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Expression of MAD2L1 and miR-28-5p in gastric cancer tissues and their clinical significance

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Abstract: Objective To explore the expression levels of mitotic arrest deficient 2 like 1(MAD2L1) and micro RNA (miR)-28-5p in gastric cancer tissues and their relationship with the clinicopathological characteristics of gastric cancer patients, in order to provide a reference for early diagnosis and treatment. **Methods** The differentially expressed genes in gastric cancer were downloaded from the Gene Expression Omnibus (GEO) database, and MAD2L1 was identified as the target gene for this study. Based on the LinkedOmics website, the miRs related to MAD2L1 were screened, and whether there was a targeting relationship between MAD2L1 and miR-28-5p was verified using Targetscan. Tumor tissues surgically resected from 94 gastric cancer patients at the Affiliated Hospital of Xuzhou Medical University were collected from May to November 2024, along with corresponding adjacent tissues obtained at least 5 cm away from the tumor margin. The expression levels of MAD2L1 and miR-28-5p were detected by immunohistochemistry and reverse transcription quantitative polymerase chain reaction (RT-qPCR), respectively. The correlation between the two was analyzed by the Spearman correlation coefficient, and their relationship with the clinicopathological characteristics of gastric cancer patients was analyzed by the chi-squared test. **Results** Bioinformatics analysis revealed that MAD2L1 was upregulated and miR-28-5p was downregulated in gastric cancer. MAD2L1 had a targeting relationship with miR-28-5p. The immunohistochemical results showed that the expression rate of MAD2L1 in gastric cancer tissues (56.38%) was significantly higher than that in adjacent tissues (39.36%) (χ^2 =5.457, P < 0.05). The RT-qPCR results showed that the expression level of miR-28-5p in gastric cancer (0.26±0.08) was significantly lower than that in adjacent tissues (1.14±0.53) (t=16.048, P<0.01). The expression of MAD2L1 was negatively correlated with the expression of miR-28-5p in gastric cancer tissues (r=-0.369, P<0.01). The expression of MAD2L1 in gastric cancer tissues was associated with the clinical stage, lymph node metastasis, degree of differentiation, nerve invasion and vascular invasion (P< 0.05), and the expression of miR-28-5p was associated with the degree of differentiation (P<0.05). **Conclusion** The expression of MAD2L1 and miR-28-5p is closely related to the clinicopathological characteristics of gastric cancer patients, and both can be used as potential biomarkers in the diagnosis and treatment of gastric cancer in clinical practice.

Keywords: Gastric cancer; Mitotic arrest deficient 2 like 1; miroRNR-28-5p; Gene Expression Omnibus database; Clinicopathological characteristics

Gastric cancer is the fifth most common malignant tumor worldwide, posing a significant threat to human life and health. It is estimated that there are over 1 million new cases of gastric cancer diagnosed annually [1]. Due to the lack of typical symptoms in the early stages of gastric cancer, the disease has often progressed to advanced stages at the time of diagnosis, resulting in high mortality and high recurrence rates, making it the third most common cause of malignancy-related death [2]. Therefore, early detection of gastric cancer is particularly important. The pathogenesis of gastric cancer is closely associated with multiple factors, including unhealthy dietary habits, *Helicobacter pylori* infection, genetic factors, and smoking [3]. Among these, genetic factors play a crucial role. Investigating the influencing factors of gastric carcinogenesis and progression at the gene and molecular levels, understanding the mechanisms of disease progression, and exploring novel and effective intervention targets can prolong the survival of patients and improve their prognosis. In this study, key genes in gastric cancer based on database analysis using bioinformatics approaches were screened, and mitotic arrest deficient 2 like 1 (MAD2L1) was finally selected as the target gene. Through analysis, microRNA (miR)-28-5p showed a targeted regulatory relationship with MAD2L1. Therefore, by detecting the expression levels of MAD2L1 and miR-28-5p in gastric cancer tissues, this study aimed to analyze the correlation between their expressions in gastric cancer and their relationship with the clinicopathological characteristics of gastric cancer patients.

1 Materials and Methods

1.1 Bioinformatics Analysis

1.1.1 Gastric cancer microarray data acquisition

Gene expression profiles of gastric cancer and paired adjacent normal gastric tissues were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo). Datasets were

searched using the keyword "gastric cancer" with the following inclusion criteria: (1) Each group contains no less than 5 samples; (2) All included specimens are obtained from adult patients; (3) All cases are histopathologically confirmed as adenocarcinoma. Three datasets (GSE13911, GSE56807, and GSE79973) were ultimately enrolled for subsequent analysis.

1.1.2 Identification of differentially expressed genes (DEGs) and target gene screening

Differential expression analysis was performed using the limma package. DEGs were identified with the threshold set as $|\log_2 FC| > 1.0$ (FC: fold change) and adjusted $P < 0.05$. Common DEGs were obtained by intersecting the DEG lists derived from the three datasets (GSE13911, GSE56807, and GSE79973). Three algorithms in Cytoscape software, including Maximum Neighborhood Component (MNC), Density of Maximum Neighborhood Component (DMNC), and Maximum Clique Centrality (MCC), were applied to score the top 21 hub genes, and the overlapping genes from the three algorithm outputs were extracted. A protein-protein interaction (PPI) network was constructed, and the target gene for this study was selected based on the connectivity degree among genes in the network. The Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn) was utilized to generate boxplots to verify the upregulated expression pattern of the target gene in gastric cancer. The predictive value of the target gene was evaluated using receiver operating characteristic (ROC) curve analysis.

1.1.3 Selection of target gene-associated miRNAs

The LinkedOmics platform (https://www.linkedomics.org) was employed to screen miRNAs that are significantly correlated with the target gene. The TargetScan database (https://www.targetscan.org) was used to validate the targeted binding relationship between the target gene and candidate miRNAs. The upstream regulatory miRNA targeting MAD2L1 was finally determined based on bioinformatics prediction results and previously published literature.

1.2 General Characteristics and Methods

1.2.1 General characteristics

A total of 94 pairs of surgically resected gastric cancer tissues and matched adjacent non-tumor gastric tissues (harvested at least 5 cm from the tumor margin) were collected from patients with gastric cancer who underwent surgical resection at the Affiliated Hospital of Xuzhou Medical University between May 2024 and November 2024.

Inclusion criteria:
(1) Histopathologically confirmed primary gastric adenocarcinoma;
(2) Newly diagnosed gastric cancer without prior history of other malignancies;
(3) No clinically significant abnormalities in routine blood tests, urinalysis, fecal examination, serum biochemistry, coagulation function, or electrocardiogram.

Exclusion criteria:
(1) Received preoperative therapy including radiotherapy, chemotherapy, or targeted therapy;
(2) With severe chronic comorbidities;
(3) Diagnosed with stage IV gastric cancer.

This study was reviewed and approved by the Medical Ethics Committee of the Affiliated Hospital of Xuzhou Medical University (Approval No. XYFY2024-KL279-01), and written informed consent was obtained from all participants.

1.2.2 Immunohistochemical staining

Tissue specimens were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5-μm-thick consecutive sections. Anti-human MAD2L1 primary antibody (No. 10337-1-AP) was purchased from ProteinTech Group (Wuhan Sanying Biotechnology Co., Ltd.). Paraffin sections were sequentially deparaffinized in xylene and rehydrated through a graded ethanol series. Antigen retrieval was performed by immersing sections in pre-boiled sodium citrate buffer (pH 6.0) and maintaining boiling for 10 min, followed by natural cooling to room temperature. Sections were then rinsed three times with phosphate-buffered saline (PBS, pH 7.4). To block endogenous peroxidase activity, sections were incubated with 3% hydrogen peroxide solution at room temperature for 25 min. After washing with PBS, non-specific binding sites were blocked by incubation with 3% bovine serum albumin at room temperature for 30 min. Subsequently, sections were incubated with primary antibody diluted at 1:150 in a humidified chamber at 4 °C overnight. After washing with PBS, horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody was applied and incubated at room temperature for 50 min. Following three washes with PBS, immunoreactivity was visualized using 3,3'-diaminobenzidine (DAB) chromogenic substrate for 5 min. Sections were then counterstained with hematoxylin for 3 min, dehydrated through an ascending ethanol series, cleared in xylene, and mounted with neutral balsam.

1.2.3 Analysis of reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Fresh gastric cancer tissues and paired adjacent normal tissues were immediately snap-frozen in liquid nitrogen and stored at -80 °C until further processing. For RNA extraction, frozen tissues were pulverized into fine powder in liquid nitrogen, and total RNA was isolated using Trizol reagent. Complementary DNA (cDNA) was synthesized from total RNA using a reverse transcription kit. Specific PCR primers were designed and synthesized by Wuhan Servicebio Technology Co., Ltd. (detailed primer sequences are listed in **Table 1**). U6 small nuclear RNA was used as the endogenous reference gene for normalization of miR-28-5p expression by quantitative PCR. All reactions were run in triplicate, and the average threshold cycle value was used for quantification.

Tab.1 Primer sequences for qRT-PCR

Item	Upstream primer（5'→3'）	Downstream primer（5'→3'）
U6	CTC GCT TCG GCA GCA CA	AAC GCT TCA CGA ATT TGC GT
miR-28-5p	ACA CTC CAG CTG GG A AGG AGC TCA CAG TCT	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG CTC AAT AG

1.2.4 Outcome Evaluation

Immunohistochemistry scoring: Positive staining was identified as the presence of distinct brown-yellow granules within cells observed under an optical microscope at ×400 magnification. Staining intensity was scored as follows: 0 for no staining, 1 for pale yellow staining, 2 for brown-yellow staining, and 3 for dark tan staining. The proportion of positively stained cells was scored as: 0 (≤5%), 1 (6%–25%), 2 (26%–50%), and 3 (>50%). The immunoreactive score (IRS) = the staining intensity score × the positive cell percentage score. An IRS ≥ 3 was defined as high MAD2L1 expression.

RT-qPCR quantification: Based on the obtained cycle threshold (Ct) values, the relative expression level of miR-28-5p in each tissue specimen was calculated using the $2^{-\Delta\Delta Ct}$ relative quantification method.

1.2.5 Statistical Analysis

All statistical analyses were performed using SPSS software version 25.0. Categorical variables were presented as case(%). The chi-square (χ^2) test was used to compare MAD2L1 expression between groups and to analyze the associations of MAD2L1 and miR-28-5p expression with the clinicopathological characteristics of gastric cancer patients. Continuous variables were expressed as $\bar{x}\pm s$. The independent samples t-test was applied for comparison of miR-28-5p expression between two groups. Spearman's rank correlation analysis was performed to evaluate the correlation between MAD2L1 and miR-28-5p expression levels. A P value < 0.05 was considered statistically significant.

2 Results

2.1 Results of Bioinformatics Analysis

2.1.1 Identification of DEGs

A total of 109 overlapping DEGs were identified across the GSE13911, GSE56807, and GSE79973 datasets (**Figure 1A**), and their interaction relationships were visualized using a PPI network (**Figure 1B**).

2.1.2 Selection of the target gene

The top 21 candidate genes were scored using Cytoscape software, and 6 hub genes were identified by intersecting the results of three algorithmic analyses (**Figure 2A**), followed by construction of the corresponding PPI network (**Figure 2B**). MAD2L1, which exhibited the highest connectivity with other genes in the network, was selected as the target gene for this study. In all three datasets, the area under the ROC curve (AUC) for MAD2L1 was > 0.80 (**Figure 2C**), indicating high diagnostic accuracy for gastric cancer. The box plot of MAD2L1 expression demonstrated significantly higher expression in gastric cancer tissues compared with adjacent non-tumor tissues (P < 0.01, **Figure 2D**).

2.1.3 Screening of MAD2L1-associated miRNAs

Fifty miRNAs showing significant negative correlation with MAD2L1 expression were preliminarily screened (**Figure 3A**), and their potential targeting relationships were further verified using the TargetScan database (**Figure 3B**). Given that Hell *et al.* [4] have previously confirmed the direct targeted interaction between miR-28-5p and MAD2L1 via luciferase reporter assay, miR-28-5p was selected as the upstream regulatory miRNA targeting MAD2L1.

2.2 Experimental Validation Results

2.2.1 Expression of MAD2L1

Immunohistochemical staining showed that MAD2L1-positive cells exhibited tan-colored granules, predominantly localized in both the nucleus and cytoplasm (**Figure 4**). Among the 94 gastric cancer tissue specimens, 53 cases (56.38%) were classified as high MAD2L1 expression, while only 37 cases (39.36%) of adjacent non-tumor tissues showed high MAD2L1 expression. The expression level of MAD2L1 was significantly higher in gastric cancer tissues compared with paired adjacent non-tumor tissues (χ^2 = 5.457, P = 0.019).

2.2.2 Expression pattern of miR-28-5p

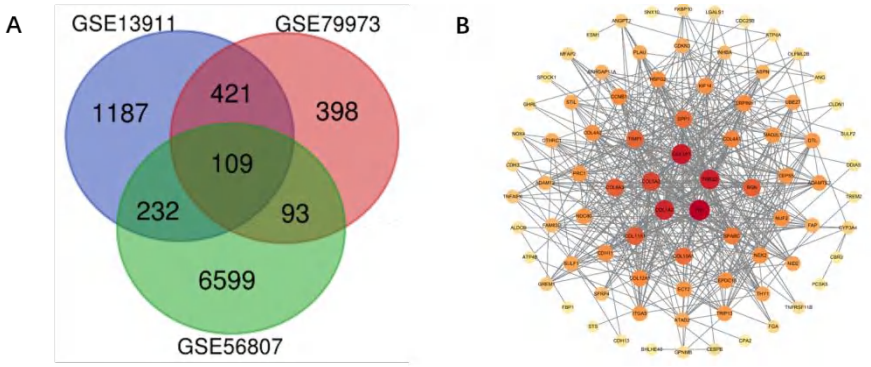
RT-qPCR analysis revealed that the relative expression level of miR-28-5p was 0.26 ± 0.08 in gastric cancer tissues and 1.14 ± 0.53 in adjacent non-tumor tissues. The expression of miR-28-5p was significantly downregulated in gastric cancer tissues compared with adjacent non-tumor tissues (t = 16.048, P < 0.01).

2.2.3 Correlation between miR-28-5p and MAD2L1 expression

Spearman correlation analysis demonstrated a significant negative correlation between miR-28-5p and MAD2L1 expression levels in gastric cancer tissues (r = -0.369, P < 0.01).

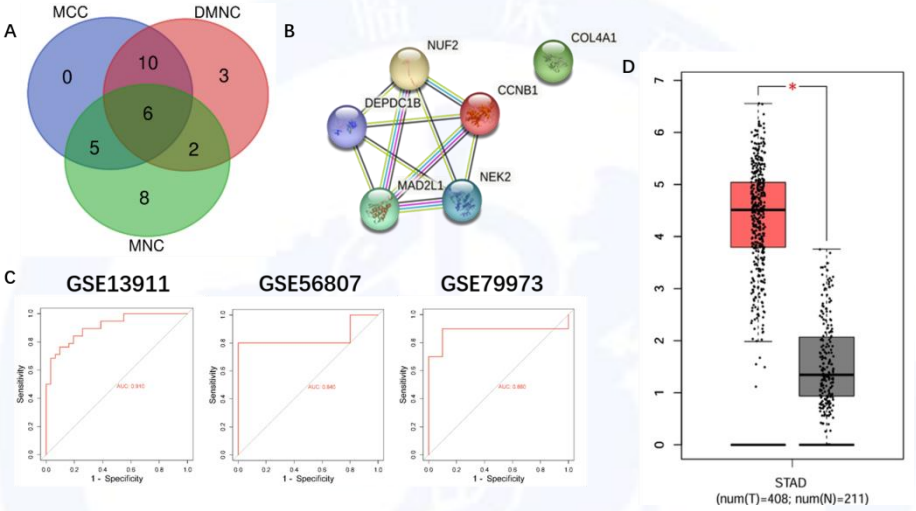
2.2.4 Associations of MAD2L1 with miR-28-5p expression and clinicopathological characteristics

Gastric cancer patients were stratified into two groups based on the median expression level of miR-28-5p (0.23) in cancer tissues: the low expression group (≤ 0.23) and the high expression group (> 0.23). Statistical analysis revealed that high MAD2L1 expression was significantly associated with advanced clinical stage, positive lymph node metastasis, poor differentiation, perineural invasion, and lymphovascular invasion (all P < 0.05). The expression level of miR-28-5p was significantly correlated with the histological differentiation grade of gastric cancer (P < 0.05) (**Table 2**).



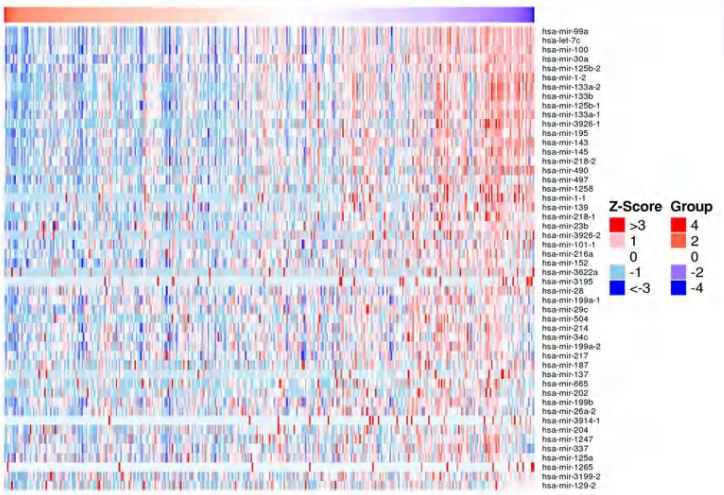
Note: A, the overlapping DEGs identified from the GSE13911, GSE56807, and GSE79973 datasets; B, the visualization of the PPI network using these overlapping DEGs.

Fig.1 Identification of DEGs



Note: A, the intersection of the top 21 hub genes scored by three algorithms implemented in cytoscape software: MCC, DMNC, and MNC; B, the PPI network of the identified hub genes; C, the ROC curves of MAD2L1 across the three included datasets; D, the comparison of MAD2L1 expression levels between gastric cancer tissues and paired adjacent non-tumor tissues.

Fig.2 Screening of hub genes



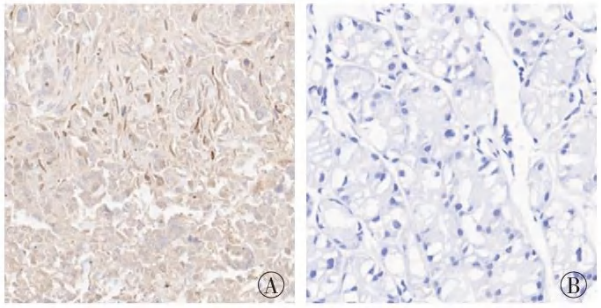
	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P_{CT}	Predicted relative K_D
Position 471-477 of MAD2L1 3' UTR	5' ...GAAAUAUUGCUGUAUAGCUCCUU...	7mer-m8	-0.30	97	-0.30	0.163	N/A	-5.702
hsa-miR-28-5p	3' GAGUUAUCUGACACUCGAGGAA							

Note: A, a heatmap of miRNAs showing significant negative correlation with MAD2L1, screened from the LinkedOmics database; B, the targeted binding relationship between miR-28-5p and MAD2L1, verified via the TargetScan database.

Fig.3 Identification of MAD2L1-related miR

Tab.2 Association between the expression of MAD2L1/miR-28-5p and clinicopathological characteristics in patients with gastric cancer

Clinicopathological characteristic	*n*	MAD2L1expression				miR-28-5p expression			
		High	Low	χ^2 value	*P* value	High	Low	χ^2 value	*P* value
Sex				0.667	0.414			0.009	0.923
Male	67	36	31			33	34		
Female	27	17	10			13	14		
Age (years)				0.209	0.647			0.082	0.774
≥60	62	36	26			31	31		
<60	32	17	15			15	17		
Maximum tumor diameter				1.785	0.182			0.218	0.640
≥5cm	37	24	13			17	20		
<5cm	57	29	28			29	28		
Clinical stage				7.133	0.008			1.284	0.257
Stage I–II	34	13	21			14	20		
Stage III	60	40	20			32	28		
Lymph node metastasis				4.740	0.029			1.075	0.300
Positive	66	42	24			30	36		
Negative	28	11	17			16	12		
Tumor differentiation				4.017	0.045			31.015	<0.001
Well-moderate differentiation	44	20	24			35	9		
Poor differentiation	50	33	17			11	39		
Perineural invasion				4.794	0.029			2.686	0.101
Present	51	34	17			21	30		
Absent	43	19	24			25	18		
Lymphovascular invasion				5.457	0.019			0.354	0.552
Present	54	36	18			25	29		
Absent	40	17	23			21	19		



Note: A, positive MAD2L1 expression in gastric cancer tissue (×400 magnification); B, negative MAD2L1 expression in adjacent non-tumor gastric tissue (×400 magnification). Immunohistochemical staining.

Fig.4 Expression level of MAD2L1 in gastric cancer tissues and adjacent non-tumorous tissues

3 Discussion

Despite substantial advancements in the clinical diagnosis and treatment of gastric cancer in recent decades, the overall prognosis of affected patients remains poor. The underlying causes are twofold: most gastric cancer cases are diagnosed at advanced stages, and the precise molecular mechanisms driving disease progression remain incompletely understood. With the rapid development of high-throughput sequencing technologies and growing insights into the molecular basis of gastric carcinogenesis, the identification of novel diagnostic and prognostic biomarkers has emerged as a major research priority. Therefore, in-depth exploration of gastric cancer pathogenesis at the molecular level and discovery of reliable biomarkers are critically important for improving early detection and therapeutic outcomes of this lethal malignancy.

Cell cycle regulation is a fundamental biological process that governs normal cellular proliferation, differentiation, and maintenance of genomic stability. Dysregulation of the cell cycle control machinery leads to uncontrolled cell proliferation, a hallmark of malignant transformation [5].

The spindle assembly checkpoint (SAC) is a conserved surveillance mechanism that ensures accurate sister chromatid segregation during anaphase, thereby guaranteeing equal chromosomal distribution to daughter cells and preserving genomic integrity [6]. MAD2L1 (mitotic arrest deficient 2 like 1), a core component of the SAC and a member of the MAD2 protein family [7], plays an essential role in monitoring chromosome segregation during mitosis to maintain chromosomal stability [8]. In mammalian cells, either overexpression or ablation of MAD2L1 impairs SAC function, leading to aneuploidy and tumorigenesis. Additionally, MAD2L1 dysregulation contributes to chromosomal instability, a well-recognized driver of tumor initiation and progression across multiple cancer types [9]. Accumulating evidence demonstrates that MAD2L1 is significantly upregulated in various human malignancies, including breast cancer [10], gastric cancer [11], hepatocellular carcinoma [12-13], and lung cancer [14]. A previous study in breast cancer reported that MAD2L1 expression levels were closely correlated with tumor differentiation grade, but showed no significant association with patient age, tumor size, lymph node metastasis, distant metastasis, or clinical stage. Importantly, high MAD2L1 expression was identified as an independent prognostic factor for poor survival in breast cancer patients

[15]. Similarly, elevated MAD2L1 expression has been associated with unfavorable clinical outcomes in patients with gastric cancer [11] and hepatocellular carcinoma [13]. In the present study, immunohistochemical analysis revealed that MAD2L1 expression was significantly higher in gastric cancer tissues compared with paired adjacent non-tumor tissues, suggesting that MAD2L1 overexpression may contribute to gastric carcinogenesis and progression. This observation may be attributed to the high proliferative rate and aggressive nature of gastric cancer cells. Furthermore, MAD2L1 expression was significantly increased in gastric cancer patients with lymph node metastasis, perineural invasion, and lymphovascular invasion.

miRNAs are a class of small non-coding RNAs approximately 22 nucleotides in length that post-transcriptionally regulate gene expression, modulating diverse cellular processes including proliferation, apoptosis, differentiation, invasion, and metastasis [16, 17]. Previous studies have demonstrated that miR-28-5p is significantly downregulated in gastric cancer, and ectopic overexpression of miR-28-5p inhibits gastric cancer cell proliferation, invasion, and migration *in vitro* and *in vivo* [18]. In our study, RT-qPCR analysis confirmed that miR-28-5p expression was significantly lower in gastric cancer tissues compared with adjacent non-tumor tissues, suggesting that miR-28-5p may function as a tumor suppressor in gastric carcinogenesis. We further observed that miR-28-5p expression was significantly lower in poorly differentiated gastric cancers compared with well/moderately differentiated tumors, and the proportion of poorly differentiated cases was significantly higher in the low miR-28-5p expression group, indicating that high miR-28-5p expression may suppress the malignant phenotype of gastric cancer cells.

Notably, Hell *et al.* [4] previously validated the direct targeting interaction between miR-28-5p and MAD2L1 using luciferase reporter assays in clear cell renal cell carcinoma. This regulatory relationship involves miR-28-5p-mediated translational repression of MAD2L1, which is triggered by inactivation of the von Hippel-Lindau (VHL) tumor suppressor. In the context of VHL deficiency, miR-28-5p overexpression inhibits MAD2L1 protein synthesis, leading to mitotic checkpoint dysfunction and chromosomal instability, demonstrating a negative regulatory relationship between these two molecules. Based on this evidence, it was hypothesized that miR-28-5p may similarly target and negatively regulate MAD2L1 expression in gastric cancer. Supporting this hypothesis, the Spearman correlation analysis revealed a significant negative correlation between MAD2L1 and miR-28-5p expression levels in gastric cancer tissues (r = -0.369, P<0.01), consistent with the findings of Hell *et al.* [4].

Several limitations of the present study should be acknowledged. First, all enrolled patients were newly diagnosed with relatively short follow-up duration, precluding comprehensive prognostic analysis at this stage. Long-term follow-up of this cohort is ongoing to further evaluate the prognostic value of MAD2L1 and miR-28-5p in gastric cancer. Second, the direct targeting interaction between miR-28-5p and MAD2L1 was not experimentally validated in the study system, but was inferred from previous literature. Future studies employing luciferase reporter assays, RNA immunoprecipitation, and functional rescue experiments are warranted to confirm this regulatory relationship in gastric cancer models. Such investigations will provide deeper insights into the mechanistic roles of the miR-28-5p/MAD2L1 axis in gastric carcinogenesis, potentially facilitating the development of novel targeted therapeutic strategies for this devastating disease.

Conflict of Interest None

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·消化道肿瘤专题·论著·

MAD2L1和miR-28-5p在胃癌组织中的表达及临床意义

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摘要：目的 探讨胃癌组织中有丝分裂阻滞缺陷蛋白2同源蛋白1（MAD2L1）和微小RNA（miR）-28-5p的表达水平及其与胃癌患者临床病理特征的关系，以期为胃癌的早期诊断及治疗提供参考。**方法** 从基因表达综合数据库（GEO）下载胃癌差异表达基因，确定MAD2L1作为此次研究的靶基因。基于LinkedOmics网站，筛选MAD2L1相关miR，利用Targetscan验证MAD2L1与miR-28-5p有无靶向关系。收集2024年5月至11月徐州医科大学附属医院94例胃癌患者手术切除的肿瘤组织，同时获取距离肿瘤边缘5 cm及以上的对应癌旁组织。通过免疫组化和逆转录定量聚合酶链式反应（RT-qPCR）分别检测MAD2L1和miR-28-5p的表达水平，采用Spearman相关系数分析二者的相关性；通过χ^2检验分析MAD2L1和miR-28-5p的表达水平与胃癌患者临床病理特征之间的关系。**结果** 生物信息学分析显示，在胃癌中，MAD2L1高表达，miR-28-5p低表达，MAD2L1与miR-28-5p具有靶向关系。免疫组化结果显示，胃癌组织中MAD2L1高表达率（56.38%）高于癌旁组织（39.36%）（χ^2=5.457，P<0.05）。RT-qPCR结果显示，胃癌中miR-28-5p的表达水平（0.26±0.08）低于癌旁组织（1.14±0.53）（t=16.048，P<0.01）。胃癌组织中MAD2L1表达与miR-28-5p表达呈负相关（r=−0.369，P<0.01）。胃癌组织中MAD2L1表达与临床分期、淋巴结转移、分化程度、神经侵犯及脉管侵犯有关（P<0.05），miR-28-5p表达与分化程度有关（P<0.05）。**结论** MAD2L1和miR-28-5p表达与胃癌患者临床病理特征密切相关，两者可作为胃癌诊疗的潜在生物标志物。

关键词： 胃癌；有丝分裂阻滞缺陷蛋白2同源蛋白1；miR-28-5p；基因表达综合数据库；临床病理特征
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Expression of MAD2L1 and miR-28-5p in gastric cancer tissues and their clinical significance

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Abstract: Objective To explore the expression levels of mitotic arrest deficient 2 like 1 (MAD2L1) and microRNA (miR)-28-5p in gastric cancer tissues and their relationship with the clinicopathological characteristics of gastric cancer patients, in order to provide a reference for early diagnosis and treatment. **Methods** The differentially expressed genes in gastric cancer were downloaded from the Gene Expression Omnibus (GEO) database, and MAD2L1 was identified as the target gene for this study. Based on the LinkedOmics website, the miRs related to MAD2L1 were screened, and whether there was a targeting relationship between MAD2L1 and miR-28-5p was verified using Targetscan. Tumor tissues surgically resected from 94 gastric cancer patients at the Affiliated Hospital of Xuzhou Medical University were collected from May to November 2024, along with corresponding adjacent tissues obtained at least 5 cm away from the tumor margin. The expression levels of MAD2L1 and miR-28-5p were detected by immunohistochemistry and reverse transcription quantitative polymerase chain reaction (RT-qPCR), respectively. The correlation between the two was analyzed by the Spearman correlation coefficient, and their relationship with the clinicopathological characteristics of gastric cancer patients was analyzed by the chi-squared test. **Results** Bioinformatics analysis revealed that MAD2L1 was upregulated and miR-28-5p was downregulated in gastric cancer. MAD2L1 had a targeting relationship with miR-

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28-5p. The immunohistochemical results showed that the high expression rate of MAD2L1 in gastric cancer tissues (56.38%) was significantly higher than that in adjacent tissues (39.36%) (χ^2=5.457, P < 0.05). The RT-qPCR results showed that the expression level of miR-28-5p in gastric cancer (0.26 ± 0.08) was significantly lower than that in adjacent tissues (1.14 ± 0.53) (t=16.048, P<0.01). The expression of MAD2L1 was negatively correlated with the expression of miR-28-5p in gastric cancer tissues (r=−0.369, P<0.01). The expression of MAD2L1 in gastric cancer tissues was associated with the clinical stage, lymph node metastasis, degree of differentiation, nerve invasion and vascular invasion (P<0.05), and the expression of miR-28-5p was associated with the degree of differentiation (P<0.05). **Conclusion** The expression of MAD2L1 and miR-28-5p is closely related to the clinicopathological characteristics of gastric cancer patients, and both can be used as potential biomarkers in the diagnosis and treatment of gastric cancer.

Keywords: Gastric cancer; Mitotic arrest deficient 2 like 1; MicroRNA-28-5p; Gene Expression Omnibus database; Clinicopathological characteristics

胃癌是全球第五大常见恶性肿瘤，是人类生命健康的一大威胁。据估计，胃癌每年新发病例超过100万[1]，而且由于胃癌早期缺乏典型症状，确诊时病情常常已进展至晚期，死亡率高，且容易复发，是恶性肿瘤相关死亡的第三大常见原因[2]。因此，胃癌的早期发现尤为重要。胃癌的发病与多种因素密切相关，比如不良饮食习惯、幽门螺杆菌感染、遗传因素及吸烟等[3]。其中，遗传因素发挥着重要的作用，从基因和分子水平上探讨胃癌发生发展的影响因素，了解疾病进展机制，发掘新型、有效的干预靶点，能够延长患者生存期，改善预后。本研究通过生物信息学分析，基于数据库筛选胃癌关键基因，最终选择细胞周期相关基因有丝分裂阻滞缺陷蛋白2同源蛋白1（mitotic arrest deficient 2 like 1，MAD2L1）作为靶基因，且分析显示微小RNA（microRNA，miR）-28-5p与MAD2L1具有靶向关系。因此，通过检测胃癌组织内MAD2L1、miR-28-5p表达量，分析二者在胃癌中表达的相关性及二者与胃癌患者临床病理特征的关系。

1 资料与方法

1.1 生物信息学分析

1.1.1 胃癌微阵列数据信息 胃癌和邻近胃组织的数据信息来源于基因表达综合数据库（Gene Expression Omnibus，GEO）（https://www.ncbi.nlm.nih.gov/geo）。以“胃癌”作为关键词搜索相关基因表达数据集，选择标准：（1）每组至少包含5个样本；（2）纳入的标本均为成人样本；（3）均为腺癌。最终纳入GSE13911、GSE56807、GSE79973三个数据集。

1.1.2 差异表达基因（differentially expressed genes，DEGs）的鉴定及靶基因的筛选 采用limma差异分析，设定$|\log_2FC|>1.0$（FC：fold change），将P<0.05作为筛选标准后得到DEGs，将GSE13911、GSE56807、GSE79973三个数据集中的DEGs取交集后得到共同DEGs，用Cytoscape软件中最大邻域组件（maximum of neighborhood component，MNC）、最大邻域组件的密度（density of maximum neighborhood component，DMNC）、最大团中心性（maximum clique centrality，MCC）三种算法对前21个基因计算后取交集，并作蛋白质-蛋白质相互作用（protein-protein interaction，PPI）图，根据基因与基因之间的关联程度选定本研究的靶基因。采用基因表达谱交互分析（Gene Expression Profiling Interactive Analysis，GEPIA）数据库（http://gepia.cancer-pku.cn）绘制箱线图预测靶基因在胃癌中高表达，采用受试者工作特征（receiver operating characteristic，ROC）曲线评价其预测价值。

1.1.3 靶基因相关miR的选择 采用LinkedOmics（https://www.linkedomics.org）筛选与靶基因具有相关性的miR，利用TargetScan（https://www.targetscan.org）验证靶基因与miR的靶向关系，并通过文献报道选定靶向MAD2L1的上游调控miR。

1.2 一般资料及方法

1.2.1 一般资料 收集2024年5月至11月徐州医科大学附属医院94例胃癌患者手术切除的肿瘤组织，同时获取距肿瘤边缘5 cm及以上的对应癌旁胃组织。纳入标准：（1）经病理确诊为原发性胃癌；（2）胃癌初诊，且无其他恶性肿瘤病史；（3）血常规、尿常规、粪便常规、生化、凝血功能、心电图无严重异常。排除标准：（1）手术前接受过放疗、化疗、靶向等治疗；（2）合并严重慢性基础疾病；（3）Ⅳ期患者。本研究经徐州医科大学附属医院医学伦理委员会审批（批准号：XYFY2024-KL279-01），所有患者均知情同意。

1.2.2 免疫组化染色 用4%多聚甲醛固定标本，石蜡切片厚度5 μm。人MAD2L1抗体（批号：10337-1-AP）购自武汉三鹰生物技术有限公司。将石蜡切片逐步进行脱蜡和酒精水化，然后放入加热至沸腾的

枸橼酸钠缓冲液中维持 10 min，待切片自然冷却后，将其浸入 pH 7.4 的磷酸盐缓冲溶液中进行抗原修复。随后，把切片放入 3%双氧水溶液中，在室温条件下孵育 25 min，以此阻断内源性过氧化物酶。完成洗涤步骤后，在切片上滴加 3%牛血清白蛋白，于室温下封闭 30 min。紧接着，在切片上滴加按 1∶150 比例稀释的一抗，放入 4 ℃环境中孵育过夜。再次洗涤后，滴加辣根过氧化物酶标记的山羊抗兔二抗，室温孵育 50 min。经过洗涤，加入二氨基联苯胺显色液染色 5 min，冲洗后用苏木素复染 3 min。最后进行脱水处理，完成封片操作。

1.2.3　逆转录定量聚合酶链反应（reverse transcription quantitative polymerase chain reaction，RT-qPCR）检测　收集胃癌组织及对应的癌旁组织，置于−80 ℃冰箱冻存。开始检测时，先把冻存组织置于液氮环境中研磨成粉末状，借助 Trizol 法提取组织总 RNA，并通过逆转录得到 cDNA。PCR 扩增引物由武汉赛维尔生物科技设计（具体引物信息见表 1）。以 U6 作为内参基因，运用 PCR 试剂盒对 miR-28-5p 展开定量检测。每个样本重复 3 次，取平均值。

表 1　RT-qPCR 引物序列

Tab.1　Primer sequences for RT-qPCR

名称	上游引物序列（5′→3′）	下游引物序列（5′→3′）
U6	CTC GCT TCG GCA GCA CA	AAC GCT TCA CGA ATT TGC GT
miR-28-5p	ACA CTC CAG CTG GGA AGG AGC TCA CAG TCT	CTC AAC TGG TGT CGT GGA GTC GGC AAT TCA GTT GAG CTC AAT AG

1.2.4　结果判读　免疫组化：在光学显微镜 400 倍视野下，阳性细胞表现为具有明显的棕黄色颗粒。按染色强度评分：无着色、浅黄色、棕黄色、黄褐色依次计为 0 分、1 分、2 分、3 分；按阳性细胞所占百分比评分：≤5%计 0 分，6%~25%计 1 分，26%~50%计 2 分，>50%计 3 分。根据免疫反应积分（immunoreactive score，IRS）=染色强度×阳性染色细胞百分比，≥3 分定义为高表达。RT-qPCR：根据所得 Ct 值，采用相对定量法 $2^{-\Delta\Delta Ct}$ 分别计算被检测组织中 miR-28-5p 的表达量。

1.2.5　统计学方法　采用 SPSS 25.0 版软件进行统计分析。计数资料以例（%）表示，MAD2L1 两组间比较以及 MAD2L1 和 miR-28-5p 表达与胃癌患者临床病理特征的关系比较采用 χ^2 检验；计量资料以 $\bar{x}\pm s$ 表示，miR-28-5p 两组间比较采用独立样本 t 检验。MAD2L1 和 miR-28-5p 的相关性采用 Spearman 相关系数分析。P<0.05 为差异有统计学意义。

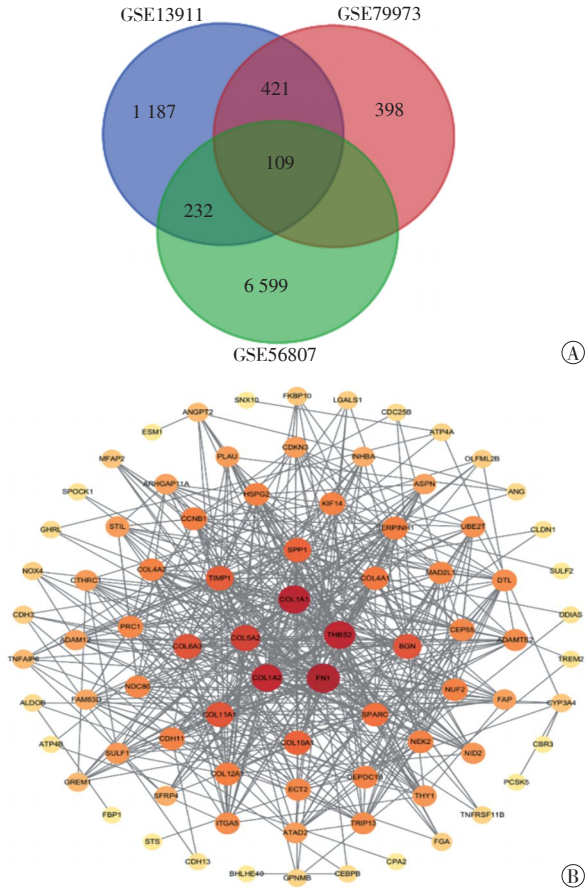
2　结　果

2.1　生物信息学分析结果

2.1.1　DEGs 的鉴定　在 GSE13911、GSE56807、GSE79973 数据集中共鉴定出 109 个共同 DEGs（图 1A），并通过 PPI 网络可视化（图 1B）。

2.1.2　靶基因的筛选　用 Cytoscape 软件计算前 21 个基因，得到 6 个枢纽基因（图 2A）并作 PPI 图（图 2B），选定与其他基因关联性最强的 MAD2L1 作为本研究的靶基因。在三个数据集中，MAD2L1 的 ROC 曲线下面积（area under the curve，AUC）均>0.80（图 2C），显示 MAD2L1 对胃癌诊断的预测准确性高。绘制 MAD2L1 表达箱线图，显示其在胃癌组织中表达高于癌旁组织（P<0.01，图 2D）。

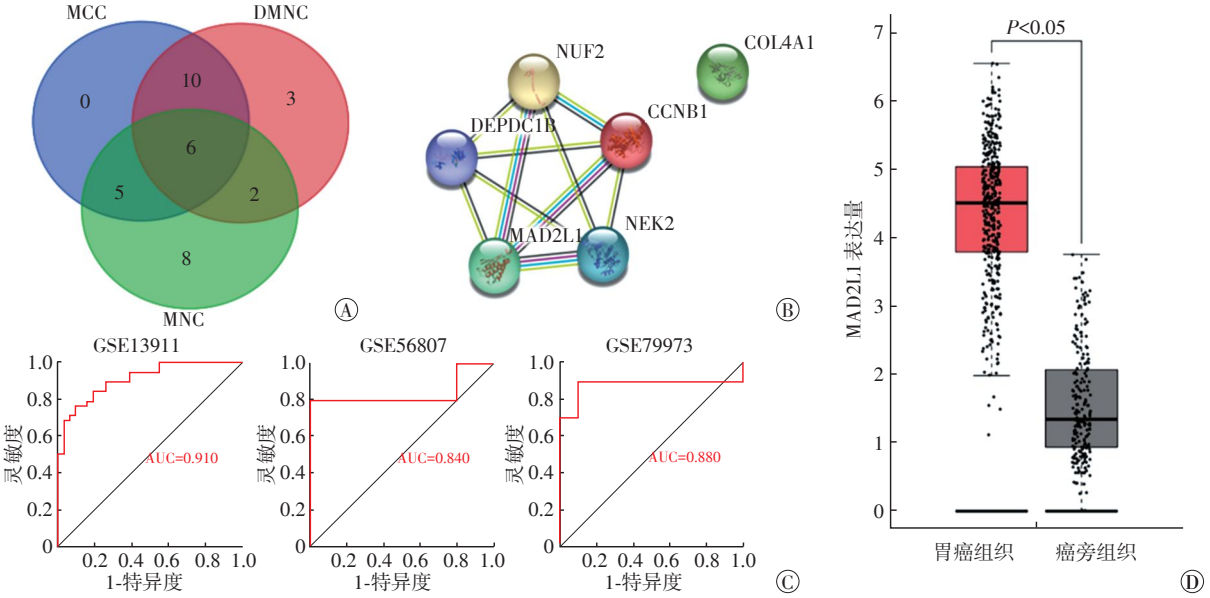
2.1.3　MAD2L1 相关 miR 的筛选　筛选出 50 个与 MAD2L1 具有负相关的 miR（图 3A），结合 TargetScan 数据库进行靶向分析（图 3B）。由于 Hell 等[4]已经证明 miR-28-5p 和 MAD2L1 具有靶向关系，故选择 miR-28-5p 作为靶向调控 MAD2L1 的 miR。



注：A 为 GSE13911、GSE56807、GSE79973 数据集的共同 DEGs；B 为共同 DEGs 的 PPI 网络可视化。

图 1　DEGs 的鉴定

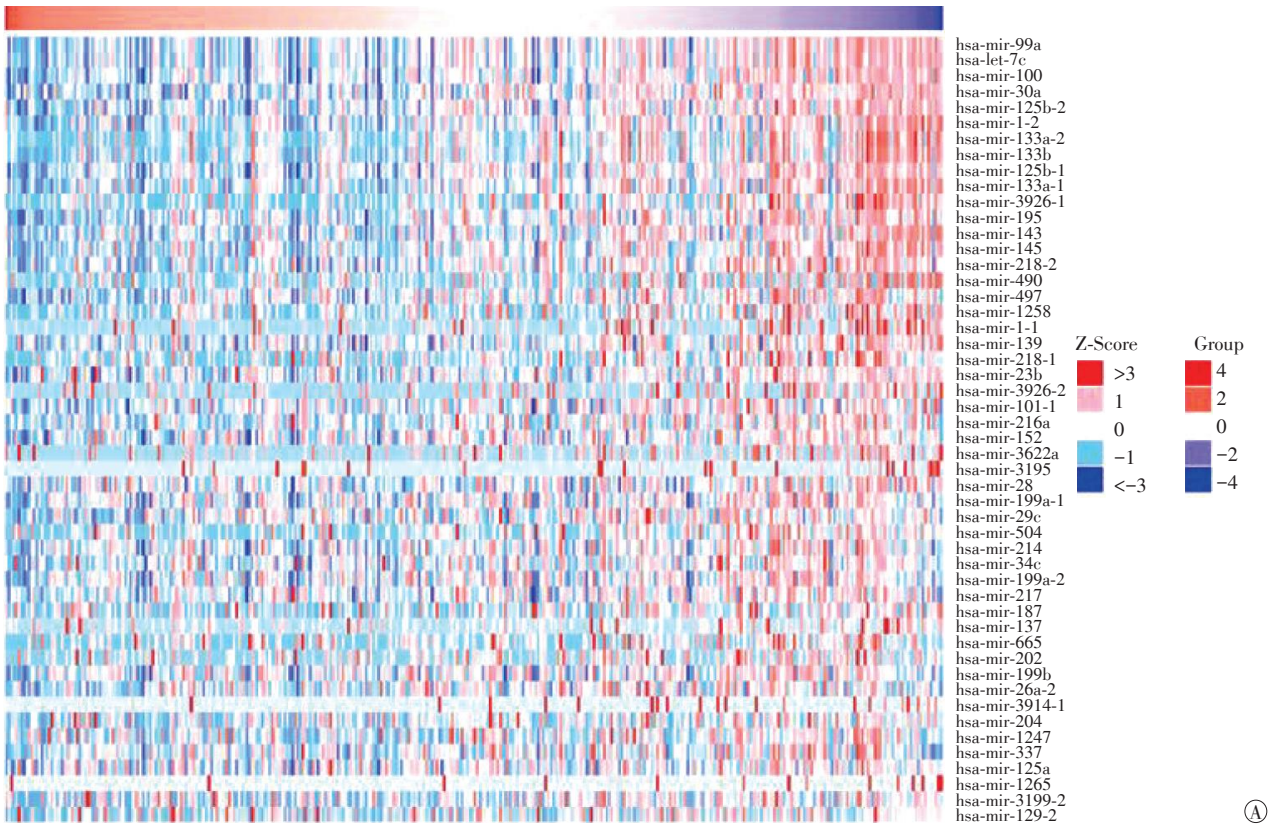
Fig.1　Identification of DEGs



注：A 为用 Cytoscape 软件的 MCC、DMNC、MNC 三种算法对前 21 个基因计算后取交集；B 为枢纽基因的 PPI 网络图；C 为 MAD2L1 在三个数据集中的 ROC 曲线；D 为 MAD2L1 在胃癌组织和癌旁组织中表达的比较。

图 2　枢纽基因的筛选

Fig.2　Screening of hub genes



	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P_{CT}	Predicted relative K_D
Position 471-477 of MAD2L1 3' UTR	5' ...GAAAUAUUGCUGUAUAGCUCCUU...	7mer-m8	-0.30	97	-0.30	0.163	N/A	-5.702
hsa-miR-28-5p	3' GAGUUAUCUGACACUCGAGGAA							

Ⓑ

注：A 为在 LinkedOmics 中筛选的与 MAD2L1 具有负相关的 miR 热图；B 为用 TargetScan 验证 miR-28-5p 和 MAD2L1 具有靶向关系。

图 3　筛选 MAD2L1 相关 miR

Fig.3　Identification of MAD2L1-related miR

2.2　实验结果

2.2.1　MAD2L1 的表达　MAD2L1 阳性细胞着色为黄褐色，位于细胞核和细胞质（图 4）。胃癌组织中有 53 例呈 MAD2L1 高表达（56.38%）；癌旁组织中 MAD2L1 高表达有 37 例（39.36%）。胃癌组织中 MAD2L1 高表达率高于癌旁组织（χ^2=5.457，P=0.019）。

2.2.2　miR-28-5p 的表达　RT-qPCR 结果表明，胃癌组织中 miR-28-5p 的表达水平为 0.26±0.08，在癌旁组织中的表达水平为 1.14±0.53。胃癌组织中 miR-28-5p 的表达水平低于癌旁组织（t=16.048，P<0.01）。

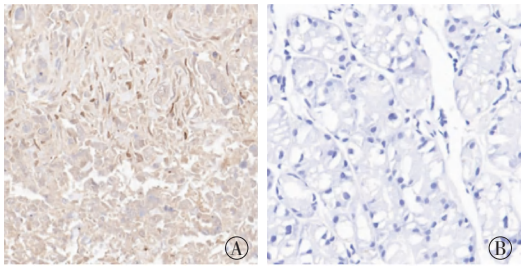
2.2.3　miR-28-5p 和 MAD2L1 的相关性分析　胃癌组织中，miR-28-5p 和 MAD2L1 的表达呈显著负相关（r=−0.369，P<0.01）。

2.2.4　MAD2L1 和 miR-28-5p 的表达与临床病理特征的关系　以胃癌组织中 miR-28-5p 表达水平的中位数（0.23）为标准将胃癌患者分为两组，≤0.23 为低表达组，>0.23 为高表达组。结果显示，MAD2L1 表达与胃癌的临床分期、淋巴结转移、分化程度、神经侵犯及脉管侵犯有关（P<0.05）；miR-28-5p 表达与胃癌的分化程度有关（P<0.05）（表 2）。

表 2　MAD2L1 和 miR-28-5p 表达与胃癌患者临床病理特征的关系　（例）

Tab.2　Association between the expression of MAD2L1/miR-28-5p and clinicopathological characteristics in patients with gastric cancer　(case)

临床病理特征	例数	MAD2L1				miR-28-5p			
		高表达	低表达	χ^2值	P值	高表达	低表达	χ^2值	P值
性别									
男	67	36	31	0.667	0.414	33	34	0.009	0.923
女	27	17	10			13	14		
年龄									
≥60 岁	62	36	26	0.209	0.647	31	31	0.082	0.774
<60 岁	32	17	15			15	17		
肿瘤最大直径									
≥5 cm	37	24	13	1.785	0.182	17	20	0.218	0.640
<5 cm	57	29	28			29	28		
临床分期									
Ⅰ~Ⅱ期	34	13	21	7.133	0.008	14	20	1.284	0.257
Ⅲ期	60	40	20			32	28		
淋巴结转移									
是	66	42	24	4.740	0.029	30	36	1.075	0.300
否	28	11	17			16	12		
分化程度									
高/中分化	44	20	24	4.017	0.045	35	9	31.015	<0.001
低分化	50	33	17			11	39		
神经侵犯									
是	51	34	17	4.794	0.029	21	30	2.686	0.101
否	43	19	24			25	18		
脉管侵犯									
是	54	36	18	5.457	0.019	25	29	0.354	0.552
否	40	17	23			21	19		



注：A 为胃癌组织中 MAD2L1 阳性表达（×400）；B 为癌旁组织中 MAD2L1 阴性表达（×400）。免疫组化染色。

图 4　胃癌组织和癌旁组织中 MAD2L1 表达水平

Fig.4　Expression levels of MAD2L1 in gastric cancer tissues and adjacent non-tumorous tissues

3　讨　论

目前，临床对胃癌的诊断和治疗已经取得了很大的进展，但胃癌的总体预后仍然较差，其根本原因是大多数胃癌不能在早期被发现，且对胃癌进展的确切分子机制的了解仍然有限。随着基因检测技术的发展和对胃癌发生发展的分子机制的研究深入，许多新型生物标志物成为当前研究热点。因此，在分子水平上深入探索胃癌发病机制，寻找新的生物标志物，对胃癌的早期诊断及治疗至关重要。

细胞周期调控是细胞生命活动的基本过程，在维持细胞正常增殖、分化和遗传稳定性中起着关键的作用。一旦细胞周期控制系统出现异常，细胞增殖将失去控制，导致肿瘤的发生[5]。

纺锤体组装检查点（spindle assembly checkpoint，SAC）主要是通过保证在细胞周期的后期，姐妹染色单体能够被准确地拉向细胞的两极，实现染色体的平均分配，确保子代细胞中遗传物质的稳定性[6]。MAD2L1 是 SAC 的核心蛋白，是 MAD2 家族的一员[7]，其功能就是在有丝分裂过程中对染色体分离起监督作用，确保染色体的稳定性[8]。在哺乳动物细胞中，MAD2L1 过表达或缺失一方面会导致 SAC 功能受损，导致非整倍体和肿瘤的发生；另一方面可导致染色体不稳定，而染色体不稳定是肿瘤发生的重要驱动因素，进而导致一些肿瘤的发生[9]。MAD2L1 在一些肿瘤中明显高表达，包括乳腺癌[10]、胃癌[11]、肝细胞癌[12-13]、肺癌[14]等。有研究发现，MAD2L1 表达水平和乳腺癌的分化程度紧密相关，但是与患者年龄、肿瘤大小、淋巴结转移、远处转移以及临床分期等因素并没有明显关联。同时，MAD2L1 高表达往往预示着乳腺癌患者的预后较差[15]。在胃癌[11]和肝癌[13]患者群体里，MAD2L1 高表达同样意味着预后不理想。本研究通过免疫组化实验发现，MAD2L1 在胃癌中表达

显著高于癌旁组织，提示其高表达可能参与胃癌的发生发展，与胃癌恶性程度高、肿瘤细胞增殖快有关。伴随淋巴结转移、神经侵犯及脉管侵犯的胃癌患者MAD2L1表达更高。

miR是长度约为20个核苷酸的非编码RNA分子，调节包括细胞增殖、凋亡、分化、侵袭和转移在内的许多细胞过程[16-17]。研究表示，胃癌中的miR-28-5p表达明显降低，过表达miR-28-5p可抑制胃癌细胞的增殖、侵袭和迁移[18]。本研究通过RT-qPCR实验，发现miR-28-5p在胃癌中表达显著低于癌旁组织，提示其高表达或许对胃癌的发生发展起到抑制作用。低分化胃癌患者miR-28-5p表达低于高/中分化者，且当miR-28-5p低表达时，低分化胃癌患者占比显著升高，这说明miR-28-5p高表达可能抑制肿瘤细胞增殖。

此外，Hell等[4]在研究肾透明细胞癌时，通过荧光素酶测定法已证明miR-28-5p和MAD2L1具有靶向关系，这种靶向关系是由miR-28-5p介导的MAD2L1翻译抑制，由希佩尔-林道肿瘤抑制因子（von Hippel-Lindau tumor suppressor，VHL）失活触发的。当VHL失活时，miR-28-5p过表达可以抑制MAD2L1蛋白翻译，使有丝分裂检查点功能障碍，导致染色体不稳定，此研究表明两者具有负相关的靶向关系。因此本研究假设，MAD2L1和miR-28-5p在胃癌发生发展过程中也具有靶向关系，并通过Spearman相关系数分析显示MAD2L1和miR-28-5p表达呈负相关（r=-0.369，P<0.01）。这与既往研究结果相符合[4]。

本研究存在一定的局限性。一方面，由于纳入的患者均为新发病例，随访时间尚短，目前无法获取足够信息进行疾病的预后分析。后续需持续跟踪患者病程发展，为全面评估预后提供依据；另一方面，MAD2L1和miR-28-5p靶向关系的确定主要参考其他文献的荧光素酶实验结果，本研究缺乏独立的验证。后续有必要通过荧光素酶实验等方法，在本研究体系内对二者的靶向关系开展深入验证。从而更全面地了解MAD2L1和miR-28-5p在胃癌发生发展中的作用机制，推动研究成果向临床应用转化。

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