The early diagnostic value of combined detection of plasma miR-181b, miR-196a and miR-210 in pancreatic cancer

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Abstract

Background This study aimed to investigate the effect of combination of plasma miR-181b, miR-196a and miR-210 on early diagnosis of pancreatic cancer (PC).Methods In our study, the plasma was isolated from patients with PC and healthy individuals, respectively. The expressions of miR-181b, miR-196a and miR-210 were detected by qRT-PCR. Moreover, the level of carbohydrate antigen 199 (CA199) was measured by electrochemiluminescence (ECL) assay. Furthermore, the area under the receiver operating characteristic (ROC) curve (AUC) was used to analyze the diagnostic efficacy of miR-181b, miR-196a, miR-210 and CA199, as well as the combination of thses miRNAs in early PC patients and healthy individuals.Results The expressions of miR-181b, miR-196a and miR-210 were significantly upregulated in PC patients. In addition, the level of CA199 was also significantly upregulated in the plasma of PC patients. The expressions of miR-181b, miR-196a and miR-210 were closely associated with lymph nodes metastasis, clinical stage and vascular invasion, but had no correlation with the patient's age, gender and tumor size. Moreover, miR-181b, miR-196a and miR-210 have lower AUC than CA199 in PC patients. The combinations miR-181b + miR-196a, miR-181b + miR-210, miR-196a + miR-210 also have lower AUC than CA199 in PC patients. It is worth noting that the combinations miR-181b + miR-196a + miR-210 have higher AUC than CA199 in PC.Conclusions Our study demonstrated that the combination of plasma miR-181b, miR-196a and miR-210 had good value for PC early diagnosis.

Background

Pancreatic cancer (PC), one of the most common gastrointestinal malignancies and ranks the sixth leading cause of cancer-related deaths in our country. [1,2] Because PC lacks early specific clinical symptoms, most patients are already in advanced stages when diagnosed with PC. [3] Although the therapeutic techniques have greatly improved, the survival rate of PC patients still remains poor. [4] The median survival time of PC is about 5 to 8 months, and the 5-year survival rate is only about 4%. [5] Therefore, identifying specific biomarkers is very important for the early diagnosis of PC, especially for the early stage of tumors.

microRNAs (miRNAs) are a class of small endogenous noncoding RNAs with 18-25 nucleotides in length, which modulate gene expression at post-transcriptional level. [6,7] More and more evidences indicated that miRNAs exert important roles in the oncogenesis and metastasis of numerous tumors, [8-10] thus the alteration of certain miRNAs provides biomarkers to detect early tumors. Under normal physiological conditions, miRNAs levels are stable in the plasma and serve as diagnostic biomarkers. The abnormal expression of miRNAs has been detected in various tumors. Previous studies have indicated that plasma miR-10b, miR-21-5p, miR-30c and miR-106b are highly expressed in PC patients, suggesting that those miRNAs may have potential to be used as new biomarkers for PC screening. [11] It is reported that fecal miR-181b, miR-196a and miR-210 are highly expressed in PC patients, suggesting that those miRNAs may be used as biomarkers for PC diagnosis. [12] However, the diagnostic value of the combinations of miR-181b, miR-196a and miR-210 in the plasma of PC has not been fully elucidated.

In this study, the effects of plasma miR-181b, miR-196a and miR-210 in the early diagnosis of PC were investigated. The expressions of plasma miR-181b, miR-196a and miR-210 were measured by qRT-PCR. The level of classic tumor markers including carbohydrate antigen 199 (CA199) was detected by electrochemiluminescence assay. In addition, the receiver operating characteristic curve (ROC) was used to reveal the effect of plasma miR-181b, miR-196a and miR-210 in clinical diagnose value of PC. We hoped to explore the detail molecular mechanism of plasma miR-181b, miR-196a and miR-210 in the early diagnosis of PC.

Methods

Clinical samples

From March 2016 to January 2019, a total of 40 plasma samples were collected from PC patients (male 22 and female 18) at our hospital. Normal plasma samples (n = 40) were collected from healthy volunteers (male 22 and female 18). None of the patients and volunteers received chemotherapy and radiotherapy. The characteristics of PC patients and healthy volunteers were presented in Table 1. This study was approved by the Ethics Committee of our hospital and it was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient and volunteers.

Real-Time fluorogenic PCR assays

Total RNA from plasma of PC patients and healthy volunteers was extracted using TRIZOL (Invitrogen, USA). Then, cDNA was synthesized from total RNA using Revert Aid First Strand cDNA Synthesis Kit (Thermo, USA). Subsequently, the qRT-PCR was performed using SYBR green qPCR Master Mix (Thermo Scientific, USA) according to the manufacturer's protocol. Primers used in this study were as follows: miR-181b (forward): 5'-GCCGTAAAGTGCTGACAGT-3', (reverse): 5'-GTGCAGGGTCCGAGGTAT-3'; miR-196a (forward): 5'-GAAGATCTTTCCTTGGCGGCGACA-3', (reverse): 5'-CCCAAGCTTGATGGCCCGCCTA-3'; miR-210 (forward): 5'-TATACAAGGGCAAGCTCTCTGT-3', (reverse): 5'-AGAGAGCTTGCCCTTGTATATT-3'; U6 (forward): 5'-CTCGCTTCGGCAGCACA-3', (reverse): 5'-AACGCTTCACGAATTTGCGT-3'.

Electrochemiluminescence assay

The plasma of PC patients and healthy volunteers was collected. The level of CA199 were measured by using electrochemical luminescence apparatus (CobasE601, Roche, Sweden). The detection threshold of CA199 was 27 U/mL.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 statistical software (Chicago, IL). The results were presented in the form of mean ± standard deviation (SD). The two-tailed t test was used for comparison between two groups, while one-way ANOVA was selected for comparison among multiple groups. The chi-square test was used to compare the counting data groups. The clinical diagnostic value of miRNA

detection was analyzed by the area under the ROC curve (AUC). P < 0.05 was considered to be statistically significant.

Results

The levels of miR-181b, miR-196a, miR-210 and CA199 are upregulated in PC patients

The results of qRT-PCR showed that the levels of miR-181b, miR-196a and miR-210 in the plasma of PC patients were higher than those in healthy volunteers (P < 0.001) (Figure 1A-C). In addition, we also found that CA199 level in the plasma of PC patients was markedly higher than that in healthy volunteers (P < 0.001) (Figure 1D). All the results suggested that the levels of miR-181b, miR-196a, miR-210 and CA199 are upregulated in PC patients.

The expressions of miR-181b, miR-196a and miR-210 are associated with clinical parameters in PC

The expressions of miR-181b, miR-196a and miR-210 were markedly correlated with lymph nodes metastasis (P < 0.001, P < 0.01 and P < 0.01, respectively), clinical stage (P < 0.01, P < 0.05 and P < 0.01) and vascular invasion (P < 0.001, P < 0.01 and P < 0.01) (Table 2). However, the expressions of miR-181b, miR-196a and miR-210 had no significantly correlation with age, gender and tumor size in PC patients (P > 0.05) (Table 2).

Plasma miR-181b, miR-196a and miR-210 have diagnostic efficacy in the early diagnosis of PC

To further investigate the diagnostic value of these enrolled plasam markers, an integrated ROC curve analysis based on the combined ratio of miR-181b, miR-196a, miR-210, miR-181b + miR-196a, miR-181b + miR-210, miR-196a + miR-210, miR-181b + miR-196a + miR-210 and CA199 was performed on PC (Fig. 2A-H). The results showed that miR-181b, miR-196a and miR-210 have lower diagnostic value (AUC) than CA199 in PC patients (Table 3) (all P < 0.0001). In addition, the combinations miR-181b + miR-196a, miR-181b + miR-210, miR-196a + miR-210 also have lower AUC than CA199 in PC patients (Table 4) (all P < 0.0001). It is worth noting that the combination of plasma miR-181b + miR-196a + miR-210 have higher AUC than CA199 in PC patients (Table 4) (P < 0.0001). Furthermore, the combination of plasma miR-181b + miR-196a + miR-210 significantly improved the value of each indicator in individual diagnosis (sensitivity: 95.00, specificity: 97.50) (Table 4).

Discussion

In recent years, PC has been a therapeutic challenge due to difficulties and delays in diagnosis, which leads to its poor prognosis. Up to now, the main treatment methods for PC are surgical operation, chemotherapy and radiotherapy. Nevertheless, The median survival time of PC is about 5 to 8 months, and the 5-year survival rate is only about 4%. [5] In this study, we explored the early diagnostic value of plasma miR-181b, miR-196a and miR-210 in PC patients and found that combined detection of plasma miR-181b, miR-196a and miR-210 has early diagnostic value for PC.

Accumulating evidence has reported that a variety of miRNAs could play important roles in PC. [13-16] For example, miR-381, a tumor suppressor, inhibits cell proliferation, migration and invasion, and induces apoptosis in PC. [17] Zhou et al. have reported that miR-340 is downregulated in PC and could suppress PC cell growth and reduce tumor size. [18] Xu et al. [19] have indicated that miR-143 is lowly expressed in PC and could regulate PC process by promoting PC cell apoptosis and inhibiting migration and invasion. In addition, Xu et al. [19] have also confirmed that miR-143 expression is closely associated with the tumor size, clinical staging and lymph nodes metastasis. Previous researches have indicated that the expressions of miR-196a and miR-210 are upregulated in the plasma of PC patients, indicating miR-196a and miR-210 could serve as potential biomarker for PC diagnosis. [20] Ren et al. [12] have reported that fecal miR-181b, miR-196a and miR-210 are highly expressed in PC patients, suggesting that those miRNAs may be used as biomarkers for PC diagnosis. In the present study, we found that the expressions of miR-181b, miR-196a and miR-210 were closely associated with lymph nodes metastasis, clinical stage and vascular invasion, but had no correlation with the patient's age, gender and tumor size. Therefore, we speculate that the combinations of miR-181b, miR-196a and miR-210 may be used as biomarkers for PC diagnosis.

Recently, some diagnostic biomarkers such as CA199, CA242, MIC-1 and Lectins can be detected in the plasma of PC patients and have been accessed for PC diagnosis. [21-24] However, CA199 is the best available biomarker among the diagnostic biomarkers. [21,25,26] In the present study, the level of CA199 was significantly upregulated in the plasma of PC patients. Up to now, more and more studies have indicated that the combination of plasma miRNAs with CA199 is effective for diagnosis and can distinguish PC. [21,27] However, CA199 is not a reasonable marker for early diagnosis of PC. Thus, it is important to explore the more effective diagnostic biomarkers for PC. In this study, we demonstrated that the expressions of miR-181b, miR-196a and miR-210 were significantly upregulated in PC patients. In addition, the results of the receiver operating characteristic (ROC) curve showed that miR-181b, miR-196a and miR-210 have lower AUC than CA199 in PC. The combinations miR-181b + miR-196a, miR-181b + miR-210, miR-196a + miR-210 also have lower AUC than CA199 in PC. It is worth noting that the combinations miR-181b + miR-196a + miR-210 have higher AUC than CA199 in PC. Moreover, miR-181b + miR-196a + miR-210 significantly improved the value of each indicator in individual diagnosis.

Conclusions

In conclusion, our work confirmed that the expressions of miR-181b, miR-196a and miR-210 were significantly upregulated in PC patients. In addition, the present study also demonstrated that the combination of plasma miR-181b, miR-196a and miR-210 had good value for PC early diagnosis. Our research provides new theoretical foundation for investigating novel biomarkers for PC early diagnosis.

Abbreviations

Pancreatic cancer, PC

microRNAs, miRNAs

receiver operating characteristic curve, ROC

Declarations

Ethics approval and consent to participate: This study was conducted after obtaining Dongying People's Hospital's ethical committee approval and written informed consent from the patients.

Consent for publication: Not applicable.

Availability of data and material: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: GPL designed and analyzed the experiment, and was a major contributor in writing the manuscript. CHS, AYL, XBZ, XJG and JGL performed the experiment. All authors read and approved the final manuscript.

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Tables

Table 1 Clinical characteristics of the patients with pancreatic cancer (PC) and healthy volunteers

	Healthy（N = 40）	Pancreatic cancer（N = 40）	P
Age (y)	60.20 ± 10.70	58.25 ± 9.67	
< 60	17	21	0.3376
≥ 60	23	19	
Gender			
Male	22	22	0.5809
Female	18	18	
TNM stage			
Ⅰ	-	9	0.2312
Ⅱ	-	11	
Ⅲ	-	13	
Ⅳ	-	6	
CA199(U/mL)	15.69 ± 11.37	139.40 ± 129.40	P < 0.001

Table 2 Correlation between the expression of miR-181b, miR-196a and miR-210 and clinical indicators in PC patients

Clinical indicators	case	miR-181b expression	P value	miR-196a expression	P value	miR-210 expression	P value
Age (y)	40						
< 60	21	1.940±0.833	0.7603	2.040±0.808	0.9349	2.020±0.851	0.9901
≥ 60	19	1.860±0.810		2.020±0.722		2.02±0.861	
Gender							
Male	22	1.830±0.773	0.5477	1.940±0.692	0.4376	1.930±0.796	0.4633
Female	18	1.990±0.895		2.130±0.840		2.130±0.911	
Lymph nodes metastasis							
Yes	23	2.490±0.618	0.0010***	2.290±0.618	0.0008**	2.050±0.511	0.0013**
No	17	1.647±0.635		1.428±0.356		1.528±0.355	
clinical stage							
□-□	21	2.330±0.768	0.0048**	2.390±0.698	0.0319*	2.520±0.730	0.0027**
□-□	19	1.620±0.630		1.820±0.611		1.670±0.623	
Vascular invasion							
Yes	20	2.260±0.594	0.0002***	2.340±0.479	0.0016**	2.230±0.533	0.0019**
No	20	1.550±0.369		1.728±0.593		1.628±0.348	
Tumor size (cm)							
< 2	14	1.930±0.884	0.9703	2.040±0.838	0.9779	2.050±0.973	0.9722
2-4	13	1.930±0.818		2.050±0.723		2.030±0.801	
> 4	13	1.860±0.826		1.990±0.764		1.980±0.0.804	

Notes: $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 3 The detail information for the clinical diagnostic value of plasma markers in patients with PC

or markers	Cut off	Sensitivity (%)	Specificity (%)	AUC (Area±Std. Error)	95% CI	P value
ıiR-181b	1.45	77.50	85.00	0.789 ± 0.055	0.681-0.898	< 0.0001
ıiR-196a	1.56	72.50	92.50	0.865 ± 0.044	0.779-0.951	< 0.0001
ıiR-210	1.46	82.50	80.00	0.834 ± 0.045	0.745-0.923	< 0.0001
CA199	41.33	82.50	99.00	0.947 ± 0.023	0.902-0.993	< 0.0001

Notes: AUC, area-under-the-curve; CI, confidence intervals.

Table 4 The detail information for the clinical diagnostic value of plasma markers in patients with PC

Tumor markers	Sensitivity (%)	Specificity (%)	AUC (Area±Std. Error)	95% CI	P value
miR-181b + miR-196a	92.50	90.00	0.944 ± 0.029	0.887-1.001	< 0.0001
miR-181b + miR-210	80.00	70.00	0.830 ± 0.045	0.743-0.917	< 0.0001
miR-196a + miR-210	87.50	80.00	0.888 ± 0.042	0.805-0.970	< 0.0001
miR-181b + 196a+210	95.00	97.50	0.968 ± 0.022	0.924-1.011	< 0.0001
CA199	82.50	99.00	0.947 ± 0.023	0.902-0.993	< 0.0001

Notes: AUC, area-under-the-curve; CI, confidence intervals.

Figures

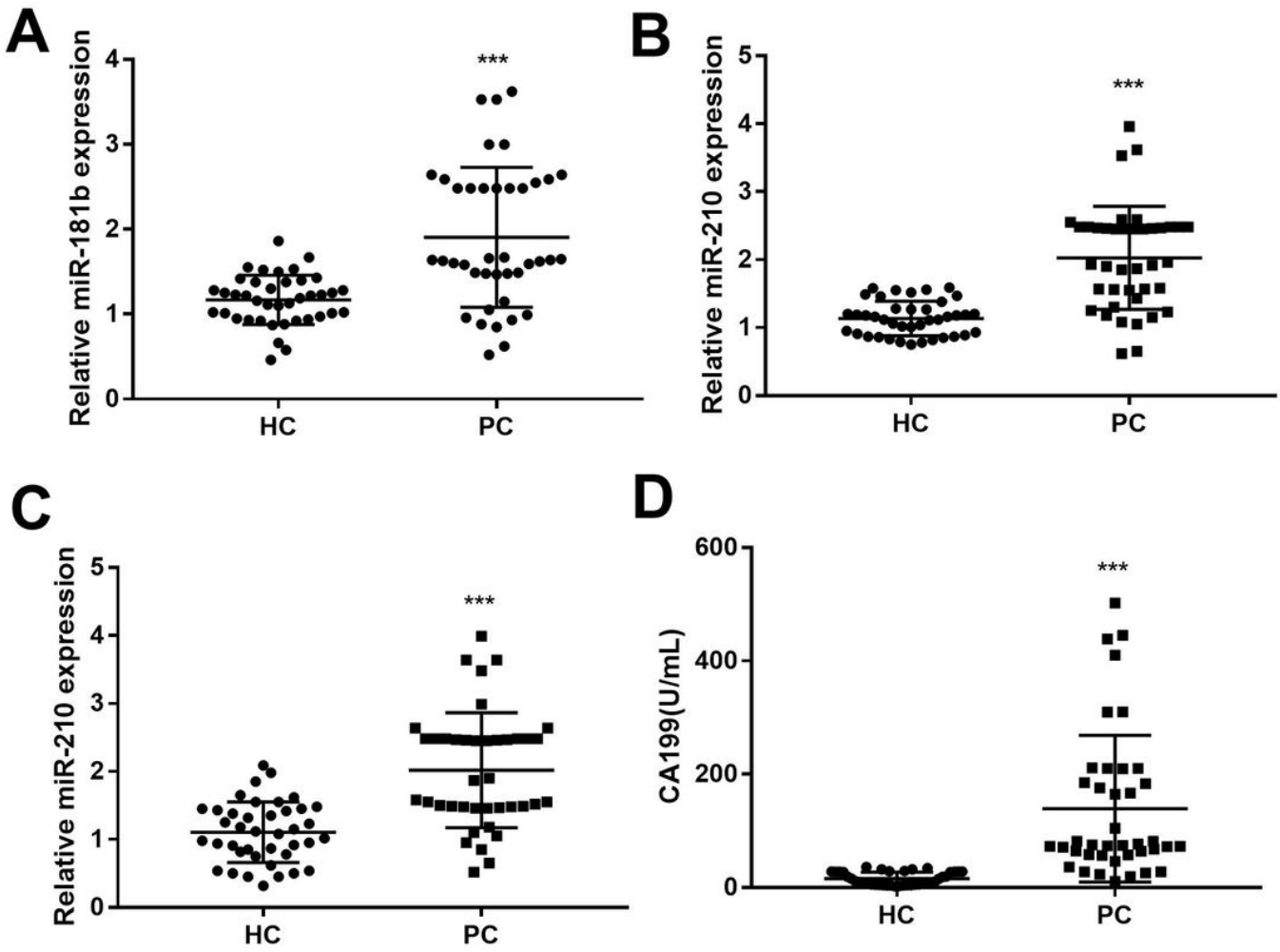

Figure 1

The expressions of plasma miR-181b, miR-196a, miR-210 and CA199 in pancreatic cancer (PC) patients and healthy volunteers. (A) The expression of plasma miR-181b was detected by qRT-PCR. (B) The expression of plasma miR-196a was detected by qRT-PCR. (C) The expression of plasma miR-210 was detected by qRT-PCR. (D) The level of plasma CA199 was detected by electrochemiluminescence assay. ***P < 0.001, vs. HC group.

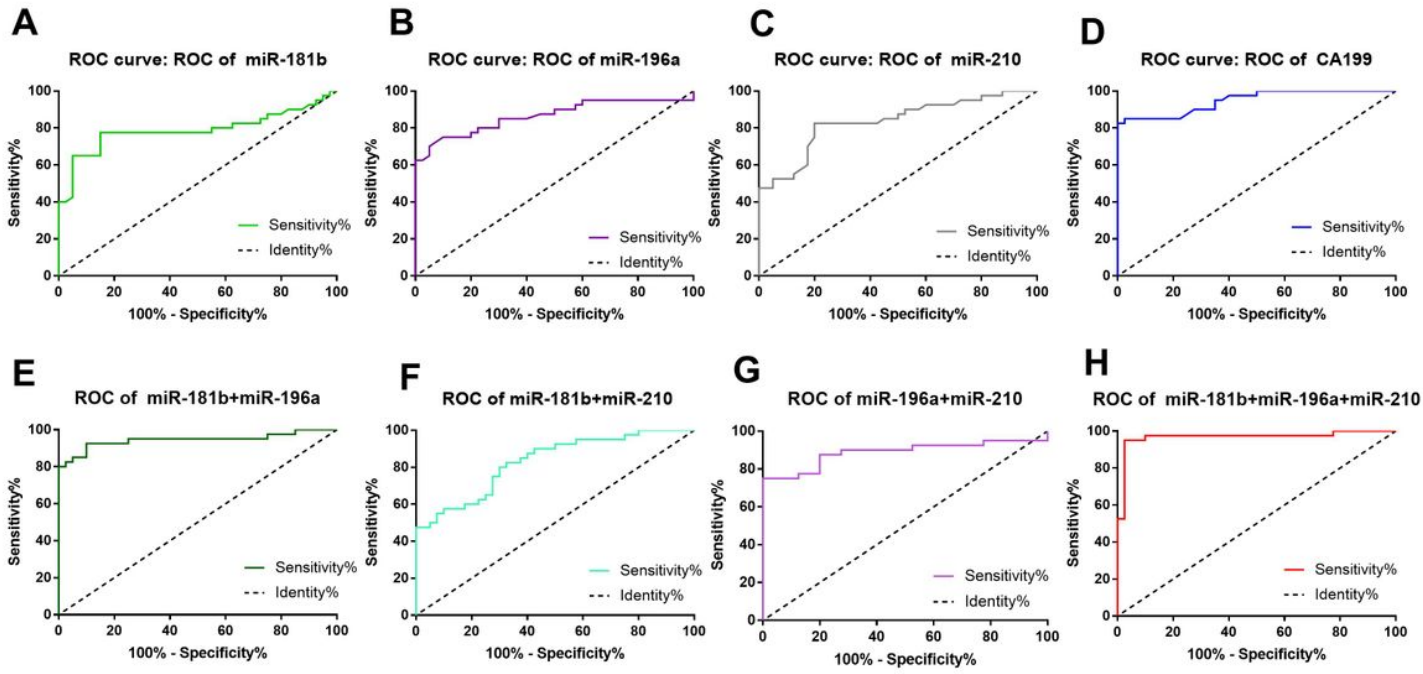

Figure 2

ROC curve of plasma miR-181b, miR-196a, miR-210 and CA199 in patients with PC. (A) The ROC of miR-181b. (B) The ROC of miR-196a (C) The ROC of miR-210. (D) The ROC of CA199. (E) The ROC of miR-181b + miR-196a. (F) The ROC of miR-181b + miR-210. (G) The ROC of miR-196a + miR-210. (H) The ROC of miR-181b + miR-196a + miR-210. $P < 0.05$ was considered as statistically significant.