Original Article

Analysis the expression of a gene panel in patients with colorectal cancer

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Abstract

Background: Colorectal cancer (CRC) is one of the most common cancers in the world. Statistically, this cancer is the fourth most common cancer worldwide, and the third, though with an increasing trend, in Iran. One of the problems encountered in the treatment of CRC and other cancers is delayed diagnosis. CRC is usually diagnosed in advanced stages. One of the objectives of cancer research is to find biomarkers to determine the prognosis and risk of metastasis. The aim of this study was to investigate the expression changes of non-coding *HOTAIR*, *AF147447*, *miR-141*, *miR-34c*, and their target genes; *PRC2* and *MUC2*, in patients with CRC. **Methods:** This cross-sectional study was performed on 50 CRC patients referred to the colonoscopy clinic of Baqiyatallah Hospital in Tehran. Punched biopsy samples obtained during colonoscopy were subjected to extract mRNAs for gene expression analysis using real time PCR. **Results and conclusion:** According to the expression patterns of the assessed genes in CRC tumor and marginal tissues, *MUC2*, *PRC2* and *HOTAIR* can be considered as prognostic genes in this cancer. Also, the results of this study suggested the miR-34c as a promising prognostic marker in CRC patients. Further studies are required to confirm our findings.

Keywords: Colorectal cancer, Metastasis, long non-coding RNA

Introduction

Colorectal cancer (CRC) is one of the most common cancers in the world, sitting on the fourth rank [1, 2]. Unlike many other malignancies, CRC is a potentially preventable and curable cancer as high-risk adenomas can be detected and eliminated in the early stages of the disease [3]. The survival rate of patients depends to a large extent on the stage of the disease at the time of diagnosis. Usually, only 40% of CRC cases are detected during early stages The overall 5-year survival rate of patients with CRC has been estimated close to 65% which declines in those who are late-diagnosed [4]. Regarding that CRC is often asymptomatic in early stages, it is essential to establish screening programs to early diagnose the disease [5].

The advances made in molecular biology over the past three decades have led to the identification of some carcinogenesis mechanisms underlying CRC. Many cases of CRC occur due to sporadic mutations or epigenetic changes. Until a few years ago, our knowledge on the molecular pathogenesis of CRC was limited to two main pathways of genomic (namely chromosomal) and microsatellite instabilities. Recent advances in the field of genetics have highlighted the potential role of epigenetic changes in the development of CRC. The role of highly hypertrophic CpG di-nucleotides in the pathogenesis of CRC was first suggested in 2000. In recent years, numerous genes have been proposed as epigenetic markers to detect CRC [6, 7].

Despite the recent advances in treatment options, the early detection and resection of adenomas are still the most effective therapeutic ways to reduce the mortality rate of CRC. More recently, screening methods to early detect CRC, especially non-invasive approaches, have received much attention in both research and clinical phases. In researches on CRC, the most desirable goal has been to reach to a reliable, non-invasive, diagnostic and prognostic biomarker [2, 8].

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The aim of the present study was to investigate the expressions of non-coding RNAs; *HOTAIR*, and *AF147447*, *miR-141* and *miR-34c*, as well as their target genes; *PRC2* and *MUC2* in CRC tumors and their surrounding tissues to determine any association between patterns of gene expression of these genes and CRC status in patients.

METHODS

Study design and patients

The subjects in this study were those referring to the colonoscopy clinic of Baqiyatallah Hospital in Tehran. After obtaining informed consent, biopsy tissue samples were taken from masses suspected as colorectal tumors through colonoscopy by a gastroenterologist. The tissues were then placed into an RNA preserving reagent. Patients' information such as age, sex, family history of CRC, family history of polyps, history of diabetes, hematuria, and smoking habits were recorded into a pre-designed questionnaire.

Two groups (case and control) were included in this study. In the case group, 50 subjects with CRC confirmed by colonoscopy and biopsy examination were enrolled. Pathologic examinations for all the patients were performed in the pathology department of Baqiyatallah Hospital. In order to include a homogenous population of patients, only those with the pathological diagnosis of adenocarcinoma CRC were included in the study. As the control tissues, 50 samples of tumor margins were collected from the same subjects.

Gene expression study

Fresh tumor tissues were frozen in liquid nitrogen and stored at -70 ° C until use. To obtain an adequate amount of tissue (8-10 ng) to extract RNA, tissue sections were cut into a special plate on dry ice and then weighed. RNAs from the tissue samples were extracted using a miRNA extraction kit (MN Company, Catalog No. 740304, Germany). In order to synthesize cDNA from the extracted RNA, Quantitative Reverse Transcription Kit, (Qiagen –Germany) was used. Specific primers and probes (Table1) for the target genes were designed using the Gene Runner software (Version 3.05). The qRT-PCR reaction was performed using a 2x Master Mix (Primer Design Co.) in 15-microliter reaction mixtures in triplicate. The temperature cycles included 10 min at 95 ℃ following by 40 cycles of 95 ℃ (15 seconds), 60 ℃ (30 seconds), and 72 ℃ (20 seconds). The fluorescence was read by Eco Biosystems, and the initial analysis of data was done by the software system ver.5.0.

Table 1. The sequences of primers and probes.

Gene name	Primer/probe sequence	Product size
MIR141-F	CGTAACACTGTCTGGTAAAGATGGA	**108**
MIR141-R	GTGCAGGGTCCGAGGT	
MIR141-P	CTTTTGCTCTGCAGTCAGTAAA	
HOTAIR -F (100124700)	GGGTGTTGGTCTGTGGAACT	**105**
HOTAIR -R	CAGTGGGGAACTCTGACTCG	
HOTAIR -P	CGCCATGTAGTGTTTCCTACTTT	
U6 RNA-F (26827)	CTCGCTTCGGCAGCACATATAC	**95**
U6 RNA-R	AATATGGAACGCTTCACGAATTTG	
U6 RNA-P	CTAAAATTGGAACGATACAG	
GAPDH-F (2597)	GAAAGCCTGCCGGTGACTAA	**150**
GAPDH-R	CTGCGCTCCTGCCTCGATGG	
GAPDH -P	AGGAAAAGCATCACCCGGAG	
PRC2-F (23512)	AGAGCTTCCAGCCAGAAGAA	**118**
PRC2-R	GCAGTTCACTCTTCGTTGGACA	
PRC2-P	AATGTCCAATAAGCAAGAAA	
MUC2-F (4583)	ATGCCCTTGCGTCCATAACA	**70**
MUC2-R	GCAGGTATTGCAGTCCACCT	
MUC2-P	CGACCTGTATTCTTCCGGCGCC	
-F miR-34c	GACCTATACGTCTTCCGGC	**104**
-R miR-34c	AATGTTACCTAGCAAGCA	
-P miR-34c	CTCGCTTCGACTCGGGAGACC	
AF147447-F	ATTCGCGCGTATAACCG	**133**
AF147447-R	TTTAGCGCTAGCGCGTAA	
AF147447-P	AATCGGGGCGCGTAAATA	

F: Forward, R: Reverse, P: Probe

Data analysis

The expression of the target genes in the tumor and marginal tissues was determined by calculating the threshold cycles (C_T) of the target and housekeeping (ctU6) genes. Then using the standard formula, relative gene expression was calculated. According to the default definition of the software system ver.5.0, a <50% difference was considered as a non-significant alternation. The reductions or elevations of the C_T were considered as either up- or down-regulations of the genes, respectively. Finally, significant differences in the expression of the genes between the two groups were determined by appropriate statistical tests.

Results

Demographic data

In this study, 50 patients with CRC were studied. In terms of gender distribution, 22 and 28 of the patients were male and female, respectively. The mean age of the subjects was 62.56 years within a range of 41 to 86 years. In terms of a family history of cancer, 13 patients mentioned a history of cancers in their relatives. Furthermore, 13 patients were smokers, and 37 had no history of smoking.

Gene expression data

The relative fold-changes of the target genes have been summarized in table 2. As the results showed, all the genes were upregulated in the tumor compared with the marginal tissues.

Table 2. Relative gene expressions in colorectal cancer tumor and marginal tissues

gene	Tumor / Margin fold change	Significance	P value
miR-141	0.29	ns	.21
HOTAIR	2.49	***	<.001
PRC2	3.11	***	<.001
MUC2	4.77	***	<.001
miR-34c	0.12	ns	.359
AF147447	0.15	ns	.138

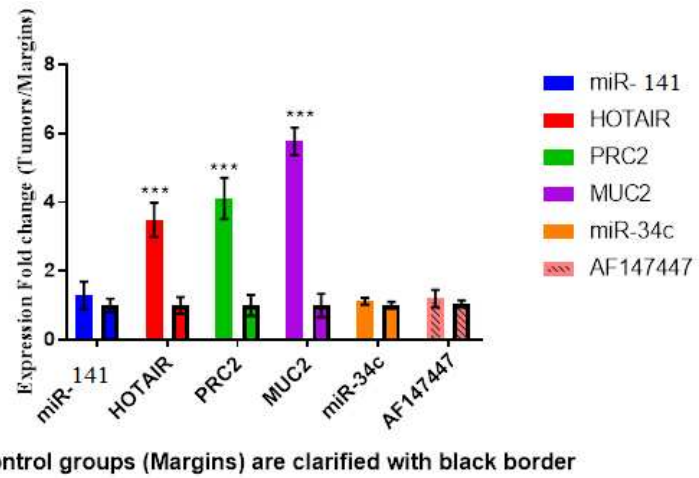

Figure 1. Relative gene expressions in colorectal cancer tumor and marginal tissues

Discussion

The role of epigenetic changes in altering gene expression and increasing the frequency of genetic mutations has been suggested in the early stages of CRC development. This reflects the high potential of epigenetic changes as diagnostic biomarkers in CRC [9-11]. The aim of the present study was to investigate the expressions of non-coding RNA genes of *HOTAIR* and *AF147447*, *miR-141* and *miR-34c*, as well as their target genes; *PRC2* and *MUC2* in CRC tumor and marginal tissues to determine their possible prognostic values in CRC patients. In the present study, the results showed that the expression of *MUC2* gene was significantly higher in the tumor than marginal tissues. With regard to a 4.77-fold change and upregulation of *MUC2* in the tumor relative to the marginal tissues, it seems that this gene can be used as a biomarker to predict prognosis in patients with CRC.

Another marker studied here was the *AF147447* non-encoding RNA. Studies have shown that the expression of *AF147447* in tumor tissues induces an upregulation of this gene in the marginal tissues. Nevertheless, the difference in the expression of this gene between tumor and marginal tissues was not statistically significant in the present study. Regarding the non-significant change in the expression of

AF147447 and contrary to the initial hypothesis and some similar studies, our observation suggested that this gene cannot be considered as a prognostic marker in CRC. In a study, Zhou X et al. investigated the role of AF147447 lncRNA in gastrointestinal cancers, especially gastric cancer. The results of that study showed that AF147447 lncRNA has a negative effect on MUC2 and plays an important role in tumor suppression and its expression has decreased in gastrointestinal cancers [12]. The results of our study showed that *miR-34c* was upregulated in the tumor tissues; however, this increase (0.12 fold change) was not statistically significant. In a similar study conducted by Gu J et al., the results showed that the high expression of miR-34c could prevent metastasis in CRC [13]. Considering that the expression of miR-34c was higher than that of the tumor margins, this gene may present a promising marker to estimate the risk of metastasis in CRC.

Another important marker assessed in the present study was the *PRC2* gene. Our experiments showed that the expression of the *PRC2* gene was significantly higher in the tumor compared to the adjacent tissues. Regarding the 3.11-fold chanhe higher expression of this gene in the tumor compared with the marginal tissues and considering the role of the *PRC2* gene in metastasis, it seems that this gene can also be a prognostic marker predicting metastasis in patients with CRC. One of the genes studied here was the *HOTAIR* non-coding RNA. Our results showed that the expression of *HOTAIR* was significantly higher in the tumor than surrounding tissues. Considering the role of *HOTAIR* in metastasis in various cancers, and the 2.49-fold change and upregulation of this gene in CRC tumors observed here, this gene can be suggested as a prognostic and metastatic marker in CRC. Finally, we also investigated the expression of *miR-141* gene. The results showed that *miR-141* was upregulated in the tumor compared to its adjacent tissues, but this difference was not statistically significant. Therefore, our results did not support a prognostic role for *miR-141* in CRC.

Conclusion

Overall, the results of this study performed on 50 patients with CRC showed that *MUC2*, *PRC2*, and *HOTAIR* were significantly upregulated in the tumors compared to their marginal tissues. This observation suggests these genes as potential prognostic biomarkers in CRC. Also, the results of this study suggested the *miR-34c* as a promising gene in determining the prognosis of CRC. The recent observation; however, requires further studies to be confirmed. Regarding the results of this study, *MUC2*, *PRC2*, and *HOTAIR* can be regarded as prognostic genes in CRC. It is suggested to further investigate the role of these genes in a case-control study on metastatic and non-metastatic CRC patients.

Ethical approve

This study has been approved by Research Ethics Committee of Baqiyatallah medical sciences university (Tehran, Iran) with ethics code: IR.BMSU.REC.1397.134.

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