES-1 and BGC-823 cells stimulated by TGF-β were significantly enhanced. At 48 h, the number of cells transfected with TGF-β through the chamber was smaller than the number of untreated cells. The number of cells was significantly increased and the difference was statistically significant (P<0.01).

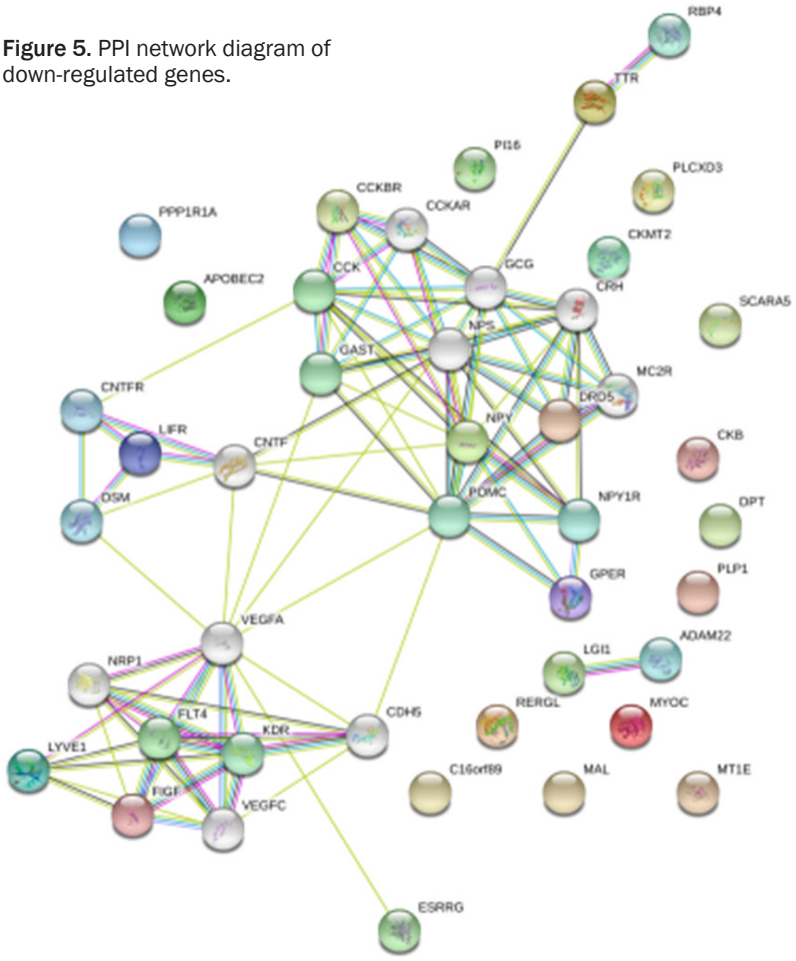

Figure 5. PPI network diagram of down-regulated genes.

Changes in expression levels of EMT-related markers

The Western-blot method was used to detect the expression of epithelial stromal phenotype markers in the GES-1 and BGC-823 cells. The results showed that E-cadherin was up-regulated in the BGC-823 cells, the N-cadherin and vimentin proteins were down-regulated, and E-cadherin was down-regulated in the TGF-β-induced BGC-823 cells, and the N-cadherin and vimentin proteins were up-regulated. After analyzing the relative expression levels, the results showed that the difference was statistically significant (P<0.05), as shown in **Figure 9**.

LncRNA MT1JP expression level

The expression levels of LncRNA MT1JP in the GES-1, GES-1 TGF-β, BGC-823, and BGC-823 TGF-β cells was determined by RT-PCR. The results showed that the expression level of LncRNA MT1JP was significantly lower in the TGF-β-induced cell group than it was in the uninduced cell group, and the difference was most pronounced in the BGC-823 cell group (P<0.01), suggesting that a low expression of LncRNA MT1JP may promote the invasion of gastric cancer cells. And transfer (**Figure 10**).

Analysis of the relationship between the expression levels of the LncRNA MT1JP and EMT markers

In order to further clarify the intrinsic relationship between LncRNA MT1JP expression and epithelial mesenchymal phenotypic marker expression, it is suggested that there may be potential regulatory roles between the two, based on the LncRNA MT1JP PCR detection results and Western-blot detection The expression levels of E-cadherin, N-cadherin, and vimentin were analyzed by Spearman's correlation. The results showed that the expression of LncRNA MT1JP was positively correlated with the expression of E-cadherin (r=0.7403, P<0.0001) and negatively correlated with the expression of N-cadherin (r=0.7202, P<0.0001) and vimentin (r=0.9537, P<0.0001). As shown in **Figure 11**.

Discussion

GC is currently the fifth most common malignancy in the world. There were approximately 95,160 new cases in 2012 [15]. Despite the decreasing mortality rate in recent years, GC is still the third leading cause of cancer-related death [13]. However, currently available tumor markers such as CEA and CA-199 have low sensitivity and specificity when used in the diagnosis of gastric cancer, which cannot meet the clinical needs well and pose certain challenges for the early diagnosis of gastric cancer [16]. Therefore, it is necessary to further explore the

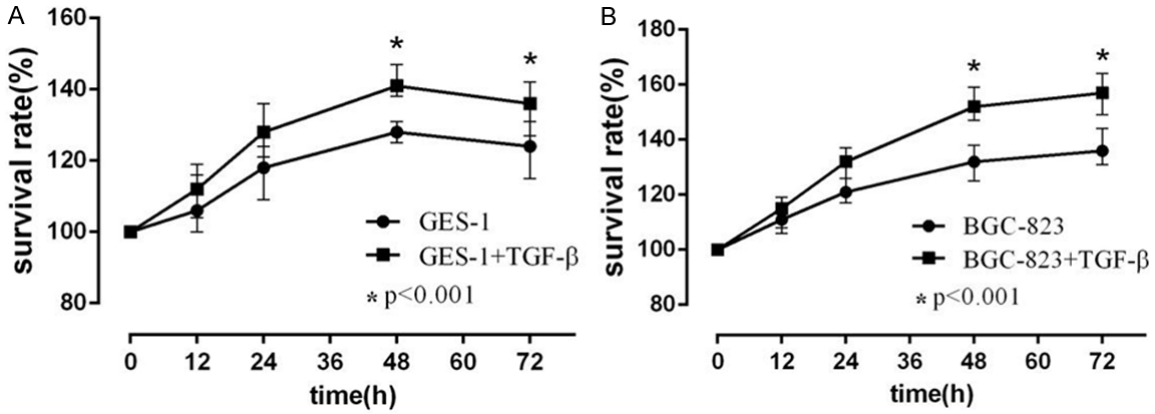

Figure 6. MTT assay results: A. The growth promoting effect of TGF-β on GES-1 cells; B. The growth promoting effect of TGF-β on BGC-823 cells.

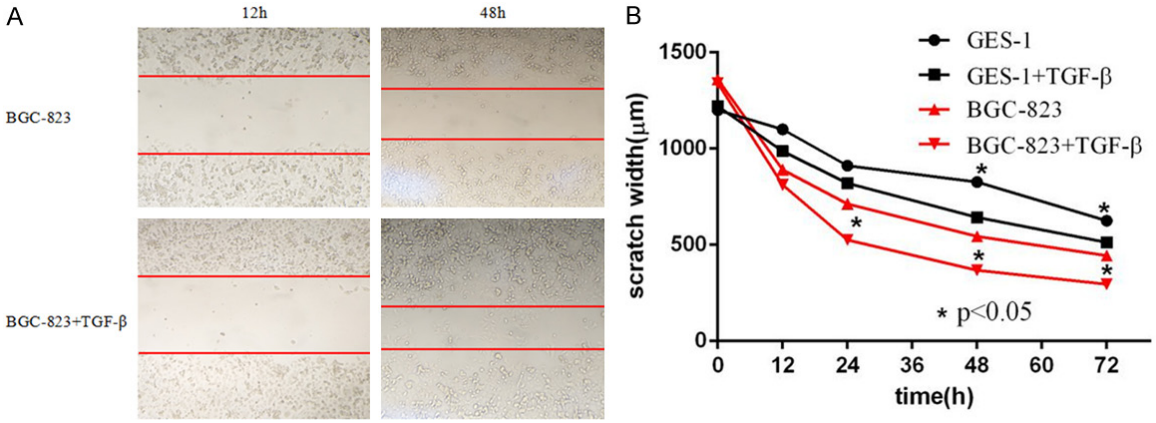

Figure 7. Cell scratch test results: A. The effect of TGF-β on the migration of BGC-823 cells; B. Changes in the scratch width of the cells in each group.

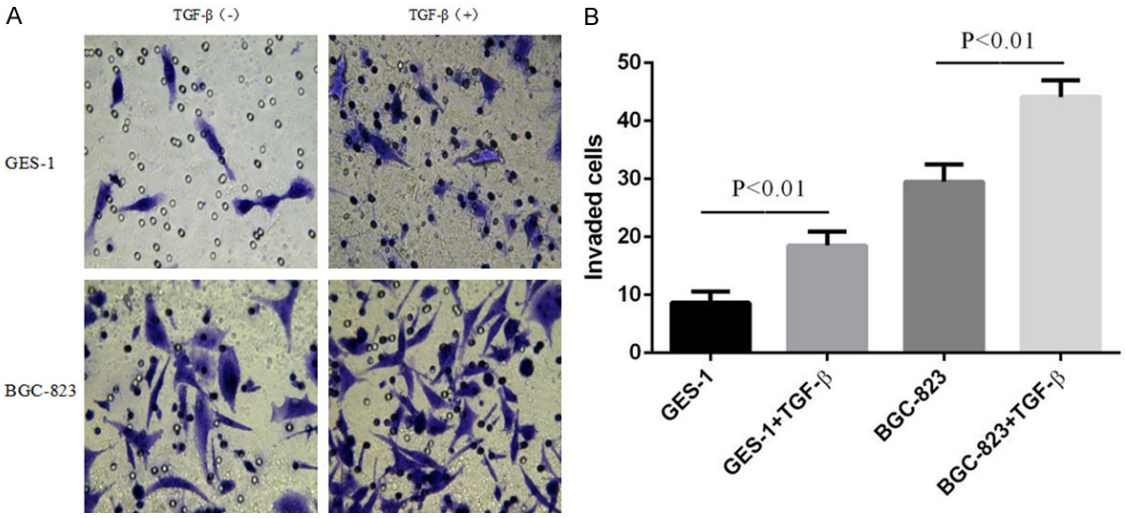

Figure 8. Transwell experimental results: A. The effect of TGF-β on the invasive ability of BGC-823 cells; B. A comparison of the number of cells passing through the transwell cell.

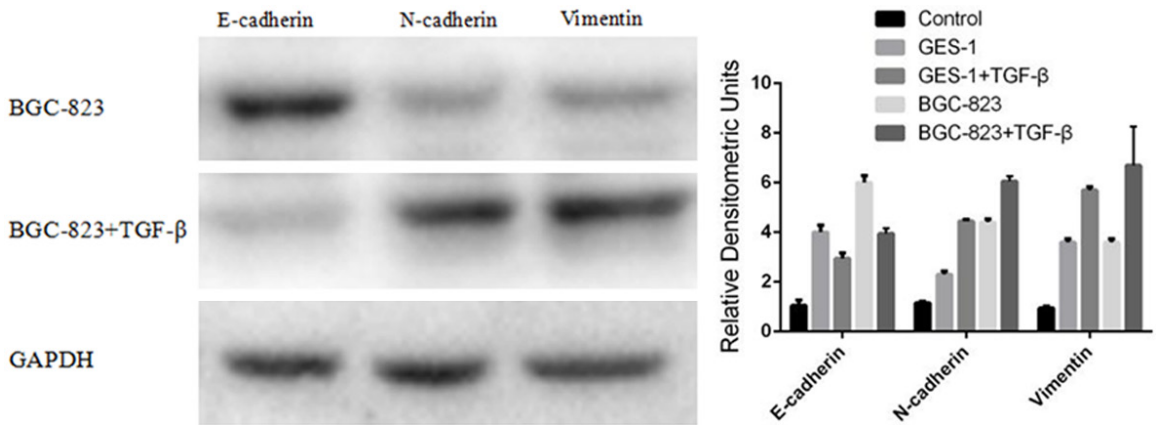

Figure 9. Changes in the expression levels of the EMT related markers.

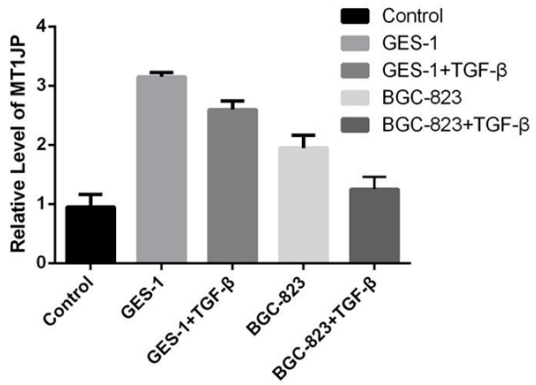

Figure 10. Relative expression levels of LncRNA MT1JP.

molecular mechanisms involved in the occurrence and development of gastric cancer to create more opportunities for its diagnosis and treatment.

Long-chain noncoding RNAs are a group of RNAs over 200 nt in length and lacking an open reading region. Compared to mRNAs, LncRNAs can be found in various parts of the nucleus and cytoplasm, and their expression folds in cells are lower than mRNAs [17]. With the development and popularization of gene high-throughput technology and the continuous update of gene chip technology, more LncRNAs have been recognized. Up to now, about 1,000 LncRNAs have been identified and found to be involved in tumor invasion and metastasis. Wait for each step [18]. The study found that some LncRNAs have a high clinical value as a serological marker for the early diagnosis of gastric cancer. Disorders of LncRNA expression are closely connected with the pathogenesis and prognosis of gastric cancer. The expression level of the LncRNA may indicate the disease's outcome in gastric cancer patients. LncRNA HOTAIR, GHET1, etc. show a high expression in gastric cancer tissues, so the overall survival of these patients is relatively poor [19]. The expression levels of other LncRNAs were found to be related to the clinicopathological features of gastric cancer patients, such as LINC00152 [20]. In addition to the application of LncRNA to the diagnosis and prognosis of gastric cancer patients, some long-chain noncoding RNAs can serve as targets for the treatment of gastric cancer [21]. Liu et al. [22] reported the LncRNA MT1JP for the first time. After detecting the expression of LncRNA MT1JP in 76 pairs of gastric cancer tissues and paracancerous tissues, it was found that it showed a low expression in tumor tissues and participated in the cell cycle, apoptosis and cell proliferation and other processes. When the expression of LncRNA MT1JP is downregulated, the expression of the p53 gene is also down-regulated, which in turn promotes cell deterioration and tumor formation. These findings suggest that LncRNA MT1JP is essential for the maintenance of the normal life activities of cells, but whether long-chain non-coding RNA MT1JP is involved in the invasion and metastasis of gastric cancer and related mechanisms still needs to be further explored.

In this study, biological information mining was used to analyze the data of the existing gene chips, and the data (such as LncRNAs, etc.) that the authors were not interested in were re-

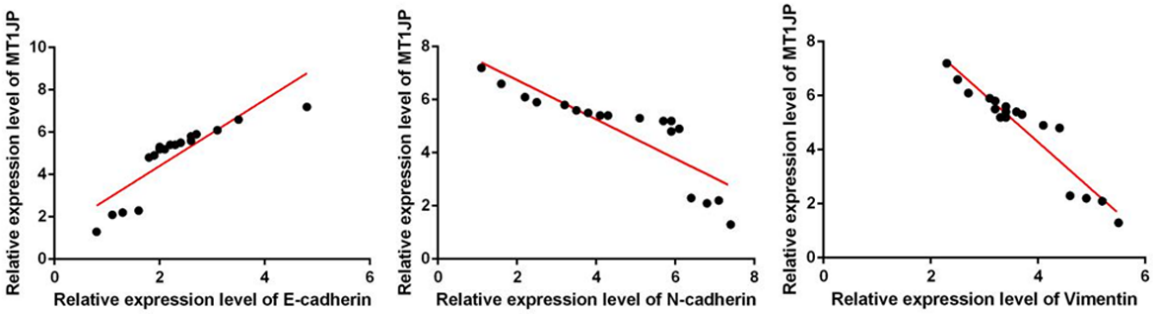

Figure 11. The correlation between LncRNA MT1JP expression levels and the EMT markers.

examined. Affymetrix Human Exon 1.0 ST array contains approximately 6.5 million independent gene probes designed throughout the entire length of the gene and linked to the 3 ends, providing a good platform for the study of long-chain non-coding RNAs [23]. This study used the above platform and applied R software to call the relevant bioinformatics analysis software package. A total of 65 differentially expressed LncRNAs were screened out, of which 40 were up-regulated and 25 were down-regulated. Although these long-chain non-coding RNAs can be annotated by the DAVID website, their related functions and the molecular mechanisms involved are not yet clear. At the same time, in this study, GES-1 and BGC-823 cells were cultured and the cells were stimulated with TGF-β at a concentration of 5 ng/ml to establish the EMT cell model and evaluate the changes in cell proliferation, migration and invasiveness. WB technology was used to detect cells. The expression levels of E-cadherin, N-cadherin, and vimentin were detected by qrt-PCR, and a correlation analysis was used to evaluate the relationship between the expression of LncRNA MT1JP and the expression of LncRNA MT1JP. The experimental data showed that LncRNA MT1JP was lowly expressed in gastric cancer cells and highly expressed in gastric mucosal epithelial cells. The proliferation and invasion of the GES-1 and BGC-823 cell lines were significantly enhanced by TGF-β-induced EMT. The expression of LncRNA MT1JP was significantly decreased, and the expression level of E-cadherin was significantly decreased in TGF-β induced BGC-823 cells ($P< 0.01$). However, the expression of N-cadherin and vimentin increased significantly ($P<0.01$). It suggests that a low expression level of LncRNA MT1JP may promote the EMT process of gastric cancer cells.

At present, the clinical application of LncRNA is still more limited, and the biological function of long-chain noncoding RNA is not yet clear, and LncRNA is diverse in its transcription level and post-transcriptional modification, and it is also difficult to detect at different stages of the disease [24]. Although it has been found that the expressions of certain lncRNAs are related to the occurrence and development of gastric cancer, their clinical application value still needs further investigation. All of the above problems have led to the limitations of long-chain non-coding RNAs in the diagnosis and prognostic evaluation of gastric cancer as molecular markers. However, as more research is carried out, lncRNA and its related target genes are expected to become novel molecular markers for the diagnosis of gastric cancer, its postoperative prognosis, and molecular targeted therapy.

It can be concluded that LncRNA MT1JP's low expression level may promote the EMT process of gastric cancer cells, LncRNA may play an inhibitory role in the invasion and metastasis of gastric cancer, the high expression of LncRNA MT1JP may be an effective way to curb the occurrence and development of gastric cancer, but the precise mechanisms of these actions need further study.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (no. 81560391).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Mingxu Da, Department of Surgical Oncology, Gansu Provincial Hos-

pital, Lanzhou 730000, Gansu Province, China. E-mail: hxdamingxu@hotmail.com

References

[1] Torre LA, Siegel RL, Ward EM and Jemal A. Global cancer incidence and mortality rates and trends--an update. Cancer Epidemiol Biomarkers Prev 2016; 25: 16-27.

[2] Fujitani K, Yang HK, Mizusawa J, Kim YW, Terashima M, Han SU, Iwasaki Y, Hyung WJ, Takagane A, Park DJ, Yoshikawa T, Hahn S, Nakamura K, Park CH, Kurokawa Y, Bang YJ, Park BJ, Sasako M and Tsujinaka T. Gastrectomy plus chemotherapy versus chemotherapy alone for advanced gastric cancer with a single non-curable factor (REGATTA): a phase 3, randomised controlled trial. Lancet Oncol 2016; 17: 309-318.

[3] Ma J, Shen H, Kapesa L and Zeng S. Lauren classification and individualized chemotherapy in gastric cancer. Oncol Lett 2016; 11: 2959-2964.

[4] Hess LM, Michael D, Mytelka DS, Beyrer J, Liepa AM and Nicol S. Chemotherapy treatment patterns, costs, and outcomes of patients with gastric cancer in the United States: a retrospective analysis of electronic medical record (EMR) and administrative claims data. Gastric Cancer 2016; 19: 607-615.

[5] Xue M, Chen W and Li X. Urothelial cancer associated 1: a long noncoding RNA with a crucial role in cancer. J Cancer Res Clin Oncol 2016; 142: 1407-1419.

[6] Mouraviev V, Lee B, Patel V, Albala D, Johansen TE, Partin A, Ross A and Perera RJ. Clinical prospects of long noncoding RNAs as novel biomarkers and therapeutic targets in prostate cancer. Prostate Cancer Prostatic Dis 2016; 19: 14-20.

[7] Cai X, Liu Y, Yang W, Xia Y, Yang C, Yang S and Liu X. Long noncoding RNA MALAT1 as a potential therapeutic target in osteosarcoma. J Orthop Res 2016; 34: 932-941.

[8] Zhang F, Zhang L and Zhang C. Long noncoding RNAs and tumorigenesis: genetic associations, molecular mechanisms, and therapeutic strategies. Tumour Biol 2016; 37: 163-175.

[9] Kamel MM, Matboli M, Sallam M, Montasser IF, Saad AS and El-Tawdi AHF. Investigation of long noncoding RNAs expression profile as potential serum biomarkers in patients with hepatocellular carcinoma. Transl Res 2016; 168: 134-145.

[10] Wu Y, Wang YQ, Weng WW, Zhang QY, Yang XQ, Gan HL, Yang YS, Zhang PP, Sun MH, Xu MD and Wang CF. A serum-circulating long noncoding RNA signature can discriminate between patients with clear cell renal cell carcinoma and healthy controls. Oncogenesis 2016; 5: e192.

[11] Li L, Zhang L, Zhang Y and Zhou F. Increased expression of LncRNA BANCR is associated with clinical progression and poor prognosis in gastric cancer. Biomed Pharmacother 2015; 72: 109-112.

[12] Heery R, Finn SP, Cuffe S and Gray SG. Long non-coding RNAs: key regulators of epithelial-mesenchymal transition, tumour drug resistance and cancer stem cells. Cancers (Basel) 2017; 9.

[13] Li T, Mo X, Fu L, Xiao B and Guo J. Molecular mechanisms of long noncoding RNAs on gastric cancer. Oncotarget 2016; 7: 8601-8612.

[14] Diepenbruck M and Christofori G. Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? Curr Opin Cell Biol 2016; 43: 7-13.

[15] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-386.

[16] Fang XY, Pan HF, Leng RX and Ye DQ. Long noncoding RNAs: novel insights into gastric cancer. Cancer Lett 2015; 356: 357-366.

[17] Guttman M and Rinn JL. Modular regulatory principles of large non-coding RNAs. Nature 2012; 482: 339-346.

[18] Duan F, Jiang J, Song C, Wang P, Ye H, Dai L, Zhang J and Wang K. Functional long non-coding RNAs associated with gastric cancer susceptibility and evaluation of the epidemiological efficacy in a central Chinese population. Gene 2018; 646: 227-233.

[19] Xu ZY, Yu QM, Du YA, Yang LT, Dong RZ, Huang L, Yu PF and Cheng XD. Knockdown of long non-coding RNA HOTAIR suppresses tumor invasion and reverses epithelial-mesenchymal transition in gastric cancer. Int J Biol Sci 2013; 9: 587-597.

[20] Pang Q, Ge J, Shao Y, Sun W, Song H, Xia T, Xiao B and Guo J. Increased expression of long intergenic non-coding RNA LINC00152 in gastric cancer and its clinical significance. Tumour Biol 2014; 35: 5441-5447.

[21] Song H, Sun W, Ye G, Ding X, Liu Z, Zhang S, Xia T, Xiao B, Xi Y and Guo J. Long non-coding RNA expression profile in human gastric cancer and its clinical significances. J Transl Med 2013; 11: 225.

[22] Liu L, Yue H, Liu Q, Yuan J, Li J, Wei G, Chen X, Lu Y, Guo M, Luo J and Chen R. LncRNA MT1JP functions as a tumor suppressor by interacting with TIAR to modulate the p53 pathway. Oncotarget 2016; 7: 15787-15800.

[23] Barnes MG, Tsoras M, Thompson SD, Martinez H, Iverson B, Nuñez R. Ambient temperature stabilization of purified RNA in GenTegra™ for use in affymetrix human exon 1.0 ST arrays. Biotechniques 2010; 23: 468-469.

[24] Tsai MC, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E and Chang HY. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010; 329: 689-693.