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Investigating the relationship between the expression of DDX21 and mir200a genes with the prognosis of colorectal cancer patients

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Abstract

Colorectal cancer (CRC) is a significant health problem as the third most common cancer in the world and the second deadliest cancer in the world. The aim of the present study was to evaluate expression of two genes (DDX21 and mir200a) in Iranian CRC patients tissue samples. In this study, 60 patients with colorectal cancer and 60 patients with no evidence of CRC were enrolled. RNA extraction from The paraffin blocks of colorectal tissue samples and polyps was performed and expression level of DDX21 and mir200a genes was assessed using qPCR method. The results showed that mean age of patients was 58.3 ± 9.82 years and 53.7 ± 8.65 years in the case and control groups respectively. The expression of DDX21 was significantly increased in CRC patients (with a mean fold-change of 1.8, $P<0.001$). Additionally, the expression of miR-200a was significantly increased in CRC patients (with a mean fold-change of 2.5, $P<0.001$). In conclusion, DDX21 and mir200a seem to be valuable markers in prognosis of CRC.

Keywords: DDX21

miR-200

Colorectal cancer Prognosis

Introduction

Colorectal cancer (CRC) has been the focus of researchers in the field of medical sciences in recent years due to its high prevalence and known complications such as mortality (1, 2). CRCs are in the third place among the known malignancies in the world in terms of prevalence and also the cancer related death. The survival rate in these patients is directly related to the stage of the disease, so that in patients with stage I, the 5-year survival rate has been reported to be more than 90%, but this statistic has decreased to less than 15% in metastatic and stage IV patients (3). In the past strong evidences, scientists have pointed out that along with the nutritional status such as the consumption of red meat in rich societies and the increase in the use of food preservatives, genetic changes have played an important role in the development and prognosis of colorectal cancer. Therefore, colorectal cancer can originate from a specific genetic basis, like almost other cancers, and environmental factors such as nutrition can accelerate this process (4).

Three underlying genetic etiologies has been introduced for progression of colorectal cancer cells including microsatellite instability, chromosomal mutations and CpG methylator phenotype. One of the most important mechanisms that can increase cancer cell growth and metastatic involvement is epithelial mesenchymal changes (EMC). The miR-200 line has a cornerstone role in EMC in these patients, but knowing the different aspects of this genetic category need more research (5).

The miR-200 category is a class of microRNAs compromising of five subtypes; miR-200a, *miR-200b*, miR-200c, miR-141 and miR-429. This genetic category has been evaluated in multiple investigation specially due to its role in metastatic behavior of CRC & surveillance. In 2008, two investigation groups established the important role of the miR-200 line in the regulation of EMC, *through* the ZEB1/E-Cadherin Street. ZEB1 attaches directly to the common promoter section of the miR-200 category and motivates replication repression. Generally, increased expression of the miR-200 category motivates post-transcriptional repression of ZEB1 *via* 3′ UTR binding (6-9).

Recent knowledge has been established the long non-coding RNA-ZFAS1 as chief protein modulators involved in different malignancies. Despite the study of this molecular pathway has not been properly identified in colorectal cancer, Wang X et al. reported the mechanism of DDX21 modulation in CRCs onset and deterioration. Specifically, ZFAS1 enforced its carcinogenic action and was dramatically upregulated related to elevated DDX21 malignant cells, which more closely associated with definite weak clinical surveillances (10).

In different genetic studies, these two genetic lines have been mentioned as prognostic factors for the progression of the disease and its transformation to higher stages. Another importance of identifying different genetic pathways is their role in creating appropriate treatment. So that the role of molecular and cellular targeted treatments on the survival rate of patients with colorectal cancer has been mentioned in various studies (11, 12). This study was conducted to determine the relationship between the expression of DDX21 and mir200a genes with the prognosis of colorectal cancer patients.

Methods

Study Design and Patients

In this study, 60 patients with colorectal cancer and 60 controls (with no evidence of CRC) were enrolled. The paraffin blocks of colon and rectum tissue samples in the case group and polyps in the control group, were prepared by the Pathology Department of Imam Hossein hospital (Tehran, Iran). The results of histopathological studies confirmed CRC diagnosis in the case group patients.

RNA isolation and qPCR

Isolation of RNA performed in using the MiRJia Kit (Rojeh Technology, Iran) and were used for cDNA synthesis with random hexamers and Superscript III Reverse Transcriptase according to manufacturer's instructions (ThermoFischer). cDNA was used for quantitative PCR analysis in a Step one real-time PCR cycler (Applied Biosystem), using SYBR Green Master Mix and gene-specific primers. The DDX21 gene primer sequences were (Forward): CGGAGGACGGGGTGAAGAT and (Reverse): AAGAAGGTTCTGAACAGCTGGTTAG. Primer sequences of miR-200a were (Forward): UAACACUGUCUGGUAACGAUGU and (Reverse): UAACACUGUCUGGUAACGAUGU. qPCR set-up has showed in tables 1 and 2.

Table 1. qPCR set-up

Component	Volume per reaction
SYBR Green Master	10 µL
Forward-Reverse primer mix (1:1)	1 µL
cDNA sample	2 µL
Nuclease free Water	2 µL
Final Volume	15 µL

Table 2. qPCR thermal cycles

Step	Temperature	Time	cycles
Activation	95°C	10 min	1
Denaturation **Annealing/extension**	95°C 60°C	10 sec 30 sec	40
Melt curve	65-95 °C	5 sec/step	1

Expression analysis and statistics

The expression of the target genes in the tumor tissues was determined by the calculation of the threshold cycles (C_T) of the target and housekeeping (ctU6) genes. Then, relative gene expression was calculated using the standard formula. According to the default definition of the software system (version 5.0), a value < 50% difference was considered a

non-significant change. Finally, significant differences in the expression of the target genes between the two groups were determined by appropriate statistical tests.

Results

The present study investigated 60 patients with CRC versus 60 non-cancerous controls. Results showed that mean age of patients was 58.3 ± 9.82 and 53.7 ± 8.65 years in the case and control groups respectively. 65% of patients and 53% of controls were men. Demographic results have shown in table 1.

Table 3. Demographic results

Group / Variable	Case (n=60)	Control (n=60)
Age	58.3 ± 9.82 y	53.7 ± 8.65 y
Gender male (%)	39 (65%)	32 (53%)
Smoking (positive)	22 (36%)	17 (29%)
Family history of cancer	8 (13%)	4 (7%)

Gene expression data

The results showed that the expression of DDX21 was significantly increased in CRC patients (with a mean fold-change of 1.8, P<0.001). Additionally, the expression of miR-200a was significantly increased in CRC patients (with a mean fold-change of 2.5 , P<0.001), which is shown in Fig. 1-2.

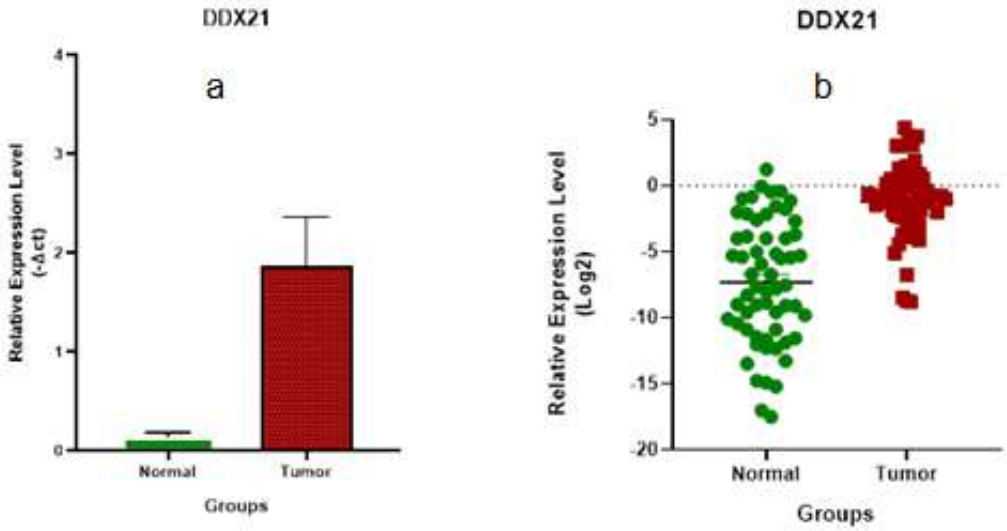


Figure 1. Gene expression data of DDX1. a: Relative Expression Level of DDX1 compared between case and control groups and fold change of 1.8 was obtained. b:

distribution of DDX1 gene expression compared between case and control groups showed higher expression of this gene in the case group.

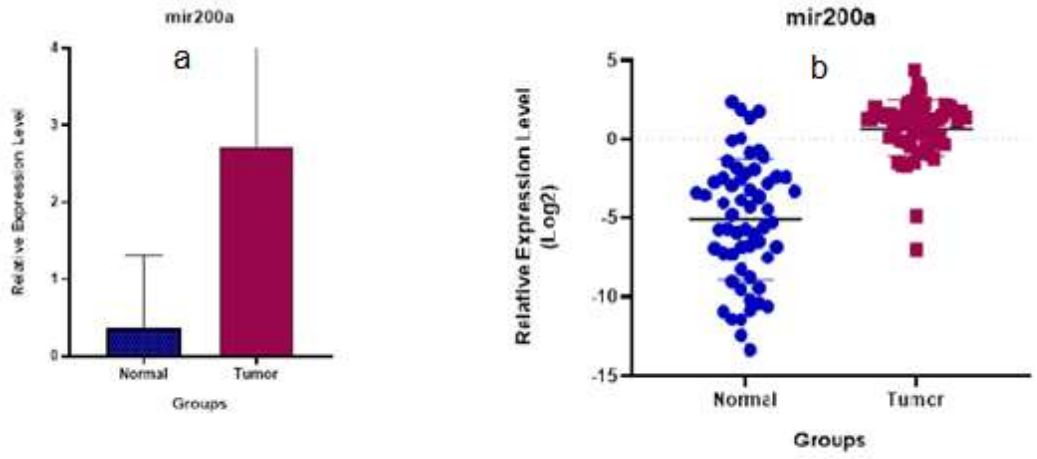


Figure 2. Gene expression data of miR-200a. a: Relative Expression Level of miR-200a compared between case and control groups and fold change of 2.5 was obtained. b: distribution of miR-200a gene expression compared between case and control groups showed higher expression of this gene in the case group.

Discussion

Colorectal cancer (CRC), which includes colon or rectal cancer, is a significant health problem as the third most common cancer in the world and the second deadliest cancer in the world. In 2020, approximately 147,000 individuals diagnosed with CRC and 53,200 died from the disease (13). However, due to the significant rise in the number of cases detected in the elderly population, it is estimated that the global incidence of CRC will more than double by 2035 (14). The most effective way to prevent CRC and reduce its related deaths in the whole population is to screen people at moderate risk. Therefore, most European countries, Canada, certain regions in North and South America, Asia and the Pacific have started population-based screening programs (15). Recent research shows various clinical, histological, and molecular features have been reported as useful markers for CRC prognosis, diagnosis and treatment and the clinical value of these factors may potentially be independent or dependent on each other (16). The biological characteristics of tumors can be used to predict the state of the disease and choose the appropriate treatment approach. More recent studies show that molecular biomarkers are tools with potential in better cancer management.

Our results showed that the expression of DDX21 was significantly increased in CRC patients. Additionally, the expression of miR-200a was significantly increased in CRC patients. In similar studies, Lu et al., showed that DDX21 interacted with WDR5 to promote colorectal cancer cell proliferation by activating CDK1 expression, suggesting that targeting DDX21 may be an alternative new strategy for CRC treatment (17). In another study by Gao et al., demonstrated that higher expressions of DDX21 and MCM5 is significantly correlated

with poor survival outcomes of stage III and IV CRC patients, indicating the importance of this mechanism in CRC late and metastatic stage (18). In conclusion, our study indicates that DDX21 and miR-200a expression is significantly high in CRC patients in comparison with healthy patients and indicated that these markers are potential target in CRC prognosis.

Ethics approval and consent to participate

This study was approved in the ethics committee in the school of medicine, Shahid Beheshti University of medical sciences with research ethics approval ID IR. SBMU.RETECH.REC.1399.886. All patients signed an informed consent form before surgery and agreed to their tissue samples being used in the research project. All ethical requirements were observed during sample collection.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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