Int J Clin Exp Pathol 2017;10(7):7356-7361
www.ijcep.com /ISSN:1936-2625/IJCEP0042950

Original Article

Plasma *microRNA-320* is a potential diagnostic and prognostic bio-marker in gastric cancer

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Received October 27, 2016; Accepted January 10, 2017; Epub July 1, 2017; Published July 15, 2017

Abstract: Objective: *MicroRNA-320* (*MiR-320*) had been reported to be down-regulated in several cancers. However, its clinical significance in gastric cancer remained unknown. In this study, we aimed to detect the expression of *miR-320* and its clinical significance in gastric cancer. Methods: The relative expression levels of *miR-320* in plasma of gastric cancer patients and healthy controls were detected using quantitative real-time polymerase chain reaction (qRT-PCR). A receiver operating characteristic (ROC) curve was established to estimate the diagnostic value of *miR-320* in gastric cancer. Moreover, its prognostic value was assessed via Kaplan-Meier and Cox regression analyses. Results: Compared with healthy individuals, the plasma *miR-320* expression in patients with gastric cancer was significantly decreased ($P<0.001$). The low plasma *miR-320* expression was closely associated with TNM stage and lymph node metastasis ($P<0.05$). Furthermore, plasma *miR-320* could be used to distinguish gastric cancer patients from healthy controls with an area under the curve (AUC) of 0.861. The sensitivity and specificity were 82.4% and 75.9%, respectively. Kaplan-Meier analysis revealed that patients with high expression of *miR-320* had an obviously longer overall survival than those with low *miR-320* expression (log rank test, $P=0.003$). *MiR-320* could be an independent prognostic factor for patients with gastric cancer via univariate and multivariate analyses. Conclusions: Plasma *miR-320* is down-regulated and correlated with the progression of gastric cancer. What's more, *miR-320* may be a potential bio-marker for the diagnosis and prognosis of gastric cancer patients.

Keywords: Gastric cancer, *MiR-320*, diagnosis, prognosis

Introduction

Gastric cancer becomes one of the most frequent malignant tumors in the world [1]. And it remains a lethal cancer, although the morbidity rate is decreased [1, 2]. The surgical resection is a curative therapeutic method for gastric cancer patients with early stage. However, lack of typical symptoms in early stage of gastric cancer, most patients were at the advanced stages with diagnosis [3]. In the current, gastroscopic screening and some serum molecules (carcinoembryonic antigen) are the major methods for early detection of gastric cancer [4]. Nevertheless, there were big limitations, such as high cost, invasive nature and unsatisfied sensitivity as well as specificity [4-6]. A large number of studies had reported that the prognosis of gastric cancer was very poor and the 5-year overall survival was less than 25% [7, 8]. Therefore, identifying novel non-invasive biomarkers for early diagnosis and prognosis of gastric cancer will provide an important clinical relevance.

MicroRNAs (MiRNAs), as small non-coding RNA molecules, had been reported to be aberrantly expressed in several human tumors, and correlated with carcinogenesis and tumor progression [9, 10]. Recently, miRNAs had been proven to be highly stable in human blood, including plasma and serum, supporting their potency as blood-based bio-markers for the diagnosis, treatment and prognosis of cancers [11, 12]. Among these miRNAs, *miR-320* was identified as a tumor suppressor and reduced between miR-320 expression a variety of tumors [13, 14]. It was shown to suppress cell proliferation and metastasis, suggesting the correlation between *miR-320* expression and the progression of tumors [15]. Increasing evidences revealed that the expression of *miR-320* could

Table 1. Relationship between *miR-320* expression and clinical characteristics of gastric cancer patients

Clinical Features	Cases (n=116)	*MiR-320* expression		x^2	*P*
		High (n=50)	Low (n=66)		
Age (years)				0.933	0.334
≤61	66	31	35		
>61	50	19	31		
Gender				0.755	0.385
Male	77	31	46		
Female	39	19	20		
Tumor size (cm)				1.386	0.239
≤4	60	29	31		
>4	56	21	35		
Histological grade				3.325	0.068
Well/Moderate	63	32	31		
Poor	53	18	35		
TNM stage				4.691	0.030
I-II	68	35	33		
III-IV	48	15	33		
Lymph node metastasis				6.153	0.013
Negative	66	35	31		
Positive	50	15	35		

be served as potential diagnosis bio-marker and therapy target of tumors [16, 17]. However, the diagnostic and prognostic value of *miR-320* remains to be known in gastric cancer.

In this study, we detected the plasma expression level of *miR-320* in gastric cancer patients and healthy controls. And the relationship between plasma *miR-320* expression and clinical factors of gastric cancer patients was analyzed. Meanwhile, we also estimated the clinical significance of *miR-320* in gastric cancer.

Materials and methods

Patients and samples

In this study, a total of 116 patients with confirmed histopathological diagnosis of gastric cancer were enrolled from Cangzhou Central Hospital. None of patients had received any chemotherapy, radiotherapy or other treatments before surgery. Additionally, 85 healthy donors were collected as controls and they had not detected with any tumors previously. This study was approved via the Ethical Committee of the hospital and written informed consents were provided by all participants in advance.

10 mL blood samples from each participator were collected into EDTA tubes. Then these samples were centrifuged for 10 min at 1500 rpm and the supernatant plasma was frozen at -80°C until use. The detailed clinicopathologic characteristics of patients were shown in **Table 1**. A 5-years' follow-up was performed using a telephone or questionnaires, and patients who died from other disease or unexpected events were excluded from this study.

RNA extraction and qRT-PCR analysis

Total RNA from plasma samples was severally isolated using Trizol Reagent (TaKaRa. Japan) according to the instructions of manufacturer. TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, US) was used to conduct reverse transcription. Then the RT-PCR reaction was performed by TaqMan Universal Master Mix (Applied Biosystems, US). Using small nuclear RNA *U6* as the internal control, the sequences of primers for *miR-320* and *U6* were as follows: *miR-320*, forward-5'-AAAAGCTGGGTTGAGAGGGCGA-3', and reverse-5'-GCGAGCACA- GAATTAATACGAC-3'; *U6*, forward-5'-CGCTTCGGCAGCACATATACTA-3', and reverse-5'-CGCTTCACGAATTTGCGTGTCA-3'. All samples were detected in triplicate and the relative mRNA quantification of *miR-320* expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All statistical analyses were performed with the SPSS 18.0 software and the data were expressed as mean ± standard deviation (SD). Student's t test was used to evaluate the differences between two groups. The relationship between *miR-320* expression and clinical characteristics was analyzed by Chi-square test. Receiver operating characteristic (ROC) was applied to estimate the diagnostic value of *miR-320* in gastric cancer. The overall survival of patients with different *miR-320* expression was compared via Kaplan-Meier analysis with log

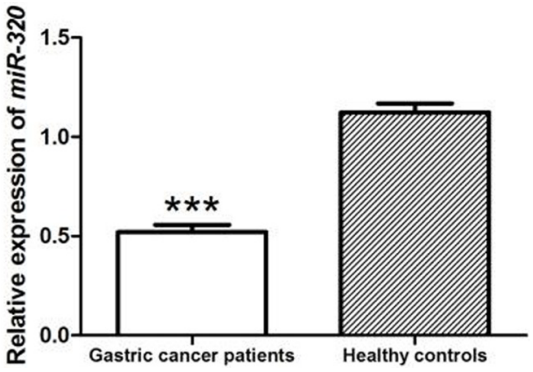

Figure 1. The relative mRNA expression of *miR-320* in plasma of gastric cancer patients and healthy individuals. Compared with healthy controls, the plasma *miR-320* expression was significantly down-regulated in gastric cancer patients (P<0.001).

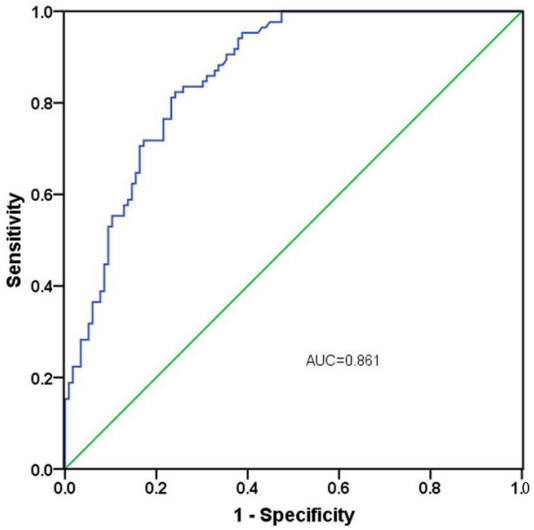

Figure 2. The ROC curve analysis for the diagnosis of gastric cancer using *miR-320* expression. It indicated that the AUC was 0.861 with a sensitivity of 82.4% and a specificity of 75.9%.

rank test. Univariate and multivariate analysis with Cox regression was used to assess the prognostic value of *miR-320* in gastric cancer. P<0.05 was considered as statistically significant.

Results

Demographic characteristics of the study subjects

The clinical characteristics of gastric cancer patients were shown in **Table 1**. There were 39 females and 77 males in gastric cancer group,

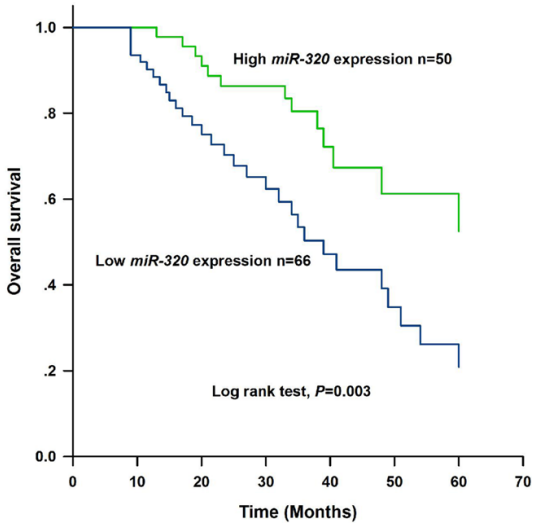

Figure 3. Kaplan-Meier analysis for estimating the overall survival of patients with gastric cancer. Patients with low expression of *miR-320* had shorter overall survival than those with high *miR-320* expression (log rank test, P=0.003).

with a mean age of 61 years (age rang, 42-87 years). The tumor size of 60 patients was less than 4 cm, and the others were more than 4 cm in size. According to the TNM staging system, 68 patients at stage I-II, and 48 patients of stage III-IV These patients were classified based on histological grade as follows: well/moderate, n=63 and poor, n=53. Lymph node metastasis of gastric cancer patients occurred in 50 of 116 patients. Another group of 85 healthy people were consisted of 38 female and 47 male with a mean age of 58 years (age range, 41-82 years).

The expression of miR-320 was down-regulated in gastric cancer patients

The relative mRNA expression levels of *miR-320* in plasma of gastric cancer patients and healthy controls were detected by qRT-PCR. The results indicated that *miR-320* expression was significantly lower in gastric cancer patients than that in healthy individuals (P<0.001, **Figure 1**).

Relationship between miR-320 expression and clinical features of patients with gastric cancer

According to the mean expression level of *miR-320*, 116 patients were divided into high *miR-320* expression group and low *miR-320* expression group to explore whether *miR-320* was

Table 2. Univariate and multivariate analyses with Cox regression for the overall survival in gastric cancer patients

Factors	Univariate analysis			Multivariate analysis		
	HR	95.0% CI	*P*	HR	95.0% CI	*P*
MiR-320 (Low vs High)	2.562	1.333-4.924	0.005	2.046	1.023-4.093	0.043
Age (year) (>61 vs ≤61)	1.020	0.548-1.899	0.951	-	-	-
Gender (Male vs Female)	1.108	0.598-2.053	0.744	-	-	-
Tumor size (cm) (>4 vs ≤4)	0.796	0.437-1.450	0.455	-	-	-
Histological grade (Poor vs Well/Moderate)	2.097	1.114-3.947	0.022	-	-	-
TNM stage (III-IV vs I-II)	2.470	1.337-4.564	0.004	1.933	1.013-3.689	0.046
Lymph node metastasis (Positive vs Negative)	2.139	1.109-4.126	0.023	-	-	-

Note: - indicated no data.

linked with the development of gastric cancer. Then we analyzed the association between *miR-320* expression and clinical characteristics of gastric cancer patients. As shown in **Table 1**, the low plasma *miR-320* expression was closely correlated with advanced TNM stage (*P*=0.030) and lymph node metastasis (*P*=0.013). However, no obvious association was found between *miR-320* expression and age, gender, tumor size or histological grade (all, *P*>0.05, **Table 1**).

The diagnostic value of miR-320 in gastric cancer patients

To explore the diagnosis significance of *miR-320* in patients with gastric cancer, ROC curve was established. The outcome demonstrated that *miR-320* could be a valuable bio-marker to discriminate gastric cancer patients from healthy controls with an area under the curve (AUC) of 0.861 (95% CI=0.811-0.910, *P*<0.001) combing with the sensitivity of 82.4% and the specificity of 75.9% (**Figure 2**). Meanwhile, the ideal cutoff value of *miR-320* expression was 0.765.

The prognostic value of miR-320 in gastric cancer patients

To investigate the prognostic value of *miR-320* in gastric cancer patients, a 5 years' follow-up was carried out. Based on the data from follow-up, Kaplan-Meier analysis with log rank test revealed that the gastric cancer patients with low *miR-320* expression had a shorter overall survival than those with high expression of *miR-320* (log rank test, *P*=0.003, **Figure 3**). Besides, the result of Cox regression analysis showed that *miR-320* expression (HR=2.046, 95% CI=1.023-4.093, *P*=0.043) as well as TNM stage (HR=1.933, 95% CI=1.013-3.689, *P*=0.046) were both correlated with the prognosis of gastric cancer patients and they may be independent prognostic factors for gastric cancer (**Table 2**).

Discussion

It was reported that the expression of *miR-320* was decreased in a variety of cancers, revealing that *miR-320* may be involved in the carcinogenesis and progression of tumor [13, 14]. Meanwhile, previous studies had demonstrated that altered expression of circulating miRNAs in serum and plasma could be potential bio-markers for tumors as well as other diseases [18, 19]. Currently, the clinical significance of *miR-320* in gastric cancer still remains unclear.

In the present study, the expression levels of *miR-320* in plasma of gastric cancer patients and healthy controls were detected. The results revealed that plasma *miR-320* expression was significantly down-regulated in gastric cancer patients compared to that in healthy individuals which was similar to the previous studies [20, 21]. This result suggested that *miR-320* could function as a tumor suppressor in gastric cancer. Then we analyzed the correlation between *miR-320* expression and clinical features of patients to explore whether *miR-320* was involved in the progression of gastric cancer. The outcome showed that the low plasma *miR-320* expression was strongly associated with TNM stage and lymph node metastasis, which were clinical factors representing progression and metastasis of tumor. These findings were consistent with what had been found in cervical cancer [22]. Taken together, *miR-320* may par-

ticipate in tumorigenesis and the progression of gastric cancer.

Furthermore, we investigated the clinical significance of *miR-320* in gastric cancer. ROC curve analysis demonstrated that the low plasma *miR-320* expression was significantly associated with the early diagnosis of gastric cancer. It was indicated to be useful to discriminate gastric cancer from healthy controls with high values of AUC, sensitivity and specificity. Consistent with our result, accumulated evidence had confirmed that *miR-320* could be used as a potential bio-marker for the diagnosis of diseases, including tumors [23-25]. For example, Neuro et al. showed that *miR-320* was significantly associated with a glioblastoma multiforme diagnosis [23]. Liu et al. have reported that the expression of *miR-320* was down-regulated in retinoblastoma patients samples and it may be considered as a valuable diagnostic biomarker in RB [24]. Moreover, in the study of Jiang et al., they proved that the expression levels of *miR-320* may be useful for the diagnosis of erectile dysfunction in patients with diabetes [25].

Previous study had reported that the expression of *miR-320* was related to the probability of recurrence-free survival in colon cancer [26]. However, the prognostic value of *miR-320* in human tumors is still poorly known. Next, the result of Kaplan-Meier analysis indicated that the overall survival of patients with high expression of *miR-320* was markedly longer than those with low *miR-320* expression, which reveled *miR-320* was associated with the prognosis of gastric cancer. Then univariate and multivariate analysis showed that *miR-320* was an independent prognostic factor of gastric cancer. Besides, our outcome demonstrated TNM stage was also an independent factor.

MiR-320 had been proven to inhibit stem cell-like properties of prostate cancer cells through modulating Wnt/beta-catenin signaling pathway [27]. Previous study demonstrated that *miR-320* suppress cell proliferation of osteosarcoma via regulating the expression of fatty acid synthase [28]. To our knowledge, *Helicobacter pylori* is one of the strongest risk for gastric cancer. A study of Noto et al. mentioned that *H. pylori* could suppress the expression of *miR-320*, indicating that *miR-320* was a tumor suppressor in gastric cancer [29]. However, little is known about the exact mechanisms by which *miR-320* regulates gastric cancer, which is still required more researches.

In summary, *miR-320* is decreased in gastric cancer and correlated with the progression of this tumor. It may be a potential and non-invasive bio-marker for the diagnosis and prognosis of gastric cancer. However, due to the limitation of sample size and its source, further studies may be urgently needed.

Acknowledgements

Key project in medical science research of Hebei health and family planning commission (20150340).

Disclosure of conflict of interest

None.

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