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Original Article

Decreased expression of miR-378 is associated with local invasion, lymph node metastasis and poor prognosis in gastric cancer

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Abstract: MicroRNAs (miRNAs) play important roles in various cancers, are associated with tumor genesis, progression and prognosis. MiR-378 has important roles in inhibiting gastric cancer cell proliferation, migration and invasion. However, the relationships between miR-378 expression and clinicopathological features, and prognosis are not yet investigated. In this study, we investigated the expression of miR-378 in 87 pairs of primary gastric cancer and their corresponding nontumorous tissues, and analyzed its clinical significance. The study showed that miR-378 was frequently downregulated in gastric cancer, and the reduced expression of miR-378 was associated with increased local tumor invasion and advanced stages. Patients with lymph node metastasis displayed a lower miR-378 expression than gastric cancer without lymph node metastasis. Univariate analysis identified low expression of miR-378 as a poor prognostic factor. These results suggest that miR-378 has a potential to serve as a clinical biomarker for predicting disease invasiveness and prognosis of patients with gastric cancer.

Keywords: Gastric cancer, mir-378, invasion, lymph node metastasis, prognosis

Introduction

Gastric cancer is one of the most common malignant tumors in China. Although multidisciplinary approaches have been applied to gastric cancer therapy, the long-term survival for these patients remains poor [1]. MicroRNAs (miRNAs) are a class of small non-coding RNA molecules, cumulative evidence suggests that miRNAs play important roles in gastric cancer by regulating gene expression or acting as diagnostic and prognostic biomarkers [2-4]. It has been reported that miR-378 is downregulated in gastric cancer and inhibits gastric cancer cell proliferation, migration and invasion [5-7]. However, the relationships between miR-378 expression and clinicopathological features, and prognosis are not yet well known. In this study, we examined the miR-378 expression in 87 pairs of gastric cancer and matched nontumorous tissues, found that miR-378 was significantly down-regulated in gastric cancer, and decreased expression of miR-378 was associated with local invasion, lymph node metastasis, advanced stage and poor survival.

Materials and methods

Patients and tissue samples

Gastric cancer and their corresponding nontumorous tissues were obtained from 87 patients with gastric adenocarcinoma who underwent surgery between 2008 and 2009 at the Department of Gastric Cancer and Soft Tissue Sarcomas, Shanghai Cancer Center of Fudan University. Written informed consent was obtained from all patients, and research protocols were approved by the Clinical Research Ethics Committee of Fudan University Shanghai Cancer Center.

The overall survival was defined as the time from surgery to death. The date of the recent follow-up was the July 2015, 52 of the 87 patients died during the follow-up period.

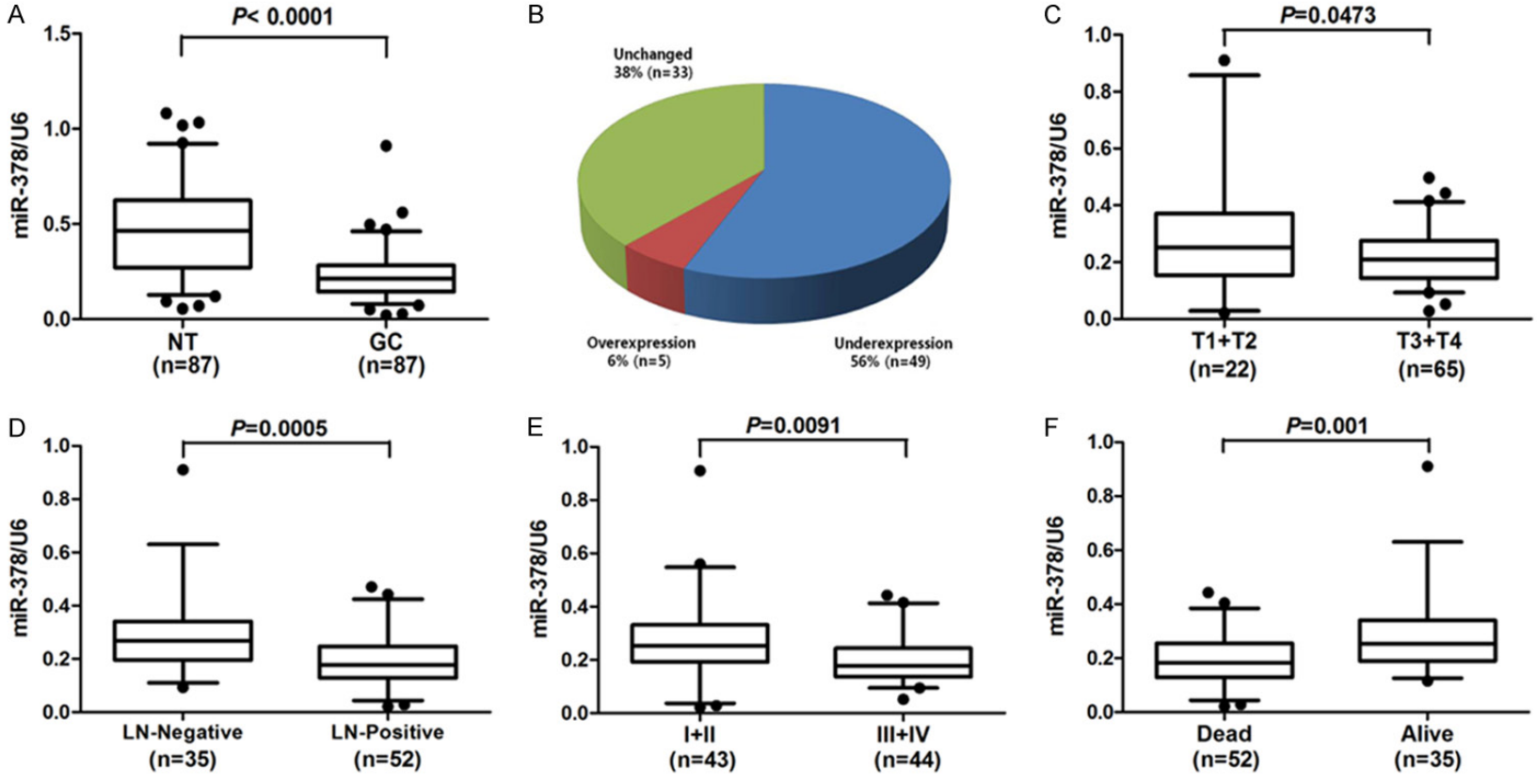

Figure 1. The expression and clinical significance of miR-378 in gastric cancer. A. The expression of miR-378 in 87 pairs of primary gastric cancer (GC) and their corresponding nontumorous tissues (NT) by TaqMan real-time PCR, U6 RNA as an endogenous control. B. miR-378 was frequently downregulated in gastric cancer, the fold change of relative miR-378 expression (GC/NT) >2 or <1/2 was considered as overexpression or underexpression. C. miR-378 expression in T1+T2 and T3+T4 groups according to the depth of tumor invasion. D. miR-378 expression in the lymph node (LN)-metastasis negative group and positive group. E. miR-378 expression in stage I+II group and stage III+IV group, patients were staged in accordance with the 7th Edition of the American Joint Committee on Cancer TNM Classification. F. Downregulation of miR-378 was associated with prognosis.

Table 1. The correlations of miR-378 expression levels with clinicopathologic features in gastric cancer

Clinicopathologic features	Cases	Median expression of miR-378	*P*
Age, years			
≤60	50	0.2365±0.02119	0.7908
>60	37	0.2289±0.01639	
Gender			
Male	64	0.2253±0.01773	0.3436
Female	23	0.2555±0.01866	
Diameter, cm			
≤5	57	0.2335±0.01446	0.9810
>5	30	0.2328±0.03017	
Location			
Distal third	55	0.2464±0.01882	0.4075
Middle third	11	0.2282±0.03162	
Proximal third	21	0.2016±0.02521	
Differentiation			
Poor	44	0.2155±0.01549	0.2001
Well and moderate	43	0.2514±0.02326	
Local invasion			
T1+T2	22	0.2808±0.04117	0.0473
T3+T4	65	0.2172±0.01211	
Lymph node metastasis			
No	35	0.2908±0.02535	0.0005
Yes	52	0.1945±0.01374	
TNM stage			
I+II	43	0.2697±0.02362	0.0091
III+IV	44	0.1977±0.01339	
Prognosis			
Dead	52	0.1965±0.01321	0.0010
Alive	35	0.2879±0.02628	

RNA extraction and quantitative real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, CA, USA). TaqMan miRNA assays (Applied Biosystems, CA, USA) were used to detect the expression level of mature miR-378 according to the provided protocol, and the small nuclear RNA U6 was selected as an internal control.

Statistical analysis

Data were presented as mean ± SEM, comparisons were analyzed by Student *t* test for two groups or ANOVA for more than two groups. Survival curves were estimated by the Kaplan-Meier method using the SPSS software (version 20), the Cox proportional hazards model was applied for univariate and multivariate analyses. *P* values <0.05 were considered statistically significant.

Results

Expression and clinical significance of miR-378 in gastric cancer

It has been reported that miR-378 is downregulated in gastric cancer [5, 7], however, the relationship between miR-378 expression and clinicopathological features is not yet well known. In this study, we firstly detected the expression of miR-378 in 87 pairs of primary gastric cancer and their corresponding nontumorous tissues by TaqMan real-time PCR, the results shown that miR-378 expression in gastric cancer was significantly suppressed (**Figure 1A**, *P*<0.0001), 56% (49/87) of the gastric cancer had at least 2-fold reduced expression of miR-378, while only 6% (5/87) of the gastric cancer were overexpressed more than 2 folds compared with their corresponding nontumorous tissues (**Figure 1B**), suggesting downregulation of miR-378 is a frequent event in gastric cancer. Moreover, gastric cancer with lymph node metastasis displayed a lower miR-378 expression than gastric cancer without lymph node metastasis (**Figure 1D**), its expression was decreased with the elevation of the depth of tumor invasion (**Figure 1C**) and stages (**Figure 1E**). We also found that miR-378 was associated with prognosis of patients with gastric cancer, patients who were alive had a higher expression of miR-378 (**Figure 1F**). No significant correlations between miR-378 expression and age, gender, tumor size, location, and differentiation were observed (**Table 1**).

Decreased expression of miR-378 is associated with poor overall survival of patients with gastric cancer

To determine the prognostic value of the decreased miR-378 in gastric cancer, the 87 patients with gastric cancer were followed up, the survival time ranged from 3 to 89 months, 52 of the 87 patients died during follow-up. The Kaplan-Meier survival analysis revealed that deeper invasion (T3+T4), lymph node metasta-

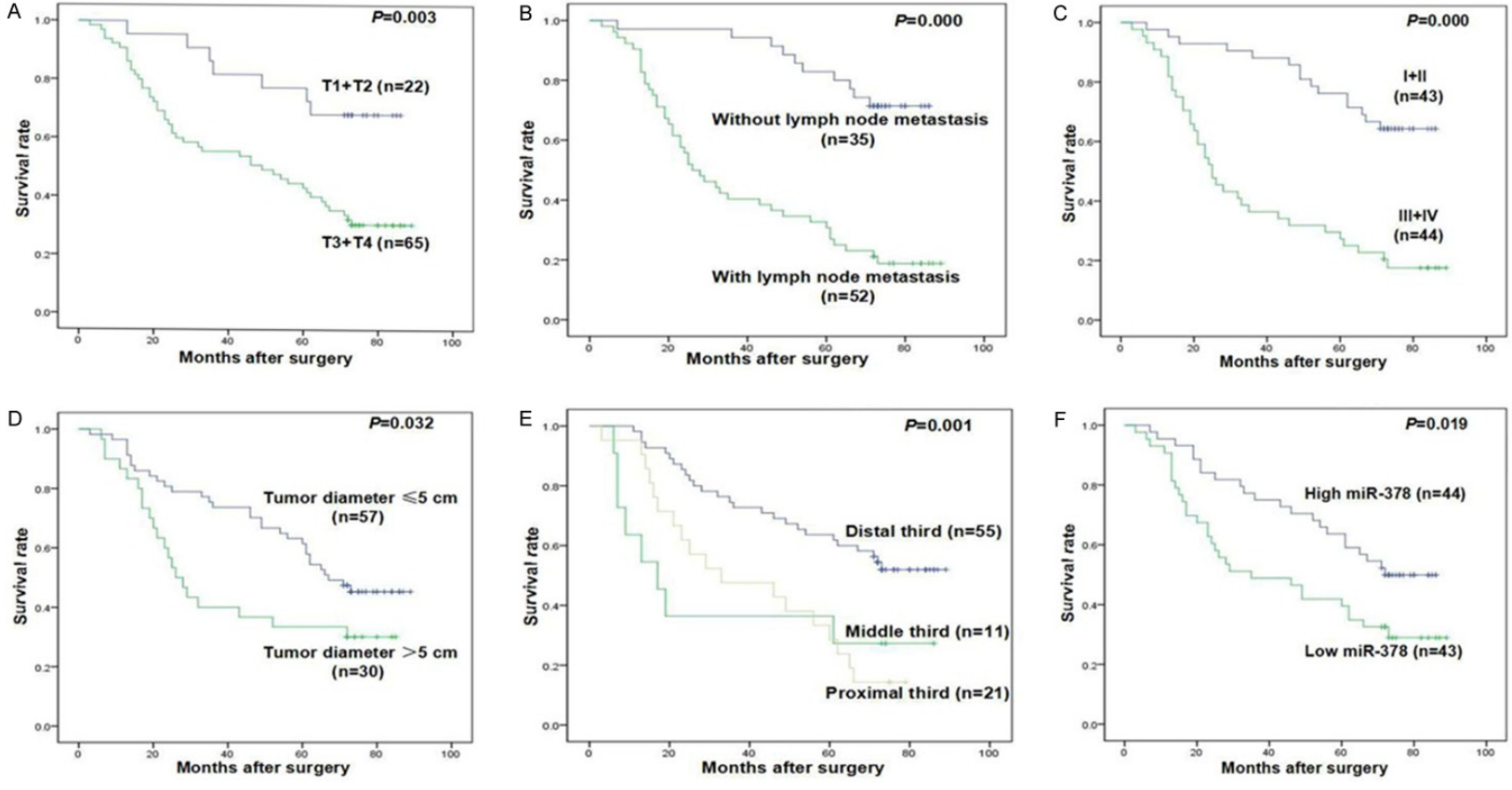

Figure 2. Kaplan-Meier curves of prognostic factors for overall survival. The relationships between depth of tumor invasion (A), lymph node metastasis (B), stage (C), tumor diameter (D), location (E), and miR-378 expression (F) and survival rate after operation were analyzed. The patients were classified into high miR-378 group and low miR-378 group according to the relative levels of miR-378.

Table 2. Univariate and multivariate analyses of prognostic factors for survival in patients with gastric cancer

Clinicopathologic features	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age	1.322	0.766-2.283	0.316			
Gender	1.001	0.542-1.848	0.998			
Diameter	2.09	1.169-3.736	0.013	1.877	1.041-3.386	0.036
Location	1.638	1.218-2.204	0.001	1.728	1.274-2.345	0.000
Differentiation	0.894	0.514-1.555	0.692			
Local invasion	2.531	1.036-6.179	0.041			
Lymph node metastasis	4.985	2.413-10.299	0.000	4.765	2.339-9.708	0.000
TNM stage	4.076	2.242-7.412	0.000			
MiR-378 expression	2.005	1.141-3.523	0.016			

HR: hazard ratio, CI: confidence interval.

sis, advanced stage, large tumor, and proximal and middle location were correlated with adversely overall survival (**Figure 2A-E**). Interestingly, when the patients were classified into high miR-378 group and low miR-378 group according to the relative levels of miR-378, we found that the low expression of miR-378 was associated with short overall survival (**Figure 2F**). Univariate analysis identified tumor size, location, local invasion, lymph node metastasis, stage and low expression of miR-378 as prognostic factors, whereas age, gender and differentiation were not significantly associated with overall survival (**Table 2**). However, multivariate analysis shown that low expression of miR-378 was not an independent prognostic factor, which may need more samples to investigate the correlation. We detected that the tumor size, location and lymph node metastasis were independent predictors for prognosis of gastric cancer (**Table 2**).

Discussion

Gastric cancer is a major cause of cancer related death in the world, identifying biomarkers for predicting gastric cancer progression and prognosis is beneficial for evaluating the disease severity [8], therefore we can select optimal and aggressive treatments for these patients. In this study, we found that miR-378 was significantly downregulated in gastric cancer. Moreover, to our knowledge, we firstly reported that decreased expression of miR-378 was associated with deep tumor invasion, lymph node metastasis, advanced stage and poor prognosis. Our results highlight the potential use of miR-378 in predicting tumor invasion, lymph node metastasis and prognosis in gastric cancer.

Local invasion and lymph node metastasis are essential characters of gastric cancer and associated with patient's survival [9]. Therefore, it is essential to devote more research to investigate the molecular mechanism or explore predictive biomarker. Accumulating studies have documented that miRNAs play an essential role in gastric tumorigenesis and progression [10-13]. Previously, we have reported that miR-148a and miR-409 suppress gastric cancer cell migration, invasion and metastasis by targeting ROCK1 and radixin respectively [10, 11]. Notably, certain miRNAs correlate with local invasion, lymph node metastasis, TNM stage and patient prognosis, such as miR-125a-3p [14], miR-181c [15], miR-10b [16] and miR-630 [17]. Besides, the report from Imaoka et al. notes that circulating miR-203 from patient's serum serves as a noninvasive marker for lymph node, peritoneal, and distant metastasis and prognosis in gastric cancer [18]. In this study, we found that the expression of miR-378 was significantly suppressed in gastric cancer, down-regulation of miR-378 was associated with deep local invasion, lymph node metastasis, advanced tumor stage and low survival rate. Our results suggest that loss of miR-378 expression correlates with aggressive disease phenotype in gastric cancer.

Overexpression of miR-378 has been observed in nasopharyngeal carcinoma [19] and breast cancer [20], and studies show miR-378 has oncogenic activities [19, 21]. However, in other tumors such as glioma [22], colorectal cancer

[23, 24] and gastric cancer [5-7], miR-378 serves as a tumor suppressor. The study from Slaby *et al.* shows that the expression of miR-378 is gradually decreased in normal esophageal mucosa, Barrett's esophagus and esophageal adenocarcinoma [25], suggesting downregulation of miR-378 may be involved in oncogenesis of esophagus. In colorectal cancer, miR-378 is frequently downregulated, as a tumor suppressor inhibits cell proliferation and invasion, and increases L-OHP-induced apoptosis. Moreover, low expression of miR-378 is significantly associated with poor prognosis [23, 24]. Interestingly, miR-378 is overexpressed in plasma of colorectal cancer patients, its levels are decreased after surgery, can be used as a biomarker to discriminate colorectal cancer patients from healthy individuals [26]. In gastric cancer, studies have shown that miR-378 is downregulated, it has been proved that miR-378 suppresses gastric cancer cell growth, migration and invasion [6, 7]. Deng *et al.* find that the low expression of miR-378 is due to the presence of promoter CpG island methylation, administration with 5-aza-dC can restore the expression of miR-378 in gastric cancer cells [7]. However, the relationship between miR-378 expression and clinicopathological features is less well known. In this study, our results indicate that the lower expression of miR-378 is associated with deeper tumor invasion, more lymph node metastasis and worse prognosis. Interestingly, Liu *et al.* report that the expression of miR-378 is elevated in serum of gastric cancer patients compared with healthy control, miR-378 can be used as a valuable biomarker for early detection of gastric cancer [27]. Combined with our results, miR-378 shows discrepant expression patterns between patient's serum and tumor tissues in gastric or colorectal cancer, which may depend on different backgrounds and the mechanism that miRNA can be selectively released by cancer cells or normal cells [28]. The finding of differences raises interesting possibilities that extracellular miRNA may not originate from the tumor cells, and the extracellular miRNA and cellular miRNA have different biological roles, further studies are required to clarify the mechanism that regulates miRNA circulation. Taken together, miR-378 not only functions as a tumor suppressor in gastrointestinal tumors, but also has a strong potential to act as a new predictive biomarker in early detection, invasiveness and prognosis of gastric cancer.

In conclusion, our results show that miR-378 is frequently downregulated, and decreased expression of miR-378 is associated with deep tumor invasion, lymph node metastasis, advanced stage and poor prognosis in gastric cancer. These findings suggest that miR-378 has a potential to serve as a clinical biomarker for predicting disease invasiveness and prognosis in gastric cancer, and these patients with loss of miR-378 expression may need additional treatments and close follow-up.

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Disclosure of conflict of interest

None.

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