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The Importance of MicroRNA 29a as a Biomarker in Colon Cancer

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ABSTRACT

Introduction: *Colon cancer (CC) has become one of the most common diseases in recent years.The incidence of (CC) varies greatly worldwide, depending on lifestyle, environment, and genetic causes. Endoscopy is invasive and expensive for early detection. Therefore, there is a need to develop reliable and non-invasive markers for the early diagnosis of CC. MicroRNAs (miRNAs) have attracted attention as promising biomarkers in other cancers In this study, our aim is to determine whether miRNAs are biomarkers in the early diagnosis of colon cancer.*

Materials and Methods*: Twenty patients diagnosed with colon cancer and twenty healthy individuals of the same age and gender were selected as the control group. Four miRNAs (let-7g, miR-29a, miR-155, miR-200c) selected and MiR 181 and miR 192 used as the endogenous control group in line with their binding potentials and gene expression levels. They were measured with StepOne ™ Real-Time PCR.*

Results: *mir29a was significantly high AUCs, thus the sensitivity and specificity values of was 100.0% and 64.3%. Common to all studies was MiR29a as sensitive and spesific like in our study.*

Conclusion: *miR29a, is a hopeful miRNA in the early diagnosis of colon cancer, prognosis and treatment of the disease. Further validation studies are needed.*

Keywords
Colon Cancer, Biomarker, microRNA, miR29a.

Introduction

Colon cancer (CC) has become one of the most common diseases in recent years [1]. The incidence of (CC) varies greatly worldwide, depending on lifestyle, environment, and genetic causes. In the last two decades, the introduction of screening programs such as colonoscopy, stool immunochemical testing (FIT), stool occult blood testing (FOBT), stool DNA testing and CT colonography (virtual colonoscopy) flexible sigmoidoscopy has led to the early detection [2]. Endoscopy is invasive and expensive, whereas the less invasive and less expensive FOBT had a sensitivity of only 47-73% in case-control studies. Therefore, there is a need to develop reliable and non-invasive markers for the early diagnosis of CC [3].

MicroRNAs (miRNAs) have attracted attention as promising biomarkers in other cancers. MiRNAs are 18-25 nucleotide non-coding RNAs that regulate gene expression post-transcriptionally and control various cellular mechanisms including the development of various types of cancer. Recent studies have shown that there is an abundant circulating miRNA load in the systemic circulation [4,5].

In this study, our aim is to determine whether miRNAs are biomarkers in the early diagnosis of colon cancer.

Materials

Twenty patients diagnosed with colon cancer twenty 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 Akdeniz University. Ethics committee approval number and date 471 and 17.08.2016.

Four miRNAs (let-7g, miR-29a, miR-155, miR-200c) 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. After miRNA was measured by QUBIT 3 FLUOROMETER device (Invitrogen by Thermo Fisher Scientific-Qubit ™ microRNA Assay Kit). The miRNA samples, whose concentration was used by using Thermal Cycler (Applied Biosystems by Life Technologies-TaqMan Advanced miRNA c DNA Synthesis Kit). 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 C_T 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.

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

Twenty patients with colon cancer included in the study, eleven male and nine female of them, the mean age and range of them was 60.81 ± 16.3 and 49-72 years: In the control group, there was eleven male and nine female , their mean age 60.3 ± 17.7 and range 47-72 years.

Four prominent microRNAs in the literature (let-7g, miR-29a, miR-155, miR-200c) were compared in patients with CC and control group according to endogen control miR181 and 192. While the level of miR-29a and miR155 were upregulated (p: 0,044) and (p: 0,029) respectively in CC and miR200c was downregulated (p: 0,026) according to Delta181CT and miR-29a was upregulated in CC according to Delta192CT(p: 0,006) . miR-29a was sensitive and specific in statistical both each group according to two endogen control (Table 1 and 2).

ROC curve analysis was performed to evaluate the diagnostic value of three miRNAs. In the ROC analysis curve, Delta192CTmir29a was significantly heigh AUCs, The sensitivity and specificity values of Delta192CTmir29a, was 100.0% and 64.3% (Table 3).

Discussion

Colon Cancer (CC) is the 3rd leading cause of cancer-related death worldwide [2]. The majority of CC is sporadic, though approximately 20-30% of CC patients carry inherited mutations [7].

El-Daly et.al. compared the diagnostic performance of their chosen miRNAs with the traditional tumor biomarkers CEA and CA 19-9. In the results of their study, they revealed that the expression levels

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

miRNA			
	Mean ± SD†	Median	p
Let7g			
Colon ca (n=16)	-0.16040062913 ± 2.302189578046	-0.51396656050	0.769*
Control (n=17)	0.11179139447 ± 2.915786952133	-0.11234283400	
Mir29a			
Colon ca (n=17)	-3.33114926953 ± 3.923921569083	-3.32902336100	**0.044***
Control (n=14)	-0.64706718779 ± 2.961541393105	-0.57290499900	
Mir200c			
Colon ca (n=9)	-11.14411735567 ± 10.876971399122	-8.25358200100	**0.026***
Control (n=10)	-1.04590203700 ± 4.660186116216	1.12148666400	
Mir155			
Colon ca (n=15)	2.05515213040 ± 4.088546569572	2.10597419700	**0.02**9†
Control (n=15)	0.51292268780 ± 3.107787871742	0.74118995700	

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

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

miRNA			
	Mean ± SD†	Median	p
Let7g			
Colon ca (n=11)	3.73334988673 ± 3.483342644337	4.89503479000	0.985*
Control (n=15)	3.76736605833 ± 5.017948074987	2.89736938500	
Mir29a			
Colon ca (n=11)	2.06935258336 ± 1.123042052073	2.11389350900	**0.006***
Control (n=14)	-0.64706718779 ± 2.961541393105	-0.57290499900	
Mir200c			
Colon ca (n=5)	-4.87582855280 ± 13.856832087673	2.16968727100	0.273*
Control (n=10)	3.05283547050 ± 3.924713152748	2.04644584650	
Mir155			
Colon ca (n=10)	7.01828269960 ± 3.076227105427	7.15880966200	0.154*
Control (n=14)	4.73393418229 ± 4.129070770205	6.06174728400	

* Independent samples t test

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

miRNAs	AUCs	Sensitivity (%)	Specificity (%)	P value
Delta192CTmir29a	0.812	100.0	64.3	0.009
Delta181CTmir155	0.733	80.0	80.0	0.029

of these miRNAs changed dynamically according to the tumor development status.They also found that serum miR-15b, miR-21 and miR-29a performed the best in terms of diagnostic power [4].

Shiosaki et al. showed that serum levels of miR-21, miR-29a and miR-92a are statistically important in the early stage of CC [8].

Orosz et al. investigated miR-21,miR-29a, miR-30a, miR-34a miR-155 and miR-221, as a biomarker using the α reverse transcription polymerase chain reaction (RT-PCR) method. They published that miR-155, miR-34a and miR-29a were downregulated in all patients with cancer compared to controls [9].

In all three studies, miRNAs were investigated by realtime PCR method. The same method was used in our study. Common to all studies was MiR29a as sensitive and spesific like in our study, all three studies were found miR29a to be downregulated compared to controls. The expression of miR29 is also important in colorectal adenomas.Uratani et.al. observed that the expression of four miRNAs, miR-21, miR-29a, miR-92a, and miR-135b, was significantly higher in CC [10]. MiR29a is crucial in the recurrence of CC. Viessmann-Brenner et al. showed that miR-29a has a significant effect on the risk of relapse in stage II patients (p:0.028) [11].

There is the role of miRNAs in the prognosis and therapy of colon cancer. Yuan et.al. published that plasma samples were collected at diagnosis, 6, 12, and 24 months after diagnosis from 144 patients in a prospective CC cohort study. They asssayed miRNAs by Taqman qRT-PCR and. showed miR-29a, 200b, 203, and 31 are being potential CC prognosis biomarkers [12].

It has been published that mir29a can also be used as a candidate molecule in cancer treatment. B7-H3, a member of the B7/CD28 immunoglobulin superfamily, was shown to be overexpressed in several solid malignant tumors, including CC. MiR-29a downregulates B7-H3 expression and accordingly inhibits CC progression, invasion, and migration, thus miR-29a and

B7-H3 might represent novel molecular targets for advanced immunotherapy in CC [13].

Limitations

The small number of cases in our study was the most important limitation. In addition, miRNAs were not separated according to the pathological diagnoses of the cases.In the validation study, it is planned to increase the number of patients and to evaluate the pathological diagnoses in detail.

Conclusion

miR29a, was found significant and sensitive in our study, it is a hopeful miRNA in the early diagnosis of colon cancer, prognosis and treatment of the disease. Further validation studies are needed.

Main Points

Endoscopy is invasive and expensive for early detection. Therefore, there is a need to develop reliable and non-invasive markers for the early diagnosis of CC. MicroRNAs (miRNAs) have attracted attention as promising biomarkers in other cancer.

In this study, our aim is to determine whether miRNAs are biomarkers in the early diagnosis of colon cancer. Common to all studies was MiR29a as sensitive and spesific like in our study.

MiR29a is a hopeful miRNA in the early diagnosis of colon cancer, prognosis and treatment of the disease.

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