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Expression and clinical significance of SYNE1 and MAGI2 gene promoter methylation in gastric cancer

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Abstract

Gastric cancer is the fifth most common cancer and the third leading cause of cancer-related mortality globally. Abnormal DNA methylation is closely related to gastric cancer. The purpose of the study was to investigate the methylation of the SYNE1 and MAGI2 gene promoter and its relationship with the clinical-pathological factors, chemotherapy efficacy, and survival, thus providing a new biomarker for the prognosis and chemotherapy efficacy in gastric cancer.

The methylation status of SYNE1 and MAGI2 in gastric cancer and adjacent tissues was detected by MSP method in 70 cases of advanced gastric cancer paraffin specimens.

The methylation rate of the SYNE1 and MAGI2 gene promoter region was higher in gastric cancer tissues compared with adjacent tissues. The methylation status of SYNE1 was associated with the age at diagnosis and the size of the primary tumors, but no clinical or pathological factors have been found to be related with the methylation status of MAGI2 promoter. A high level of SYNE1 promoter methylation was associated with poorer chemotherapy efficacy in recurrent patients with gastric cancer. Thirty-three percent of the 70 patients exhibited highly methylated MAGI2; in this group, the median progression-free survival time was 4.1 months, shorter than those with negative methylated MAGI2 whose PFS was 5.1 months.

MAGI2 is more methylated in gastric cancer than in adjacent tissues suggesting that hypermethylation changes in MAGI2 may be one of the mechanisms of tumorigenesis in gastric cancer. The methylation status of the SYNE1 and MAGI2 promoter regions may affect the chemotherapy efficacy of advanced gastric cancer. The prognosis of MAGI2-negative patients was better than that of positive ones, suggesting that MAGI2 may be an independent prognostic factor for PFS in patients with advanced gastric cancer.

Abbreviations: CR = complete remission, MAGI2 = membrane-associated guanylate kinase protein 2, MSP = methylation-specific PCR, OS = overall survival, PD = disease progression, PFS = progression-free survival, PR = partial remission, SD = stable disease, SYNE1 = nuclear envelop spectrin repeat protein, Nesprin1.

Keywords: chemotherapeutic efficacy, gastric cancer, methylation, prognosis, SYNE1 and MAGI2

1. Introduction

Although it is steadily declining in incidence, cancer of the stomach (also known as gastric cancer) remains one of the most common and deadly neoplasms in the world. According to the data of the epidemiology of gastric cancer published in 2019, gastric cancer is the 5th most commonly diagnosed cancer in the world. Over 1 million cases of gastric cancer are diagnosed each year around the world. Gastric cancer accounts for 783,000 deaths each year, making it the third most deadly cancer among males worldwide.[1,2] Surgery remains the only way to cure the early disease. Early detection and treatment at early stage remains considerable to improve population survival outcome of gastric cancer. But the majority of patients are already in advanced disease at the time of diagnosis, leading to a poor 5-year survival rate of 20% to 25%.[3] In patients with recurrent and unresectable disease, chemotherapy is the most common therapeutic method to prolong the survival.[4,5] However, a considerable number of patients have low efficiency of chemotherapy, but have to endure the adverse effects of chemotherapy that may affect the quality of life. Therefore, it is significant to sake definitely efficient biomarkers for the early detection of gastric cancer and chemotherapy efficiency.

It has been found that epigenetic alterations, including DNA methylation of CpG islands, are involved in the development of gastric cancer.[6–8] DNA methylation is a modification in which a methyl group is added to the cytosine residue at 5-Carbon in a CpG dinucleotide.[9] Promoter methylation plays an important role in regulating gene expression.[7,10]

Studies have confirmed that abnormal methylation of *SYNE1* (nuclear envelop spectrin repeat protein, Nesprin1) and *MAGI2* (Membrane-associated guanylate kinase protein 2) gene, which is a member of the membrane-bound guanylate kinase family, is closely related to colorectal cancer,[11–13] lung cancer,[14,15] acute

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lymphocytic leukemia,[16] and other cancers.[17,18] But few studies have explored the abnormal DNA methylation of the 2 genes in gastric cancer.

The purpose of the present study was to investigate the methylation status of the *SYNE1* and *MAGI2* gene in the cancerous and adjacent tissues of patients with gastric cancer. The association between the SYNE1 and MAGI2 methylation patterns and clinical characteristics, chemotherapy efficacy, progression-free survival (PFS), and OS times in patients with advanced gastric cancer was evaluated.

2. Materials and methods

2.1. Patients and sample collection

Seventy paraffin-embedded tissue samples were collected from advanced gastric cancer patients, who were hospitalized at the Second Hospital of Dalian Medical University (Liaoning, China) between January 2010 to August 2017. And the adjacent tissues of 20 of 70 patients with gastric cancer were selected as controls. The inclusion criteria were as follows: The patient's clinical data were complete; gastric cancer could be confirmed histopathologically by surgical pathology; the patients had received at least 2 cycles of first-line chemotherapy in our hospital, and has not received other radiotherapy, chemotherapy, biological therapy; there were lesions that could be measured by imaging machines such as CT or MRI; the ECOG scores was $\leq$2 points. The exclusion criteria included are as follows: the patients were complicated with severe metabolic diseases or with severe liver and kidney function and bone marrow dysfunction before chemotherapy; the patients were concurrent with other malignant tumors; and the patients had previously received anti-cancer treatment.

The deadline for follow-up is February 1, 2019, with a total follow-up of 2.7 to 71 months. The median follow-up time was 10.55 months. The present study was approved by the Ethics Committee of the medical university and the patients provided written informed consent.

All patients conducted at least one efficacy evaluation after 2 to 3 cycles of chemotherapy. According to the RECIST (response evaluation criteria in solid tumors) standard, the disease changes were divided into complete remission (CR), partial remission (PR), and stable disease (SD) and disease progression (PD).

2.2. Methylation-specific PCR (MSP)

SYNE1 and *MAGI2* gene promoter methylation was detected using MSP. Methylation-specific primers were designed using Sequenom Assay Design 3.1 software (Sequenom) and the sequences are listed in Table 1. Genomic DNA isolation was performed according to the manufacturers' protocols. The MSP reaction (10 μL) included 4 μL modified DNA template, 0.6 μL methylation-specific or nonmethylation-specific primers, 1 μL 10X Buffer I, 0.1 μL HsTaq DNA polymerase mixture, and 3.5 μL ddH_2O. PCR was performed as the following thermocycling conditions: 95°C for 5 minutes; 35 cycles of 95°C for 30 seconds, 63°C for 30 seconds, and 72°C for 30 seconds, and then 72°C for 10 minutes according to the manufacturers' protocols. Gel electrophoresis was used to extract and sequenced the PCR products.

Amplification using methylation-specific primers was considered to indicate a positive result for methylation. No amplification using methylation-specific primers, or amplification using nonmethylation-specific primers was considered as negative methylation. In addition, amplifications using methylation-specific and nonmethylation-specific primers that also exhibited partial methylation were considered as positive methylation.

Table 1

Sequences and amplicon sizes of primers used for MAGI2 and SYNE1 methylation-specific PCR.

Primer	Sequence	PCR products size, bp
MAGI2-MF	5' GAGTTGTTGTTGGAGGTGAAC 3'	146/146
MAGI2-UF	5' GAGTTGTTGTTGGAGGTGAAT 3'	
MAGI2-R	5' CCC(A/G)ACAAAACACTAAACAAAC 3'	
SYNE1-MF	5' GGGTTTT(C/T)GTAGTTTTGTAGAT(T/C)GC 3'	279/279
SYNE1-UF	5' GGGTTTT(C/T)GTAGTTTTGTAGAT(T/C)GT 3'	
SYNE1-R	5' CCCAAC(G/A)TACAAACACAAACTTAC 3'	

MAGI2 = membrane-associated guanylate kinase protein 2, SYNE1 = nuclear envelop spectrin repeat protein, Nesprin1.

3. Results

3.1. Characteristics of patients

Among the 70 gastric cancer patients, 49 were male, and 21 were female. The ages of the 70 patients ranged from 30 to 83 years, with a median age of 61.5 years. The patients were divided into a palliative group comprising 38 patients and a relapse group comprising 32 patients with recurrence after radical gastrectomy. Of the 70 patients with advanced gastric cancer, 66 had undergone lymph node dissection, 34 patients with N0-N2 and 32 patients with N3. The tumors were divided by histology into simple adenocarcinoma and non-simple adenocarcinoma (including adenocarcinoma with other types of gastric cancer and non-adenocarcinoma gastric cancer); 40 cases and 29 cases in the 2 groups, respectively. Regarding the degree of differentiation, 35 patients had low differentiation, and 32 had high-medium differentiation. Concerning tumor size, 35 patients had primary tumors with a maximum diameter of $\leq$5 cm, and 32 patients had primary tumors with a maximum diameter $>$5 cm. After chemotherapy, a total of 18 patients had their target lesions reduced to the PR standard, while 37 patients had SD and 15 patients had PD.

3.2. Methylation status of SYNE1 and MAGI2 in cancer tissues and adjacent tissues

Of the 70 gastric cancer tissues, 47 were negative for SYNE1 methylation, 17 were positive, and 6 were missing. Among the 20 paracancerous tissues, 15 were negative for SYNE1 methylation, 4 were positive, and 1 was missing. Of the 70 gastric cancer tissues, 46 were negative for MAGI2 methylation, 23 were positive, and 1 was missing. Among the 20 cases of tissues adjacent to the cancer, 18 were negative for MAGI2 methylation, and 2 were positive. Selected results of representative gastric cancer samples using methylation-specific PCR assays of MAGI2 and SYNE1 are shown in Figure 1.

χ^2 test was used to analyze the methylation status of SYNE1 and MAGI2 in the cancer tissues and adjacent tissues. In the 70 gastric cancer tissues, the positive rate of SYNE1 methylation was 26.6% (17/64); in the adjacent tissues, the positive rate of SYNE1 methylation was 21.1% (4/19). In addition, no statistically difference was found ($P = .628$). In the 70 gastric cancer tissues,

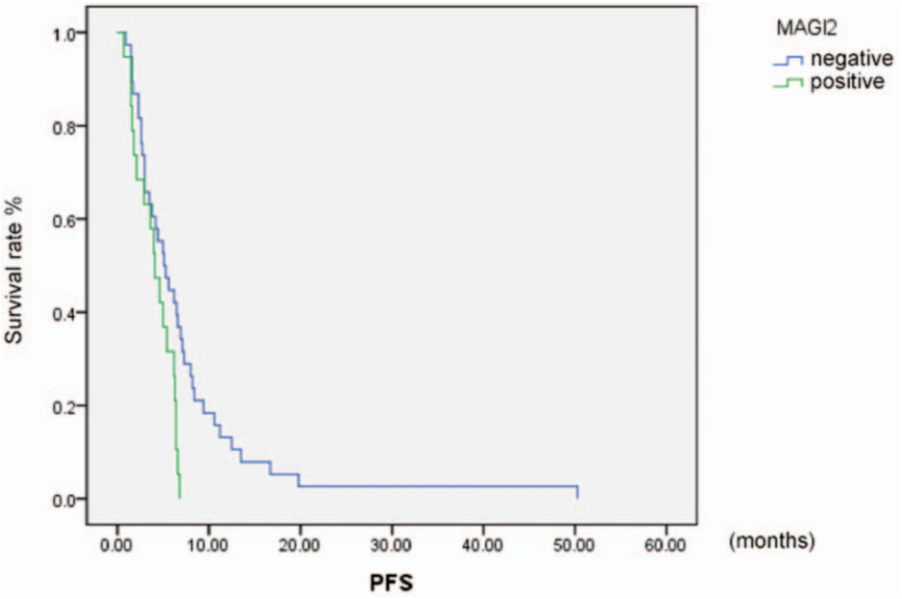

Figure 1. Representative gastric cancer samples using methylation-specific PCR assays of MAGI2 (A) and SYNE1 (B). M = methylated alleles; MS = methylated DNA Standard; M1000 = DNA marker; NMS = nonmethylated DNA Standard; NTC = no template control; U = unmethylated alleles.

the positive rate of MAGI2 methylation was 33.3% (23/69); in the adjacent tissues, the positive rate of MAGI2 methylation was 10.0% (2/20). In addition, a statistically significant difference was found ($P=.041$) (see Table 2 for details).

3.3. Relationship between SYNE1 and MAGI2 methylation status and clinicopathological factors

χ^2 test was used to analyze the correlation of the methylation status of SYNE1 and MAGI2 in the tumor tissues of patients with gastric cancer with age, sex, primary tumor size, degree of differentiation, number of lymph node metastases, recurrence and metastasis/advanced palliative, gastric cancer case classification, and other clinic-pathological factors. We found that the methylation status of SYNE1 was related to age and tumor size, and the methylation positive rate in the SYNE1 promoter region was higher than that in patients aged ≤60 years (37.8% vs 11.1%; $P=.017$). The methylation positive rate of the SYNE1 promoter region in the primary tumor lesion with a maximum diameter of ≤5 cm was higher than that in the primary tumor lesion with a maximum diameter of > 5 cm (40.0% vs 16.1%; $P=.038$). No clinicopathological factors were found to be related to MAGI2 methylation (see Table 3 for details).

3.4. Short-term efficacy

The short-term curative effect of chemotherapy was evaluated on the basis of the first curative effect evaluation. CR and PR are jointly defined as an effective group for chemotherapy, SD and PD are collectively defined as an ineffective group; those with CR, PR, and SD are defined as a disease control group, and PD is defined as a progressive group. According to the chemotherapy regimen, oxaliplatin combined with tigeo or capecitabine or 5-FU was defined as platinum/fluorine group and paclitaxel or docetaxel combined with tigeo or 5-FU was defined as the taxane/fluorine group. χ^2 test was used to analyze the correlation between various factors and the efficacy of chemotherapy.

After examination, the methylation status of SYNE1 and MAGI2 showed no apparent relationship with the effectiveness of chemotherapy and disease control rate.

Because different clinicopathological factors and chemotherapy programs have certain effects on the efficacy of chemotherapy, after stratification with different clinicopathological factors and chemotherapy programs and other factors, the relationship between SYNE1 and MAGI2 and the efficacy of chemotherapy was further analyzed. After stratified examination, we found that, in 29 relapsed patients, the methylation status of SYNE1 was related to the efficacy of chemotherapy. The SYNE1-negative patients showed a higher chemotherapy efficiency than the

Table 2

Methylation status of SYNE1 and MAGI2 in cancer tissues and adjacent tissues.

	SYNE1			MAGI2		
	Negative	Positive	*P*	Negative	Positive	*P*
Cancer tissues	47	17	.628	46	23	.041
	73.4%	26.6%		66.7%	33.3%	
Adjacent tissues	15	4		18	2	
	78.9%	21.1%		90.0%	10.0%	

MAGI2 = membrane-associated guanylate kinase protein 2, SYNE1 = nuclear envelop spectrin repeat protein, Nesprin1.

Table 3

Relationship between methylation status of SYNE1 and MAGI2 and clinical pathological.

	SYNE1			MAGI2		
	Negative	Positive	*P*	Negative	Positive	*P*
Total	47	17		46	23	
Sex						
Male	32	12	.849	32	16	1
Female	15	5		14	7	
Age						
≤60	24	3	.017	22	9	.494
>60	23	14		24	14	
Recurrence/Palliative surgery						
Recurrence	21	8	.866	23	8	.231
Palliative surgery	26	9		23	15	
Lymph node staging						
N0-2	22	7	.613	25	10	.332
N3	22	9		18	12	
Degree of differentiation						
Poor	24	7	.349	24	10	.871
Moderate or well	20	10		22	10	
Pathological type						
Simple adenocarcinoma	25	13	.111	26	14	.577
Other type	21	4		20	8	
Tumor size						
≤5 cm	18	12	.038	22	13	.485
>5 cm	26	5	.038	22	9	

MAGI2 = membrane-associated guanylate kinase protein 2, SYNE1 = nuclear envelop spectrin repeat protein, Nesprin1.

SYNE1-positive patients (47.6% vs 0%; $P=.027$) (see Table 4 for details).

In the stratification of chemotherapy and other clinicopathological factors, no statistically significant differences were found between SYNE1 and MAGI2 methylation and chemotherapy efficacy. After stratification, we found that the 2 genes were still not significantly related to disease control.

At the same time, the different methylation states of SYNE1 and MAGI2 were used for stratification to analyze whether the clinicopathological factors and chemotherapy regimen in patients with different methylation levels were related to the efficacy of chemotherapy. We found that, among SYNE1-negative patients, relapsed patients were more effective in chemotherapy than palliative patients (47.6% vs 7.7%; $P=.020$). Among SYNE1-positive patients, patients with nonsimple adenocarcinoma had a higher rate of chemotherapy than those with simple adenocarcinoma (50% vs 0%; $P=.044$). Among MAGI2-negative patients, those with fewer lymph node metastases had a higher rate of chemotherapy than those with more lymph node metastases (45.8% vs 5.3%, $P=.030$) (see Table 5 for details).

No statistically significant differences were found in the efficacy of the chemotherapy regimens and other clinicopathological factors in different methylation states.

3.5. Survival and prognosis

3.5.1. Single-factor survival analysis. The Kaplan–Meier method was used to draw survival curves, and the Log-Rank test was used to test whether there was a statistically significant difference between PFS and overall survival (OS).

Seventy patients with advanced gastric cancer had a median PFS of 4.8 months and a median OS of 10.3 months. The median PFS of those who tested negative for MAGI2 methylation was 5.1 months, and the median PFS of those who tested positive was 4.1 months ($P=.012$), which was statistically significant (see Fig. 2 for the survival curve). The median OS of MAGI2 methylation-negative patients was 11.0 months, which was longer than 10.0 months for positive patients, but with no statistically significant difference. The median PFS of SYNE methylation-negative and SYNE methylation-positive patients were 4.6 and 4.0 months, respectively, and the median OS were 9.6 and 11.6 months, respectively, with no statistically significant difference (see Table 6 for details).

3.6. Analysis of prognostic factors

In the Cox multivariate regression analysis model, the methylation status of SYNE1 and MAGI2 and the chemotherapy regimen of clinicopathological factors were used as variables to

Table 4

Relationship between SYNE1 methylation status and chemotherapy efficacy in patients with recurrence disease.

	SYNE1		
Chemotherapy efficacy	Positive	Negative	*P*
Effective (CR+PR)	0	10	.027
Non-effective (SD+PD)	8	11	

MAGI2 = membrane-associated guanylate kinase protein 2, SYNE1 = nuclear envelop spectrin repeat protein, Nesprin1.

Table 5

Relationship between clinicopathological factors and the effectiveness of chemotherapy in patients with different methylation status.

	Chemotherapy efficacy		
	Noneffective (PD+SD)	effective (CR+PR)	*P*
SYNE1 negative			
Recurrence/ Palliative			
Recurrence	11	10	.020
Palliative	24	2	
SYNE1 positive			
Pathological type			
Simple adenocarcinoma	13	0	.044
Other	2	2	
MAGI2 negative			
Lymph node staging			
N0-2	13	11	.030
N3	18	1	

CR = complete remission, MAGI2 = membrane-associated guanylate kinase protein 2, PD = disease progression, PR = partial remission, SD = stable disease, SYNE1 = nuclear envelop spectrin repeat protein, Nesprin1.

analyze the independent prognostic factors of PFS and OS. After including gender, age, number of lymph nodes, and methylation status, we found that the methylation status of the MAGI2 promoter region may be an independent prognostic factor for PFS [*P* = .017; hazard ratio (HR) = 2.245; 95% confidence interval (95% CI): 1.154–4.369] (see Table 7 for details). However, no statistically significant association was found between the methylation status of the SYNE1 promoter region and PFS and OS.

Table 6

Log-rank test for single-factor survival analysis of survival time.

	P (PFS)	*P* (OS)
Methylation status of SYNE1	.879	.979
methylation status of MAGI2	.012	.315

MAGI2 = membrane-associated guanylate kinase protein 2, OS = overall survival, PFS = progression-free survival, SYNE1 = nuclear envelop spectrin repeat protein, Nesprin1.

4. Discussion

Epigenetics means that the function and expression level of a gene are changed without changing the gene's DNA sequence of the cell gene.[7,19] This change is reversible and can be inherited through mitosis and meiosis. Epigenetic regulation mechanisms mainly include ncRNA regulation, DNA methylation modification, and histone modification, chromatin remodeling, etc.[19,20] DNA methylation is the most widely studied epigenetic modification and mainly refers to the use of S-adenosylmethionine as a methyl donor under the action of DNA methyltransferase (DNMT).[9] The methyl group is transferred to the 5' carbon atom of cytosine in the CpG dinucleotide, thereby forming 5-methylcytosine. Methylated cytosine regulates gene expression and protects related DNA sites from degradation by specific restriction enzymes. In epigenetics, DNA methylation is characterized by genome-wide hypomethylation and CpG island hypermethylation in the occurrence and development of tumors. Abnormal methylation of DNA in the gene promoter region can lead to the inactivation of tumor suppressor genes and other tumor-related genes in cells, and it is currently the clearest epigenetic marker in gastric cancer.[21–24]

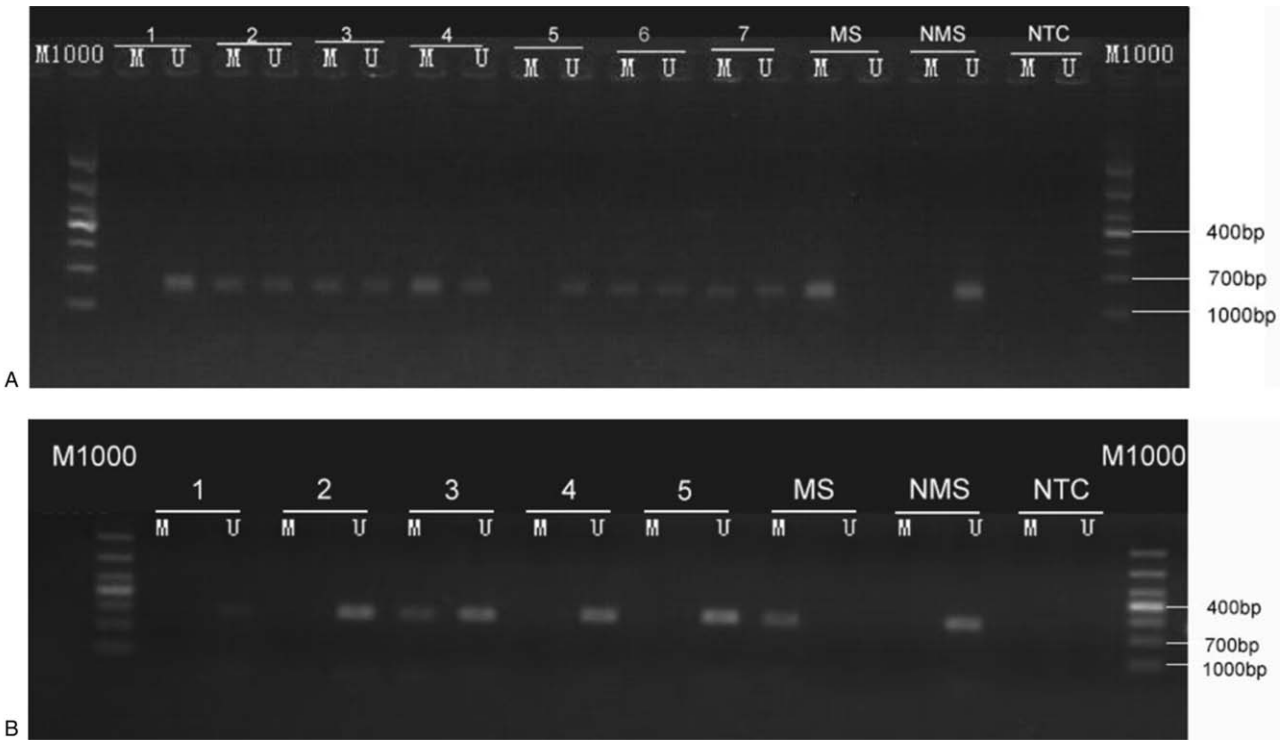

Figure 2. Kaplan–Meier survival plot of PFS based on the methylation status of MAGI2 (*P* = .029, HR = 2.619, 95% CI: 1.106–6.204).

Table 7

Multivariate prognostic analysis of MAGI2 methylation status and PFS.

	B	SE	Wald	df	Sig.	HR	95% CI	
							lower	upper
MAGI2	0.809	0.340	5.671	1	0.017	2.245	1.154	4.369

MAGI2 = membrane-associated guanylate kinase protein 2.

Liu Yue's research used the Affymetrix Human U133 Plus2.0 expression profile chip to screen out genes with differential expression in gastric cancer tissues, in order to describe the characteristic gene expression profile of gastric cancer, and further combined with the clinicopathological data of patients, found that it can be used to supplement pathology TNM staging molecular markers, including SYNE1 and MAGI2. This research revealed that the higher the gene methylation values of SYNE1 and MAGI2 are, the lower the 5-year survival rate of patients is, suggesting that there is a certain correlation between the 2 markers and the occurrence and development of gastric cancer.[25]

4.1. Differences in the methylation status of SYNE1 and MAGI2 in gastric cancer and adjacent tissues

In this study, the positive rate of MAGI2 methylation in gastric cancer tissues was higher than that in adjacent tissues (33.3% vs 10.0%, $P=.041$), suggesting that MAGI2 may inhibit the occurrence of gastric cancer. Chang et al[26] found that *MAGI2* gene hypermethylation in endometrial cancer and ovarian cancer may be associated with the occurrence of the respective cancers. Feng et al[27] found that, in patients with cervical cancer CIN3+ lesions, MAGI2 was significantly hypermethylated. The previous research results in other tumors were similar to the results of this study. Because the mechanism by which MAGI2 inhibits tumorigenesis and development in various tumors is not exactly the same, its mechanism in gastric cancer remains unclear. However, the mechanism of action is similar to that of MAGI1. Studies have found that MAGI1 inhibits the expression of MMPs (particularly MMP7 and MMP9 expression) by blocking the MAPK/ERK signal transduction pathway, and changes the expression of typical EMT molecules (inhibiting N-cadherin expression and enhancing E-cadherin expression), which ultimately inhibit the migration and invasion of gastric cancer cells.[28,29] The results of this study provide a feasible way to further explore the mechanism by which MAGI2 inhibits the development of gastric cancer.

At the same time, the positive rate of SYNE1 methylation in gastric cancer tissue was slightly higher than that in adjacent tissue (26.6% vs 22.1%), but this result was not statistically significant ($P=.628$). Papadia et al[11] found that patients with positive methylation of the *SYNE1* gene are at a greater risk of developing dysplasia and colorectal cancer. Ling et al[30] found that *SYNE1* gene methylation was more frequent in high-risk precancerous lesions and carcinoma in situ, suggesting that *SYNE1* gene methylation is expected to become a more stable biomarker for the early diagnosis of colorectal cancer and high-risk adenoma. Tessem et al[14] found that the *SYNE1* gene shows abnormal methylation in lung cancer and may play a key role in promoting sporadic lung cancer. Almamun et al[16] found that the *SYNE1* gene shows abnormal methylation in acute lymphocytic leukemia, providing a direction for the selection of leukemia cells. The results of this study were somewhat different from previous study findings in other tumors. The cause for this result may be related to the sample size, regional differences, experimental methods, and specimen quality.

4.2. Correlation between the methylation status of SYNE1 and MAGI2 and clinical pathological factors

In this study, SYNE1 expression was related to age and tumor size. The positive rate of SYNE1 methylation was higher in those aged > 60 years and primary tumor lesions ≤ 5 cm, suggesting that age and the size of primary tumor lesions may affect the methylation status of the *SYNE1* gene and may be related to the proliferation of gastric cancer cells. Ling et al[30] found no clinical pathological factors related to positive SYNE1 methylation in the study of SYNE1 methylation in colorectal cancer, a finding that is inconsistent with our study. In the study by Mokarram et al,[31] all the colorectal cancer specimens tested showed positive results of SYNE1 methylation; thus, no clear differences were found in the clinical pathological factors. Considering that Ling et al[30] did not conduct a stratified study of age and tumor size, and that the clinical manifestations and pathological characteristics of gastric cancer and colorectal cancer were quite different, so the difference between our results and previous studies may be related to the characteristics of these 2 different tumors, stratification of clinical pathological factors, and experimental methods.

No statistically significant differences were found between the clinical pathological factors and the methylation status of MAGI2. Previous studies have explored the mechanism of action between MAGI2 methylation and the molecular biological mechanism of tumor cell lines to explore its role in tumorigenesis and development. Few studies were related to the correlation between the MAGI2 methylation status and clinicopathological factors. Therefore, the relationship between MAGI2 methylation and the clinicopathological factors of various malignant tumors remains unclear.

The relationship between the methylation of *SYNE1* and *MAGI2* genes and the clinicopathological factors of gastric cancer needs to be further verified after expanding the sample size. The relationship between the methylation of the *SYNE1* and *MAGI2* genes and clinical pathological factors of other malignant tumors also needs further research and exploration.

4.3. Relationship between SYNE1 and MAGI2 and the efficacy of chemotherapy

For patients with gastric cancer who have lost the opportunity for radical surgery or have recurrence or metastatic disease, it is generally believed that comprehensive treatment based on systemic chemotherapy should be considered the first choice. Fluorouracil, platinum taxane, anthracycline drugs, and irinotecan are the most commonly used chemotherapy drugs for

advanced gastric cancer. No clear and unified standard exists for the choice of chemotherapy. Due to the low efficiency of single drugs in the treatment of gastric cancer, 2 or 3 drugs are usually used in combination to inhibit the progression of gastric cancer.[32] The 5-FU based chemotherapy regimen occupies a dominant position in the treatment of gastric cancer in China. However, due to its serious adverse reactions and long administration time, it cannot be accepted by all patients in clinical use. Although adverse reactions may affect patient compliance and affect the efficacy, the overall chemotherapy efficacy of the drug is still the most concerning result of clinicians and patients. Therefore, it is important to predict the efficacy of chemotherapy to identify patients with poor chemotherapy, thus helping them to avoid of chemotherapy related adverse reactions.

Few studies have investigated the relationship between the methylation of SYNE1 and MAGI2 in gastric cancer and chemotherapy efficacy. This study found no connection between MAGI2 and the efficacy of chemotherapy. Because no previous study has verified this finding, this conclusion needs further confirmation. Different clinicopathological factors and chemotherapy schemes will also affect the final efficacy of chemotherapy. Thus, after stratifying different clinicopathological factors and chemotherapy schemes and other factors, the relationship between the methylation of SYNE1 and MAGI2 and chemotherapy efficacy would be analyzed again. This study found that, in 29 patients with recurrent disease, SYNE1 was related to the efficacy of chemotherapy for the first efficacy evaluation. SYNE1-negative patients had a higher chemotherapy efficiency than SYNE1-positive patients (47.6% vs 0%; $P = .027$). Overall, SYNE1-negative patients had more effective chemotherapy and more pronounced tumor regression. This result suggests that SYNE1-positive patients may have tumors that are more prone to migration and metastasis, which affects the efficacy of chemotherapy to a certain extent.

In the stratification of chemotherapy and other clinicopathological factors, no statistically significant differences were found between SYNE1 and MAGI2 methylation and chemotherapy efficacy. However, we found that, in patients with negative SYNE1 methylation, relapsed patients had higher chemotherapy efficiency than palliative patients. Among patients with positive SYNE1 methylation, patients with non-simple adenocarcinoma have a higher rate of chemotherapy efficiency than patients with simple adenocarcinoma. Among the patients with negative MAGI2 methylation, the number of lymph node metastases was lower than those with more lymph node metastases. Thus, under different methylation states, the relationship between the efficacy of chemotherapy and clinical pathological factors is not the same. This result suggests that different methylation states may affect the efficacy of chemotherapy.

4.4. Survival and prognosis

Of the 70 patients with advanced gastric cancer in this study, the median PFS was 4.6 months and the median OS was 10.3 months. Liu Yue's research classified the primary gastric cancer lesions according to the TNM stage, and found that MAGI2 and SYNE1 were closely related to the survival time of patients in the primary gastric cancer stage III group.

We found that the median PFS of MAGI2 methylation-negative patients was 5.1 months and that of positive patients was 4.1 months ($P = .012$), and the PFS of methylation-negative patients in the MAGI2 promoter region was relatively longer than that of methylation-positive patients ($P = .029$; HR = 2.619; 95% CI: 1.106–6.204). The median OS of MAGI2 methylation-negative and methylation-positive patients were 11.0 and 10.3 months, respectively. The median OS of negative patients was relatively longer, but with no statistically significant difference. This result suggests that negative MAGI2 methylation may have a better prognosis than the positive MAGI2 methylation. The median PFS of SYNE1 methylation-negative and positive patients were 4.6 and 4.0 months, respectively, and the median OS were 9.6 and 11.6 months, respectively. A difference was found in the survival time between negative and positive patients, but this difference was not statistically significant. This finding is inconsistent with that of Liu Yue's research results because the OS is affected by various factors, including clinicopathological factors, chemotherapy options, and patient compliance, which are important factors that affect survival.

5. Conclusion

MAGI2 is more methylated in gastric cancer than in adjacent tissues suggesting that hypermethylation changes in MAGI2 may be one of the mechanisms of tumorigenesis in gastric cancer. The methylation status of the SYNE1 and MAGI2 promoter regions may affect the chemotherapy efficacy of advanced gastric cancer. The prognosis of MAGI2-negative patients was better than that of positive ones, suggesting that MAGI2 may be an independent prognostic factor for PFS in patients with advanced gastric cancer.

Author contributions

Data curation: Na Gao.
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Methodology: Na Gao, Tao Wu.
Project administration: Tao Wu.
Software: Yanjun Qu.
Validation: Tao Wu.
Writing – original draft: Yanjun Qu.
Writing – review & editing: Yanjun Qu.

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