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High miR-718 Suppresses Phosphatase and Tensin Homolog (PTEN) Expression and Correlates to Unfavorable Prognosis in Gastric Cancer

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Background: Phosphatase and tensin homolog (PTEN) is a kind of phosphatase which has been demonstrated to suppress progression of gastric cancer. Many micro-RNAs (miRNAs), such as miR-106b, miR-93, and miR-200c, could inhibit expression of PTEN in cell lines; and many miRNAs including miR-21, miR-22, miR-18a, and miR-222 are related to the progression and prognosis of gastric cancer. However, among these miRNAs, the clinical significance of miR-718 has not yet been elucidated.

Material/Methods: The expression of PTEN and miR-718 in 141 gastric cancer tissues were detected by immunohistochemistry and quantitative real-time PCR respectively. The correlation between PTEN, miR-718, and the clinicopathological factors was analyzed by χ^2 test. The prognostic significance of PTEN and miR-718 was evaluated by univariate and multivariate analysis. Luciferase reporter assay was performed to evaluate the regulation of PTEN by miR-718. The effect of miR-718 on gastric cancer proliferation and invasion was investigated by MTT assay and Transwell assay.

Results: Low expression of PTEN and high expression of miR-718 were both significantly associated with unfavorable prognosis, and both were identified as biomarkers predicting poorer prognosis of patients with gastric cancer. Increased miR-718 expression could decrease PTEN expression, thus enhancing phosphatidylinositide 3-kinases/protein kinase B (PI3K/Akt) signaling. Moreover, the abilities of proliferation and invasion of gastric cells transfected with miR-718 were promoted significantly compared with those transfected with control miRNA.

Conclusions: Low expression of PTEN and increased expression of miR-718 in gastric cancer tissues were both independent unfavorable prognostic factors of gastric cancer. Upregulation of miR-718 could increase PI3K/Akt signaling by directly downregulating PTEN, thus promoting the proliferation and invasion of gastric cancer cells.

MeSH Keywords: MicroRNAs • Prognosis • PTEN Phosphohydrolase • Stomach Neoplasms

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Background

Gastric cancer is the fourth most common cancer in frequency and the third leading cause of cancer-related deaths worldwide, causing 723 100 deaths in 2012 [1]. Although the surgical equipment and adjuvant therapies have shown remarkably developed over the years, the overall 5-year survival rate of gastric cancer is still unfavorable, remaining lower than 30%, because most patients with gastric cancer are diagnosed at an advanced stage and the recurrence rate is very high [2,3]. In recent years, many predictive and prognostic biomarkers of gastric cancer have been discovered, resulting in the application of new drugs like Herceptin for use the adjuvant therapy. However, many gastric cancer patients are insensitive to chemotherapy and normally have a lower survival rate than average. Thus, further identification of risk factors and exploration for new drug targets are still urgently needed to control the prevalence and recurrence of gastric cancer.

MicroRNAs (miRNAs) are short (~22 nucleotide) non-protein-coding RNAs which regulate gene expression post-transcriptionally by translational repression or transcriptional degradation [4]. MiRNAs are involved in many cellular processes including apoptosis, differentiation, and proliferation. Ectopic miRNA levels are also observed in several diseases, especially in tumors [5]. Because of tissue-specific expression, miRNAs have great potential as cancer biomarkers, which has been demonstrated in numerous studies [6,7]. The aberrant expression of miRNAs in neoplasms has been observed, and the functions of miRNAs in cell lines has been verified by previous studies [8,9]. This feature of miRNAs as biomarkers could help guide individualized therapy for cancer patients. In gastric cancer, many miRNAs have been demonstrated to be correlated to the progression and prognosis of gastric cancer, including miR-21, miR-22, miR-18a, and miR-222 [10,11].

In gastric cancer, phosphatase and tensin homolog (PTEN) has been identified as a tumor suppressor that represses tumor proliferation and invasion [12]. PTEN protein is widely expressed in most gastric cancer cell lines and gastric cancer patients [12]. In normal stomach tissues, PTEN has also been detectable with immunohistochemistry (IHC). PTEN deficiency could lead to the gastric tumorigenesis and the progression of gastric cancer [13]. The expression of PTEN has been reported to be regulated by several kinds of miRNAs, including miR-718, miR-93, miR-200c, and miR-106b [14–17]. Among these miRNAs, miR-718 was a newly identified miRNA thought to modulate PI3K/Akt signaling by directly downregulating PTEN [14]. However, whether miR-718 could target PTEN in gastric cancer is still unknown.

In our study, we detected the level of miR-718 as well as the expression of PTEN in gastric tumor tissues, and we analyzed their correlation with the survival rate. With experiments *in vitro*, we further detected whether miR-718 targeted PTEN directly in gastric cancer cells, and we explored the influence of miR-718 on the proliferation and invasion of gastric cancer.

Material and Methods

Patients and follow-ups

From 2006 to 2012, a total of 424 patients underwent radical resection of gastric adenocarcinoma in Yishui Central Hospital and Qianfoshan Hospital. The final diagnosis of gastric adenocarcinoma was confirmed by routine pathology. Then 141 patients were further enrolled following the criteria: 1) available tissue samples and medical records, 2) available follow-ups more than 5 months after operation, and 3) no severe perioperative complications and other tumors. Another 12 pairs of fresh tumor tissue and adjacent tissue were collected for the detection of PTEN mRNA level. All the samples were obtained with prior patients' consents, and the approval of the Institutional Clinical Ethics Review Board of Yishui Central Hospital and Qianfoshan Hospital. The study was supervised by the Ethics Committee of Yishui Central Hospital and Qianfoshan Hospital. The tumor TNM stage was confirmed according to the guideline of 7th American Joint Committee on Cancer/Union for International Cancer Control.

Immunohistochemistry and evaluation

Sections of formalin-fixed, paraffin-embedded gastric cancer tissues were immunohistochemically (IHC) stained to evaluate the PTEN expression as previous described [18,19]. Briefly, the formalin-fixed and paraffin-embedded specimens were deparaffinized and rehydrated in graded ethanol. Optimal antigen retrieval was performed by heating in citrate buffer (pH = 6.0), and endogenous peroxidase activity was blocked by incubation with 3% hydrogen peroxide. After blocking unspecific antigen binding with 5% fetal bovine serum, the primary antibody of PTEN (#9559, Cell Signaling Technology, MA, USA) at a dilution of 1: 200 was used to soak the slides overnight. After rinsing the slides with phosphate buffered saline, the secondary antibody labeled by biotin was used to incubate the specimens. The antigens' visualization was realized by the incubation with 3, 3'-diaminobenzidine substrates.

The evaluation of IHC results of PTEN was carried out by 2 independent pathologists unaware of the clinical data of the patients. The score system of IHC results was performed as previous described [20,21]. IHC score consisted of 2 parts: the staining intensity and the positive cell number. The score for staining intensity was defined as: 0 for no staining, 1 for weak staining, 2 for moderate staining, and 3 for strong staining.

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The score for the positive cell percentage was defined as: 0 for no positively stained cells, 1 for <25% positively stained cells, 2 for 25–50% positively stained cells, 3 for 50–75% positively stained cells, and 4 for more than 75% positively stained cells. The final IHC score was the product of the score for staining intensity multiplied by the score for the positive cell percentage, ranging from 0 to 12. The cohort was divided into PTEN high-expression and low-expression group according the cut-off of IHC score, which was set as the point with the highest specificity and sensitivity in the receiver operating characteristic curve (ROC) curve.

RNA extraction and quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was used to detect the RNA levels of miR-718 and PTEN as previously reported [22]. Total RNA was first extracted from gastric tissues or adjacent tissues with the agent TRIzol (Invitrogen, Carlsbad, USA) according to the manual, and dissolved in 10 μL DEPC-treated water. For the detection of PTEN mRNA, the complementary DNA (cDNA) synthesis and quantitative PCR were conducted with SYBR Green Master Mix and StepOnePlus system (Applied Biosystem, Waltham, MA, USA) according to the manual. The level of GAPDH was set as the internal control for PTEN mRNA detection. The sequences of primers for qRT-PCR were as follows: PTEN: forward 5'-GCTATGGGATTTCCTGCAGAA-3', reverse 5'-GGCGGTGTCATAATGTCTTTCA-3'; GAPDH: forward 5'-GGATTTGGTCGTATTGGG-3', reverse 5'-GTGGCTGGGG CTCTACTTC-3'. For the detection of mature miR-718 levels, RNA was reverse-transcribed with miRNA-specific primers and a TaqMan microRNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). The small noncoding RNA U6 was used as the internal control for miR-718. The ROC curve was used to set the cutoff of miR-718 to divide the cohort into high-level or low-level miR-718 group.

Cells and reagents

Gastric adenocarcinoma cell line MKN-7 was purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and cultured in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum, and 100 U/mL penicillin and 100 μg/mL streptomycin (Thermo Fisher Scientific Waltham, MA, USA) in 5% CO_2 resuscitation. All antibodies without special instruction were purchased from Cell Signaling Technology (MA, USA). All reagents without special instruction were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Lipofectamine 2000 was purchased from Invitrogen (Carlsbad, CA, USA).

Western blotting

Cells were scraped with phosphate buffered saline and lysed with ice-cold RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitor cocktail (Roche, Welwyn Garden City, UK). The lysates were centrifuged at 10 000×g for 15 minutes at 4°C and the precipitate was discarded. Equal amount protein (about 10 μg) was electrophoresed in sodium dodecyl sulfate-polyacrylamide gel and then the proteins in gel were transferred to a polyvinylidene difluoride membrane (Merck Millipore, Kenilworth, USA). The primary antibodies including anti-PTEN, anti-AKT, anti-p-Ser473-AKT and anti-β-actin, as well as the corresponding secondary antibodies were used to incubate the membrane, respectively. The bands were finally visualized with the use of an ECL substrate kit (Thermo Scientific, Waltham, MA, USA).

MTT assay

MTT assay was used to evaluate the proliferation of gastric adenocarcinoma cells as described be a previous study [23]. Briefly, cells with or without miR-718 transfection were passaged into 96-well plates at a density about 4000 cells per well. After starvation for 6 hours, the test subgroups had added 100 ng/mL epidermal growth factor (EGF), while the control group instead had added normal medium. Finally, 10 μL MTT at the concentration of 5 mg/mL was added to terminate the reaction and 100 μL DMSO was used to dissolve the crystal. The optical density (OD) at 570 nm was measured using a spectrophotometer (Molecular Devices Company, USA). The proliferation ratio of other groups was the ratio of OD570 to the baseline.

Matrigel invasion assay

The invasion ability of gastric cancer cells was evaluated by the Matrigel Transwell assay [24]. MKN7 cells about 10^5 with or without miR-718 transfection were seeded into 8-μm-core Matrigel-precoated Transwell chamber (BD Biosciences Company, Franklin Lakes, NJ, USA) and incubated for 24 hours with 10% fetal bovine serum and 100 ng/mL EGF as chemo-attractants in the lower compartment. The invaded cells in the lower surface were stained with crystal violet. The presented data was the average of triplicate experiments.

Statistical analysis

All data in the study were analyzed with software SPSS 21 (SPSS Inc., Chicago, IL, USA). The correlation between PTEN expression and clinicopathological factors was analyzed by χ^2 test. Student's *t*-test was used to analyze the differences in demographic factors between cases and controls. ROC curve was used to determine the cutoff of miR-718 and IHC score of PTEN. The overall survival curve was displayed by Kaplan-Meier

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method and the statistical significance between subgroups was compared by the log-rank test. The independent prognostic factors were identified with the Cox regression proportional hazards model. A value of $P<0.05$ was considered statistically significant.

Results

Level of PTEN and miR-718 in gastric cancer and adjacent tissues

The level of PTEN in gastric cancer was detected by IHC and the miR-718 level was investigated by qRT-PCR. With the cut-off of IHC score and qRT-PCR score respectively, our cohort was divided into high- and low-expression PTEN subgroups or high- and low-level miR-718 subgroups. In our study, the percentage of high-expression PTEN and high-level miR-718 were 63.1% (89 out of 141) and 43.3% (61 out of 141), respectively. The expression of PTEN was mainly observed in the cell cytoplasm in our experiments (Figure 1A, 1B).

Besides the detection of PTEN expression with IHC, we detected the mRNA level of PTEN in a prospective cohort of 12 fresh pairs of gastric cancers and their adjacent tissues with qRT-PCR to quantify the correlation between PTEN and miR-718. The data for the prospective cohort of 141 cases and the prospective cohort with 12 patients are displayed in Supplementary Table 1. In our study, the PTEN mRNA in adjacent tissues was significantly higher compared with that in gastric cancers, suggesting a role for PTEN as a tumor suppressor (Figure 1C). On the contrary, miR-718 levels in adjacent tissues were remarkably lower than that in gastric cancers. This indicated that miR-718 may play an oncogenic role in gastric cancer tumor genesis and progression (Figure 1D).

Correlation between PTEN, miR-718 and clinicopathological features

After dividing the cohort into high- and low-expression PTEN subgroups and high- and low-level miR-718 subgroups, we further analyzed the correlations between expression of PTEN, level of miR-718, and other clinicopathological factors including patients' age, sex, tumor size, tumor infiltration (T stage), lymphatic invasion (N stage), metastasis status (M stage), TNM stage, and tumor cell differentiation (Table 1). With χ^2 test, we demonstrated that the expression of PTEN was significantly associated with miR-718 level. Patients with higher level of miR-718 appeared to have lower expression of PTEN, indicating that miR-718 could inhibit PTEN expression in gastric cancer. No other substantial correlations between PTEN, miR-718, with clinicopathological factors were observed in our study.

Prognostic value of PTEN and miR-718

PTEN has been confirmed as a tumor suppressor in gastric cancer in previous studies. In our study, we verified the prognostic significance of PTEN and evaluated the prognostic value of miR-718 simultaneously. The correlation between PTEN, miR-718, and the 5-year overall survival time was analyzed by the univariate analysis using Kaplan-Meier method (Table 2). The T stage, N stage, M stage, TNM stage and tumor differentiation, were all proven to be significantly associated with the 5-year overall survival rate in our study, which was similar to previous study finding (Figure 2A–2E). In our study, PTEN was also identified as a tumor suppressor of gastric cancer. High expression of PTEN led to the favorable prognosis of patients with gastric cancer ($P=0.005$) (Figure 2F). On the contrary, high level of miR-718 was significantly associated with the unfavorable prognosis ($P=0.008$) (Figure 2G).

The multivariate analysis was further performed to identify the independent prognostic factors in the survival of patients with gastric cancer (Table 3). All factors confirmed to be related to the survival rate were enrolled into the Cox-regression hazard model, including the T stage, N stage, M stage, tumor differentiation, PTEN expression and miR-718 level. The TNM stage was naturally excluded because of the known interaction with T stage, N stage and M stage. In our test, all enrolled factors were proven to be the independent prognostic factors of gastric cancer. Although PTEN expression and miR-718 were demonstrated to be significantly correlated, they were both independent prognostic factors of gastric cancer. High expression of PTEN ($P=0.004$, hazard ratio (HR)=0.41, 95% CI=0.22–0.75) alone could predict the favorable prognosis, while the high level of miR-718 ($P=0.018$, HR=2.14, 95% CI=1.14–3.99) was a high-risk biomarker of poorer prognosis to patients with gastric cancer.

miR-718 could promote gastric cell proliferation and invasion by targeting PTEN

In the clinical analyses, we observed that a high level of miR-718 was significantly associated with low PTEN expression in gastric cancer tissues, so we further performed function assays to evaluate the role of miRNA in tumor progression. PTEN is a well-known suppressor to PI3K-AKT signaling pathway, therefore, we further explored the effect of miR-718 on regulating PI3K-AKT signaling (Figure 3A). As a generally acknowledged stimulator of PI3K-AKT signaling, EGF was applied in our study to activate PI3K signaling. In our study, miR-718 could substantially decrease the expression of PTEN, thus increasing the phosphorylation of Ser473 of AKT under stimulation of EGF. These results suggested that miR-718 could facilitate the activation of PI3K-AKT signaling by degrading PTEN. To detect the influence of miR-718 on tumor cell function, we

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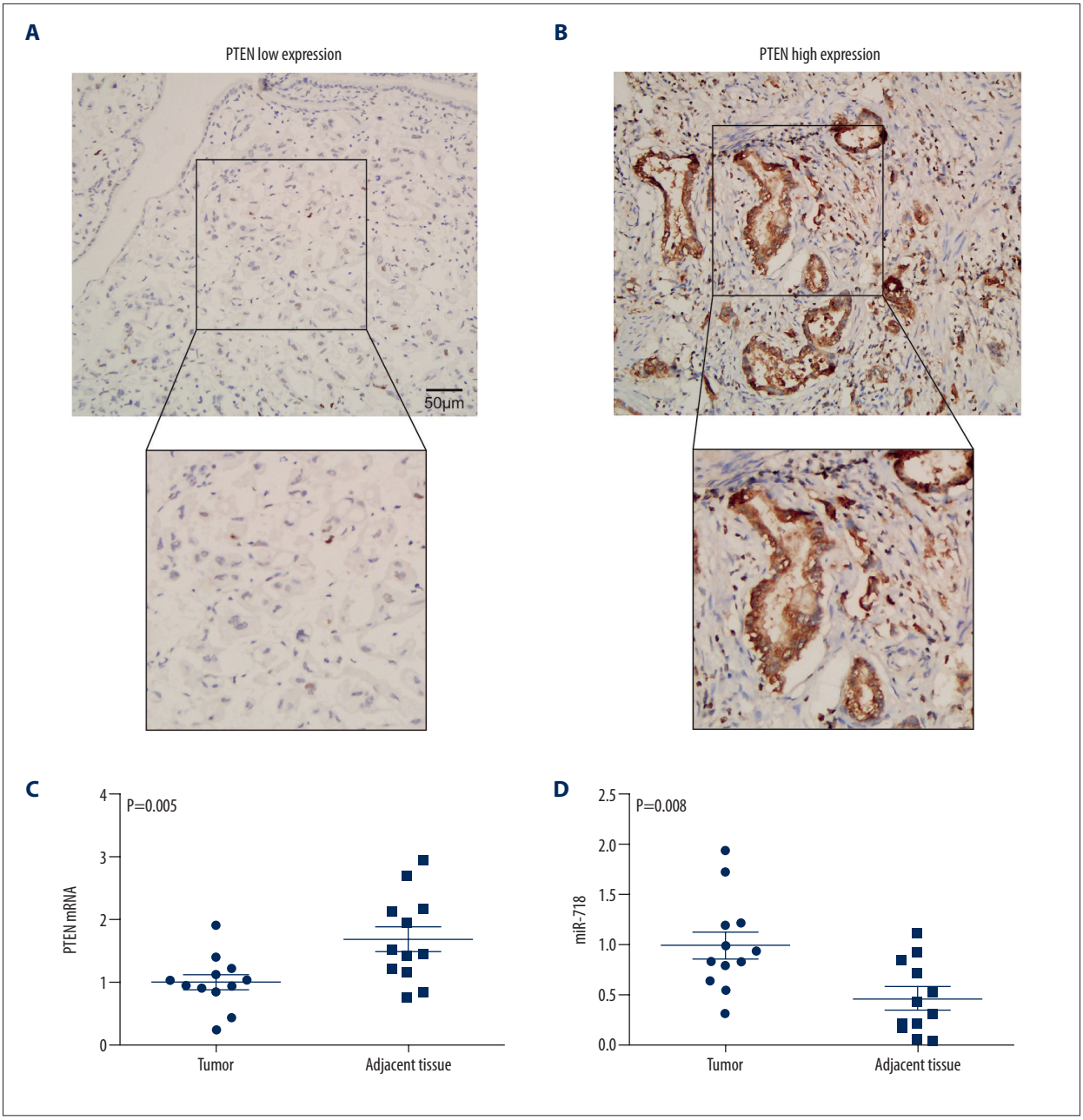

Figure 1. The representative images of low expression and high expression of PTEN. (**A**) The representative image and magnified image for PTEN low expression. The score of staining intensity is 1 and the score of positive cells is 1. Total IHC score is 1 and defined as the low-expression of PTEN. The relative ratio of miR-718 in this case was 2.43. (**B**) The representative image and magnified image for PTEN high expression. The score of staining intensity is 3 and the score of positive cells is 4. Total IHC score is 12 and defined as the low-expression of PTEN. The relative ratio of miR-718 in this case was 0.83. (**C, D**). The level of PTEN mRNA (**C**) and miR-718 (**D**) in the 12 pairs of fresh gastric cancers and their adjacent tissues was detected with qRT-PCR. The PTEN mRNA in adjacent tissues was significantly higher compared gastric cancers, while miR-718 level in adjacent tissues was remarkably lower than gastric cancers.

performed MTT assay and Transwell assay to evaluate the proliferation and invasion of gastric cancer cell. In MTT assay, MKN7 cells with miR-718 treatment exhibited an accelerated proliferation compared to the cells without miR-718 treatment (Figure 3B). Similar results were also observed in Transwell assay. Cells with miR-718 treatment had more aggressive ability of invasion compared with those without miR-718 treatment (Figure 3C). These results indicated that miR-718

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Table 1. Correlations between clinicopathologic factors and PTEN or miR-718.

Characters	PTEN			P*	miR-718		P*
	Number	Low (52)	High (89)		Low (80)	High (61)	
Sex							
Male	102	37	65	0.847	61	41	0.258
Female	39	15	24		19	20	
Age							
<60	59	24	35	0.481	30	29	0.301
≥60	82	28	54		50	32	
Tumor diameter (cm)							
≤5	62	24	38	0.727	32	30	0.307
>5	79	28	51		48	31	
Differentiation							
Well+moderate	78	33	45	0.162	41	37	0.307
Poor	63	19	44		39	24	
Tumor invasion							
T1+T2	37	10	27	0.169	25	12	0.176
T3+T4	104	42	62		55	49	
Lymph node metastasis							
No (N0)	54	20	34	1.000	36	18	0.080
Yes (N1/2/3)	87	32	55		44	43	
Distant metastasis							
M0	130	49	81	0.746	72	58	0.350
M1	11	3	8		8	3	
TNM stage							
I–II	64	22	42	0.603	37	27	0.865
III–IV	77	30	47		43	34	
PTEN							
Low	52				21	31	0.005
High	89				59	30	
miR-718							
Low	80	21	59	0.005			
High	61	31	30				

* Means calculated by χ^2 test. PTEN – phosphatase and tensin homolog.

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Table 2. Univariate analysis.

Characters	5-year survival rate	P*	Characters	5-year survival rate	P*
Sex			Distant metastasis		
Male	47.4	0.129	M0	50.2	**0.018**
Female	50.1		M1	36.4	
Age			TNM stage		
<60	43.9	0.856	I–II	54.5	**0.043**
≥60	52.6		III–IV	41.2	
Tumor diameter (cm)			Differentiation		
≤5	41.0	0.208	Well+moderate	54.1	**0.017**
>5	58.6		Poor	42.2	
Tumor invasion			PTEN		
T1+T2	79.0	**0.014**	Low	34.0	**0.005**
T3+T4	38.6		High	59.5	
N stage			miR-718		
No (N0)	59.0	**0.021**	Low	65.5	**0.008**
Yes (N1/2/3)	35.3		High	27.3	

* Means calculated by log-rank test. PTEN – phosphatase and tensin homolog.

in gastric cancer cells could promote the proliferation and invasion processes.

Discussion

Gastric cancer is still a major public health problem and a medical financial burden worldwide. The discovery of new biomarkers would help broaden the insights of gastric cancer progression and help find effective drug targets. Elucidation of mechanisms underlying how human epidermal growth factor receptor-2 (HER2) led to poor prognosis of gastric cancer directly resulted in the application of trastuzumab in the treatment of gastric cancer and improved patient survival time. Similarly, the discovery of miRNAs could expand the understanding of carcinogenesis and progression of gastric cancer. Recently, many miRNAs have been demonstrated to be associated with the progression and outcome of gastric cancer, including miR-206, miR-421, and miR-22 [25–27]. Chang et al. revealed that many potential miRNAs were associated with the outcome of gastric cancer patients following chemotherapy using a high-throughput miRNA microarray analysis [28]. Accumulating evidence shows that miRNAs are effectively predictive and prognostic biomarkers in gastric cancer [29,30]. MiRNAs have the potency to predict prognosis and guide the treatment. Some miRNAs could help predict sensitivity to chemotherapy and help find effective chemo-drugs, and some miRNAs could help predict the prognosis of patients [31]. Exploration of more prognostic miRNAs in gastric cancer could help distinguish the patients with higher risk for more potential adjuvant therapy, thus defining appropriate treatment options for patients.

As a dual-specificity protein phosphatase, PTEN is a well-acknowledged tumor suppressor in many kinds of cancers [32,33]. The tumor suppressor role of PTEN was also elucidated in several previous studies. PTEN was involved in many processes in gastric cancer, including tumor genesis, progression, chemical drug sensitivity, and prognosis [12,34–36]. Since PTEN could suppress PI3K-AKT signaling, the loss of PTEN function resulting from gene mutation or deletion, would increase phosphatidylinositol 3-phosphate (PIP3), which is the product of the PI3K pathway [33]. The accumulation of PIP3 could activate a signaling cascade leading to the activation and phosphorylation of AKT and the downstream proteins, by which PTEN is involved in many cellular processes such as cell survival, proliferation, and invasion [37]. The mRNA of PTEN is featured with its large 3'UTR (about 3.3kbp), which could be targeted by several miRNAs including miR-21, miR-214, miR-216a, miR-217, and miR-26a [38–42]. Recently, miR-718 was reported to repress the pro-inflammatory cytokine production through

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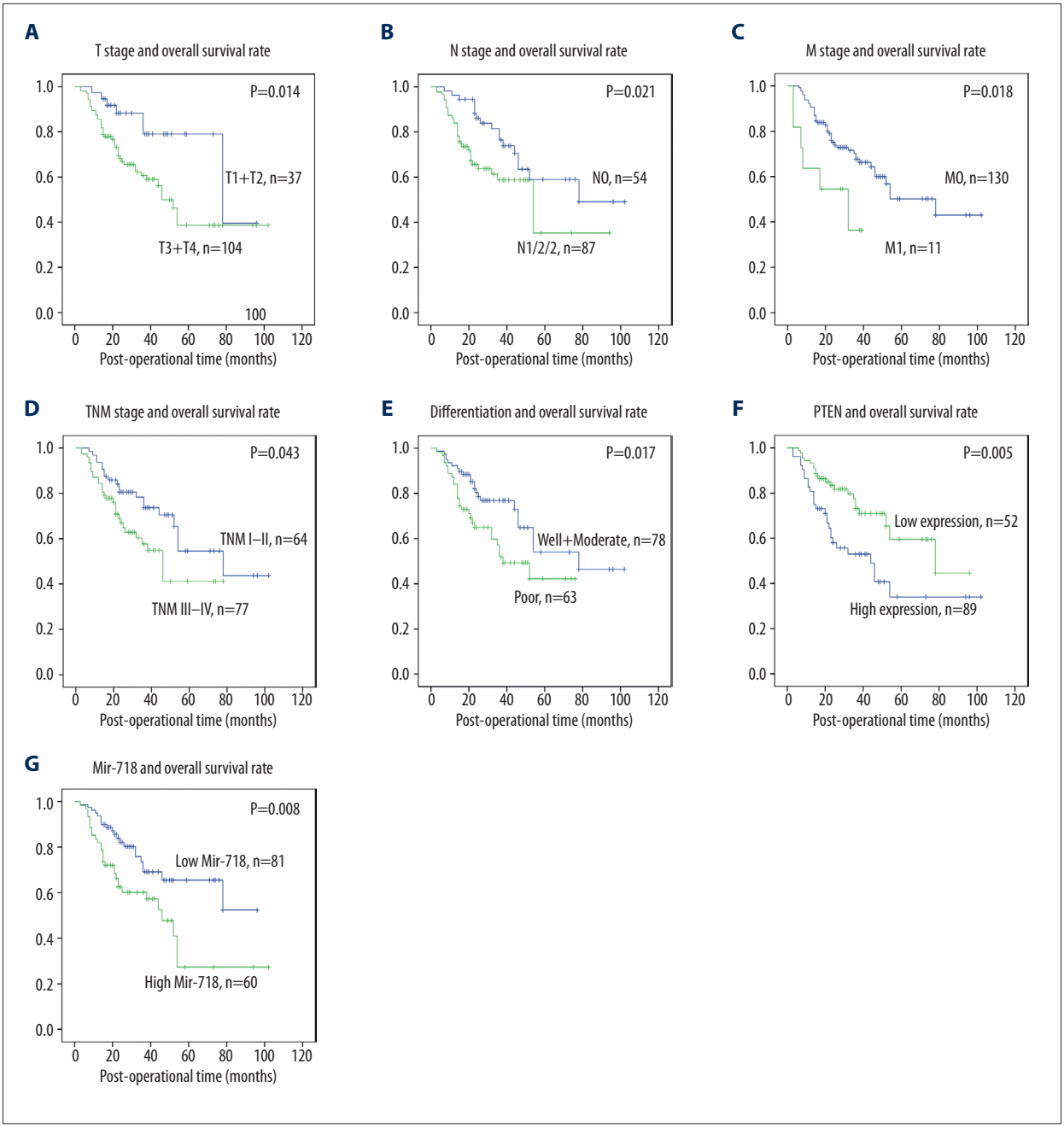

Figure 2. The survival curves of clinicopathological factors, PTEN and miR-718. (**A**) The survival curves of group T1+T2 and group T3+T4. (**B**) The survival curves of group N0 and group N1/2/3. (**C**) The survival curves of group M0 and group M1. (**D**) The survival curves of group TNM I+II and group TNM III+IV. (**E**). The survival curves of poor differentiation and good/moderate differentiation. (**F**) The survival curves of high-expression and low-expression of PTEN. (**G**) The survival curves of high level and low level of miR-718.

targeting PTEN, and it has been suggested that miR-718 could function as an oncogene [14]. In our study, we demonstrated that miR-718 could promote gastric cell proliferation and invasion via targeting PTEN mRNA, and that high level miR-718 was an independent prognostic risk factor for a poorer prognosis of gastric cancer. These results add to the study on gastric cancer progression and treatment and could help stratify patients more preciously for individual treatment.

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Table 3. Multivariate analysis.

Characters	HR	95%CI	P*
Tumor invasion			
T1+T2	1		
T3+T4	2.79	1.23–6.32	**0.014**
Lymph node invasion			
No (N0)	1		
Yes (N1/2/3)	1.94	1.06–3.55	**0.031**
Distant metastasis			
M0	1		
M1	5.18	2.04–13.14	**0.001**

Characters	HR	95%CI	P*
Differentiation			
Well+Moderate	1		
Poor	3.38	1.80–6.37	**P<0.001**
PTEN			
Low	1		
High	0.41	0.22–0.75	**0.004**
miR-718			
Low	1		
High	2.13	1.14–3.99	**0.018**

* Means calculated by Cox-regression model. PTEN – phosphatase and tensin homolog.

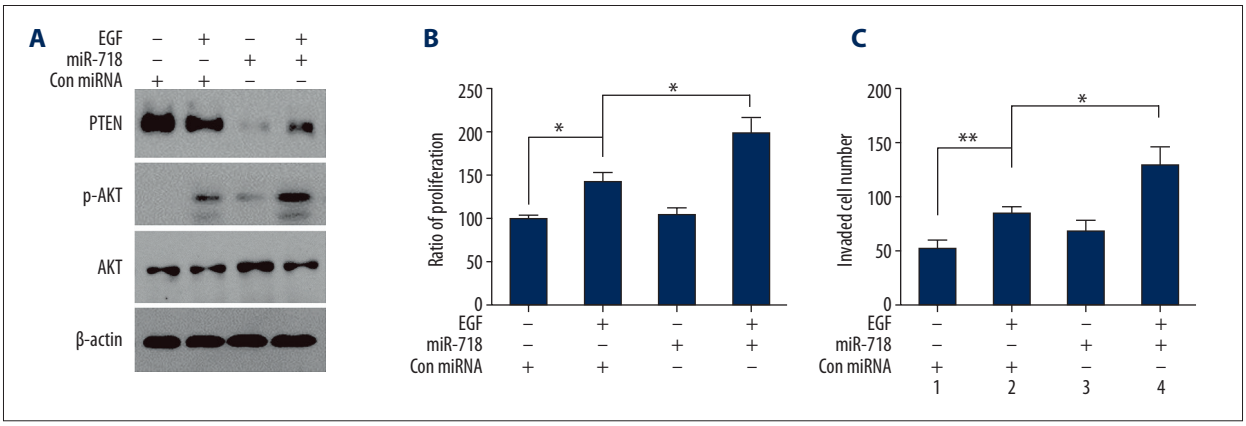

Figure 3. miR-718 could activate PI3K/Akt signaling by targeting PTEN. (**A**) The transcription of PTEN in gastric cell line was suppressed by miR-718. The normalized firefly luciferase activity of PTEN was detected 48 hours after the transfection with the PTEN-3'UTR-Luc reporter construct with the absence of miR-718 expression vector or the scrambled control miRNA vector in gastric cancer cell MKN7. Data were from 3 independent experiments and displayed by the mean ±SEM. (**B**) PI3K/Akt signaling in MKN7 could be activated by miR-718. The effect of miR-718 on Akt signaling of MKN7 cells was evaluated by western blotting. EGF at 100 ng/mL was used to incubate the cells for 10 minutes, 48 hours after transfection of expression vector of miR-718 or scrambled control miRNA. Cells were then lysed, and western blotting was performed to detect the levels of PTEN, phosphor-Ser473-Akt, total Akt, and β-actin. (**C**) miR-718 could accelerate the proliferation of MKN7. The MTT assay was used to detect the proliferation index of MKN7 48 hours after transfection of expression vector of miR-718 or scrambled control miRNA. Data were from 3 independent experiments and displayed by the mean ±SEM. (**D**) miR-718 could enhance the invasion of MKN7; 48 hours after transfection of expression vector of miR-718 or scrambled control miRNA, MKN7 was seeded into the Matrigel-precoated chamber and incubated for 24 hours with 10% fetal bovine serum and 100 ng/mL EGF as a chemoattractant. Cells at the bottom compartment were fixed and stained with crystal violet. Invaded cells were counted in at least 8 random visual fields. Data were from 3 independent experiments and displayed by the mean ±SEM. (**E**) Representative images of invaded cells in D. Image 1, 2, 3, 4 represent the groups with/without EGF stimulation or with/without miR-718 transfection.

Conclusions

We demonstrated that expression of PTEN and miR-718 were significantly correlated in patients with gastric cancer. Low expression of PTEN and high level of miR-718 were notably associated with a lower 5-year overall survival rate. Both PTEN and miR-718 were identified as prognostic factors of gastric cancer. With experiments *in vitro*, we proved that miR-718 could increase PI3K/Akt signaling by directly downregulating PTEN, thus promoting the proliferation and invasion of gastric cancer cells.

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Conflicts of interest

None.

Supplementary Table

Supplementary Table 1. Basic information of the retrospective cohort and prospective cohort.

Characters	Retrospective cohort		Prospective cohort	
	Number	Percentage	Number	Percentage
Sex				
Male	102	72.3%	9	75.0%
Female	39	27.7%	3	25.0%
Age				
<60	59	41.8%	3	25.0%
≥60	82	58.2%	9	75.0%
Tumor diameter (cm)				
≤5	62	44.0%	5	41.7%
>5	79	56.0%	7	58.3%
Differentiation				
Poor	78	55.3%	6	50.0%
Well+moderate	63	44.7%	6	50.0%
Tumor invasion				
T1+T2	37	26.2%	5	41.7%
T3+T4	104	73.8%	7	58.3%
Lymph node metastasis				
No (N0)	54	38.3%	6	50.0%
Yes (N1/2/3)	87	61.7%	6	50.0%
Distant metastasis				
M0	130	92.2%	11	91.7%
M1	11	7.8%	1	8.3%
TNM stage				
I–II	64	45.4%	5	41.7%
III–IV	77	54.6%	7	58.3%

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