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Evaluation of the Methylation Status of the *MEIS1* Promoter Gene in Colorectal Cancer

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Abstract

Background: Colorectal cancer, the third most common type of cancer is a major cause of mortality worldwide. If colorectal cancer is detected at the early stages, the 5-year survival rate is 90%. *MEIS1* homeobox gene is implicated in numerous solid tumors and hematological malignancies. Therefore, the present study aims to investigate the methylation status of the *MEIS1* gene in colorectal cancer.

Methods: We used real-time quantitative methylation-specific PCR to detect *MEIS1* promoter methylation in 42 colorectal cancer tissues and 42 normal colorectal tissues.

Results: Methylation was observed only in the positive control samples - CG Genome Universal Unmethylated DNA and CG Genome Universal Methylated DNA. There was no change observed in *MEIS1* promoter methylation status in 42 patients.

Conclusion: The results of the current study indicated that the *MEIS1* gene promoter was not methylated in the cases. Gene expression study confirmed the unmethylated status of the *MEIS1* gene in the colorectal cancer process among the studied population.

Keywords: Colorectal cancer, Methylation, *MEIS1*

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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide.[1] A rapid increase has been reported in the CRC incidence in Iran.[2,3] In studies conducted between 2000-2005, the CRC survival rate was estimated at 41% in Iran which has compared to some developed countries.[4] The lack of early primary symptoms cause patients to present with advanced stage disease at the time of diagnosis.[5] Epigenetic changes play an important role in the initiation and progression of colorectal and other cancers. DNA methylation is one of the major epigenetic changes with an important role in gene expression regulation. Hypermethylation of the promoter of tumor suppressor genes can play an important role by stopping transcription, suppression of the *MEIS1* gene, and expression of the corresponding genes in carcinogenesis.[6]

The *MEIS1* gene is located on the short arm of chromosome 2 (2p14) and composed of 137360 bp in 13 exons. It encodes a 390 amino acid product with a molecular weight of 43 kDa, which is physiologically expressed in various tissues such as bone marrow, stem cells, the pancreas, and colon. In addition, it is found in numerous cancers such as leukemia, neuroblastoma, pancreatic cancer, and colon cancer.[7]

Recent studies indicate that the promoter of this gene is hypermethylated in several cancers including breast, lung, myeloid leukemia, and colorectal.[8-10] Because its expression is suppressed, hence this gene is introduced as a tumor suppressor gene.

Recently, the assessment of hypermethylation has opened new paths toward molecular markers. Molecular analysis of epigenetic changes may play an important role in the early detection and prognosis of diseases.

Given the global prevalence and mortality of CRC and the importance of screening and early detection of this type of cancer, we intended to study the expression and methylation status of the *MEIS1* gene and its role in the development of CRC.

Materials and Methods

Patients and DNA extraction

This case-control study included 42 patients (25 men and 17 women) with CRC. This study was approved by the ethics committee at Mashhad University of Medical Sciences. All patients were enrolled after obtaining informed consent. (Approval No.Ir.mums.res.1393.244). DNA samples of colorectal tissue from the 42 patients were selected and processed by bisulfite. Methylation of the *MEIS1* gene was investigated. Sampling was performed by a gastroenterologist at the Center for Endoscopy-Colonoscopy at Ghaem Hospital, Mashhad Iran, affiliated with Mashhad University of Medical Sciences, Mashhad, Iran. Biopsy specimens were collected. The samples were confirmed at the Department of Pathology for CRC and the tumor grade was determined DNA was extracted with a GeNet Bio kit.

Methylation of the MEIS1 gene

DNA methylation was assessed according to the sodium bisulfite-based method. In this method, we evaluated the differences between methylated and non-methylated nucleotides. The bisulfite conversion of DNA was conducted using EpiTect 96 Bisulfite Kit (QIAGEN, Venlo, The Netherlands). The CpGenome Universal Methylated DNA (S7821, Millipore, Billerica, MA) was used as a positive control, and the CpGenome Universal Unmethylated DNA (S7822, Millipore, Billerica, MA) was used as a positive control.

Table 1. Primers used for PCR of *MEIS1*-M and *MEIS1*-U.

Gene	Primer	PCR product
MEIS1-M (Methylated)	Forward:5'-CGTTTCGCGTATTTATTTTTGTC-3'	
	Reverse: 5'-GCTTACAATCCCCGTACGTA-3'	168 base pairs
MEIS1-U (Un-methylated)	Forward:5'-GAGTTTGTTTTGTGTATTTATTTTTGTTG-3'	
	Reverse: 5'-CCCACTTACAATCCCCATACATA-3'	176 base pairs

According to Table 1, two pairs of specific primers were used for methylation and non-methylation. We used a real-time PCR thermocycler (one step ABI).[11] The real-time PCR reaction was conducted with a SYBR Green Real-Time PCR Master Mix and 2 µl of Bisulfite-treated DNA, with a final stage as the melting curve to show the specificity of the PCR product.

Results

The average age of the subjects was 52.42±14.97; the minimum age was 27 years and maximum age was 89. There were 17 (40.5%) The frequency of females and males was 17(40.5%) and 25(59.5%), respectively.

Several pathological factors including cancer stage, grade of tumor, tumor location, extent and depth infiltration of tumor, and lymph node involvement were investigated in this study.[12] Among all patients, 32 cases (76.2%) were at stage A, 7 patients (16.7%) were at stage B and 3 patients (7.1%) were at stage C; the majority of subjects had stage A disease. 18 patients (42.8%) were in grade 1, 23 patients (54.8%) were in grade 2 and 1 patient (2.4%) was in grade 3; most of the patients showed moderately differentiated tumors, in grade 2. The tumor was located at distal side of colon in 34 cases (81%) and in 8 cases (19%) it was in proximal. Analysis of primary tumor indicated that 12 (28.6 %) patients had T2 disease, whereas 25 (59.5%) had T3 and 5 (11.9 %) patients were T4. The majority of patients had T3 disease. We assessed for regional lymph node involvement. In 27 (64.3%) patients, there was no lymph node involvement reported (N0), 12 (28.6%) patients had 1-3 lymph nodes (N1), and 3 (7.1%) patients had metastases in more than 4 lymph nodes (N2).

Methylation of the MEIS-1 gene

After DNA extraction and analysis, the bisulfite process was performed. We used four tubes to assess for methylation of the *MEIS1* gene - two tubes for methylation and two for non-methylation, followed by quantitative methylation-specific real-time PCR (qMSP). According to the results, there was no methylation of the *MEIS1* gene observed.

Discussion

In recent years, the epigenetic changes have been highly regarded because of their fundamental role in the regulation of gene expression. Epigenetic changes, especially DNA methylation in tumor initiation and progression, are very effective and important.

Recent studies indicate that the promoter of *MEIS1* gene is frequently hypermethylated. Its expression is suppressed in some cancers including breast, leukemia, lung and CRC.

Based on the findings of the current study, we observed no methylation of *MEIS1* in 42 patients. Methylation was observed only in the positive control. Assessment of *MEIS1* expression showed no significant differences between the control group and the patients. The most common tumor localization was the distal side which was inconsistent with recent reports.

Laohavinij et al. studied the prognostic factors for survival in patients with CRC and analyzed the traits of pre-/post-treatment in 287 patients with stages I-IV CRC. They assessed 15 clinical variables for survival. Their results on five-year survival showed that 2% of patients with stage IV, 100% of stage I, 68% of stage II, and 44% of stage III patients survived.[13] Based on the findings of other studies, hypermethylation of genes mostly were observed in stages II and III of colon cancer,[14,15] which indicated that methylation in higher cancer stages could be considered a risk factor for predicting patient survival. It can be stated that the *MEIS1* is methylated in higher stage of colon cancer. However, the current study patients had early stages of the disease.

Dihal et al. identified DNA methylation changes associated with the BRAFp.V600 mutation.[11] They tested DNA isolated from cancerous epithelial cells and normal tissue. The researchers concluded that *MEIS1* was the methylated gene compared with normal BRAF and BRAF P.v600E; a correlation existed between the methylation of *MEIS1* and BRAF P.v600E.

Our study showed no reduction in *MEIS1* methylation expression in samples from patients with CRC (data not shown). The methylation gene was absent in these patients, hence it could be said that these cases had good prognosis because methylation has been considered a bad prognostic factor. The patients should be investigated for the BRAF gene mutation. In terms of drug resistance they would be screened as the BRAF would show appropriate feedback to anti-EGFR treatment. These patients must be followed after treatment, because a mutation in the BRAF gene is indicative of a poor prognosis and possibly *MEIS1* methylation would appear at higher cancer stages. Another study that includes additional samples, including patients resistant to anti-EGFR treatment or those with BRAF gene mutations, should be considered. *MEIS1* methylation is suggested to be of prognostic value in patients with CRC.

Musialik et al. investigated the methylation and hematopoietic gene expression among patients with acute leukemia of lymphoblastic B cell progenitors. In patients with acute lymphoblastic leukemia analyzed the promoter methylation of DNA and gene expression levels of *HOXA4, HOXA5, MEIS1, TAL1, IRF8*, and *IRF4* in blood samples of 38 children with acute lymphoblastic leukemia and 20 normal subjects. DNA was treated by sodium bisulfite. DNA methylation levels were assessed for *HOXA4, HOXA5, MEIS1, TAL1, IRF8* and *IRF4*. They found aberrant methylations of *TAL1* (6.3%), *IRF8* (7.9%), *MEIS1* (5.3%), and *IRF4* (2.6%) from the patient group. However, there was no methylation observed in the control group. In the control group, 16% had methylated *HOXA4* and 5% of participants had hypermethylated *HOXA5*. There were less expression levels of *IRF8, MEIS1*, and *TAL1* in patients compared to the control group.[16] They reported an inverse relation between *MEIS1* expression and the number of white blood cells.

In 2014, Mitchell et al. survey a group of genes that cause CRC via hypermethylation. They concluded, in addition to *SEPT9*, *VIM1* and *TMEFF2* genes which played high fraction of methylation and carcinoma, other genes had a high frequency for methylation and colon cancer. *MEIS1*, *EDIL3,* and *SDC2* genes had low methylation rates in non-neoplastic colon tissue.[17] According to the above studies, *MEIS1* methylation was lower than other genes for different cancers. In the current study, we observed less methylation due to the small sample size and low rate of levels of CRC.

In 1997, researchers studied a non-invasive method on patients' blood samples for early diagnosis and detection of tumor metastasis due to CRC. In this study, different cancer stages were diagnosed. This method was introduced as noninvasive, sensitive, and specific for early detection.[18]

Nichita et al. studied the gene expression in mononuclear cells of peripheral blood for early detection of CRC, treatment of adenomatous colorectal polyps and CRC, and reduced mortality rate. This test was acceptable for the majority of patients because of its non-invasiveness. They sought to determine if gene expression of mononuclear cells from peripheral blood could detect the presence of adenomatous polyps and CRC. From a total of 85 samples, there were 41 normal control subjects, 21 cases with adenomatous polyps, and 23 cases had CRC. The qPCR method and statistical models of logistic regression were used to analyze the data. They reported a sensitivity of 78% for CRC, 46% for adenomatous polyps, and 92% specificity for both cases. This study has indicated that the test is associated with developmental potential and minimally invasive for patients.[19]

Ciarloni et al., in a similar study, used a panel that consisted of 29 genes to study gene expression in mononuclear cells. They reported a sensitivity of 59% for patients with adenomatous polyps and 75% for CRC cases. The specificity was 91%.[20]

Several outcomes can be concluded for the current study. The lack of methylation is due to the low disease stage, with 76.20% at stage A, 16.70% at stage B, and 7.10% at stage C.

Despite the lack of methylation in tumor

samples and non-decreasing gene expression in peripheral cells (data not shown), it can be concluded that the patients in this study had good prognosis. Methylation is a poor prognostic factor and should be considered for mutation in others gene for CRC patients.

The second possibility is that the *BRAF* gene is normal and does not allow *MEIS1* to methylate and expression to be suppressed. In order to determine overall survival and examine the amount of methylation, as well as the lack of methylation and its relationship with longevity and response to treatment, we propose that a clinical trial be conducted.

Conflict of Interest

No conflict of interest is declared.

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