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Up-Regulation of Plasma miR-23b is Associated with Poor Prognosis of Gastric Cancer

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Data Interpretation D
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Background: Gastric cancer (GC) is a common malignant disease and microRNAs (miRNAs) have been shown to play important roles in GC tumorigenesis. As the clinical outcome of GC is closely correlated with the clinical stage at the time of diagnosis, early detection and prevention are crucial. This study was designed to evaluate the expression level of plasma miR-23b in patients with GC and investigate the relationship between plasma miR-23b expression level and the prognosis of GC.

Material/Methods: We recruited 138 patients diagnosed with GC and 50 healthy volunteers. Quantitative real-time PCR (qRT-PCR) was performed to evaluate the expression level of plasma miR-23b in all participants. The association between miR-23b expression and clinicopathological factors as well as survival rates was analyzed. Receiver operator curve (ROC) analysis was carried out to evaluate the diagnostic performance of plasma miR-23b for GC.Univariate and multivariate Cox regression analyses were conducted to determine whether plasma miR-23b was an independent predictor of survival.

Results: The expression levels of miR-23b were upregulated in plasma samples from GC patients ($P<0.01$) and were significantly associated with T stage, distant metastasis, and differentiation. Significantly shorter 5-year overall survival (OS) and disease-free survival (DFS) were observed in patients with higher expression of the miR-23b ($P<0.01$). The area under the curve (AUC) of high expression of plasma miR-23b to diagnose GC was 0.80 (95% CI: 0.74–0.86, $P<0.001$). Multivariate analysis revealed that enhanced expression of plasma miR-23b was an independent predictor of OS ($P=0.015$) and DFS ($P=0.004$).

Conclusions: Our results indicated that plasma miR-23b was overexpressed in GC patients and high plasma miR-23b expression was associated with poor clinical outcome. Thus, plasma miR-23b may serve as a potential diagnostic biomarker and therapeutic target for GC.

MeSH Keywords: MicroRNAs • Plasma • Prognosis • Stomach Neoplasms

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Background

Gastric cancer (GC), a malignant tumor arising from the lining of the stomach, is the fourth most common cancer globally and the second most frequent cause of cancer-related death [1]. GC development is a complex multistep process involving a number of genetic alterations and epigenetic modifications. Currently, the major treatment options for GC are surgery, chemotherapy, radiotherapy, and targeted therapy. The clinical outcome of GC is mainly dependent on the stage of the disease. Unfortunately, most GC patients are diagnosed at moderate and advanced stage, leading to a poor 5-year overall survival rate [2]. Thus, it is extremely urgent to screen novel biomarkers that can be used to detect GC at an early stage and predict the prognosis of this deadly disease.

MicroRNAs (miRNAs), which function mainly by suppressing targeted mRNA translation at the post-transcriptional level, are a group of small, highly conserved non-coding RNAs [3,4]. MiRNAs are involved in most, if not all, important physiological and pathological processes [5]. Dysregulation of miRNA expression is a common character of GC, indicating that miRNAs might play indispensable roles in the development of this malignancy [6]. Jiang et al. showed that miR-874 was downregulated in GC tissues and its expression level was correlated with various clinical features. Moreover, miR-874 overexpression inhibited the proliferation, migration, and invasion capacity both *in vitro* and *in vivo*, whereas the opposite was seen on miR-874 suppression [7]. Su et al. reported that plasma miR-18a was significantly upregulated in patients with GC. In addition, plasma miR-18a overexpression indicated poor survival rates and prognosis of GC, suggesting that miR-18a might function as an oncogene in GC [8].

The concrete role of miR-23b in carcinogenesis is complex and might depend on cancer types.

Majid et al. showed that miR-23b played a tumor-suppressive role in prostate cancer by repressing Src kinase expression [9]. However, miR-23b can act as an oncogene in renal carcinoma via downregulating the expression level of proline oxidase [10]. Tissue miR-23b has been reported to be upregulated in patients with GC, and it was correlated with various important clinicopathological parameters [11]. However, the clinical significance of plasma miR-23b in GC patients remains unknown. The purpose of this study was to evaluate the association between plasma miR-23b expression level and clinical features, as well as the prognosis of GC.

Material and Methods

Study population

We recruited 138 patients with GC and 50 healthy volunteers. The project was approved by the Ethics Committee of The Second Affiliated Hospital Xi'an Jiaotong University and written informed consent was obtained from all participants. The diagnosis of GC was confirmed by histological evaluation in all GC patients. None of the patients received any kind of treatment prior to sample collection. All plasma samples were handled anonymously according to ethical and legal standards. Peripheral blood (5 ml) samples were obtained following an overnight fast and placed at room temperature for 60 min. To obtain plasma, the samples were centrifuged at 1000 g for 10 min at 4°C followed by centrifugation of the supernatant at 4°C at 12 000 g for 2 min. All plasma samples were stored at –80°C until use. The clinical features of GC patients are summarized in Table 1.

RNA isolation and quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was isolated from 400 µL of plasma using the mirVana PARIS miRNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. The yield and purity of RNA (A260/280 >2.0, A260/230 >1.8) were determined using the NanoDrop 1000 spectrophotometer (Thermo Scientific Wilmington, DE, USA). Reverse transcription reactions were prepared using the RT-PCR kit (Qiagen, Hilden, Germany). Real-time PCR was performed by ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The amplification conditions were 94°C for 5 min, followed by 40 cycles of 95°C for 55 s, 60°C for 1 min, and 72°C for 1 min. U6 small nuclear RNA (RNU6B) was used as internal control and fold change of miR-23b was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

The Mann-Whitney U test was performed to compare the expression level of plasma miR-23b between GC patients and healthy controls. Associations between clinicopathological parameters and plasma miR-23b expression were evaluated using χ^2 tests. The overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method. The area under the receiver operating characteristic ROC curve (AUC) was used as to determine the diagnostic accuracy of plasma miR-23b for the diagnosis of GC. The variables that were statistically significant by univariate analysis were included in multivariate Cox proportional hazards regression models to identify the independent prognostic values. Statistical analysis was performed using SPSS version21.0 for Windows (SPSS Inc, IL, USA) and *P*-value<0.05 was considered statistically significant.

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Table 1. Plasma miR-23b level and clinicopathological parameters in patients with GC.

Variable	n	Plasma miR-23b expression (n)		P
		Low	High	
Gender				0.757
Male	85	44	41	
Female	53	26	27	
Age				0.237
<60	74	41	33	
≥60	64	29	35	
T stage				0.003
T1–T2	60	39	21	
T3–T4	78	31	47	
Lymph node metastasis				0.368
No	56	31	25	
Yes	82	39	43	
Distant metastasis				0.010
No	115	64	51	
Yes	23	6	17	
Differentiation				0.002
Well/moderate	63	42	21	
Poor	75	28	47	
Tumor size (cm)				0.171
<5	67	38	29	
≥5	71	32	39	
Site				0.530
Gastric cardia	42	23	19	
Non-cardia	96	47	49	

Results

Plasma miR-23b expression was upregulated in GC patients

Our qRT-PCR results showed that average expression of plasma miR-23b was upregulated significantly in GC patients compared with healthy volunteers ($P<0.01$) (Figure 1).

Clinicopathologic significance of plasma miR-23b in GC

The fold-change in the expression of miR-23b in plasma from 138 patients with GC was calculated by comparison with the median of 50 healthy control samples. Then the patients were divided into high and low expression groups by the median expression level of plasma miR-23b (3.92-fold). The results revealed that miR-23b expression was associated with T stage ($P=0.003$), distant metastasis ($P=0.010$), and differentiation ($P= 0.002$) (Table 1).

Relationship between plasma miR-23b expression and GC patients' survival

Kaplan-Meier analysis showed that patients with higher levels of miR-23b had significantly lower overall survival than those with lower expression of this miRNA in patients, with a 5-year OS of 29.4% and 75.7%, respectively ($P<0.01$) (Figure 2). Moreover, the high miR-23b expression group also exhibited a significantly lower 5-year DFS than the low miR-23b expression group. Five-year DFS was 72.9% in the low miR-23b expression group and 19.1% in the high miR-23b expression group ($P<0.01$) (Figure 3).

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Figure 1. The expression level of plasma miR-23b in GC patients. The results showed that the plasma miR-23b expression level in patients with GC was significantly higher than that in healthy controls (P<0.01).

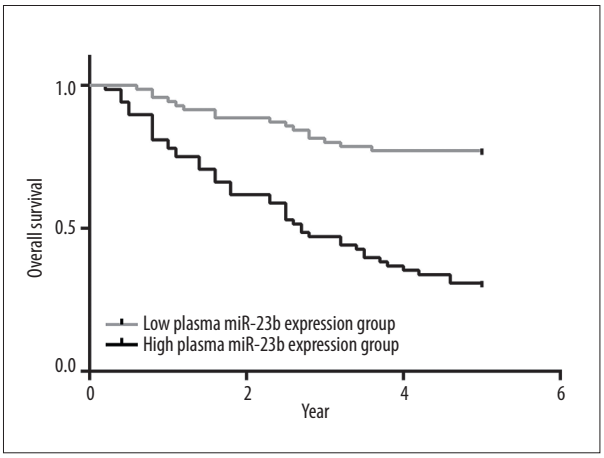

Figure 2. The association between plasma miR-23b expression and OS. The survival analysis revealed that the GC patients with higher expression level of plasma miR-23b had worse 5-year OS (P<0.01).

The sensitivity and specificity of plasma miR-23b for diagnosing GC

A ROC curve was performed to evaluate the diagnostic proficiency of plasma miR-23b for GC. The AUC in terms of size was 0.80 (95% CI: 0.74-0.86, P <0.001; cut-off value: 1.86). The sensitivity and specificity at the optimal cutoff were 71.0% and 74.0%, respectively (Figure 4).

Plasma miR-23b expression level was independent prognosis factor of OS and DFS for GC

Multivariate analysis revealed that T stage (P=0.026), differentiation (P=0.008), and plasma miR-23b expression (P=0.015) were independently associated with the OS.

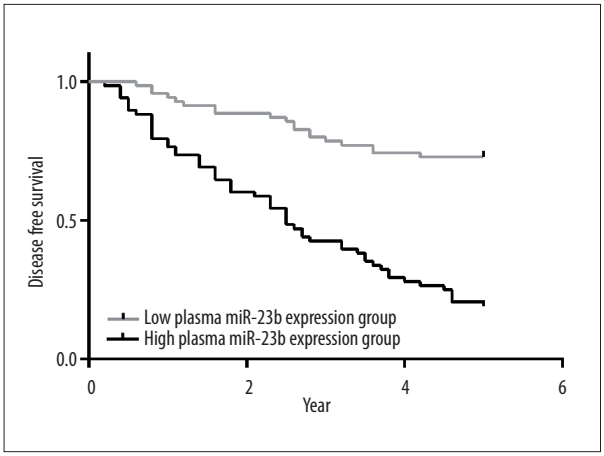

Figure 3. The correlation between plasma miR-23b expression and DFS. The GC patients in the high plasma miR-23b expression group had significantly lower 5-year DFS rate than those in the low plasma miR-23b expression group (P<0.01).

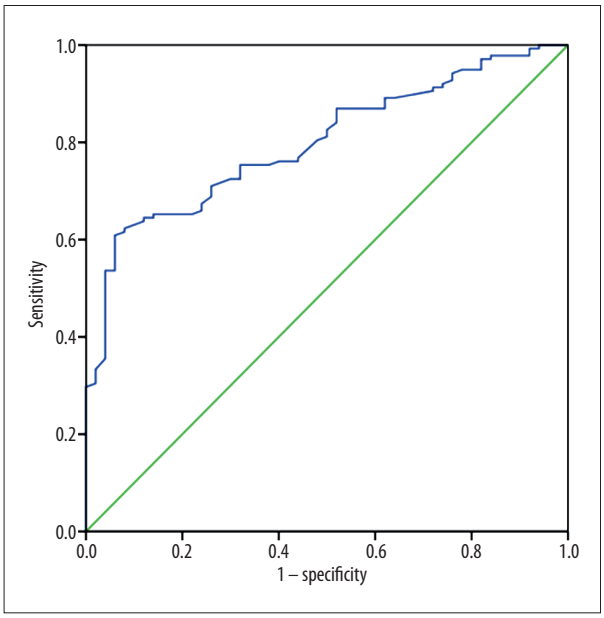

Figure 4. The diagnostic value of plasma miR-23b for GC. The results of ROC analysis showed that plasma miR-23b had moderate diagnostic value for the identification of GC (AUC=0.80, 95% CI: 0.74–0.86, P<0.001)

As regards to DFS, differentiation (P=0.012) and plasma miR-23b (P=0.004) expression were independent prognosis factors (Table 2).

Discussion

Although the incidence of GC has declined rapidly over the past few decades, GC remains a leading cause of cancer death.

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Table 2. Multivariate analysis of 5-year overall and disease-free survival in patients with GC.

Variable	Overall survival			Disease-free survival		
	HR	95%CI	*P*	HR	95%CI	*P*
T stage (T3,T4 *vs.* T1,T2)	2.352	0.912–4.863	0.026	1.675	0.752–3.024	0.085
Distant metastasis (Yes *vs.* No)	1.273	0.633–1.942	0.385	1.428	0.871–2.763	0.278
Differentiation (Poor *vs.* Well/moderate)	3.246	1.312–6.232	0.008	3.161	1.475–5.632	0.012
Plasma miR-23b (High *vs.* Low)	2.614	1.035–5.457	0.015	3.578	1.764–6.726	0.004

The malignant disease causes more than 700 000 deaths globally each year and China has a high incidence of GC [12,13]. Currently, clinical indicators, such as TNM staging system and histological evaluation, are the main prognostic factors for GC. However, these clinical indictors lack specificity and contribute little to the early detection of GC. Thus, it is crucial to explore new biomarkers for diagnosing GC at an early stage and to predict the prognosis of GC.

A large amount of studies have showed that alterations in miRNAs expression were implicated in the initiation and progression of many cancers including gastric cancer [14,15]. As body fluids contain a number of important substances that can be used for disease detection and diagnosis, they exhibited great potential for actual clinical use. The advantages of diagnosing diseases using body fluids are obvious. First, body fluids such as blood, urine and saliva are easily accessible and less invasive than tissue biopsy. In addition, it is convenient for the clinical doctors to classify the patients into different subgroups and monitor the therapy response in real-time by analyzing the body fluids. What is more, screening the biomarkers in the body fluids might help us detect the diseases at an early stage. Previous studies have reported that miR-23b was detectable in various types of body fluids. Wu et al. showed that miR-23b was upregulated significantly in plasma samples of patients with breast cancer using SOLiD sequencing method [16]. Recently miR-23b was also reported to increase in the saliva of pancreatic cancer patients compared with healthy controls [17].

Our results showed that the expression level of plasma miR-23b was overexpressed in GC patients compared with healthy controls. In addition, plasma miR-23b expression was associated with multiple clinical parameters, including T stage, distant metastasis, and differentiation. GC patients who were in the high plasma miR-23b expression group had worse 5-year OS and DFS. The ROC analysis results demonstrated that plasma miR-23b had a moderate diagnostic value for GC with the AUC of 0.80. Moreover, plasma miR-23b was an independent risk factor of GC. Consistent with previous observations, tissue miR-23b expression level was increased in the biopsy samples from patients with GC, and higher miR-23b expression was correlated with worse clinical outcome [11]. Dong et al. revealed that suppressing β-catenin could reduce miR-23b expression, indicating miR-23b might regulate the development of GC via interaction with the β-catenin signaling pathway [18]. There was a reciprocal repression correlation between tumor suppressor candidate 7 (TUSC7) and miR-23b. TUSC7 acted as a tumor suppressor by partially inhibiting miR-23b, suggesting miR-23b can promote the progression of GC [19]. However, Huang et al. recently showed that miR-23b inhibited various tumorigenesis processes, such as growth, viability, migration, and invasion, by targeting Notch2 receptor in GC cell lines [20]. Moreover, ectopic expression of miR-23b-3p can reduce the resistance of gastric cancer cells to multiple chemotherapeutic agents *in vitro* and sensitize tumors to chemotherapy *in vivo* [21]. The evidence presented above suggests that miR-23b might also function as a tumor suppressor gene in GC. The contradictory findings regarding the role of miR-23b in GC might due to several reasons. Firstly, the function of miR-23b itself is very complex. Whether miR-23b acts as an oncogene or a tumor suppressor gene might be closely correlated with tumor microenvironment and the numerous regulatory signals it receives. Secondly, the performance of miR-23b might be different among gastric cancer cell lines, animal models, and humans. Thirdly, it is possible that the concrete role of miR-23b depends on different subtypes of gastric carcinoma and GC cell lines investigated. Fourthly, miR-23b might even function differently in different clinical stages of GC.

MiR-23b was shown to play contradictory roles in gastric cancer, thus it is no wonder that miR-23b function differently in different types of cancers. Pellegrino et al. revealed that miR-23b is a metastatic suppressor microRNA in breast cancer, as suppressing miR-23b led to an increase of cell migration and metastatic spread *in vivo*. In addition, low expression level of miR-23b was associated with metastasis in breast cancer patients [22]. However, the expression level of tissue miR-23b was upregulated in patients with renal cell carcinoma (RCC) and correlated with survival rates. Down-regulation of miR-23b could induce apoptosis and inhibit invasion in RCC cell lines, indicating miR-23b plays a proto-oncogenic role in RCC [23]. Further studies are needed to elucidate the complex regulatory role of miR-23b in cancer.

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Conclusions

The high stability of plasma miRNAs gives them great potential for clinical use. These preliminary data suggest that plasma miR-23b is a promising diagnostic and prognostic biomarker for patients with GC. However, the molecular mechanisms of regulation and action of miR-23b in GC need further investigation.

Conflict of interest

None declared.

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