

Evaluation of *FOXF1* promoter DNA methylation as a promising epigenetic biomarker for colorectal cancer in stool samples

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Abstract

Colorectal cancer pathogenesis is influenced by gene promoter region methylation, a key epigenetic mechanism. This study investigated whether *FOXF1* promoter methylation in stool samples could serve as a biomarker for colorectal cancer. *FOXF1* promoter methylation was analyzed in stool samples from 50 patients with colorectal cancer and 50 healthy controls. The percentage of methylation reference was determined for each sample. *FOXF1* promoter methylation levels were higher in patients with colorectal cancer than in healthy controls ($p < 0.001$). The median percentage of methylation reference was 0.06 (95% confidence interval, 0.01–13.1) in patients and 0.01 (95% confidence interval, 0.0–0.02) in controls. Receiver operating characteristic curve analysis demonstrated a sensitivity of 59% and a specificity of 96% for *FOXF1* promoter methylation in stool samples. Increased *FOXF1* promoter methylation in stool samples suggests that it may serve as a noninvasive biomarker for the detection of colorectal cancer independent of sex, age, or disease stage.

Keywords: Colorectal cancer, Stool samples, DNA methylation, *FOXF1* gene, Epigenetic biomarker

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Introduction

Colorectal cancer (CRC) is the third most frequent malignancy worldwide and one of the leading causes of cancer-related deaths. The burden of CRC is expected to rise to 60% by 2030^[1,2]. In Iran, CRC ranks as the fourth most prevalent cancer^[3], with its incidence expected to double by 2040. While CRC rates remain very low among the aging Iranian community, there is a concerning rise among younger Iranians^[4]. CRC develops as genetic and epigenetic changes gradually accumulate in precursor lesions such as adenomas and serrated lesions. These changes lead to the inactivation of tumor suppressor genes, chromosomal instability, and oncogene activation^[5,6]. CRC screening can remarkably improve tumor detection in the early stages^[7]. Colonoscopy remains the gold standard tool for CRC diagnosis; however, its invasiveness and high cost limit its use for population screening^[8,9]. The fecal occult blood test (FOBT) is a more affordable and noninvasive alternative, but results in a high rate of false-positive tests and requires frequent repetition^[10]. Unfortunately, the majority of CRC patients are diagnosed at an advanced stage, underscoring the urgent requirement for the rapid identification of diagnostic and prognostic biomarkers^[11,12].

Colorectal cancer can potentially be identified through the detection of genomic biomarkers in stool or blood samples, with varying sensitivity levels^[13,14]. Biomarker-based screening offers significant advantages over classical methods such as imaging and endoscopy^[15]. There is now widespread consensus that methylation markers serve as reliable indicators for identifying early-stage CRC patients^[16]. One well-known epigenetic mechanism is the methylation of cytosine residues within CpG dinucleotide sequences, known as CpG islands, which are located in gene promoter regions. About 60% of human gene promoter regions contain CpG islands, mostly found at the 5' end of genes^[17]. Alterations in DNA methylation patterns are a hallmark of CRC and many other cancers. Studies have revealed that numerous

genes exhibit hypermethylation in early-stage CRC, directly contributing to tumor development and progression. Thus, various methylated genes may serve as valuable molecular markers for early tumor screening^[18,19].

Various studies have demonstrated that numerous members of the FOX family play an active role in cancer progression^[20]. *FOXF1* is a forkhead box (FOX) transcription factor family member. Various FOX family members have been linked to various biological processes, including cell differentiation, development, cell cycle regulation, cell metabolism, aging, cell survival, immune responses, organogenesis, DNA repair, and embryonic development^[21–23]. Early research demonstrated that *FOXF1* was found to be a factor in the progression of multiple cancers, such as osteosarcoma, breast cancer, lung cancer, and colorectal cancer^[24–28]. Notably, *FOXF1* acts as both an oncogene and a tumor suppressor in cancer development^[21].

Despite the increasing evidence pointing to *FOXF1*'s impact on cancer development, its potential as a biomarker for CRC detection in stool remains unexplored. In this study, we aimed to assess, for the first time, the potential of utilizing *FOXF1* gene promoter methylation in stool samples as a noninvasive biomarker for distinguishing CRC patients from healthy controls. Furthermore, we compared its diagnostic sensitivity and specificity with FOBT to evaluate its potential as an alternative or complementary screening method.

Materials and methods

Sample collection

A total of 50 fecal specimens were obtained from CRC patients 7 d following colonoscopy and pathological confirmation of CRC. In addition, 50 stool samples were taken from healthy people 7 d after a colonoscopy to confirm their health. Consent forms were signed by all

participants of the study. The studied population was made up of individuals who had been referred to Imam Reza Hospital, situated in Kermanshah, Iran. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Iran (Ethics code: No. 95285).

DNA extraction

DNA was extracted from fecal samples according to the manufacturer's instructions using the Gene All kit (South Korea, Cat. No. 115-150). In brief, 200 mg of the stool sample and 1 mL of PBS buffer were homogenized in a 2 mL microtube; the microtube was maintained at room temperature for several minutes, and the supernatant was separated into a 2 mL microtube, centrifuged at 13,000 r/min for 2 min, and the supernatant was discarded. Then, 1.3 mL of lysing buffer was added to the pellet and mixed. The tube was incubated at room temperature and then centrifuged at 12,000 r/min for 5 min. The supernatant was transferred to an EZ pass filter and centrifuged at 12,000 r/min for 1 min. The filter was placed on a 1.5 mL microtube, and 100 µL of elution buffer (EB) was added to the filter and centrifuged at 12,000 r/min for 5 min. We added 500 µL of PB to the microtube's contents, transferred them to a G-type spin column, and centrifuged them for 1 min at 12,000 r/min. A 500 µL sample of washing buffer was added to the spin column and centrifuged at 12,000 r/min for 1 min. We then added 50 µL of EB buffer to the column for centrifugation at 13,000 r/min for 1 min, and collected the DNA-containing solution in a new 1.5 mL microtube. The concentration of the extracted DNA was determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). DNA concentration ranged between 20 and 100 ng/µL.

Bisulfite treatment

DNA bisulfite treatment was performed to prepare DNA samples for MethyLight PCR using the EpiTect bisulfite kit in conformity with the manufacturer's protocol (Qiagen, Germany). The EpiTect bisulfite kit was used to modify 20–40 µL of DNA. DNA incubation with high concentrations of sodium bisulfite salt at low pH and high temperature leads to a change in unmethylated cytosine residues into uracil, leaving the methylcytosine unchanged.

Primer and probe design

Primers and a TaqMan probe were constructed using Beacon Designer™ (version 8.13; www.premierbiosoft.com/molecular_beacons) to specifically anneal full-methylated DNA at the *FOXF1* promoter gene. The ALU-C4 sequences were used as a reference control. All primers and probes were synthesized by Metabion Biotechnology Company (Metabion, Steinkirchen, Germany). The primer and probe sequences are shown in Table 1.

Table 1. Primer and probe sequences applied for MethyLight PCR.

Primers and probes		Sequence (5'–3')
<i>FOXF1</i>	Forward primer	5'-GTTAATCGTCGGCGGATTTAGTAG-3'
	Reverse primer	5'-AACTAACTACACCTCTACCGTCAT-3'
	Probe	5'-6-FAM-ACCACTTCTCCCGAAC GAACCGC-TAMRA-3'
<i>ALU-C4</i>	Forward primer	5'-GGTTAGGTATAGTGGTTTATATT TGTAATTTTAGTA-3'
	Reverse primer	5'-ATTAACATAAACTAATCTTAACT CCTAACCTCA-3'
	Probe	5'-6-FAM-CCTACCTTAACCTCCC-TAMRA-3'

MethyLight, a TaqMan-based methylation-specific real-time PCR technique

MethyLight PCR was carried out by an Applied Biosystems (ABI) 7500 Real-time device (Foster City, CA, USA), using the EpiTect MethyLight PCR + ROX Vial Kit (Qiagen, Germany) according to the manufacturer's protocol. Reactions were performed in a 20 µL volume including 10 µL of 1XEpiTect Methylation MasterMix, 5 µL mix including 0.4 µM forward and reverse primers and 0.2 µM TaqMan probe, 50x ROX Dye Solution 0.4 µL, 3–4 µL RNase-free water, and 1–2 µL converted DNA. Thermal cycling was performed for 45 cycles: 5 min at 95 °C for the initial PCR activation step, 15 s at 95 °C for denaturation, and 60 s at 60 °C for the annealing and extension step. Each plate consisted of 1 µL of patient DNA, full-methylated bisulfite-transformed DNA as a positive control (EpiTect PCR Control DNA, Qiagen, Germany), unmethylated human bisulfite-converted DNA (EpiTect PCR Control DNA, Qiagen, Germany), and RNase-free water, used as a negative control with each set of primers and probes. The standard curve was plotted using the Ct value of commercial methylated control DNA (EpiTect PCR Control DNA, Qiagen, Germany, Cat. No. 59655) with different concentrations vs the log value of input concentration DNA. We acquired an acceptable MethyLight PCR result, which is shown by the PCR efficiency (92%–105%) and correct slope value (–3.65 to –3.2). The standard curve was obtained by plotting the Ct values of methylated control DNA as a function of DNA concentrations.

FOBT

An alcohol pyrimidine-based FOBT was performed to assess the presence of blood in all of the stool samples. For this, we suspended a spatula of the stool in normal saline in a microtube and mixed it. Pyrimidine alcohol and 4–5 drops of 10% acetic acid were added and mixed, and 5–6 drops of 10% H₂O₂ were added as a substrate. Pyrimidine alcohol oxidizes the oxygen released from H₂O₂ decomposition by the peroxidase enzyme, causing a color change. The color change to purple indicates the presence of blood in the medium. The activity of the peroxidase enzyme or blood content in the stool determines the extent of color change intensity.

Data analysis

The PCR data were evaluated using ABI 7500 SDS software (v1.3.1, Applied Biosystems, USA). For all samples, the percentage of methylation reference (PMR) was determined using the following formula: ([*FOXF1*/ALU] sample/[*FOXF1*/ALU] positive control) × 100. Statistical analysis was performed using SPSS software (v16.0, IBM, USA). A *p*-value < 0.05 was considered statistically significant. Receiver operating characteristic (ROC) curve analysis was used to evaluate the sensitivity and specificity of *FOXF1* methylation.

Results

Demographic results

Overall, 50 stool samples were obtained from patients with CRC and 50 samples from the control group. The average age of the patients was 56 ± 12.9 years. The healthy controls were matched with the patient group in terms of age and sex. There was no significant difference in age or gender between the CRC and control groups (Table 2).

FOXF1 gene promoter methylation

Median PMR values of stool samples from patients and healthy controls were 0.06 (95% CI 0.01–13.1) and 0.01 (95% CI 0.0–0.02),

respectively (Table 3; Fig. 1). In stool samples from CRC patients, PMR values were higher than those of the control group ($p < 0.001$). PMR values showed no significant difference in demographic or pathological features between the cohorts used in the study (Table 4). The highest value of PMR in the stool samples from healthy controls was considered a threshold of methylation status, while a PMR value higher than that was considered a positive methylation status. Methylation of the *FOXF1* gene (positive results) in the age group > 50 years was compared with the age group ≤ 50 years (73.5% vs 75%) and between female and male patients (80% vs 65%) (Table 5). There was no significant difference in the level of methylation between the gender and age subgroups (Table 5).

The ability of the *FOXF1* gene PMR level as a biomarker

Furthermore, we utilized ROC curve analysis to assess the ability of the *FOXF1* gene PMR level in stool samples to distinguish between CRC patients and controls. ROC curve analysis showed that the sensitivity and specificity of *FOXF1* gene promoter methylation at the

Table 2. The demographic characteristics of the patient group with CRC and the healthy control.

Demographic features	Patient group (<i>n</i> = 50)	Control group (<i>n</i> = 50)	<i>p</i> value*
Age (years)	56 ± 12.9	54 ± 12	0.166
Gender (female:male)	3:2	9:7	0.686

* Mann-Whitney U test.

Table 3. Median PMR value results for *FOXF1* gene promoter methylation in the stool samples of patients with CRC and healthy controls.

	No. of patients	Minimum	Maximum	Percentiles**			<i>p</i> -value*
				25 th	50 th	75 th	
Stool PMR							
Patient group	50	0.0	73	0.01	0.06	13.1	< 0.001
Normal group	50	0.0	0.03	0.0	0.01	0.02	

* Mann-Whitney U test. ** Explore.

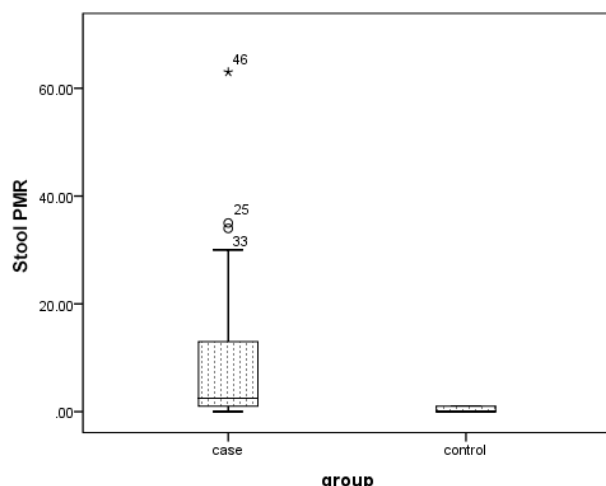


Fig. 1 Box plot for the *FOXF1* gene PMR value of stool samples in case and control groups. The boxes show the interquartile range (the interval between the 25th and 75th percentiles). The lines within the boxes indicate the median values.

optimal cut-off value, with an AUC of 0.744 ($p < 0.001$), were 59% and 96%, respectively (Fig. 2).

FOBT results

We also examined the FOBT as an early diagnostic test. The sensitivity of FOBT was 38% compared to 59% for *FOXF1* gene promoter methylation. These results showed that the sensitivity of *FOXF1* gene promoter methylation in stool samples was significantly higher than that of the FOBT.

Discussion

Colorectal cancer is a major public health concern, accounting for about 10% of worldwide incidence and mortality^[29]. In the development of CRC, there is a gradual accumulation of different genetic and epigenetic changes, with abnormal DNA methylation being a crucial

Table 4. Association of stool PMR values (percentiles) for the *FOXF1* gene with demographic and pathological characteristics of the patient group.

Characteristics		No. of patients	Stool PMR (percentiles)**			<i>p</i> value*
			25 th	50 th	75 th	
Age	≤ 50 years	16	0.99	2.64	13.37	0.5
	> 50 years	34	2.05	3.48	13.6	
Gender	Female	30	2.07	3.9	10.87	0.41
	Male	20	1.1	2.55	14.95	
Grade	Poorly and moderate	10	1.91	3.1	9.15	0.97
	Well	40	1.98	2.85	14.5	
Location	Distal	21	1.34	2.5	5.62	0.06
	Proximal	29	2.04	4.6	20.25	
Size	≤ 10 mm	25	1.91	2.65	9.02	0.31
	> 10 mm	25	1.74	4.7	17.25	
Stage	Stage I and II	28	2.2	3.65	10.1	0.13
	Stage III and IV	22	2.71	4.1	15.6	

* Mann-Whitney U test. ** Explore.

Table 5. Association between *FOXF1* gene promoter hypermethylation and clinicopathological characteristics.

Characteristics		No. of patients	Hypermethylation status**		<i>p</i> value*
			Positive (%)	Negative (%)	
Age	≤ 50 years	16	12 (75%)	4 (25%)	0.61
	> 50 years	34	25 (73.5%)	9 (26.5%)	
Gender	Female	30	24 (80%)	6 (20%)	0.14
	Male	20	13 (65%)	7 (35%)	
Histological grade	Poor and moderate	10	8 (80%)	2 (20%)	0.53
	Well	40	29 (72.5%)	11 (27.5%)	
Tumor location	Distal	21	15 (71.4%)	6 (28.6%)	0.74
	Proximal	29	22 (75.9%)	7 (24.1%)	
Tumor size	≤ 10 mm	25	17 (68%)	8 (32%)	0.34
	> 10 mm	25	20 (80%)	5 (20%)	
Tumor stage	Stage I and II	28	23 (82.1%)	5 (17.9%)	0.15
	Stage III and IV	22	19 (83.3%)	3 (16.7%)	
Methylation levels (PMR values)			0.06 (95% CI; 0.01–13.1)	–	< 0.001
Median PMR (healthy controls)			0.01 (95% CI; 0.0–0.02)	–	

* Fisher's exact test. ** Crosstabs.

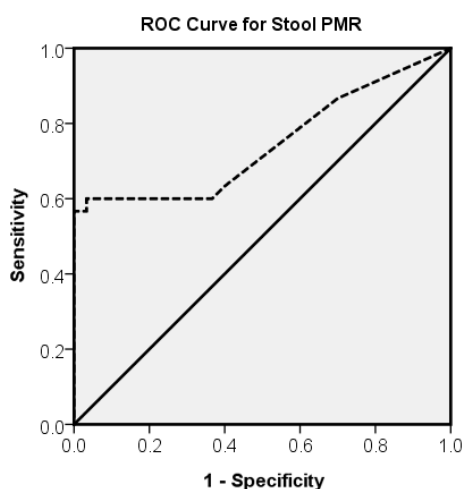


Fig. 2 ROC curve for *FOXF1* gene promoter methylation in stool samples.

mechanism^[30]. Aberrant DNA methylation patterns are commonly observed in gastrointestinal neoplasms and could serve as valuable markers for screening, diagnosis, prognosis determination, and prediction of CRC. In addition, the plentiful presence in extracellular environments, resistance to degradation, and presence in plasma, serum, fecal specimens, and other noninvasive bodily fluids facilitate quantitative analysis of methylated DNA biomarkers^[31]. Collecting bodily fluids is a non-intrusive process that allows for the early identification of cancer or disease monitoring^[32]. Numerous studies have validated stool-based methylated DNA detection as an effective method for early CRC screening^[31]. Promoter hypermethylation contributes to the inactivation of tumor suppressor genes and ultimately oncogenic processes by affecting key cellular pathways^[16,33]. Common methylated and silenced genes often seen in CRC include vimentin (VIM), cadherin-1 (CDH1), MLH1, TIMP metalloproteinase inhibitor-3 (TIMP3), hypermethylated in cancer-1 (HIC1), and secreted frizzled related protein-1 (SFRP1)^[33]. Recently, methylated syndecan-2 (mSDC2) and methylated SEPT9 (mSEPT9) have been identified as promising biomarkers for noninvasive detection of CRC^[34–37]. Researchers have studied abnormal methylation of different genes in the tissues and bodily fluids of individuals with CRC, which could be used as valuable biomarkers in screening for CRC^[16]. Cologuard is the first multitarget stool DNA test that has received FDA approval for CRC screening. It evaluates KRAS mutations, as well as BMP3 and NDRG4 methylation levels^[13,38]. Moreover, NDRG4, BMP3, and SEPT9 are now incorporated as DNA methylation markers in FDA-approved CRC screening tests^[39]. Therefore, we aimed to identify non-invasive biomarkers capable of detecting CRC in small stool samples. Analyzing single genes in epigenetic studies could provide a more cost-effective option for screening. Nevertheless, these studies have presented remarkable initial findings about these single epigenetic markers and their utility as stool DNA-based assays^[40]. Moradi et al. reported that PMR values in the stool of CRC patients were higher than those of healthy controls. Also, the analysis of SOX21 gene promoter methylation using a stool-based assay shows a notably high level of sensitivity (72.5%) and specificity (100%)^[41]. Zhang et al. showed that SPG20 promoter hypermethylation serves as a highly sensitive and specific biomarker in the detection of CRC using stool samples (80.2% and 100%, respectively)^[42]. Glöckner et al. reported that, in stage I to III CRC patients, TFPI2 methylation was found in stool DNA with a sensitivity ranging from 76% to 89% and a specificity ranging from 79% to 93%. Recognition of TFPI2 methylation in fecal DNA may serve as a biomarker for CRC screening^[40]. Muller et al. identified a sensitivity of 77% to 90% and a specificity of 77% for

SFRP2 methylation as a sensitive single DNA-based biomarker for detecting CRC in stool samples from 49 patients^[43]. According to research, using single-marker methylation-based stool markers for Vimentin and SFRP2, the sensitivity has been found to be 52% and 87% to 94%, while the specificity has been reported to be 90% and 85% to 90%, respectively^[44–47]. Various studies have indicated that the FOX family plays a crucial role in cellular proliferation, apoptosis, metastasis, the cell cycle, cellular metabolism, progression, and cancer biology in various cancers^[48–51]. In total, dysregulation of FOX proteins may impact cell fate, tumor growth, and metastasis of cancer^[52]. *FOXF1*, a transcription factor, attaches to the promoter regions of target genes to control gene expression levels and influence the function of various signaling pathways crucial for physiological and pathological processes^[20]. *FOXF1* is critical for controlling angiogenesis during embryonic development and may also be involved in angiogenesis, impacting CRC progression. Increased *FOXF1* expression has been linked to aggressive CRC features, including poor differentiation and advanced disease stages. There was a significant association between increased *FOXF1* expression and poorer prognosis in CRC patients^[28]. In contrast, *FOXF1* expression is often silenced through DNA hypermethylation in breast cancer, where it acts as a tumor suppressor^[53]. Herrera-Merchan et al. reported that *FOXF1* expression could also be silenced via promoter methylation in lung adenocarcinoma and functions as a tumor suppressor^[54]. In another study, Mitchell et al. analyzed a panel of 23 genes in the blood and tissue of CRC patients compared to a control group. They found increased DNA methylation in at least half of the CRC tissue samples compared to normal tissue. Additionally, 11 genes, including *FOXF1*, exhibited low methylation levels in peripheral blood DNA from healthy individuals^[55]. On the other hand, *FOXF1* promotes the development and maintenance of gastrointestinal stromal tumors through the regulation of KIT and ETV1 expression^[56]. In hepatocellular carcinoma, *FOXF1* serves as a suppressor of cell invasion and tumorigenesis, with its expression closely linked to patient survival rates^[57]. Furthermore, in CRC cell lines with p53 inactivation, *FOXF1* expression is silenced, leading to genomic instability in *FOXF1*-expressing CRC cells with a dysfunctional p53-p21WAF1 checkpoint. This underscores *FOXF1*'s significance in CRC development^[58]. Additionally, the competitive binding of miR-375-3p by LINC00022 could lead to the upregulation of *FOXF1*, potentially contributing to CRC progression^[59]. Lo et al. reported cytoplasmic mislocalization of upregulated *FOXF1* protein in CRC epithelial cells, which is associated with poor prognosis factors^[21]. Furthermore, *FOXF1* upregulation is related to poor outcomes, disease progression, invasion, and metastasis of CRC^[20]. Recently, it was reported that *FOXF1* gene methylation levels in the plasma of CRC patients are higher than in healthy controls^[60]. In the present study, for the first time, we determined the *FOXF1* gene promoter methylation for 50 patients with CRC and 50 healthy controls. Our results showed that the median PMR values of stool samples from patients and healthy controls were between 0.01 to 13.1 and 0.0 to 0.02, respectively. In addition, we examined the association of the PMR values obtained from stool samples with the demographic and pathologic features of the patients. There was no statistically significant correlation found between the methylation levels of the *FOXF1* gene promoter and age, gender, histological grade, tumor stage, tumor location, or tumor size in CRC patients. In our research, *FOXF1* gene promoter methylation position was assessed by the MethyLight technique, a quantitative method with high sensitivity and specificity capable of assaying a small amount of DNA. Our data revealed that the *FOXF1* gene promoter methylation had higher sensitivity and specificity than FOBT in stool samples.

Although our results offer valuable information on the potential of *FOXF1* methylation as a noninvasive biomarker for CRC detection in stool samples, certain limitations must be considered. The sample size

is relatively small and may affect the generalizability of our results. Furthermore, potential biases in patient selection and sample collection could impact the observed methylation patterns. To validate our findings and better evaluate the clinical utility of *FOXF1* methylation in CRC detection, future studies should include larger, more diverse cohorts.

Conclusions

In summary, as the first study to evaluate *FOXF1* gene promoter methylation in stool samples of patients with CRC, it can be concluded that the PMR value for *FOXF1* in CRC patients is higher than in healthy controls. The quantification of *FOXF1* gene promoter methylation in stool samples shows promise as a noninvasive biomarker for CRC screening, with a sensitivity of 59% and a specificity of 96%. These results suggest potential sensitivity advantages over FOBT. Additionally, *FOXF1* gene promoter methylation was found to be independent of age, sex, tumor grade, or stage, highlighting its potential utility in diverse patient populations. Future research with larger cohorts and more diverse populations is necessary to confirm these findings and explore their feasibility as a cost-effective screening tool.

Ethical statements

All individuals participating were tasked with signing the informed consent form. The study was approved by the Ethics Committee at Kermanshah University of Medical Sciences, Iran (Ethics Code: No. 95285) and conformed to the principles of the Helsinki Declaration.

Author contributions

The authors confirm their contribution to the paper as follows: study conception and design: Mehrjoei B, Rezvani N; sample and clinical data collection: Tahmasebi S; data analysis and interpretation: Rezvani N; manuscript preparation: Mehrjoei B; manuscript review and editing: Vaisi-Raygani A. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest

The authors declare that there is no conflict of interest.

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References

- [1] Mehrjoei B, Haghazari L, Bashiri H, Rezvani N. 2024. The diagnostic potential of miR-196a-1 in colorectal cancer. *BMC Cancer* 24(1):162
- [2] Mao Y, Wang W, Yang J, Zhou X, Lu Y, et al. 2024. Drug repurposing screening and mechanism analysis based on human colorectal cancer organoids. *Protein & Cell* 15(4):285–304
- [3] Dahouri A, Sahebhiagh MH, Gilani N. 2023. Comparison of sexual function of people with colorectal cancer with and without colostomy bag in Iran: a comparative cross-sectional study. *Scientific Reports* 13:12558
- [4] Hoseini B, Rahmatinejad Z, Goshayeshi L, Bergquist R, Golabpour A, et al. 2022. Colorectal Cancer in North-Eastern Iran: a retrospective, comparative study of early-onset and late-onset cases based on data from the Iranian hereditary colorectal cancer registry. *BMC Cancer* 22(1):48
- [5] Jung G, Hernández-Illán E, Moreira L, Balaguer F, Goel A. 2020. Epigenetics of colorectal cancer: biomarker and therapeutic potential. *Nature Reviews Gastroenterology & Hepatology* 17(2):111–130
- [6] Gupta S. 2022. Screening for colorectal cancer. *Hematology/Oncology Clinics of North America* 36(3):393–414
- [7] Li J, Li ZP, Ruan WJ, Wang W. 2024. Colorectal cancer screening: the value of early detection and modern challenges. *World Journal of Gastroenterology* 30(20):2726–2730
- [8] Cordero OJ, Mosquera-Ferreiro L, Gomez-Tourino I. 2024. Improving colorectal cancer screening programs. *World Journal of Gastroenterology* 30(22):2849–2851
- [9] Shaikat A, Levin TR. 2022. Current and future colorectal cancer screening strategies. *Nature Reviews Gastroenterology & Hepatology* 19(8):521–531
- [10] Bretthauer M. 2011. Colorectal cancer screening. *Journal of Internal Medicine* 270:87–98
- [11] Martinou E, Falgari G, Bagwan I, Angelidi AM. 2021. A systematic review on HOX genes as potential biomarkers in colorectal cancer: an emerging role of HOXB9. *International Journal of Molecular Sciences* 22(24):13429
- [12] Jia S, Zhang R, Li Z, Li J. 2017. Clinical and biological significance of circulating tumor cells, circulating tumor DNA, and exosomes as biomarkers in colorectal cancer. *Oncotarget* 8(33):55632–55645
- [13] Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, et al. 2014. Multitarget stool DNA testing for colorectal-cancer screening. *New England Journal of Medicine* 370(14):1287–1297
- [14] Church TR, Wandell M, Lofton-Day C, Mongin SJ, Burger M, et al. 2014. Prospective evaluation of methylated *SEPT9* in plasma for detection of asymptomatic colorectal cancer. *Gut* 63(2):317–325
- [15] Almeida-Lousada H, Mestre A, Ramalhete S, Price AJ, de Mello RA, et al. 2021. Screening for colorectal cancer leading into a new decade: the "roaring '20s" for epigenetic biomarkers? *Current Oncology* 28(6):4874–4893
- [16] Fatemi N, Tierling S, Es HA, Varkiani M, Mojarad EN, et al. 2022. DNA methylation biomarkers in colorectal cancer: clinical applications for precision medicine. *International Journal of Cancer* 151(12):2068–2081
- [17] Constância V, Nunes SP, Henrique R, Jerónimo C. 2020. DNA methylation-based testing in liquid biopsies as detection and prognostic biomarkers for the four major cancer types. *Cells* 9(3):624
- [18] Weisenberger DJ, Liang G, Lenz HJ. 2018. DNA methylation aberrancies delineate clinically distinct subsets of colorectal cancer and provide novel targets for epigenetic therapies. *Oncogene* 37(5):566–577
- [19] Gyparakis MT, Basdra EK, Papavassiliou AG. 2013. DNA methylation biomarkers as diagnostic and prognostic tools in colorectal cancer. *Journal of Molecular Medicine* 91(11):1249–1256
- [20] Wang S, Yan S, Zhu S, Zhao Y, Yan J, et al. 2018. *FOXF1* induces epithelial-mesenchymal transition in colorectal cancer metastasis by transcriptionally activating *SNAIL1*. *Neoplasia* 20(10):996–1007
- [21] Lo PK, Lee JS, Chen H, Reisman D, Berger FG, et al. 2013. Cytoplasmic mislocalization of overexpressed *FOXF1* is associated with the malignancy and metastasis of colorectal adenocarcinomas. *Experimental and Molecular Pathology* 94(1):262–269
- [22] Ouyang X, Li S, Ding Y, Xin F, Liu M. 2022. *Foxf1* gene increases the risk of osteoporosis in rats by inhibiting osteoblast formation and promoting

- osteoclast differentiation through the upregulation of NF- κ B pathway. *Journal of Musculoskeletal & Neuronal Interactions* 22(2):242–250
- [23] Jin Y, Liang Z, Lou H. 2020. The emerging roles of fox family transcription factors in chromosome replication, organization, and genome stability. *Cells* 9(1):258
- [24] Zhu KP, Zhang CL, Ma XL. 2017. Antisense lncRNA FOXF1-AS1 promotes migration and invasion of osteosarcoma cells through the FOXF1/MMP-2/-9 pathway. *International Journal of Biological Sciences* 13(9):1180–1191
- [25] Nilsson G, Kannius-Janson M. 2016. Forkhead Box F1 promotes breast cancer cell migration by upregulating lysyl oxidase and suppressing Smad2/3 signaling. *BMC Cancer* 16(1):142
- [26] Wei HJ, Nickoloff JA, Chen WH, Liu HY, Lo WC, et al. 2014. FOXF1 mediates mesenchymal stem cell fusion-induced reprogramming of lung cancer cells. *Oncotarget* 5(19):9514–9529
- [27] Saito RA, Micke P, Paulsson J, Augsten M, Peña C, et al. 2010. Forkhead box F1 regulates tumor-promoting properties of cancer-associated fibroblasts in lung cancer. *Cancer Research* 70(7):2644–2654
- [28] Wang S, Xiao Z, Hong Z, Jiao H, Zhu S, et al. 2018. FOXF1 promotes angiogenesis and accelerates bevacizumab resistance in colorectal cancer by transcriptionally activating VEGFA. *Cancer Letters* 439:78–90
- [29] Shen Y, Wang D, Yuan T, Fang H, Zhu C, et al. 2023. Novel DNA methylation biomarkers in stool and blood for early detection of colorectal cancer and precancerous lesions. *Clinical Epigenetics* 15(1):26
- [30] Lim SB, Joe S, Kim HJ, Lee JL, Park IJ, et al. 2023. Deciphering the DNA methylation landscape of colorectal cancer in a Korean cohort. *BMB Reports* 56(10):569–574
- [31] Hashimoto Y, Zumwalt TJ, Goel A. 2016. DNA methylation patterns as noninvasive biomarkers and targets of epigenetic therapies in colorectal cancer. *Epigenomics* 8(5):685–703
- [32] Toiyama Y, Okugawa Y, Goel A. 2014. DNA methylation and microRNA biomarkers for noninvasive detection of gastric and colorectal cancer. *Biochemical and Biophysical Research Communications* 455(1–2):43–57
- [33] Yi JM. 2021. DNA methylation change profiling of colorectal disease: screening towards clinical use. *Life* 11(5):412
- [34] Fu B, Yan P, Zhang S, Lu Y, Pan L, et al. 2018. Cell-free circulating methylated SEPT9 for noninvasive diagnosis and monitoring of colorectal cancer. *Disease Markers* 2018(1):6437104
- [35] Zhao G, Liu X, Liu Y, Li H, Ma Y, et al. 2020. Aberrant DNA methylation of SEPT9 and SDC2 in stool specimens as an integrated biomarker for colorectal cancer early detection. *Frontiers in Genetics* 11:643
- [36] Zhao G, Liu X, Liu Y, Ma Y, Yang J, et al. 2021. Methylated SFRP2 and SDC2 in stool specimens for Colorectal Cancer early detection: a cost-effective strategy for Chinese population. *Journal of Cancer* 12(9):2665–2672
- [37] Song L, Jia J, Peng X, Xiao W, Li Y. 2017. The performance of the SEPT9 gene methylation assay and a comparison with other CRC screening tests: a meta-analysis. *Scientific Reports* 7:3032
- [38] Ahlquist DA, Zou H, Domanico M, Mahoney DW, Yab TC, et al. 2012. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology* 142(2):248–256
- [39] Koch A, Joosten SC, Feng Z, de Ruijter TC, Draht MX, et al. 2018. Analysis of DNA methylation in cancer: location revisited. *Nature Reviews Clinical Oncology* 15(7):459–466
- [40] Glöckner SC, Dhir M, Yi JM, McGarvey KE, Van Neste L, et al. 2009. Methylation of *TFPI2* in stool DNA: a potential novel biomarker for the detection of colorectal cancer. *Cancer Research* 69(11):4691–4699
- [41] Moradi K, Babaei E, Ali Hosseinpour Feizi M, Safaralizadeh R, Rezvani N. 2021. Quantitative detection of SRY-Box 21 (SOX21) gene promoter methylation as a stool-based noninvasive biomarker for early diagnosis of colorectal cancer by MethyLight method. *Indian Journal of Cancer* 58(2):217–224
- [42] Zhang H, Song YC, Dang CX. 2013. Detection of hypermethylated spastic paraplegia-20 in stool samples of patients with colorectal cancer. *International Journal of Medical Sciences* 10(3):230–234
- [43] Müller HM, Oberwalder M, Fiegl H, Morandell M, Goebel G, et al. 2004. Methylation changes in faecal DNA: a marker for colorectal cancer screening? *The Lancet* 363(9417):1283–1285
- [44] Chen WD, Han ZJ, Skoletsky J, Olson J, Sah J, et al. 2005. Detection in fecal DNA of colon cancer-specific methylation of the nonexpressed vimentin gene. *Journal of the National Cancer Institute* 97(15):1124–1132
- [45] Wang DR, Tang D. 2008. Hypermethylated SFRP2 gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. *World Journal of Gastroenterology* 14(4):524
- [46] Huang Z, Li L, Wang J. 2007. Hypermethylation of SFRP2 as a potential marker for stool-based detection of colorectal cancer and precancerous lesions. *Digestive Diseases and Sciences* 52(9):2287–2291
- [47] Oberwalder M, Zitt M, Wöntner C, Fiegl H, Goebel G, et al. 2008. SFRP2 methylation in fecal DNA—a marker for colorectal polyps. *International Journal of Colorectal Disease* 23(1):15–19
- [48] Kim CG, Lee H, Gupta N, Ramachandran S, Kaushik I, et al. 2018. Role of Forkhead Box Class O proteins in cancer progression and metastasis. *Seminars in Cancer Biology* 50:142–151
- [49] Weng W, Okugawa Y, Toden S, Toiyama Y, Kusunoki M, et al. 2016. FOXM1 and FOXQ1 are promising prognostic biomarkers and novel targets of tumor-suppressive miR-342 in human colorectal cancer. *Clinical Cancer Research* 22(19):4947–4957
- [50] Cui YM, Jiang D, Zhang SH, Wu P, Ye YP, et al. 2014. FOXC2 promotes colorectal cancer proliferation through inhibition of FOXO3a and activation of MAPK and AKT signaling pathways. *Cancer Letters* 353(1):87–94
- [51] Xie R, Wang J, Liu X, Wu L, Zhang H, et al. 2017. RUFY3 interaction with FOXK1 promotes invasion and metastasis in colorectal cancer. *Scientific Reports* 7:3709
- [52] Myatt SS, Lam EWF. 2007. The emerging roles of forkhead box (Fox) proteins in cancer. *Nature Reviews Cancer* 7(11):847–859
- [53] Lo PK, Lee JS, Liang X, Han L, Mori T, et al. 2010. Epigenetic inactivation of the potential tumor suppressor Gene *FOXF1* in breast cancer. *Cancer Research* 70(14):6047–6058
- [54] Herrera-Merchan A, Cuadros M, Rodriguez MI, Rodriguez S, Torres R, et al. 2020. The value of lncRNA *FENDRR* and *FOXF1* as a prognostic factor for survival of lung adenocarcinoma. *Oncotarget* 11(13):1172–1185
- [55] Mitchell SM, Ross JP, Drew HR, Ho T, Brown GS, et al. 2014. A panel of genes methylated with high frequency in colorectal cancer. *BMC Cancer* 14(1):54
- [56] Ran L, Chen Y, Sher J, Wong EWP, Murphy D, et al. 2018. FOXF1 defines the core-regulatory circuitry in gastrointestinal stromal tumor. *Cancer Discovery* 8(2):234–251
- [57] Zhao ZG, Wang DQ, Hu DF, Li YS, Liu SH. 2016. Decreased FOXF1 promotes hepatocellular carcinoma tumorigenesis, invasion, and stemness and is associated with poor clinical outcome. *OncoTargets and Therapy* 9:1743–1752
- [58] Lo PK, Lee JS, Sukumar S. 2012. The p53–p21WAF1 checkpoint pathway plays a protective role in preventing DNA rereplication induced by abrogation of FOXF1 function. *Cellular Signalling* 24(1):316–324
- [59] Xu L, He H, Shang Y, Qu X, Sun J. 2022. LINC00022 acts as an oncogene in colorectal cancer progression via sponging miR-375-3p to regulate FOXF1 expression. *BMC Cancer* 22(1):453
- [60] Dastafkan Z, Rezvani N, Amini S. 2023. Diagnostic value of *FOXF1* gene promoter-methylated DNA in the plasma samples of patients with colorectal cancer. *The International Journal of Biological Markers* 38(3–4):194–202



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