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The role of microRNAs as biomarker in pancreas cancer

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Abstract

Objective: Pancreatic cancer (PC) is an aggressive cancer of the digestive tract Overall survival for patients with pancreatic cancer is poor mainly due to the lack of biomarkers that enable early diagnosis. Several studies have shown that miRNAs can act as potential biomarkers of PC. Our aim in this study was to investigate microRNAs as a biomarker in the early diagnosis of PC.

Patients and method: Nine patients diagnosed with pancreas cancer and nine healthy individuals of the same age and gender were selected as the control group. Six miRNAs (let-7c, miR-34a miR-125b, miR-141, miR-145, miR-155) selected and MiR 181 and miR 192 used as the endogenous control group.

Results: In our study, when compared with endogenous controls, Mir125b was found to be significantly upregulated, while Mir141 was found to be significantly downregulated. As conlusion; Mir125b and MiR-141 may have an important role in both the early diagnosis of PC. Further extensive studies are needed.

Keywords: Pancreas cancer; Biomarker MicroRNA; miR125b; miR141.

Introduction

Pancreatic cancer (PC) is an aggressive cancer of the digestive tract that has become a serious health problem worldwide. Overall survival for patients with PC is poor mainly due to the lack of biomarkers that enable early diagnosis [1]. PC has been associated with many somatic genetic abnormalities such as KRAS, CDKN2A/p16, TP53 and SMAD4. However, these genetic abnormalities do not provide benefits in the early diagnosis of PC. MicroRNAs (miRNAs) modulate proliferation in PC, some as oncomiRs to promote proliferation of PC and some as tumor suppressors of PC [2]. Thousands of miRNAs have been screened in PC and have been associated with disease proliferation, apoptosis, metastasis, chemosensitivity and radiosensitivity. Several studies have shown that miRNAs can act as potential biomarkers of PC [3,4]. Our aim in this study was to investigate microRNAs as a biomarker in the early diagnosis of pancreatic cancer.

Patients

Nine patients diagnosed with pancreas cancer and nine healthy individuals of the same age and gender were selected as the control group. After each individual included in the study was informed and signed a written consent form, 5cc EDTA blood samples were taken from the patients and the control group.

Methods

Approval was obtained from Faculty of Medicine Clinical Research Ethics Committee of XXXX University. Ethics committee approval number and date 471 and 17.08.2016.

Selected miRNAs

Six miRNAs (let-7c, miR-34a, miR-125b, miR-141, miR-145, miR-155) selected and MiR 181 and miR 192 used as the endogenous control group in line with their binding potentials and gene expression levels. MiRNA extraction and measurement from blood samples, the measurement methods of the four available miRNAs in patients and healthy individuals were performed as indicated in our previous study [5]. Blood samples were centrifuged for 15 minutes and plasma was separated. MiRNA isolation (Invitrogen by Thermo Fisher Scientific-mirVana™ miRNA Isolation Kit) was performed from plasma. The obtained miRNA was measured in ng / µl on the QUBIT 3 FLUOROMETER device (Invitrogen by Thermo Fisher Scientific-Qubit™ microRNA Assay Kit). The miRNA samples, whose concentration was found to be suitable, were obtained by using Thermal Cycler (Applied Biosystems by Life Technologies-TaqMan Advanced miRNA c DNA Synthesis Kit). cDNAs were kept at -20°C until the sufficient number was reached in two formats, 30 and 50. Gene expression levels of the component and cDNA prepared with a total volume of 20 µl in each well were measured with StepOne™ Real-Time PCR (Catalog No: 4376357 Thermo Fisher) device. Cт values automatically taken from the system are reported in the excel file. The average CT values of the duplicated samples were compared with the control group miR 181 and miR 192, and the ΔCт values were calculated. At the end of the study, the ΔCт values of individuals with colon cancer were compared with the ΔCтs of the healthy control group [5]. Statistical Method: The data were evaluated using the SPSS (Statistical Package for the Social Sciences) version 23.0 (SPSS Inc., Chicago, IL, USA) program. Descriptive findings are presented with number, percentage, mean ± standard deviation and median. Shapiro-Wilk test and skewness/kurtosis values were used to evaluate whether the data represented normal distribution. Independent samples "t" test was used if the data conformed to the normal distribution and Mann-Whitney U test was used if the data was not normally distributed. Comparisons were made between colon cancer group and healthy control group. A p value p <0.05 was considered statistically significant. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the sensitivity and specificity and diagnostic efficacy of miRNAs among the investigated groups [6].

Results

Nine patients with pancreas cancer included in the study, seven male and two female of them, the mean age and range of them was 52.60 ± 17.2 and 57-66 years: In the control group, there was seven male and two female, their mean age 51.8 ± 14.6 and range 52-63 years. MiR125b was upregulated and statistically important compared with the endogenous control miR181and miR192 for patients and healthy individuals (p:0.001) and (p: 0,010 respectively). MiR141 was downregulated and statiscally important compared with the endogenous control miR181and miR192 for patients and healthy individuals (p:0.009) and (p: 0,021 respectively) (Table 1 and 2). ROC curve analysis was performed to evaluate the diagnostic value of three miRNAs. The closer the value of area under the curve (AUC) was to 1.00, the more important was the miRNA that reflected the significant difference between pancreas cancer and healthy controls. The sensitivity and specificity values of Delta181CT-mir125b, with an optimal cut-off value of 1.0337500305, were respectively 85.7% and 100.0%. The sensitivity and specificity values of Delta181CTmir141, with an optimal cut-off value of -3.8399881515, were respectively 100.0% and 100.0%. The sensitivity and specificity values of Delta192C, Tmir125b, with an optimal cut-off value of 7.103257736, were respectively 85.7% and 100.0%. The sensitivity and specificity values of Delta192CTmir141, with an optimal cut-off value of 1.8585049975, were respectively 100.0% and 100.0% (Table 3).

Table 1: MiRNA comparison of pancreas cancer and healthy subjects according endogen control Delta181CT.

miRNA	Mean±SD†	Median	p
Let7c			
Pancreas ca (n=8)	-,61570715925±4,854053761379	1,01789379100	0,746*
Healthy control (n=9)	-,05059330089±1,636041233185	-,67729568500	
Mir34a			
Pancreas ca a (n=5)	1,01984329240±2,592818310932	-,33976745600	0,884†
Healthy control (n=8)	1,23100942800±2,143121988829	2,05993461600	
Mir125b	upregulated		
Pancreas ca (n=7)	3,65026909971±3,489568245360	4,58758163500	**0,001***
Healthy control (n=9)	-1,84643158633±1,959938615230	-1,69742202800	
Mir141	downregulated		
Pancreas ca (n=4)	-6,71910047525±2,185395750159	-6,46164798750	**0,009***
Healthy control (n=4)	-1,40825000000±1,792469874224	-1,52250000000	
Mir145			
Pancreas ca (n=8)	-1,61658096312±2,954680359925	-2,20819091800	0,789*
Healthy control (n=8)	-1,99996741688±2,654056687513	-2,38601209250	
Mir155			
Pancreas ca (n=6)	3,17267576850±1,449945505079	2,53145027150	0,086*
Healthy control (n=7)	1,22426045443±2,135280170241	1,67700000000	

* Independent samples t test, † Mann-Whitney U testi

Table 2: MiRNA comparison of pancreas cancer and healthy subjects according endogen control Delta 192CT.

miRNA	Mean±SD†	Median	p
Let7c			
Pancreas ca (n=8)	2,78869652763±5,997536307671	5,34918022150	0,564†
Healthy control (n=9)	3,06271136133±4,624356266128	2,31400871300	
Mir34a			
Pancreas ca (n=5)	3,17160644520±3,540063446031	1,33894920300	0,843*
Healthy control (n=8)	3,66085217275±4,566002808441	3,21625041950	
Mir125b	upregulated		
Pancreas ca (n=7)	7,36785425400±4,747302861212	8,59853744500	**0,010†**
Healthy control (n=9)	1,26687307578±4,759926782776	,39388084400	
Mir141	downregulated		
Pancreas ca (n=4)	-1,14032602300±,888161312928	-1,21311092400	**0,021†**
Healthy control (n=4)	5,37775000000±1,283750073028	5,25300000000	
Mir145			
Pancreas ca (n=8)	1,78782272338±3,490720068432	1,91378211950	0,859*
Healthy control (n=8)	1,45071104438±3,927406304514	2,31926467900	
Mir155			
Pancreas ca (n=6)	5,53612804417±3,595417809936	4,88042163850	0,886*
Healthy control (n=7)	5,19724653186±4,535575554134	6,19000000000	

* Independent samples t test, † Mann-Whitney U testi

Table 3: Sensitivity and Specificity of miRNAs by ROC analysis.

miRNAs	AUCs	Sensitivity (%)	Specifity (%)	P value
Delta181CTmir125b	0.937	85.7	100.0	0.004
Delta181CTmir141	1.000	100.0	100.0	0.021
Delta192CTmir125b	0.889	85.7	100.0	0.010
Delta192CTmir141	1.000	100.0	100.0	0.021

Discussion

Pancreatic cancer (PC) is a lethal disease, however current screening methods unable to achieve early diagnosis. Blood-based microRNAs (miRNAs) are promising molecular biomarkers for detecting PC. Wei et.al. published a meta analysis consist of twenty-seven eligible studies for single miRNA dysregulation, 32 miRNAs were found as upregulated and 5 miRNAs as downregulated in PC patients. Four studies identified a 2-miRNA panel, and 10 studies identified a panel consisting of 3 or more miRNAs which were used to detect PC patients. Additionally, 8 studies combined miRNA panels and carbohydrate antigen 19-9 (CA 19-9) to diagnose PC. They focused miRNAs may be used as promising diagnostic biomarkers for detection of PC [7]. Zhou et. al. have evaluated the prognostic relevance of microRNAs (miRNAs) in patients with pancreatic cancer (PC). Thirteen miRNAs were identified to be significantly related with overall survive (OS) in PC patients. Patients with high risk score suffered poor OS compared with patients who had low risk score. Their study identified a miRNA signature including 13 miRNAs which could serve as an independent marker in prognosis of PC [8].

Zhou et.al. also published an another study consist of a six-miRNA signature including up-regulated miR-122-5p, miR-125b-5p, miR-192-5p, miR-193b-3p, miR-221-3p and miR-27b-3p was identified. Plasma miR-125b-5p might act as an independent biomarker in predicting OS of PC patients [9]. In our study, MiR125b was upregulated and statiscally important compared with the endogenous control miR181and miR192 for patients and healthy individuals. Xu et al. detected expression of IGF2BP2 and miR-141 in pancreatic cancer, and Bioinformatics analyses and validation experiments showed that IGF2BP2 is a direct target of miR-141 [10]. Xu et al demonstrated that upregulated TM4SF1 and lost miR-141 expression were associated with advanced clinicopathological features and poor survival of pancreatic cancer patients [11]. In our study, we investigated six microRNAs. Mir141 was significantly downregulated in patients. When we compare it with the last two studies, we can say that Mir141 can be used as a significant biomarker in PC.

Limitations

The small number of cases in our study was the most important limitation. It is planned to increase the number of patients and to evaluate the pathological diagnoses in detail in future validation studies. As conlusion; Mir125b and MiR-141 may have an important role in both the early diagnosis. Further extensive studies are needed.

Main points

Pancreatic cancer (PCa) is a lethal disease, however current screening methods unable to achieve early diagnosis. Blood-based microRNAs (miRNAs) are promising molecular biomarkers for detecting PCa.

In this study, our aim was to detect the most specific and sensitive microRNA by studying the microRNAs in the patient and control groups. Mir125b and MiR-141 may have an important role in both the early diagnosis. Further extensive studies are needed.

Declarations

Conflict of interest: There is no conflict of interest among the authors.

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