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Original Article

High serum miR-203 predicts the poor prognosis in patients with pancreatic cancer

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Abstract: Altered expression of microRNAs (miRNAs) has been demonstrated to promote the progression of many types of malignancy including pancreatic cancer (PC). However, the correlation between serum miR-203 expression and the prognosis of PC remains unknown. In this study, a total of 96 PC patients, 20 patients with benign pancreatic tumor, 30 patients with chronic pancreatitis and 30 healthy controls were recruited. The expression level of serum miR-203 in the participants was examined by real-time PCR. Then the association between serum miR-203 levels with clinical variables as well as survival time was investigated. Our results showed that serum miR-203 levels were significantly up-regulated in patients with PC (P<0.01) and its levels were remarkably downregulated in those who received surgical resection (P<0.01). In addition, serum miR-203 levels were correlated with various clinicopathological parameters including N stage, histological grade and TNM stage. Furthermore, PC patients with serum miR-203 overexpression had poorer 5 year overall survival and disease free survival rates (P=0.016 and P=0.004, respectively). Finally, serum miR-203 expression was demonstrated to be an independent risk factor for PC (P<0.05). Overall, up-regulated expression of serum miR-203 is correlated with poor prognosis in PC and might be a promising prognostic biomarker for this malignancy.

Keywords: Biomarker, serum miR-203, pancreatic cancer, prognosis

Introduction

Pancreatic cancer (PC) is one of the most common fatal malignancy and the fourth leading cause of cancer related mortality worldwide [1]. Due to a lack of clear clinical symptoms and early detection methods, most patients are diagnosed at the terminal stage when surgical treatment is impossible. It has a dismal prognosis and the 5 year overall survival rate is consistently less than 5% [2]. Therefore, it is urgent to identify the biomarkers help in the early diagnosis and prognosis prediction for PC.

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate many biological processes such as proliferation, differentiation, survival, apoptosis and development [3]. Dysregulated miRNAs has been implicated in many human diseases including cancer [4]. MiRNAs might function as either “oncomiRs” or “tumor suppressors” in the initiation and progression of PC. For instance, Zhao et al showed that the expression level of miR-130b was significantly reduced in PC tissues and cell lines. In addition, low miR-310b was associated with poor prognosis of this malignant disease. Ectopic expression of miR-130b inhibited the oncogenic activities of cancer cells both *in vitro* and *in vivo*. Moreover, STAT3 was a downstream target of miR-130b, suggesting that miR-310b played a tumor suppressive role in PC [5]. MiR-196a was significantly increased in human pancreatic cancer cell lines. In addition, inhibition of miR-196a suppressed the proliferation and migration capacity of pancreatic cancer cells through targeting nuclear factor-kappa-B-inhibitor alpha, suggesting that miR-196a functions as an oncogene in PC [6].

MiR-203 has been proved to play an important role in pancreatic cancer cell proliferation, migration and invasion [7, 8]. Some studies have shown the prognostic role of tissue miR-203 in PC. However, whether the aberrant expression of serum miR-203 has any clinical significance was unknown. Hence the aim of our study was to explore the prognostic significance of serum miR-203 in PC.

Table 1. Relations between serum miR-203 levels and clinical characteristics of PC

Variables	Cases	Serum miR-203 expression		*P*
		Low	High	
Age				0.1571
<60	42	24	18	
≥60	54	23	31	
Gender				0.5601
Male	58	27	31	
Female	38	20	18	
CEA				0.4261
<4.3 μg/ml	31	17	14	
≥4.3 μg/ml	65	30	35	
CA19-9				0.2149
<37 U/mL	45	19	26	
≥37 U/mL	51	28	23	
N Stage				0.0038**
N0	53	33	20	
N1	43	14	29	
Histological grade				0.0319*
Well or moderate	59	34	25	
Poor	37	13	24	
Tumor diameter (cm)				0.5455
<2.5	46	24	22	
≥2.5	50	23	27	
TNM stage				0.0002***
I+II	41	29	12	
III+IV	55	18	37	

Footnote: *P<0.05, **P<0.01, ***P<0.001.

Materials and methods

Patients

In this study, 96 PC patients, 20 patients with benign pancreatic tumor and 30 patients with chronic pancreatitis who were treated in our department were enrolled. The PC patient group comprised 58 men and 38 women. The cancer stage was determined according to the TNM classification system of the International Union against Cancer (UICC). The clinicopathological parameters of tumor collection were listed in **Table 1**. The benign pancreatic tumor group comprised 14 male and 6 female aged between 27 to 61 years old. The chronic pancreatitis group comprised 19 male and 11 female aged between 31 to 64 years old. The thirty healthy controls aged between 18 to 55 years old were also recruited. The study was approved by Shanxi Cancer Hospital and the written consent was received from all the participants.

Serum sample collection

Blood samples were taken from patients before accepting any type of treatment as well as from healthy volunteers. Up to 6 mL of fasting venous blood was withdrawn from all the participants. All samples were processed within 1 h after collection and separated by centrifugation (15 min, 3000 × g). Then the separated serum was divided into aliquots and stored in cryotubes at -80°C for further use.

Quantitative real-time RT-PCR

Total RNA was isolated from 200 μL serum by using the miRVana PARIS kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. RNA purity was evaluated by the A260/A280 ratio using nano-spectrophotometer, and was inversely transcribed to first strand cDNA using the miRcute miRNA First-Strand cDNA Synthesis Kit (Tiangen Biotech, Beijing, China). Quantitative real-time PCR was carried out with the Maxima SYBR Green qPCR Kit (Thermo Scientific, CA, USA) and run on a Roche LC480II lightcycler (Roche, Indianapolis, IN, USA). Each sample was examined in triplicate. MiR-203 expression levels were normalized to those of RNU6B and relative quantification values were calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All statistical analyses were processed using GraphPad Prism 6 (GraphPad Software Inc., San Diego, California, USA). One way ANOVA was performed to evaluate the difference in serum miR-203 expression levels among different groups. Chi-square test was used to compare the categorical variables. Kaplan-Meier survival analysis plus a log rank test was used to analyze the association between serum miR-203 expression levels and overall survival (OS) as well as disease free survival (DFS). All data were presented as mean ± S.D. Statistical significance was defined as a P value less than 0.05.

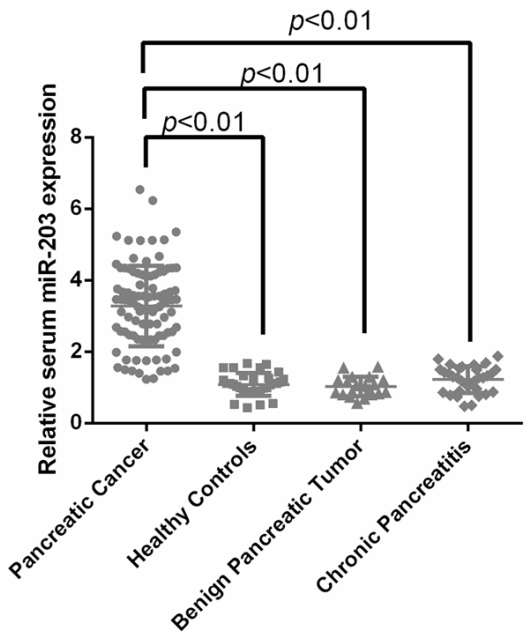

Figure 1. Serum miR-203 was upregualted in patients with PC.

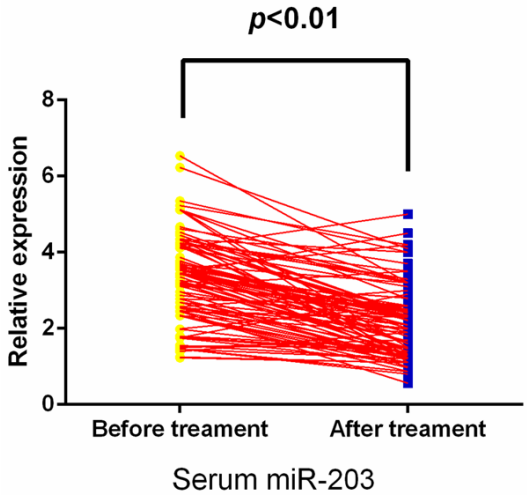

Figure 2. Serum miR-203 levels are sensitive to therapeutic response.

Results

Serum miR-203 expression was significantly upregulated in PC patients

The expression level of serum miR-203 was detected by using qRT-PCR. Our results showed that serum miR-203 expression was remarkably overexpressed in PC patients when compared with patients with benign pancreatic tumor, patients with chronic pancreatitis and the healthy controls (P<0.01). However, no significant difference in serum miR-203 levels was found among patients with benign pancreatic tumor, patients with chronic pancreatitis and the healthy controls (P>0.05) (**Figure 1**). Moreover, the expression level of miR-203 in PC patients was significantly decreased after receiving surgical resection (P<0.01), suggesting that serum miR-203 level was very sensitive to therapeutic responses (**Figure 2**).

Correlation between serum miR-203 and clinical variables of PC

We divided the PC patients into two groups based on the mean expression level of serum miR-203. The high serum miR-203 expression group had 49 cases while the low serum miR-203 expression group had 47 cases. Serum miR-203 expression level was strongly correlated with several important clinical characteristics including N stage (P=0.0038), histological grade (P=0.0319) and TNM stage (P=0.0002). However, it was not associated with gender (P=0.5601), age (P=0.1571), carcinoembryonic antigen (CEA) (P=0.4261), carbohydrate antigen 19-9 (CA19-9) (P=0.2149) and tumor diameter (P=0.5455) (**Table 1**).

The association between serum miR-203 expression and survival rates

The PC patients in high serum miR-203 expression group had a significantly shorter 5 year OS than those in the low serum miR-203 expression group (P=0.016) (**Figure 3A**). In addition, the PC patients with lower serum miR-203 expression had more favorable DFS (P=0.004) compared to those with higher serum miR-203 expression, indicating high serum miR-203 level was associated with poor prognosis of PC (**Figure 3B**).

Serum miR-203 was an independent prognostic marker in PC patients

Our multivariate analysis found out that TNM stage (OS:RR=4.53, 95% CI=1.56-7.85, P=0.003; DFS:RR=4.87, 95% CI=1.65-8.14, P=0.001), N stage (OS:RR=2.78, 95% CI=1.26-4.32, P=0.038; DFS:RR=2.65, 95% CI=1.21-4.45, P=0.032) and serum miR-203 expression

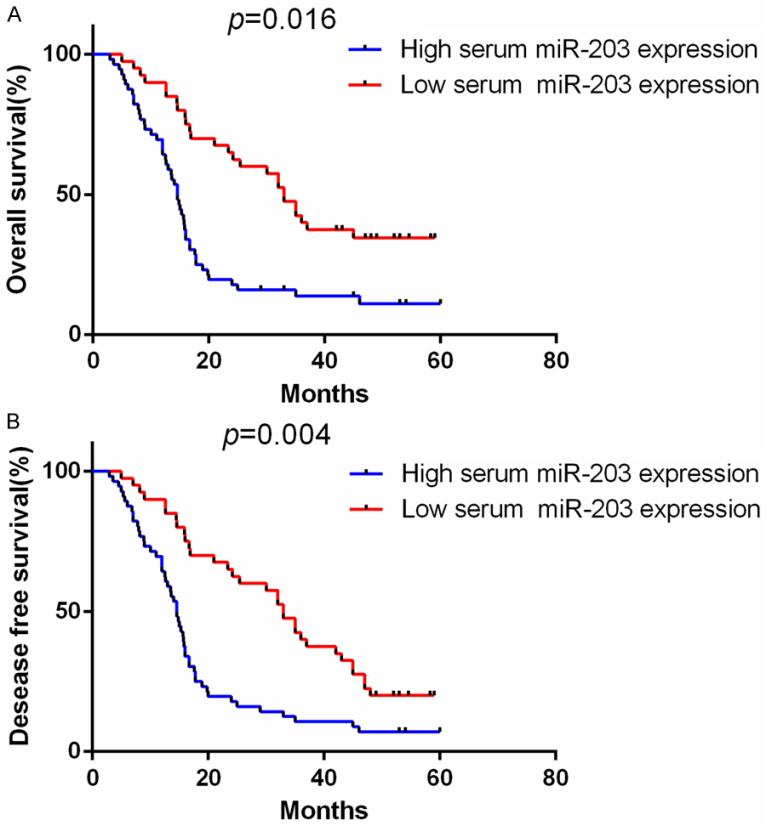

Figure 3. The association between serum miR-203 expression and survival rates.

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

Variables	Overall survival			Disease free survival		
	Relative risk	95% CI	*P*	Relative risk	95% CI	*P*
N stage	2.78	1.26-4.32	0.038	2.65	1.21-4.45	0.032
TNM stage	4.53	1.56-7.85	0.003	4.87	1.65-8.14	0.001
Serum miR-203	3.26	1.34-5.13	0.019	3.79	1.58-5.98	0.015

(OS:RR=3.26, 95% CI=1.34-5.13, *P*=0.019; DFS:RR=3.79, 95% CI=1.58-5.98, *P*=0.015) were independent risk factors for PC (**Table 2**).

Discussion

PC is a deadly disease and has become a significant public health issue worldwide [9]. Hence it is urgent to reveal the hidden mechanisms and offer efficient therapy for this malignancy. Although numerous studies have shown that many small molecules play key roles in the tumorigenesis of PC, no prognostic biomarkers with good performance are available for the clinical use.

In the current study, our results showed that serum miR-203 expression was increased significantly in PC patients. Furthermore, its expression level was positively associated with poor clinical outcome of PC, indicating miR-203 acted as an oncogene in PC. Consistent with the results in the previous study, Ikenaga et al reported that miR-203 was overexpressed in the pancreatic adenocarcinoma tissues compared with adjacent normal tissues as well as chronic pancreatitis samples. The PC patients with high tissue miR-203 expression had significantly shorter survival time than those with low tissue miR-203 expression [10]. Moreover, decreased expression of miR-203 inhibited the proliferation, migration and invasion of pancreatic cancer cells by degrading salt-inducible kinase 1 (SIK1) [11].

MiR-203 was also shown to play an oncogenic role in various types of cancers. High miR-203 expression level was able to predict the poor prognosis of breast cancer and ependymoma efficiently [12]. Overexpression of miR-203 promoted the oncogenic activities of ovarian cancer cells both *in vitro* and *in vivo*, and opposite results was observed when miR-203 was suppressed. Moreover, pyruvate dehydrogenase B (PDHB) was identified as a downstream target of miR-203 [13]. Serum miR-203 was remarkably enhanced in papillary thyroid carcinoma (PTC) patients with recurrence. In addition, high serum miR-203 was associated with poor recurrence free survival, suggesting serum miR-203 expression might be a promising prognostic factor for PTC [14]. He et al showed that miR-203 was upregulated in breast cancer

tissues and cell lines. MiR-203 downregulation suppressed colony formation, transformation and migration in breast cancer cells by targeting fibroblast growth factor 2 [15].

It is frequent to observe the phenomenon that a specific miRNA functions as an oncogene in some cancers, while acts as a tumor suppressor in other cancers. MiR-203 had been also proved to be a tumor suppressor gene in carcinogenesis. Wang et al showed that miR-203 expression was down-regulated in human hypopharyngeal cancer tissues. MiR-203 underexpression was inversely associated with cancer progression and lymph node metastasis. In addition, miR-203 suppression promoted cell viability, migration and invasion in hypopharyngeal cancer cells by enhancing the expression level of PDPN [16]. Similarly, Fu et al found that miR-203 expression was markedly reduced in colorectal cancer tissues compared with the normal tissues. Negative correlation between miR-203 levels and distant metastasis, lymph node metastasis, and TNM stage in colorectal cancer patients was observed [17]. MiR-203 exerts a tumor suppressor role in multiple myeloma by inhibiting expression of Bmi-1, which was a validated oncogene [18]. Therefore, the disputed features of miR-203 in different types of cancers reflect that the function of miR-203 might be closely linked to the tumor microenvironment.

In conclusion, this study demonstrates that serum miR-203 level is elevated in PC patients and correlated with unfavorable outcome of PC. Therefore, serum miR-203 might be a novel biomarker to predict the prognosis of PC patients.

Disclosure of conflict of interest

None.

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