Original Article

Expression of miR-143, miR-145, miR-192, Tumor Suppressor miRNAs using qPCR in Colon Cancer stage II

Stage II Kolon Kanserinde Tümör Süpressör miRNA olan miR-143, miR-145 ve miR-192'nin Ekspresyonu

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ABSTRACT

Background: In this study, it was aimed to determine the expression levels of tumor suppressor microRNAs (miRNA) including miR-143, miR-145 and miR-192 in tumor and normal colon / rectum tissues of Stage II colorectal cancer (CRC) patients by using qPCR and to compare the obtained data.

Materials and Methods: This study was performed on tumor or normal (control-clean surgical margin) colon / rectum tissues of 9 patients diagnosed with colorectal cancer as a result of clinical evaluation with laboratory and pathological findings in Gaziantep University Medical Faculty Research and Practice Hospital General Surgery Department. miRNAs isolated with miRNeasy mini kit were used for qPCR. Isolated miRNAs were converted into cDNA by miScript RT-PCR commercial kit. The miRNA expression levels of tissue samples were determined by using RT- SYBR Green qPCR kit in Rotor Gene Q.

Result: The mean level of miRNA-143 expression in 9 patients diagnosed with colorectal cancer appears to have decreased 0.096 fold compared to normal tissue and tumor tissue, while miR-145, 1.52 fold. This decrease was considered as statistically significant ($p < 0.05$). When the fold chance values of normal and tumor tissue are compared, despite being tumor supressor miRNA, miR-192 appears to have increased 3.25 fold and was not considered statistically significant ($p > 0.05$).

Conclusion: According to the findings obtained in our study, the expression levels of tumor supressor miRNA miR-143 and miR-145 in tumor colon tissues of patients with colorectal cancer have decreased. miRNAs have attracted major interest as a means to analyze the molecular pathways involved in cancer development and progression. In addition to their important cellular functions, it is possible that secreted miRNAs may be diagnostic biomarkers for cancer detection.

Keywords: miRNA, Tumor suppressor miRNA, Biomarker, Colorectal cancer

ÖZ.

Amaç: Bu çalışmada Stage II kolorektal kanserli hastalardan alınmış tümörlü ve normal kolon/rektum dokularında Tümör süpressör mikroRNA (miRNA) olan miR-143, miR-145 ve miR-192'in ekspresyon seviyelerinin qRT-PCR ile tespit edilmesi ve elde edilen verilerin karşılaştırılması amaçlanmıştır.

Materyal ve Metod: Bu çalışma Gaziantep Üniversitesi Tıp Fakültesi Araştırma ve Uygulama Hastanesi Genel Cerrahi Anabilim Dalı'nda laboratuar

ve patalojik bulgular ile klinik değerlendirme sonucundakolorektal kanser tanısı konmuş 9 hastanın tümörlü ve normal (kontrol-temiz cerrahi sınırları) kolon/rektum dokuları ile gerçekleştirilmiştir. qRT-PCR uygulaması için miRNeasy mini kit ile izole edilen miRNA'lar kullanıldı. İzole edilen miRNA'lar miScript RT-PCR ticarikit ile cDNA'yadönüştürüldü. Doku örneklerindeki miRNA ekspresyon seviyeleri RT- SYBR Green qPCR kiti kullanılarak Rotor Gene Q sistemi ile belirlendi. **Bulgular**: Kolorektal kanser teşhisi konulmuş 9 hastanın ortalama miRNA-143 ekspresyon seviyesi normal doku ve tümörlü doku karşılaştırıldığında 0.096 kat azaldığı, miR-145'in ise 1.52 kat azaldığı tespit edilmiştir. Bu artış istatiksel olarak anlamlı bulunmuştur ($p<0,05$). miR-192 tümör baskılayıcı miRNA olmasına rağmen ekpresyon miktarının normal ve tümör dokusu fold chancedeğerleri karşılaştırıldığında 3.25 kat arttığı tespit edilip istatiksel olarak anlamlı bulunmamıştır ($p>0,05$). **Sonuç**: Çalışmamızdan elde ettiğimiz bulgulara göre kolorektal kanserde tümör süpressör miRNA olan miR-143 ve miR-145'in hastaların tümörlü kolon dokularında ekspresyon seviyeleri azalmıştır. miRNA'lar, kanser gelişimi ve ilerlemesindeki moleküler yollarda önemli etkiler etmektedir. miRNA'lar hücreselbirçok işleve ek olarak, kanser tanı ve tedavisinde biyobelirteç olarak kullanılabileceği düşünülmektedir.
Anahtar Kelimeler: miRNA, Tümör baskılayıcı miRNA, Biyobelirteç, Kolon kanseri

INTRODUCTION

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide. Systematic methods for diagnosing pathological conditions might contribute to a high detection rate of patients at early stages of CRC, leading to a reduction in mortality rates. Implementation of the fecal occult blood test and flexible sigmoidoscopy as screening methods has reduced CRC mortality (1-3). However, these techniques have inherent limitations; the sensitivity of detection of the fecal occult blood test is fairly low and flexible sigmoidoscopy is invasive and uncomfortable for patients. Therefore, identification of novel prognostic biomarkers to improve patient outcome and to assess individual prognosis is required. microRNAs are being evaluated for their potential in this area.

Recent studies have demonstrated a link between the aberrant expression of a class of small noncoding RNAs, termed miRNAs and the pathogenesis of cancer (4).

MicroRNAs (miRNAs) are small non-coding RNAs as 18-25 nucleotides in lenght that downregulate or upregulate gene expression during in various cellular processes such as cell-cycle regulation, differantiation, proliferation, apoptosis and metastasis (4-6).

Bioinformatic data indicate that each miRNA can control hundreds of gene targets, underscoring the potential influence of miRNAs on almost every genetic pathway. miRNAs are RNA molecules that silence gene expression by either cleaving target mRNAs or inhibiting their translation (7,8). There are two main categories of miRNAs that are involved in cancer progression: those that enhance cell growth, survival, and proliferation [onco-microRNAs (oncomiRs)], and those that suppress these activities [tumor-suppressor (TSmiRNAs)] (8-10). The majority of miRNAs involved in

tumor promotion are TS-miRNAs (11-13). Tumor suppressor miRNAs are downregulated in cancer cells, and would normally inhibit the translation of protooncogenes (12,13).

The expression of miRNAs is reproducibly altered in CRC and their expression patterns are associated with diagnosis, prognosis and therapeutic outcome in CRC (18,19). Studies have begun to examine the association of miRNA related polymorphisms and their association with CRC incidence and prognosis as well as the possibility of using miRNA expression as non-invasive early detection biomarkers (20,21). These data suggest that miRNAs may be potential molecular classifiers, early detection biomarkers and therapeutic (22,10,3).

Table 1. Clinicopathological data for 9 patients with colorectal cancer

Characteristics (n=9)	Sex		Age (mean)	Location of tumors		Pathologic T classification		Pathologic N classification			Metastatic classification		AJCC Classification
	Male	Female		Colon	Rectum	T2	T3	N0	N1	N2	M0	M1	Stage II
Number	7	2	47	8	1	0	9	9	0	0	9	0	9

MATERIALS AND METHODS

This study was approved by the report of the ethics committee of number 74059997.050.01.04/126 issued by Medical Ethical Committee of the Harran University, Turkey.

For this study, nine CRC tissues and normal colorectal mucosa tissues were taken from patients with stage II CRC who underwent surgical excision the operating Gaziantep University Hospital. Clinicopathological data was collected prospectively and is summarised in Table 1. The biopsy materials were snap frozen in liquid nitrogen in after surgical resection then stored at −80°C temperature in a cryovial and covered with the RNALater (Applied Biosystems) solution until RNA extraction.

In our study we analyzed expression of selected mature tumor supressor miRNA (miR-143, miR-145 and miR-192) by qPCR (Table 2).To isolate miRNA, approximately 30 mg of tissue samples were homogenized in liquid nitrogen by using homogenizer (Precellys Bertin) in 1-2 mL of Qiazol (Qiagen, UK). Total RNA was extracted by using miRNeasy mini kit (Qiagen) according to the manufacturer's instructions and then purity of total RNA was measured by using nanospectrophotometer (Nanodrop Technologies Inc., USA Implen). Isolated miRNAs were

reverse transcribed to cDNA by using Miscript II RT kit(Qiagen).

For qPCR was used RT- SYBR Green kit. The PCR reaction was carried out via the first step at 95 oC for 15 min followed by 40 cycles with hybridization 15 seconds at 94 oC, annealing 30 second 55 oC and extension 30 seconds at 70 oC. RNU6 was used as endogenous control. The ΔCT method was used for calculating the relative expression of a given miRs between a paired normal and tumor sample. ΔΔCT results were evaluated using the SABiosciences web based software (Qiagen) fold-change calculated. Difference between groups was compared using student's t-test. P value less than 0.05 was considered as statistically significant.

Table 2. Detail of microRNA with roles in colon cancer and their targets (13-15)

MicroRNA name	Accession number	Tumor Suppressor and Oncogene	Example of experimentally validated microRNA Target
Hs-miR-143-1	MS00003514	Tumor Suppressor	KRAS,DNMT3A,ERK5
Hs-miR-145-1	MS00003528	Tumor Suppressor	IRS-1, c-Myc, YES1, STAT1, OCT4, SOX2, KLF4, FLI1
Hs-miR-192	MS00003689	Tumor Suppressor	No functionally verified targets
Hs-RNU6-2-11	MS00033740	Referans	

RESULT

Fold changes were calculated using the measured ΔCt values in normal and tumor tissues of 9 patients with colorectal cancer and shown in Table 3.

The mean ΔCt value for miR-143 was 1.35 in the normal tissue while it was found to be 1.58 in the tumor tissue (Figure 1). The miR-143 was observed to be 0.096 fold less expressed in the tumor tissue compared to the normal tissue. Similarly, the mean ΔCt value of miR-145 was 2.61 in the normal tissue whereas it was found to be 1.26 in the tumor tissue (Figure 1). This increase was statistically significant ($p<0.05$). The expression level of miR-145 in the tumor tissue was determined to be 1.52 fold less than the normal tissue. The data were evaluated statistically and the difference between the normal and tumor tissues was found to be significant for both miR-143 and miR-145 ($p<0.05$).

Despite it is an oncogenic miRNA, the mean expression amount of miR-192 in the normal tissue, which was 2.80, increased in the tumor tissue and found to be 4.21 (Figure 1). However, the statistical evaluation revealed that the difference between them (Fold change 3.25) was not significant ($p>0.05$).

Table 3: Mean fold change of th 6 miRNA, on the 9 colon cancer patients and healty individuals.

miRNA	Mean FC	SD (±)	p-value
miR-143-1	-0.96*	0.54	0.049
miR-145-1	-1.52*	0.87	0.026
miR-192	3.25	1.78	0.253

DISCUSSION

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths worldwide (23). The sensitivity of markers used in the early and painless diagnosis of various cancers, including CRC, remains low. Consequently, the results of this study will increase our understanding of development, progression and earlier detection and personnel treatment of colon cancer.

Studies have shown that expression of miRNA in colon carcinoma can be used as a diagnostic marker. The first study of biomarkers of miRNAs in patients with adenomas and colorectal cancers has found that miR-143 and miR-145 levels are decreased (11,12,15). In another study, miR-192 levels in plasma were found to be significantly higher in colorectal cancer patients and it could be used as a non-invasive biomarker in the diagnosis of colorectal cancer (16,17).

Recently, miRNAs have attracted major interest as a means to analyze the molecular pathways involved in cancer development and progression. In addition to their important cellular functions, it is possible that secreted miRNAs may be diagnostic biomarkers for cancer detection. miRNAs have a clear role in the initiation and progression of CRC. Future research will have to specifically address the potential role for miRNA-based classifiers and therapeutics in medicine.

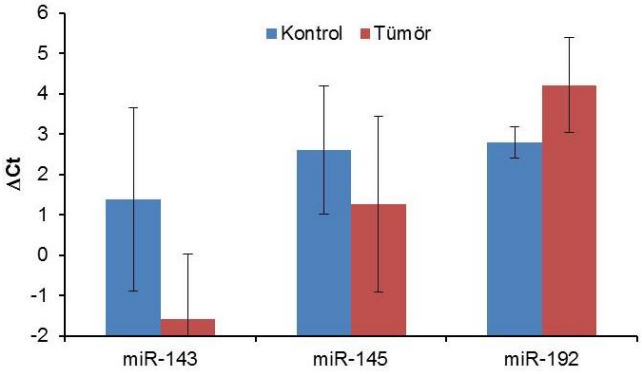

Figure 1: Graphical representation of control and tumor groups according to ΔCt averages.

The expression levels of miR-143 and miR-145 in the normal and tumor tissues were decreased and the results were found to be statistically significant in our study which was conducted with the colon/rectum tissues of nine patients with colorectal cancer.

Although colon cancer includes tumor-suppressor miRNA, the difference between normal tissue and tumor tissue in terms of the expression level of miR 192 was observed to be not statistically significant. It was considered that the icrease in the expression difference in our study result may be due to the fact that the discrimination between normal tissue and tumor tissue during surgery is imperfect.

Some questions remain to be answered in order to be able to administer the miRNA treatment method. The miRNAs that are desired to be used as biomarkers can affect the entire gene regulatory system rather than affecting a single gene product. Each of the miRNAs regulates the expression of numerous target genes and changing the expression of a single miRNA may target many unexpected genes. In contradiction to this condition, a single gene can be regulated by many miRNAs, altering the expression of a specific miRNA can affect a specific gene target productively. In order to apply the miRNA treatment method successfully, new research findings targeting overcoming these kinds of problems are needed.

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