MiR-504 and miR-205 May be Used to Evaluate Prognosis and Chemotherapeutic Effect of Pancreatic Cancer Patients

LE WANG, YINGHUI SONG, SULAI LIU, HONGYING TANG, ZHIHUA ZHANG, CHUANG PENG*, BO JIANG*

Department of Hepatobiliary Surgery, Hunan Provincial People's Hospital & The First Affiliated Hospital of Hunan Normal University, Changsha 410006, P.R.China

Abstract: To explore the value of miR-504 and miR-205 in evaluating the prognosis and chemotherapeutic effect of pancreatic cancer (PC) patients. Ninety-six PC patients who came to our hospital from March 2015 to February 2017 were selected as the research group (RG), and 96 healthy subjects during the same period were considered as the control group (CG). Serum miR-504 and miR-205 of subjects in the two groups were detected and compared by Rt-PCR, and the miR-504 and miR-205 expression levels in serum of PC patients before and after chemotherapy were analyzed, so did the relationship between miR-504, miR-205 and prognosis of patients. The miR-504 and miR-205 expression levels in serum of PC patients were dramatically higher than those of healthy people (P< 0.05), and the expression levels in serum of those in the chemotherapy-effective group were dramatically lower than those of those in the chemotherapy-ineffective group (P< 0.05). Moreover, the 3-year mortality of patients with high expression of miR-504 and miR-205 was higher than that of those with low expression (P< 0.05), and the miR-504 and miR-205 expression levels in dead patients were higher than those in living patients. miR-504 and miR-205 had high predictive value for poor prognosis of PC patients. Univariate analysis identified that their poor prognosis was related to pathological stage, lymph node metastasis, differentiation degree and expression of miR-504 and miR-205 (P< 0.05). Cox multivariate analysis identified that pathological stage, lymph node metastasis, differentiation degree and expression of miR-504 and miR-205 were independent risk factors for poor prognosis (P< 0.05).DLX6-AS1 is highly expressed in the serum of PC patients, and it can evaluate the chemotherapeutic effect and predict the prognosis. So, it may be used as a new target direction for evaluating the chemotherapeutic effect and predicting the prognosis of patients.

Keywords: miR-504, miR-205, pancreatic cancer, expression, prognosis, chemotherapeutic effect

1.Introduction

Pancreatic cancer (PC) is a high malignancy disease. The prognosis of PC patients has been weak for a long time, and the 5-year survival rate is less than 5% [1]. At present, radical resection is the first choice for those patients. However, due to the low rate of PC's early diagnosis, many patients are already in an advanced stage at diagnosis, and they, therefore, lost the opportunity of surgery but can only take conservative treatment methods such as radiotherapy and chemotherapy [2, 3]. Chemotherapy, as a standard treatment method for clinical tumors, has vital clinical significance for improving the quality of life and prognosis of PC patients [4]. However, due to the significant individual differences of patients and PC's rapid progression, it is difficult for them to obtain satisfactory results even after undergoing chemotherapy [5]. Hence, how to improve the chemotherapeutic effect and implement a comprehensive treatment plan according to patients' specific conditions are also crucial research directions at the moment.

microRNA (miRNA) is a non-coding short-chain RNA, which regulates target genes after transcription to exert its effect on cells [6]. Recently, more and more attention has been paid to miRNA's role in tumors. Its abnormality and expression are relevant to the development and progression of many tumors [7]. miR-504, as a member of miRNA, has been reported to be effective in various tumors

*email: pengchuangcn@163.com; hepatojiangbo@163.com

previously. For example, research [8] reported that it could inhibit the proliferation and invasion of non-small cell lung cancer cells by targeting LOXL2. Recent studies [9] also found that it acted as an oncogene in PC. In the past studies, miR-205 was found to be up-regulated in PC tissues and cells through microarray analysis, and it was also believed to function as an oncogene in PC [10]. However, there are few studies on the clinical significance of miR-504 and miR-205 in PC patients before and after chemotherapy and their prognostic value.

In order to better evaluate the chemotherapeutic effect of PC patients to predict their prognosis and provide reference indicators for the adjustment of clinical treatment programs, we analyzed the changes of miR-504 and miR-205 before and after chemotherapy and the predictive value of prognosis.

2. Materials and methods
2.1. Clinical data
Ninety-six PC patients who visited our hospital from March 2015 to February 2017 were selected as the research group (RG), including 51 males and 45 females. Furthermore, they were (53.75±3.62) years old on average. Meanwhile, 96 healthy subjects were enrolled as the control group (CG). Inclusion criteria: patients were diagnosed as PC by pathological diagnosis. Exclusion criteria: patients received surgery or radiotherapy and chemotherapy before; patients complicated with other malignancy diseases, serious immune system diseases, or severe liver and kidney dysfunction; all the subjects and their families agreed to take part in the test and signed an informed consent form. The Hospital Ethics Committee approved the test.

2.2. Chemotherapy protocol and efficacy evaluation
All the patients received chemotherapy based on the first-line drugs recommended by the national comprehensive cancer network (NCCN). The chemotherapy drug was gemcitabine. Moreover, the specific treatment plan was as follows: 1000 mg/m^2 gemcitabine intravenous drip, 30 min each time, once a week, three times in a row, rest for one week, four weeks as a course of treatment, six courses of continuous treatment. Efficacy evaluation: complete remission (CR): all target lesions disappeared, and tumor marker levels returned to normal; partial remission (PR): the total reduced area of the lesion was more than 30%; stable condition (SD): the focal area decreased by less than 30% or increased by less than 20%; progression of the disease (PD): the focal area increased $\geq$ 20%, and new lesions appeared. CR+PR+SD= effective number, SD= ineffective number.

2.3. qRT-PCR detects expression of miR-504 and miR-205
Altogether 5 mL venous blood of all subjects was taken on an empty stomach, and it was then centrifuged 5 min at 3000 r/min, and the supernatant was taken for detection. The total RNA was extracted by adding TRIzol (Beijing Baiolebo Technology Co., Ltd.) into the serum. Its purity, concentration and integrity were tested through ultraviolet spectrophotometer and agarose gel electrophoresis. cDNA reverse transcription was performed in view of the kit instructions (TransGen Biotech, Beijing, China). PCR amplification reaction conditions were as follows: pre-denaturation at 94 ℃ for 5 min, denaturation at 95℃ for 10 s, annealing extension at 6 ℃ for 20 s, 40 cycles in total. We employed U6 as an internal reference, the primer sequence was shown in Table 1, and the test was repeated three times.

Table 1 Primer sequence table

Factor	Upstream primer 5'-3'	Downstream primer 5'-3'
miR-504	GCTGCTGTTGGGAGACC	GCCCTCTGTATGGGAAAC
miR-205	TGGGCTGAGTCC CTCT	GAGGGACGGGGTGATGGGCAGA TTGG′
U6	GCTCGCTTCGGCAGCACAT	AAAATATGGAACGCTTCACG

2.4. Statistical methods

The collected data were statistically analyzed via SPSS19.0, and the required pictures were drawn via GraphPad 6. The counting data were expressed as percentages, the chi-square test was employed for analysis, and the measurement data were represented by mean±standard deviation. A comparison between the two groups was assessed via the independent-samples T-test, and survival was analyzed through the Kaplan-Meier curve. The risk factors for PC's poor prognosis were analyzed through the Cox regression model. There was a statistical difference when $P < 0.05$.

3. Results and discussions
3.1. Comparison of general data

There was no marked difference in gender, age, and BMI between both groups ($P > 0.05$), which was comparable (Table 2).

Table 2 General data table

Factor	RG (n=96)	CG (n=96)	X^2	P
Gender			0.021	0.885
Male	51 (53.13)	50 (52.08)		
Female	45 (46.88)	46 (47.92)		
Age (years)			0.021	0.885
≥ 53	52 (54.17)	53 (55.21)		
< 53	44 (45.83)	43 (44.79)		
BMI (kg/m^2)			0.021	0.885
≥ 23	47 (48.96)	48 (50.00)		
< 23	49 (51.04)	48 (50.00)		
Smoking history			0.021	0.883
Yes	40 (41.67)	39 (40.63)		
No	56 (58.33)	57 (59.38)		
Lymph node metastasis			-	-
Non-metastasis	38 (39.58)	-		
Metastasis	58 (60.42)	-		
Degree of differentiation			-	-
High	13 (13.254)	-		
Moderate	26 (27.08)	-		
Low	57 (59.38)	-		
Pathological staging			-	-
Stages I-II	35 (36.46)	-		
Stages III-IV	61 (63.54)	-		
Pathological typing			-	-
Ductal adenocarcinoma	79 (82.29)	-		
Acinic cell carcinoma	10 (10.42)	-		
Others	7 (7.29)	-		

3.2. Comparison of miR-504 and miR-205 expression levels

The serum miR-504 and miR-205 levels in the RG were dramatically higher than those in the CG, and the difference was statistically remarkable based on Figure 1 (P< 0.05).
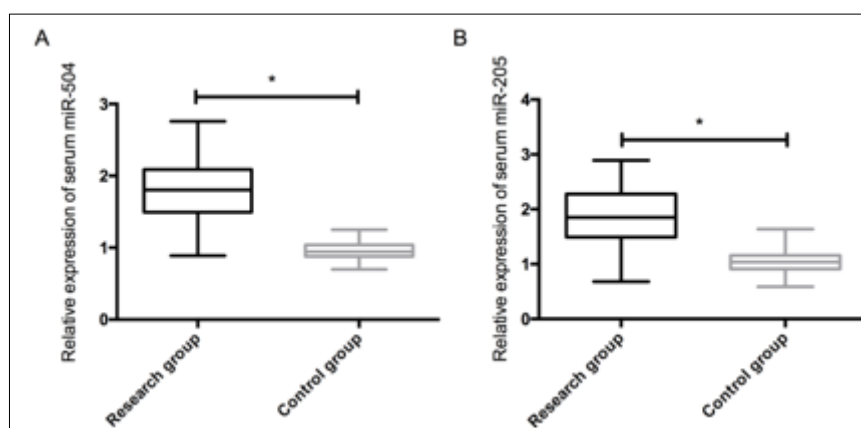

Figure 1. Comparison of miR-504 and miR-205 expression levels;
A: miR-504 is highly expressed in serum of PC patients;
B: miR-205 is highly expressed in serum of PC patients (* indicates P< 0.05)

3.3. Comparison of miR-504 and miR-205 expression levels in patients' serum before and after chemotherapy

After chemotherapy, we divided patients into the active group (EG, 71) (including CR, PR, and SD) and the ineffective group (IG, 25) (PD) based on their chemotherapeutic effect, and we compared their miR-504 and miR-205 expression levels in serum before and after chemotherapy. The results showed that there was no noticeable difference in the expression levels before chemotherapy (P> 0.05). Meanwhile, the expression levels of patients in the EG decreased dramatically (P< 0.05), and those of patients in the IG decreased slightly, but there was no marked statistical difference (P> 0.05), and the expression levels of those in the EG were dramatically lower than those in the IG based on Figure 2 (P < 0.05).
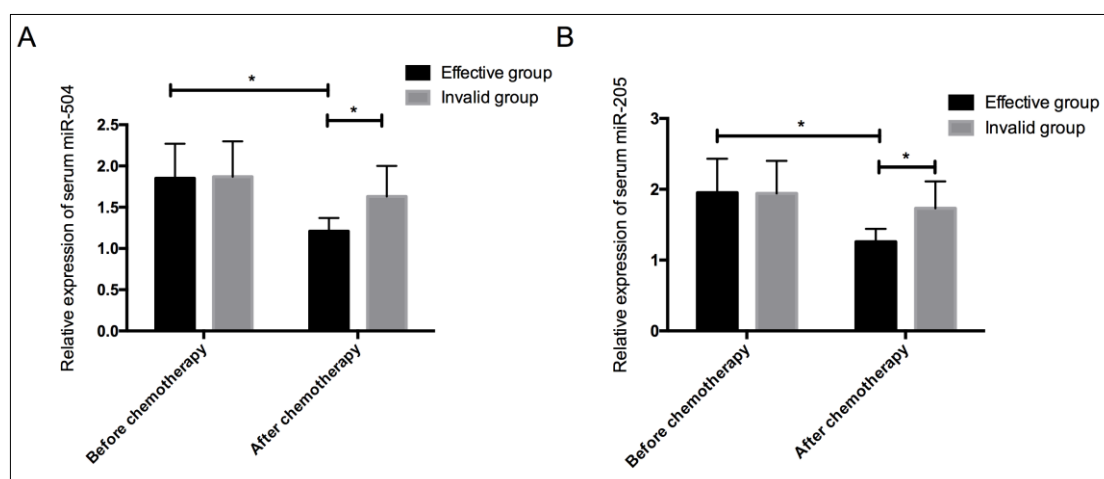

Figure 2. Comparison of miR-504 and miR-205 expression levels in patients'
serum before and after chemotherapy; **A:** comparison of miR-504 expression in patients'
serum before and after chemotherapy; **B:** comparison of miR-205 expression in patients'
serum before and after chemotherapy (* indicates P< 0.05)

3.4. Influence of miR-504 and miR-205 on survival rate and prognosis prediction of PC patients

Because of the mean expression of miR-504 and miR-205 in the serum of PC patients after chemotherapy, they were divided into miR-504 high expression group (HEG, 50 cases), miR-504 low expression group (LEG, 46 cases), miR-205 HEG (52 cases) and miR-205 LEG (44 cases). After analyzing the 3-year survival rate of all patients, we found that 45 in the miR-504 HEG died within three years, with a 3-year survival rate of 10.00%; in the LEG, 28 died within three years, about 37.5%; in the miR-205 HEG, 47 died within three years, about 13.46%; in the LEG, 26 died within three years, about 36.36%; in the miR-504 and miR-205 HEGs, the rate was dramatically lower than that in the LEG, with a statistically marked difference ($P < 0.05$). According to PC patients' prognosis, they were divided into the death group (DG, 73) and survival group (SG, 23), and the serum miR-504 and miR-205 expression levels of both groups were compared. The results manifested that the expression levels of those in the DG were dramatically higher than those in the SG. ROC analysis identified that miR-504 and miR-205 had high predictive value for poor prognosis, 0.786 and 0.830, respectively (Figure 3).
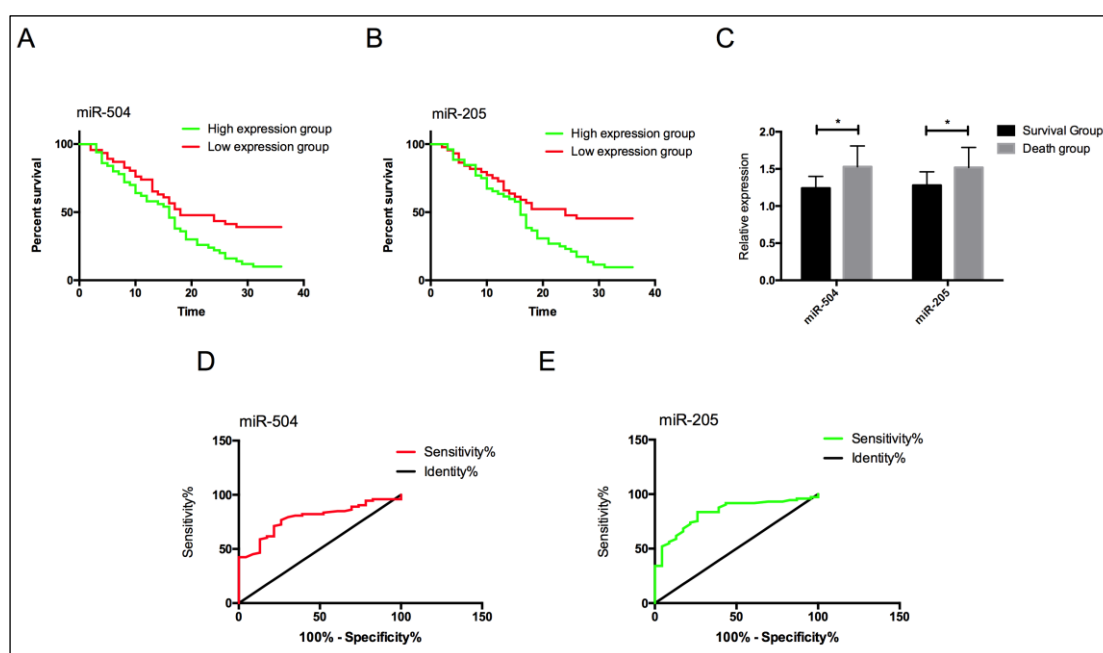

Figure 3. Effect of miR-504 and miR-205 on survival rate and prognosis prediction of PC patients; **A:** relationship between miR-504 and 3-year survival rate of PC patients; **B:** the relationship between miR-205 and 3-year survival rate of PC patients; **C:** expression of miR-504 and miR-205 in the serum of patients with different prognosis; **D:** ROC analysis of miR-504 for predicting poor prognosis of PC patients; **E:** ROC analysis of miR-205 in predicting poor prognosis of PC patients (* indicates $P < 0.05$)

3.5. Univariate analysis on the prognosis of PC patients

All the patients were divided into DG and SG in the light of their prognosis. Univariate analysis identified that tumor size, pathological stage, lymph node metastasis, differentiation degree, and miR-504 and miR-205 expression were tied to the prognosis of patients ($P < 0.05$), while age, BMI and prognosis were not related based on Table 3 ($P > 0.05$).

Table 3 Univariate analysis on prognosis of PC patients

Factor	DG n=73	SD n=23	X^2	P
Age (years)			0.048	0.826
≥ 53 (n=52)	40 (54.79)	12 (52.17)		

< 53 (n=44)	33 (45.21)	11 (47.83)			
BMI				0.732	0.392
≥ 23 (n=47)	36 (49.32)	11 (47.83)			
< 23(n=49)	37 (50.68)	12 (52.17)			
Pathological staging				33.29	<0.001
I, II（n=35）	15 (20.55)	20 (86.96)			
III-IV（n=61）	58 (79.45)	3 (13.04)			
Pathological typing				0.342	0.853
Ductal adenocarcinoma (n=79)	61 (83.56)	18 (78.26)			
Acinic cell carcinoma (n=10)	7 (9.59)	3 (13.04)			
Others (n=7)	5 (6.85)	2 (8.70)			
Degree of differentiation				24.30	<0.001
High (n=13)	3 (4.11)	10 (43.48)			
Moderate (n=26)	20 (27.40)	6 (26.09)			
Low (n=57)	50 (68.49)	7 (30.43)			
Lymph node metastasis				8.908	0.003
Non-metastasis(n=58)	38 (52.05)	20 (86.96)			
Metastasis (n=38)	35 (47.95)	3 (13.04)			

3.6. Multivariate analysis on the prognosis of PC patients

We set the pathological stage, lymph node metastasis, differentiation degree, and expression of miR-504 and miR-205 as independent variables and assign values. We employed a Cox regression analysis as dependent variable to carry out a multivariate analysis. The results manifested that those above were independent risk factors for poor prognosis of PC patients (Table 4).

Table 4 Multivariate analysis table

Factor	β	S.E	Wald	HR	95%CI	P
Pathological staging	0.342	0.164	5.472	1.468	1.014-1.883	< 0.05
Lymph node metastasis	0.578	0.121	9.483	2.692	1.776-4.314	< 0.05
Differentiationdegree	0.517	0.083	8.646	2.431	1.517-3.775	< 0.05
miR-504	0.481	0.152	7.811	1.983	1.261-2.911	< 0.05
miR-205	0.417	0.129	6.238	1.751	1.177-2.365	< 0.05

PC is a kind of tumor disease with a high degree of malignancy clinically. As a gradually developing multi-gene disease, its treatment is complicated due to the influence of chemotherapy drug resistance and tumor metastasis [11-12]. miRNA, as a small non-coding RNA, has been proved to be a potential therapeutic target in many tumors [13]. In PC, many miRNAs have been proved to be useful in its development and progression. For example, research [14] found that miR-9-5p inhibited the proliferation, invasion, and glutamine metabolism of PC cells by targeting GOT1.

miR-504, as a member of miRNA, has been shown to act as an oncogene in many tumors in the past [15, 16]. Previous studies [17] also showed that it was highly expressed in PC and might act as an oncogene. miR-205 is also a factor that has been proved to function as an oncogene in various tumors [18, 19]. Many studies have been reported on miR-205's role in PC, for example, studies [20] report that

it can promote the proliferation of PC cells by regulating APC. However, there are few studies on the clinical value of miR-504 and miR-205 in PC, and our study first analyzes the clinical significance of the two. First of all, we detected the miR-504 and miR-205 expression levels in the serum of PC patients, and we discovered that the expression levels of patients were dramatically higher than those of healthy people, which was also consistent with previous studies on their expression in PC.

For PC patients, chemotherapy plays a vital role in improving their quality of life. However, many patients are not sensitive to chemotherapy, which leads to reduced efficacy of traditional chemotherapy. Therefore, how to formulate a comprehensive and individualized treatment plan based on patients' situation is a clinical problem to be solved currently [21, 22]. Thus, we divided patients into EG and IG according to their chemotherapeutic effect, and we detected and compared the serum miR-504 and miR-205 before and after chemotherapy. The results displayed that the two patients in both groups before chemotherapy had no marked difference. However, those of patients in the EG were lower than those in the IG after chemotherapy, which suggested that they might become serum markers for evaluating the chemotherapy efficacy of PC. Subsequently, in order to further analyze the relationship between miR-504 and miR-205 and prognosis of PC patients, we divided them into high and low expression groups according to the mean expression of serum miR-504 and miR-205, and we compared their 3-year survival rates. The results showed that the 3-year survival rates of those with high expression of miR-504 and miR-205 were dramatically lower than those of those with low expression, and ROC analysis identified that miR-504 and miR-205 had high predictive value for poor prognosis. In the past, miR-205 was considered as a biomarker for predicting the prognosis of bladder cancer [23], and miR-504 was also considered to be tied to the poor prognosis of acute myeloid leukemia [24].

4. Conclusions

Our research suggested that the two might also be used as biomarkers for prognosis evaluation of PC. Finally, to further analyze the factors affecting PC's prognosis, we divided patients into SG and DG. Univariate analysis identified that pathological stage, lymph node metastasis, differentiation degree, miR-504, and miR-205 expression were related to the prognosis of PC patients. Then, further Cox analysis identified that those above were independent risk factors for their poor prognosis. Previous studies [25] found that pathological stage, lymph node metastasis, and differentiation degree were independent risk factors for their poor prognosis, which was consistent with our results. In our research, we first discovered that the high expression of miR-504 and miR-205 were also independent risk factors for poor prognosis. To sum up, miR-504 and miR-205 are highly expressed in the serum of PC patients, and they may be used as evaluation indicators of chemotherapeutic effect and have high predictive value for their prognosis, but further expansion of samples is still needed in the future.

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