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Diagnostic and therapeutic potencies of miR-18a-5p in mixed-type gastric adenocarcinoma

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Abstract

Mixed-type gastric adenocarcinoma (by Lauren Classification) has poor clinical outcomes with few targeted treatment options. The primary objective of this study was to find the prognostic factors, accurate treatment approaches, and effective postoperative adjuvant therapy strategies for patients with mixed-type gastric adenocarcinoma (GA). A microRNA sequencing data set and the corresponding clinical parameters of patients with gastric cancer were obtained from The Cancer Genome Atlas. Differentially expressed microRNAs (DEMs) of diffuse- and intestinal-type GA were, respectively, determined. Kaplan–Meier and log-rank tests were subsequently carried out to evaluate the prognostic relevance of each DEM. To study the common factors between diffuse- and intestinal-type GA, a pathway enrichment analysis was performed on the target genes of identified DEMs using the PANTHER database. After data preprocessing, we analyzed a total of 230 samples from 210 patients with GA. Eighty-six DEMs in diffuse-type GA samples and 59 DEMs in intestinal-type GA samples were, respectively, identified (p 2.0). The Kaplan–Meier survival method further screened out six prognosis-related DEMs for diffuse-type GA and seven prognosis-related DEMs for intestinal-type GA ($p < 0.05$). MiR-18a-5p was found to be the only common prognosis-related DEM between diffuse- and intestinal-type GA. The common signaling pathways further revealed that target genes of miR-18a-5p are involved in mixed-type GA progression. This study suggests that miR-18a-5p acts as a potential target for treatment, and common signal pathways provide a rich basis to seek reliable and effective molecular targets for the diagnosis, clinical treatment, and postoperative adjuvant therapy strategy of mixed-type GA.

KEYWORDS

microRNA, miR-18a-5p, mixed-type gastric adenocarcinoma, prognosis, signal pathway

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1 | INTRODUCTION

Gastric cancer (GC) is important cancer worldwide and is responsible for over 1,000,000 new cases in 2018 and an estimated 783,000 deaths, making it the fifth most frequently diagnosed cancer and the third leading cause of cancer death.[1] The outcomes are often poor with a less than 10% 5-year survival rate globally.[2] Gastric adenocarcinoma (GA) forms the majority (about 90%–95%) of GC and can be further divided into diffuse- and intestinal-type GA according to the Lauren classification.[3] The Lauren classification is widely used in clinics around the world, and in this classification system there may also be mixed-type GA, made up of both diffuse- and intestinal-type GA.[3]

Diffuse- and intestinal-type GA exhibit numerous differences in etiology, pathology, and epidemiology.[4] The diffuse type occurs equally among men and women and tends to develop at a younger age than the intestinal type.[5,6] This type of cancer usually affects the body of the stomach and presents with a shorter duration and worse prognosis compared with the intestinal type. Peritoneal metastasis of diffuse GC, without easily recognized precursor lesions, is common.[7] By contrast, the intestinal-type GA occurs more often in men than in women and more often in older people. Tumor cells exhibit adhesion and are arranged in tubular or glandular formations. This type of GC is associated with lymphatic or vascular invasion, affects the gastric antrum, and presents with a longer course and better prognosis. Surgery is often used to treat both diffuse- and intestinal-type GA. The type of surgery depends mainly on the size and location of the tumor. Gastrectomy is the most common surgery to treat GC. Radiation therapy, chemotherapy, and targeted therapy are effective supplemental treatments.[8] With recent developments in molecular biology, an increasing number of research projects have begun to investigate methods to accurately predict the prognosis of GC, and to develop more effective targeted therapy methods.[9]

MicroRNAs (miRNAs) are small noncoding RNA molecules that regulate expression of different genes by binding to mRNAs.[10–12] An increasing number of studies have found that miRNAs play an important role in the occurrence and development of malignant tumors, including the regulation of tumor cell proliferation, differentiation, and apoptosis.[13,14] Many previous studies have reported that miRNAs may be used as diagnostic biomarkers in GC. Particular miRNAs act as tumor suppressors or are oncogenic and hence considered as biomarkers for early diagnosis and accurate prognosis of GC.[15–17] Sandoval-Bórquez et al.[18] has demonstrated miR-335-5p is a potential suppressor of metastasis and invasion in GC. Cao et al.[19] has revealed that mir-381 inhibits the metastasis of Zhang et al.[20] has suggested a five-miRNA signature (miR-20a, miR-106b, miR-135b, miR-141, and miR-145) as a prognostic signature in GC patients. Jiang et al.[21] has demonstrated abnormal expression of miR-421 in early stage GC as a diagnostic biomarker. Recent research had reported that miR-18a-5p is highly expressed in renal cell carcinoma,[22] colon cancer,[23] esophageal cancer,[24] and liver cancer[25] tissues. Further research suggested that miR-18a-5p overexpression had a positive effect on cell proliferation, migration, invasion, and inhibition of apoptosis in these diseases.

However, research on miRNA in mixed-type GA had not been reported yet. In addition, many previous GA studies did not have an accurate distinction between −3p and −5p in miRNA analysis.[26] Here, mature miRNA names were translated to the latest miRBase version according to MIMAT-ID (accession), and this study analyzed abnormal miRNA expression and screened for DEMs associated with survival in diffuse- and intestinal-type GA, respectively. The common DEM suggested that miR-18a-5p (MIMAT-ID: MIMAT0000072) overexpression is associated with poor prognoses of patients with mixed-type GA. Furthermore, the common signaling pathways revealed that the target genes of miR-18a-5p are involved in mixed-type GA progression. These results may provide novel accurate treatment approaches and effective postoperative adjuvant therapy strategies for patients with mixed-type GA.

2 | MATERIALS AND METHODS

2.1 | TCGA-STAD data set preprocessing

The raw sequencing data and clinical parameters were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/, TCGA-STAD project) and handled by R (https://cran.r-project.org/, v3.5.1) program. Studies have shown that diffuse-type GA has significantly worse overall survival (OS) than intestinal-type GA.[27] Therefore, diffuse-type GA data and intestinal-type GA data were separated according to Lauren classification and then respectively analyzed. The inclusion criteria as follows: (1) the samples have complete miRNA sequencing data, clinical information, and prognostic parameters; (2) prognosis follow-up time is more than 1 week; (3) excluding patients with outlier survival times (Figure 1); (4) no patient with cancer metastasis or other cancers; (5) no duplicate patients and samples.
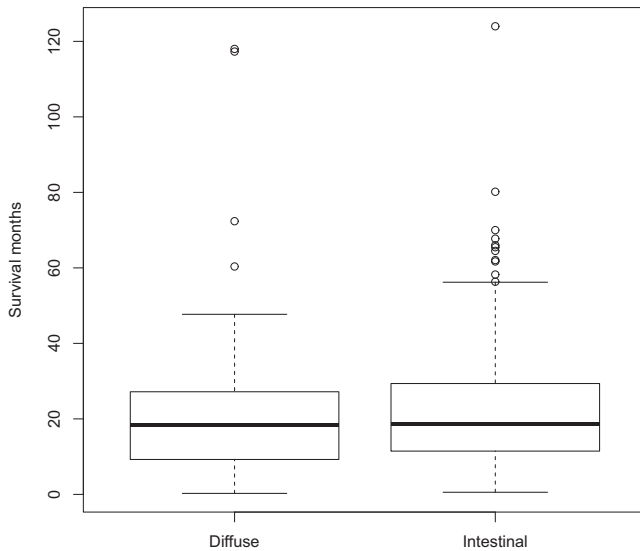
FIGURE 1 Box plots of survival time for gastric adenocarcinoma patients

2.2 | Screening for differentially expressed miRNAs (DEMs) associated with survival

The miRNA expression profiles were normalized by $\log_2$ transformed, and the sequencing expression between GA and adjacent normal tissues were then compared with identify DEGs by limma (v3.42.0) package in R. Differentially expressed miRNAs with $\log_2|FC| > 2.0$ and $p < 0.05$ were considered to be significant. The R program was used to merge data on the expression of DEMs and the corresponding clinical survival time. For each DEM, patients were effectively divided into high- and low-expression groups using the median expression value. Subsequently, specific DEMs associated with survival prognosis were determined by log-rank test ($p < 0.05$).

2.3 | Target gene prediction and signaling pathway enrichment analysis

The target genes of prognostic DEMs were predicted using miRTarBase (http://mirtarbase.mbc.nctu.edu.tw, v7.0). miR-TarBase[28] is a more comprehensively annotated, experimentally validated miRNA–target interactions database in the field of miRNA-related research. It has analyzed related articles from pubmed, integrated TargetScan,[29] and miRanda[30] for target gene prediction, and provides a more updated collection by comparing with other similar, previously developed databases. Signaling pathway enrichment analyses were performed for target genes using PANTHER (Protein ANalysis THrough Evolutionary Relationships, Annotation v14.1) on Gene Ontology (http://

geneontology.org/). Fisher's exact test type and Bonferroni correction ($p < 0.05$) for multiple testing were used, and the p-value < 0.05 and Fold Enrichment > 2 were set as the cut-off criteria. The common signaling pathways between diffuse- and intestinal-type GA were then obtained. Eventually, the R program including the ggplot2 (v3.2.1) package was used to convert the analysis results into visual images.
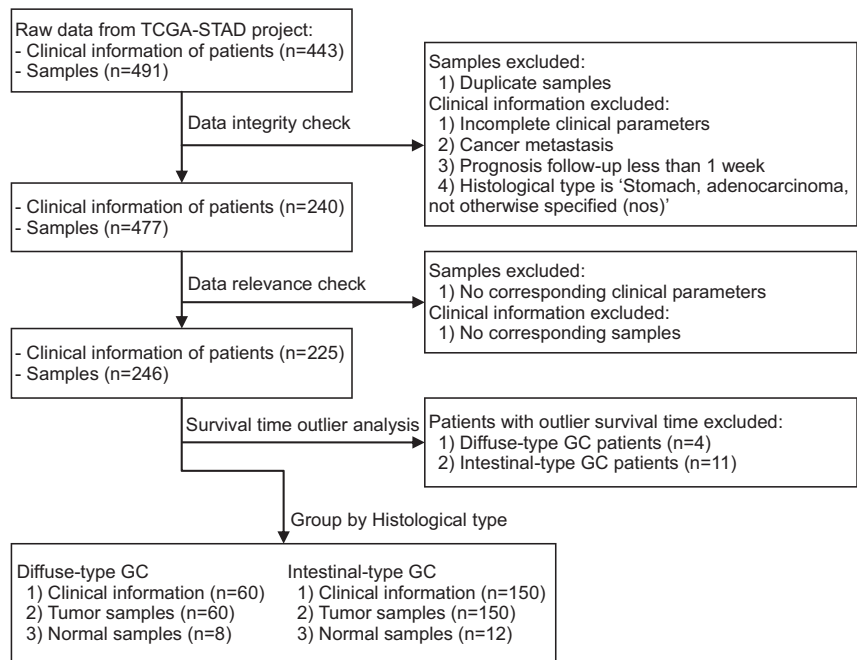
2.4 | Statistical analysis

All data processing and analyses in this study were handled using the R platform (v3.5.1, https://cran.r-project.org/). DEMs were screened using the limma package, which downloads from Bioconductor (http://bioconductor.org/). Survivors were defined as censored data and any cause of death was defined as an event. Kaplan–Meier survival curves were drawn by using the survival (v3.1-7) package and the log-rank test was applied to compare the distribution between patient subsets. Univariate analyses between clinical features and OS were compared using the log-rank test. Hazard ratio (HR) and 95% confidence interval (CI) were calculated from the Univariate Cox proportional hazards regression model. All p values were bilateral and $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Preprocessing workflow presentation

The raw miRNA expression data set and clinical information of patients with GA were obtained from the TCGA database website. The mature miRNA names were translated to the latest miRBase version according to MIMAT-ID (accession). Data preprocessing was performed for removing incomplete data and noise data (Figure 2). Eventually, a total of 60 patients with diffuse-type GA samples (including 60 cancer tissues and 8 matched normal tissues) and 150 patients with intestinal-type GA samples (including 150 cancer tissues and 12 matched normal tissues) were enrolled in this study. Clinical characteristics include age at diagnosis, gender, H pylori infection, tumor status, lymph node status, Stage, and microsatellite status were calculated by the R program (Table 1). Furthermore, the association between clinical characteristics and OS was evaluated by Univariate Cox regression analysis (Table 2). The results showed that microsatellite status was significantly associated with OS ($p = .0175$) in diffuse-type GA and tumor status was significantly associated with OS ($p = .0032$) in intestinal-type GA.

FIGURE 2 Raw data preprocessing and classification

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Raw data from TCGA-STAD project:
- Clinical information of patients (n=443)
- Samples (n=491)

        │ Data integrity check                    Samples excluded:
        │                          ──────────▶     1) Duplicate samples
        ▼                                         Clinical information excluded:
                                                   1) Incomplete clinical parameters
- Clinical information of patients (n=240)         2) Cancer metastasis
- Samples (n=477)                                  3) Prognosis follow-up less than 1 week
                                                   4) Histological type is 'Stomach, adenocarcinoma,
                                                   not otherwise specified (nos)'

        │ Data relevance check                    Samples excluded:
        │                          ──────────▶     1) No corresponding clinical parameters
        ▼                                         Clinical information excluded:
                                                   1) No corresponding samples
- Clinical information of patients (n=225)
- Samples (n=246)

        │ Survival time outlier analysis          Patients with outlier survival time excluded:
        │                          ──────────▶     1) Diffuse-type GC patients (n=4)
        │                                          2) Intestinal-type GC patients (n=11)
        ▼
        Group by Histological type

Diffuse-type GC                         Intestinal-type GC
 1) Clinical information (n=60)           1) Clinical information (n=150)
 2) Tumor samples (n=60)                  2) Tumor samples (n=150)
 3) Normal samples (n=8)                  3) Normal samples (n=12)
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TABLE 1 Clinical characteristics of gastric cancer patients

	Diffuse type	Intestinal type
Age at diagnosis		
≤median	31	78
>median	29	72
Gender		
Female/male	22/38	47/103
H. pylori infection		
No/Yes	27/5	90/9
NA	28	51
Tumor status		
T1/T2/T3/T4	0/16/24/20	9/30/73/38
Lymph node status		
N0/N1/N2/N3	62/45/29/35	40/33/42/31
NX+NA	1	4
Stage		
I/II/III/IV	5/21/31/1	19/40/81/8
NA	2	2
Microsatellite status		
Mss	43	101
Msi-L/Msi-H	7/10	21/28

Note: Median age at diagnosis: Diffuse type is 61, Intestinal type is 68.
Abbreviation: NA, not available.

3.2 | Prognosis-related differentially expressed miRNAs and target genes

We used the R program to compare gene expression levels in tumor and normal tissues and identified DEMs in diffuse- and intestinal-type GA samples. respectively (Figure 3A,B). Eighty-six miRNAs in diffuse-type GA samples and 59 miRNAs in intestinal-type GA samples were identified as DEMs ($p < 0.05$ and $\log_2$FC>2). The volcano plots of these DEMS in diffuse- and intestinal-type GA samples were visualized by the "plot" package in R. To identify the miRNAs that would be potentially associated with overall survival of diffuse- and intestinal-type patients, we evaluated the association between miRNAs expression and patients' survival using Kaplan–Meier curve and log-rank test. Six DEMs (miR-18a-5p, miR-194-3p, miR-222-3p, miR-92a-1-5p, miR-671-5p, miR-19a-3p) in the diffuse-type GA data set and seven DEMs (miR-18a-5p, miR-141-3p, miR-552-5p, miR-188-5p, miR-21-5p, miR-181b-3p, miR-500a-3p) in the intestinal-type GA data set were identified as prognosis-related DEMs (log-rank $p < 0.05$). All of these prognosis-related DEMs are upregulated miRNAs and miR-18a-5p is the only common miRNA between diffuse- and intestinal type GA (Figure 3C,D). The results showed that patients in the miR-18a-5p high-expression group have significantly worse OS than patients in the miR-18a-5p low-expression group. Thus, miR-18a-5p overexpression is associated with a poor prognosis in both diffuse- and intestinal-type GA patients.

	Diffuse subtype		Intestinal subtype	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age (>median vs.≤ median)	1.22 (0.63–2.34)	0.5602	1.17 (0.77-1.78)	0.4568
Gender (Female vs. Male)	1.08 (0.56-2.09)	0.8202	0.77 (0.49-1.2)	0.2494
Tumour status (T3+T4 vs. T1+T2)	1.08 (0.51-2.26)	0.8442	0.51 (0.32-0.8)	**0.0032**
Lymph Node status (N1-2 vs. N0)	0.54 (0.25-1.18)	0.1223	0.83 (0.53-1.32)	0.4394
Clinical Stage (III + IV vs. I + II)	0.71 (0.36-1.41)	0.3268	0.67 (0.44-1.02)	0.0592
Microsatellite Status (Mss vs. Msi-l&h)	2.41 (1.17-4.99)	**0.0175**	1.02 (0.66-1.59)	0.9195

Note: Significant data are emphasized in bold.

Abbreviations: CI, confidence interval; GA, gastric adenocarcinoma; HR, hazard ratio.

3.3 | Signaling pathway enrichment analysis and visualization

Next, enrichment analyses were, respectively, performed on two target gene sets. Between diffuse- and intestinal-type GA, the PANTHER pathways were significantly enriched in seven common pathways (Table 3): P00038 (JAK/STAT signaling pathway), P00033 (Insulin/insulin-like growth factor [IGF] pathway-protein kinase B signaling cascade), P00005 (Angiogenesis), P00052 (TGF-beta signaling pathway), P06664 (Gonadotropin-releasing hormone receptor pathway), P06959 (CCKR signaling map), and P00006 (Apoptosis signaling pathway). The signaling pathway enrichment analysis suggested that the target genes of miR-18a-5p may be involved in these pathways related to mixed-type GA. These underlying molecular mechanisms provide a basis and strategy for novel targeted therapy. Subsequently, the R program including the ggplot2 package was used to convert the enriched analysis data into visual images (Figure 4).

4 | DISCUSSION

A large number of miRNAs have been identified as regulators for a wide range of biological functions in human cancers. In this study, we identified the DEMs of diffuse- and intestinal-type GA, respectively, and further analyzed the prognosis of patients, and eventually revealed that miR-18a-5p is the only common DEM associated with the prognosis. According to the Lauren classification, this study demonstrates that miR-18a-5p plays an important role in the development and prognosis of mixed-type GA. To further investigate the possible biological functions of miR-18a-5p in GC, path enrichment analysis was carried out using target genes of prognosis-related DEMs, and there were seven common pathways in the results. These pathways reveal the effects of miR-18a-5p upregulation on the development of mixed-type GC.

Previous studies have revealed the role of angiogenesis (P00005) and angiogenic factors in GC[31] as well anti-angiogenic treatments of GC.[32,33] Angiogenesis is a critical process for tumor growth and progression, therefore, the development of angiogenesis inhibitors with efficient therapeutic has been a central focus for researchers. The new angiogenesis inhibitor, Ramucirumab, has been approved to be used in advanced gastric or gastro-oesophageal after an international, randomized, multicentre, placebo-controlled, Phase 3 trial.[34] The JAK/STAT (P00038) cascade is a principal signal transduction pathway in cytokine and growth factor signaling, regulating cell proliferation, differentiation, migration, and survival. Constitutive activation of JAK/STAT signaling is well-established in cancers. Aberrant JAK/STAT signaling also is crucial to the development of GC, in particular, aberrant STAT3 expression has been implicated in GA patients.[35–37] Huang et al. found that oxymatrine exhibits the effects via regulation of JAK/STAT signaling pathway and exhibits antitumor activity in GC.[38] The therapeutic potential of targeted inhibition of JAK/STAT in GC deserves further investigation. Transforming growth factor β signal (P00052) has been reported to promote GC metastasis[39–41] and high stromal expression plays a role as a novel marker of poor prognosis in GC.[42] He et al.[43] found that sauchinone significantly inhibited transforming growth factor-β1-induced migration and invasion in GC cells. However, only preliminary research has been conducted on the CCKR signaling map (P06959),[44–46] Insulin/IGF pathway-protein kinase B signaling cascade (P00033),[47–49] Apoptosis signaling pathway (P00006),[50,51] and gonadotropin-releasing hormone receptor pathway (P06664).[52,53] With the development of molecular biology and the progress of GA research, we
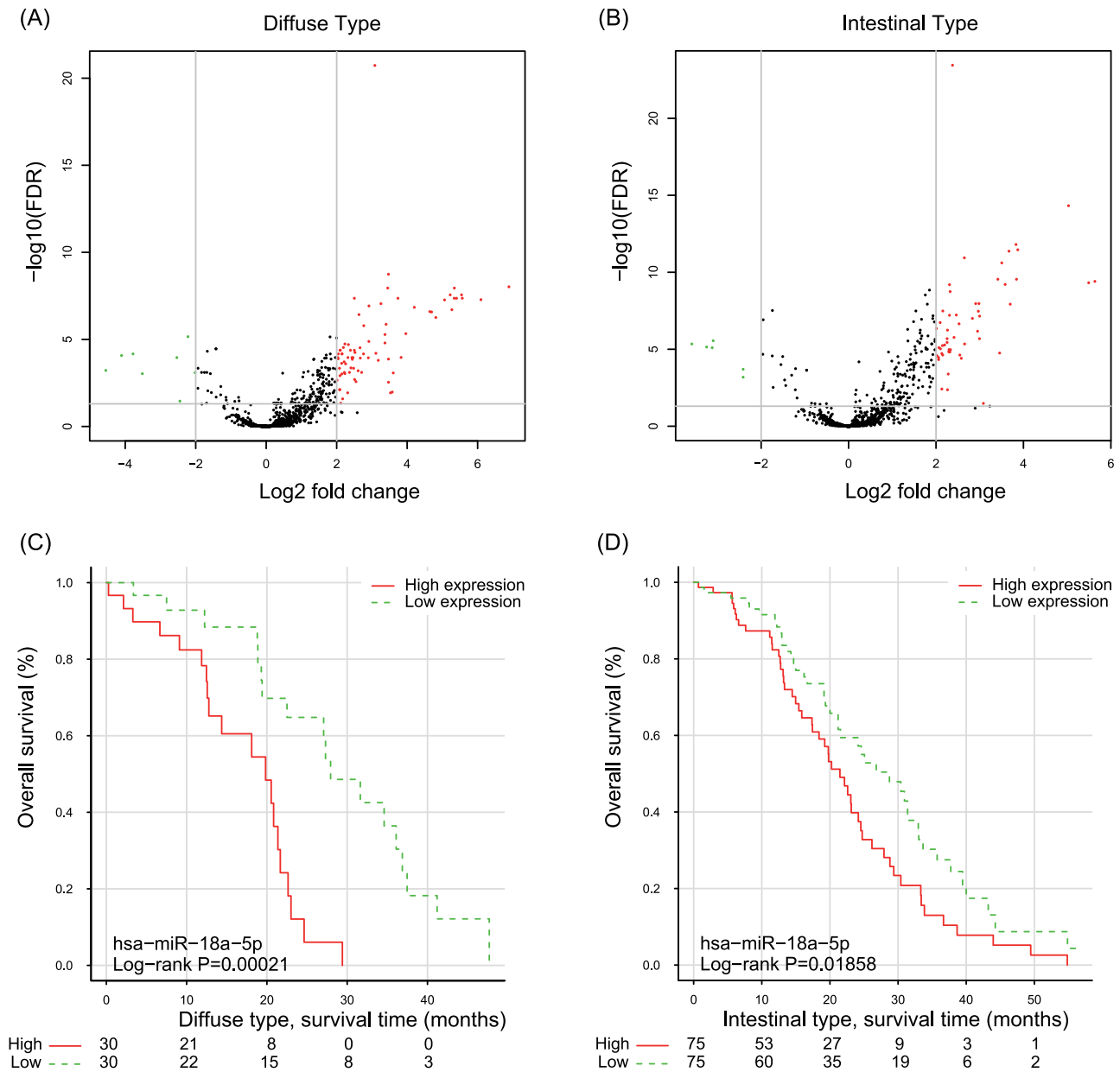
FIGURE 3 Volcano plot of differentially expressed miRNAs (DEMs) for diffuse-type (A) and intestinal-type (B) gastric adenocarcinoma. The red dot represents upregulated miRNA, and green dot represents downregulated miRNA ($p < 0.05$ and $|log2FC| > 2$). Kaplan–Meier curves for diffuse-type (C) and intestinal-type (D) gastric adenocarcinoma patients. The patients were stratified into a high (red line) expression group and a low (green dash) expression group based on the median

believe that the potential value of these pathways for treatment of GC will be found.

At present, most patients with GC who are initially treated are already in the advanced stage, and there are very few studies on mixed-type GC, which leads to the current treatment plan based on resection and chemoradiotherapy, but the prognosis is poor. Many patients undergo surgery. After recurrence and metastasis, there is an urgent need for effective and precise treatment options, as well as treatment strategies to prolong prognosis. This study provides a new program and basis for treatment and prognosis strategies

from the abnormal expression of miRNA and the enrichment analysis of target genes. It is not only effective for mixed-type GC but also for diffuse- and intestinal-type GA. The emergence of novel targeted drugs and progress in tumor molecular biology research will provide new opportunities for the comprehensive treatment of GC. Therefore, new research and developments will make it possible to improve the treatment of GC. Achieving a more accurate, effective, personalized treatment plan and postoperative adjuvant therapy strategy is critical for bringing the greatest clinical benefit to patients.

TABLE 3 Common signaling pathways between diffuse- and intestinal-type GA in the PANTHER Pathway System

| ID | Description | Diffuse | | | Intestinal | | | Count |
		Gene ratio	Fold enrichment	p value	Gene ratio	Fold enrichment	p value	
P00038	JAK/STAT signaling pathway	3/17	33.08	2.41E−02	6/17	30.88	2.61E−05	1
P00033	Insulin/IGF pathway-protein kinase B signaling cascade	4/41	18.29	1.47E−02	5/41	10.67	2.57E−02	1
P00052	TGF-beta signaling pathway	6/97	11.60	2.85E−03	11/97	9.92	6.23E−06	4
P00006	Apoptosis signaling pathway	7/118	11.12	7.24E−04	11/118	8.16	3.90E−05	2
P06664	Gonadotropin-releasing hormone receptor pathway	11/230	8.97	1.01E−05	22/230	8.37	2.06E−11	4
P06959	CCKR signaling map	8/174	8.62	8.99E−04	14/174	7.04	4.97E−06	3
P00005	Angiogenesis	7/173	7.59	7.71E−03	11/173	5.56	1.29E−03	2

Note: Count: number of matches between target genes of miR-18a-5p and reference genes of the corresponding signaling pathway.

Abbreviation: GA, gastric adenocarcinoma.
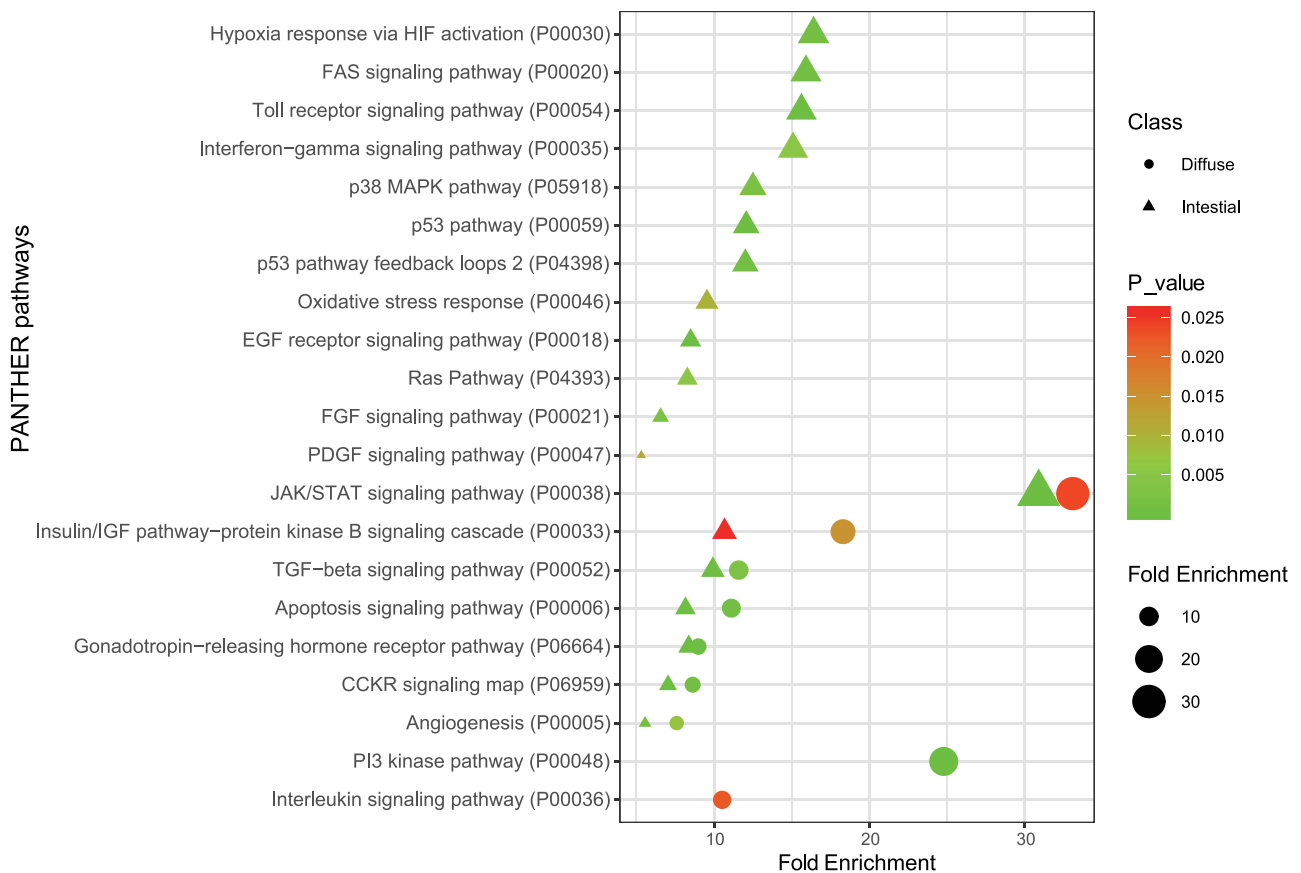
FIGURE 4 Enrichment analysis performed on DEGs using the PANTHER pathway system. DEGs, differentially expressed genes; PANTHER, Protein ANalysis THrough Evolutionary Relationships

As TCGA only entered clinical data and sequencing data of diffuse- and intestinal-type GA types, this article analyzed two types of GC and indirectly inferred the prognosis of mixed-type GA and the influence of miRNA.

To improve on these deficiencies, we plan to collect mixed-type GA tumor samples and clinical prognosis information in the future, and then we can verify our results on more adequate mixed-type GA data. It is now

necessary to conduct more studies on the molecular mechanism of miR-18a-5p in GC and further test in actual clinical applications and explore more accurate treatment options.

In conclusion, the results of this study reveal the impact of miR-18a-5p on the development and prognosis of mixed-type GA. The target genes of miR-18a-5p (such as PTEN and BCL2) and the seven common pathways (JAK/STAT signaling pathway, Insulin/IGF pathway-protein kinase B signaling cascade, etc.) further provide the relevant mechanism of mixed-type GA carcinogenesis and development. Therefore, this study provides a rich basis to seek reliable and effective molecular targets and biomarkers for the diagnosis, clinical treatment, and postoperative adjuvant therapy strategy of mixed-type GA. Further research is now needed to confirm these results for future application in clinical practice.

CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS
Study design: Li Wang, Mingxin Zhang, and Jiansheng Wang. *Data collection processing and statistical analysis*: Li Wang and Jia Zhang. *Manuscript editing*: Li Wang and Mingxin Zhang. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The original datasets analyzed during the current study are publicly available from the TCGA repository at: https://portal.gdc.cancer.gov/. All codes used during the present study are available from the corresponding author upon reasonable request.

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REFERENCES
1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424. https://doi.org/10.3322/caac.21492

2. Orditura M, Galizia G, Sforza V, et al. Treatment of gastric cancer. *World J Gastroenterol.* 2014;20(7):1635-1649. https://doi.org/10.3748/wjg.v20.i7.1635

3. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand.* 1965;64:31-49. https://doi.org/10.1111/apm.1965.64.1.31

4. Ma J, Shen H, Kapesa L, Zeng S. Lauren classification and individualized chemotherapy in gastric cancer. *Oncol Lett.* 2016;11(5):2959-2964. https://doi.org/10.3892/ol.2016.4337

5. Qiu MZ, Cai MY, Zhang DS, et al. Clinicopathological characteristics and prognostic analysis of Lauren classification in gastric adenocarcinoma in China. *J Transl Med.* 2013;11:58. https://doi.org/10.1186/1479-5876-11-58

6. Zheng H, Takahashi H, Murai Y, et al. Pathobiological characteristics of intestinal and diffuse-type gastric carcinoma in Japan: an immunostaining study on the tissue microarray. *J Clin Pathol.* 2007;60(3):273-277. https://doi.org/10.1136/jcp.2006.038778

7. Chen YC, Fang WL, Wang RF, et al. Clinicopathological variation of Lauren classification in gastric cancer. *Pathol Oncol Res.* 2016;22(1):197-202. https://doi.org/10.1007/s12253-015-9996-6

8. Isobe Y, Nashimoto A, Akazawa K, et al. Gastric cancer treatment in Japan: 2008 annual report of the JGCA nationwide registry. *Gastric Cancer.* 2011;14(4):301-316. https://doi.org/10.1007/s10120-011-0085-6

9. Li CY, Liang GY, Yao WZ, et al. Identification and functional characterization of microRNAs reveal a potential role in gastric cancer progression. *Clin Transl Oncol.* 2017;19(2):162-172. https://doi.org/10.1007/s12094-016-1516-y

10. Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. *Nature.* 2008;455(7209):64-71. https://doi.org/10.1038/nature07242

11. Mukherji S, Ebert MS, Zheng GX, Tsang JS, Sharp PA, van Oudenaarden A. MicroRNAs can generate thresholds in target gene expression. *Nat Genet.* 2011;43(9):854-859. https://doi.org/10.1038/ng.905

12. Selbach M, Schwanhausser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. *Nature.* 2008;455(7209):58-63. https://doi.org/10.1038/nature07228

13. Calin GA, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer.* 2006;6(11):857-866. https://doi.org/10.1038/nrc1997

14. Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature.* 2005;435(7043):834-838. https://doi.org/10.1038/nature03702

15. Grabsch HI, Tan P. Gastric cancer pathology and underlying molecular mechanisms. *Dig Surg.* 2013;30(2):150-158. https://doi.org/10.1159/000350876

16. Shrestha S, Hsu SD, Huang WY, et al. A systematic review of microRNA expression profiling studies in human gastric cancer. *Cancer Med.* 2014;3(4):878-888. https://doi.org/10.1002/cam4.246

17. Wang JL, Hu Y, Kong X, et al. Candidate microRNA biomarkers in human gastric cancer: a systematic review and

validation study. *PLOS One*. 2013;8(9):e73683. https://doi.org/10.1371/journal.pone.0073683

18. Sandoval-Borquez A, Polakovicova I, Carrasco-Veliz N, et al. MicroRNA-335-5p is a potential suppressor of metastasis and invasion in gastric cancer. *Clin Epigenetics*. 2017;9:114. https://doi.org/10.1186/s13148-017-0413-8

19. Cao Q, Liu F, Ji K, et al. MicroRNA-381 inhibits the metastasis of gastric cancer by targeting TMEM16A expression. *J Exp Clin Cancer Res*. 2017;36(1):29. https://doi.org/10.1186/s13046-017-0499-z

20. Zhang Z, Dong Y, Hua J, et al. A five-miRNA signature predicts survival in gastric cancer using bioinformatics analysis. *Gene*. 2019;699:125-134. https://doi.org/10.1016/j.gene.2019.02.058

21. Jiang Z, Guo J, Xiao B, et al. Increased expression of miR-421 in human gastric carcinoma and its clinical association. *J Gastroenterol*. 2010;45(1):17-23. https://doi.org/10.1007/s00535-009-0135-6

22. Zhou L, Li Z, Pan X, et al. Identification of miR-18a-5p as an oncogene and prognostic biomarker in RCC. *Am J Transl Res*. 2018;10(6):1874-1886. https://www.ncbi.nlm.nih.gov/pubmed/30018727

23. Wu CW, Dong YJ, Liang QY, et al. MicroRNA-18a attenuates DNA damage repair through suppressing the expression of ataxia telangiectasia mutated in colorectal cancer. *PLOS One*. 2013;8(2):e57036. https://doi.org/10.1371/journal.pone.0057036

24. Hirajima S, Komatsu S, Ichikawa D, et al. Clinical impact of circulating miR-18a in plasma of patients with oesophageal squamous cell carcinoma. *Br J Cancer*. 2013;108(9):1822-1829. https://doi.org/10.1038/bjc.2013.148

25. Liu WH, Yeh SH, Lu CC, et al. MicroRNA-18a prevents estrogen receptor-alpha expression, promoting proliferation of hepatocellular carcinoma cells. *Gastroenterology*. 2009;136(2):683-693. https://doi.org/10.1053/j.gastro.2008.10.029

26. Ambros V, Bartel B, Bartel DP, et al. A uniform system for microRNA annotation. *RNA*. 2003;9(3):277-279. https://doi.org/10.1261/rna.2183803

27. Stiekema J, Cats A, Kuijpers A, et al. Surgical treatment results of intestinal and diffuse type gastric cancer. Implications for a differentiated therapeutic approach? *Eur J Surg Oncol*. 2013;39(7):686-693. https://doi.org/10.1016/j.ejso.2013.02.026

28. Chou CH, Shrestha S, Yang CD, et al. miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic Acids Res*. 2018;46(D1):D296-D302. https://doi.org/10.1093/nar/gkx1067

29. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. *eLife*. 2015;4:e05005. https://doi.org/10.7554/eLife.05005

30. Betel D, Koppal A, Agius P, Sander C, Leslie C. Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome Biol*. 2010;11(8):R90. https://doi.org/10.1186/gb-2010-11-8-r90

31. Nienhuser H, Schmidt T. Angiogenesis and anti-angiogenic therapy in gastric cancer. *Int J Mol Sci*. 2017;19(1):43. https://doi.org/10.3390/ijms19010043

32. Pinto MP, Owen GI, Retamal I, Garrido M. Angiogenesis inhibitors in early development for gastric cancer. *Expert Opin Investig Drugs*. 2017;26(9):1007-1017. https://doi.org/10.1080/13543784.2017.1361926

33. Shan F, Miao R, Xue K, et al. Controlling angiogenesis in gastric cancer: a systematic review of anti-angiogenic trials. *Cancer Lett*. 2016;380(2):598-607. https://doi.org/10.1016/j.canlet.2015.12.023

34. Fuchs CS, Tomasek J, Yong CJ, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet*. 2014;383(9911):31-39. https://doi.org/10.1016/S0140-6736(13)61719-5

35. Khanna P, Chua PJ, Bay BH, Baeg GH. The JAK/STAT signaling cascade in gastric carcinoma (Review). *Int J Oncol*. 2015;47(5):1617-1626. https://doi.org/10.3892/ijo.2015.3160

36. Khanna P, Chua PJ, Wong BSE, et al. GRAM domain-containing protein 1B (GRAMD1B), a novel component of the JAK/STAT signaling pathway, functions in gastric carcinogenesis. *Oncotarget*. 2017;8(70):115370-115383. https://doi.org/10.18632/oncotarget.23265

37. Wu CS, Wei KL, Chou JL, et al. Aberrant JAK/STAT signaling suppresses TFF1 and TFF2 through epigenetic silencing of GATA6 in gastric cancer. *Int J Mol Sci*. 2016;17(9):1467. https://doi.org/10.3390/ijms17091467

38. Huang Y, Zhang J, Wang G, et al. Oxymatrine exhibits anti-tumor activity in gastric cancer through inhibition of IL-21R-mediated JAK2/STAT3 pathway. *Int J Immunopathol Pharmacol*. 2018;32:2058738418781634. https://doi.org/10.1177/2058738418781634

39. Ishimoto T, Miyake K, Nandi T, et al. Activation of transforming growth factor Beta 1 signaling in gastric cancer-associated fibroblasts increases their motility, via expression of rhomboid 5 homolog 2, and ability to induce invasiveness of gastric cancer cells. *Gastroenterology*. 2017;153(1):191-204. https://doi.org/10.1053/j.gastro.2017.03.046

40. Martinez-Campos C, Torres-Poveda K, Camorlinga-Ponce M, et al. Polymorphisms in IL-10 and TGF-beta gene promoter are associated with lower risk to gastric cancer in a Mexican population. *BMC Cancer*. 2019;19(1):453. https://doi.org/10.1186/s12885-019-5627-z

41. Zhou Q, Zheng X, Chen L, et al. Smad2/3/4 pathway contributes to TGF-beta-induced MiRNA-181b expression to promote gastric cancer metastasis by targeting Timp3. *Cell Physiol Biochem*. 2016;39(2):453-466. https://doi.org/10.1159/000445638

42. Suzuki M, Yokobori T, Gombodorj N, et al. High stromal transforming growth factor beta-induced expression is a novel marker of progression and poor prognosis in gastric cancer. *J Surg Oncol*. 2018;118(6):966-974. https://doi.org/10.1002/jso.25217

43. He Z, Dong W, Li Q, Qin C, Li Y. Sauchinone prevents TGF-beta-induced EMT and metastasis in gastric cancer cells. *Biomed Pharmacother*. 2018;101:355-361. https://doi.org/10.1016/j.biopha.2018.02.121

44. Mjones P, Nordrum IS, Sordal O, et al. Expression of the cholecystokinin-B receptor in neoplastic gastric cells. *Horm Cancer*. 2018;9(1):40-54. https://doi.org/10.1007/s12672-017-0311-8

45. Rao SV, Solum G, Niederdorfer B, Norsett KG, Bjorkoy G, Thommesen L. Gastrin activates autophagy and increases migration and survival of gastric adenocarcinoma cells. *BMC*

Cancer. 2017;17(1):68. https://doi.org/10.1186/s12885-017-3055-5

46. Smith JP, Fonkoua LK, Moody TW. The role of gastrin and CCK receptors in pancreatic cancer and other malignancies. *Int J Biol Sci.* 2016;12(3):283-291. https://doi.org/10.7150/ijbs.14952

47. Alessi DR, Andjelkovic M, Caudwell B, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J.* 1996;15(23):6541-6551. https://www.ncbi.nlm.nih.gov/pubmed/8978681

48. Ganguly S, Basu B, Shome S, et al. Dopamine, by acting through its D2 receptor, inhibits insulin-like growth factor-I (IGF-I)-induced gastric cancer cell proliferation via up-regulation of Kruppel-like factor 4 through down-regulation of IGF-IR and AKT phosphorylation. *Am J Pathol.* 2010;177(6): 2701-2707. https://doi.org/10.2353/ajpath.2010.100617

49. Pavelic K, Kolak T, Kapitanovic S, et al. Gastric cancer: the role of insulin-like growth factor 2 (IGF 2) and its receptors (IGF 1R and M6-P/IGF 2R). *J Pathol.* 2003;201(3):430-438. https://doi.org/10.1002/path.1465

50. Pistritto G, Trisciuoglio D, Ceci C, Garufi A, D'Orazi G. Apoptosis as anticancer mechanism: function and dysfunction of its modulators and targeted therapeutic strategies. *Aging.* 2016;8(4):603-619. https://doi.org/10.18632/aging.100934

51. Shang H, Cao Z, Zhao J, et al. Babao Dan induces gastric cancer cell apoptosis via regulating MAPK and NF-kappaB signaling pathways. *J Int Med Res.* 2019;47(10):5106-5119. https://doi.org/10.1177/0300060519867502

52. Ohlsson B. Gonadotropin-releasing hormone and its physiological and pathophysiological roles in relation to the structure and function of the gastrointestinal tract. *Eur Surg Res.* 2016; 57(1-2):22-33. https://doi.org/10.1159/000445717

53. Sand E, Bergvall M, Ekblad E, D'Amato M, Ohlsson B. Expression and distribution of GnRH, LH, and FSH and their receptors in gastrointestinal tract of man and rat. *Regul Pept.* 2013;187:24-28. https://doi.org/10.1016/j.regpep.2013.09.002