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The Implications of *SFRP2* Gene Hypermethylation in Colorectal Cancer

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Abstract

Background: The Wnt pathway is stimulated by the DNA hypermethylation of *SFRP2*, which inhibits gene activity, downregulates the gene expression and promotes CRC.

Objectives: This study aims to assess the prognostic impact of hypermethylation of *SFRP2* in colorectal cancer.

Methodology: The research used twenty (20) blood samples from patients with colorectal cancer detected at Sher-I-Kashmir institute of medical sciences as well as thirty (30) patients with histologically confirmed colorectal cancer who underwent surgical excision in the department of general surgery. Methylation Specific PCR (MSP) was employed to assess *SFRP2* methylation and the results were correlated with several investigated clinicopathological characteristics.

Results: Our results showed that *SFRP2* methylation levels are significantly present in tumor tissues and blood samples as compared to normal counterparts.

Conclusion: We infer that the hypermethylation of the *SFRP2* promoter in colorectal tumors may contribute to the development of cancer in normal colon and rectum cells.

Keywords: *SFRP2*; Methylation; Methylation Specific PCR (MSP); Clinicopathological; Hypermethylation

Introduction

Colorectal cancer is a very heterogeneous illness that includes a variety of tumor phenotypes with distinct genetic and morphological characteristics. Proto oncogenes, DNA repair pathway genes and tumor suppressor genes are all mutated in CRC [1]. It is currently the most frequent malignant cancer of the gastrointestinal tract, accounting for 13% of all malignant tumors [2]. It normally grows as a polyp, a tumor projecting into the lumen, in the lining of the colon and rectum. Colorectal cancer is the second most prevalent cancer to cause death in 2020, according to the GLOBOCAN 2020 database, with more than 1.9 million new cases and almost 935,000 fatalities [3].

DNA methylation abnormalities are also known to play a function in carcinogenesis [4]. Promoter hypermethylation is becoming acknowledged playing a critical part in epigenetic gene silencing in human neoplasia, including CRC. One of the primary epigenetic mechanisms known to be involved in carcinogenesis is the methylation of the cytosine residues of CpG rich sequences (CpG islands) located inside the promoter regions of genes directing cell proliferation, death and DNA repair [5]. *APC*, *MLH1*, *CDKN2A*, *VIM* and *CDH1* have aberrant promoter hypermethylation in CRC, which silences their expression and promotes to carcinogenesis [6,7]. CRC is linked to aberrant activation of the Wnt canonical signaling pathway, which also promotes tumor cell growth, proliferation and loss of apoptosis. Adenomatous Polyposis Coli (APC), a tumor suppressor that is highly mutated in colorectal cancers, is a crucial component of the Wnt canonical pathway that is involved in the degradation of β-catenin. One of the main signaling pathways in cancer, Wnt signaling controls cell proliferation, motility and differentiation [8].

Gastrointestinal cancers have high amounts of β-catenin. APC inactivation can be caused by germline and somatic mutations, as well as promoter hypermethylation. CIN can also be caused by changes to other genes in this route, including β-catenin. These mutations are detected in 48% of CRCs that do not have APC. A group of 162 Wnt pathway target genes are activated by β-catenin in a colon cancer cell line [9]. Secreted Frizzled Related Proteins (SFRPs) are a group of five secreted glycoproteins that suppress Wnt signaling both canonically and noncanonically *via* various ways. 90% of colorectal tumors have aberrant Wnt signaling as an early stage event. As a result, SFRP's role as a repressive Wnt signaling regulator could be significant in carcinogenesis and its down regulation has been linked to human malignancies [10].

In the N-terminal half of the proteins, SFRPs have a distinctive Cysteine Rich Domain (CRD) that is homologous to the CRD of the Frizzled (Fz) receptor of Wnt. In order to prevent Wnt proteins from

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attaching to Fz proteins, SFRPs may interact with them or they may create inactive complexes with Fz, to limit Wnt signaling. SFRPs have been shown to possibly block the complete canonical Wnt pathway in colon cancer cells, even in the presence of mutations that activate APC or β-catenin downstream of the Fz receptor [11]. The DNA hypermethylation of *SFRP2*, which is situated upstream of the canonical Wnt signaling pathway, causes the Wnt pathway to be activated, downregulate gene expression, block gene function and promote CRC. Additionally, these genes' DNA hypermethylation may serve as a biomarker for the detection of CRC [12]. So, this study intends to evaluate the prognostic significance of hypermethylation of *SFRP2* in colorectal cancer.

Materials and Methods

Sample collection

The study was given ethical clearance by the ethical committee of SKIMS, Srinagar, Jammu and Kashmir, India and all patients provided written consent. Thirty (30) histologically confirmed colorectal cancer people who had surgical excision in the department of general surgery, at Sher-I-Kashmir institute of medical sciences and twenty (20) blood samples from colorectal cancer diagnosed patients were incorporated into this research. Neither of the CRC patients had been given chemotherapy or radiotherapy. Amounting to 500 mg, sterile vials were immediately filled with surgically removed tumor tissue and normal tissue that was nearby and 10 cm away from the tumor and 2 ml of venous blood in EDTA vial (purple topped collection tube) were collected from the CRC patient along with control blood samples collected from healthy individuals The collected tissues and blood were stored at 80°C till further processing. Histopathological reports of the amassed tissues were acquired from the department of pathology, SKIMS.

From a tissue sample, molecular DNA was extracted by the salting-out method, and from the blood, it was done by the phenol-chloroform proteinase-k method. Utilizing an EZ DNA methylation kit, 1 µg-2 µg of extracted genomic DNA was treated to the bisulfite modification (Zymo research, Irvine, California). The primers used for the methylation and unmethylated alleles' promoter regions are listed in the Table 1. Products that had been methylated and those that hadn't were both 138 base pairs and 145 base pairs long.

CpG status	Primers (5'-3')	Annealing temperature (°C)	Product size (bp)
SFRP2 MF	GGGTCGGAGTTTTTCGGAGTTGCGC	62°C	138 bp
SFRP2 MR	CCGCTCTCTTCGCTAAATACGACTCG		
SFRP2 UF	TTTTGGGTTGGAGTTTTTTGGAGTTGTGT	58°C	145 bp
SFRP2 UR	AACCCACTCTCTTCACTAAATACAACTCA		
Note: M=Methylated; U=Unmethylated; R=Forward; R=Reverse.			

Table 1: Table is showing primers used for the methylation and unmethylated alleles' promoter regions.

Statistical analysis

For continuous variables, the independent t-test and paired t-test were used; for discrete variables, Pearson's $\chi 2$ test, Fisher's exact test or $\chi 2$ test (trend) were used. Using logistic regression analysis, Odds Ratios (ORs) and 95% Confidence Intervals (CIs) were calculated. On two sided tests, all given P values were based. $P<0.05$ was used as the significance threshold. The statistical software STATA 16 was used to conduct the tests.

Results

The study involved a total of fifty CRC cases (n=50) which included 29 (58%) males and 21 (42%) females. 15 of 50 (30%) subjects were >50 years and 35 of 50 (70%) with a mean age of ≤ 50 years, 55.32 ± 15.38. Out of 50 cases, 20 (40%) were smokers and 30 (60%) were non-smokers. 14 (28%) had colon cancer and 36 (72%) had rectal cancer. 35 (70%) exhibited stage I or stage II illness and 15 (30%) had stage III or IV illness. On the basis of the grade of differentiation 15 (30%) cases were well differentiated and 21 (42%) were moderate and 14 (28%) were poorly differentiated (Table 2).

Characteristics	Number and percentage (%)
Age	
>50	15 (30)
≤ 50	35 (70)
Gender	
Male	29 (58)
Female	21 (42)
Dwelling	

Rural	29 (58)
Urban	21 (42)
Social class	
Low	15 (30)
Middle	35 (70)
Family history	
Yes	12 (24)
No	38 (76)
Smoking status	
Smoker	20 (40)
Non-smoker	30 (60)
Lifestyle	
Active	38 (76)
Sedentary	12 (24)
Body mass	
Normal	29 (58)
Obese	13 (26)
Underweight	08 (16)
Salt tea intake	
Yes	46 (92)
No	04 (8)
Red meat consumption	
Yes	28 (56)
No	22 (44)
Sundried vegetables	
Yes	41 (82)
No	09 (18)
Junk food consumption	
Yes	07 (14)
No	43 (86)
Pesticide exposure	
Yes	08 (16)
No	42 (84)
Site of tumour	
Colon	14 (28)
Rectum	36 (72)

Tumour differentiation	
Well	15 (30)
Moderate	21 (42)
Poor	14 (28)
TNM stage	
T1	12 (24)
T2	18 (36)
T3	14 (28)
T4	06 (12)
T1+T2	30 (60)
T3+T4	20 (40)
Stage	
I+II	35 (70)
III+IV	15 (30)
Tumour grade	
1	16 (32)
2	21 (42)
3	13 (26)

Table 2: This study examined the clinicopathologic and clinicoepidemiological characteristics of patients with colorectal cancer.

Methylation status of SFR2 in tissue samples

It is showing the Figure 1.

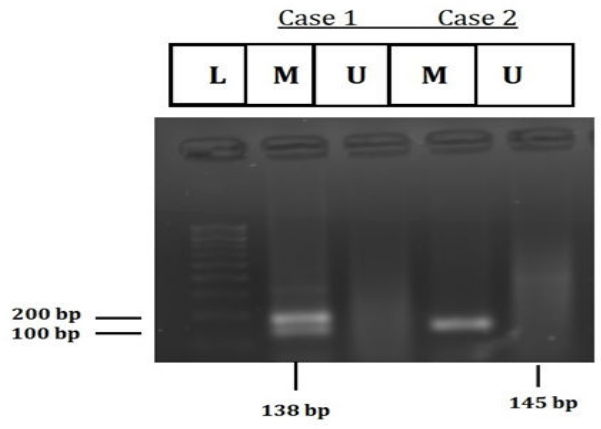

Figure 1: Representative image of *SFRP2* gene promoter methylation pattern of CRC tissue samples by MSP.

L: 100 bp unmethylated *SFRP2* is indicated by the DNA marker U: (145 bp); while methylated *SFRP2* is indicated by M: (138 bp).

In tumor tissues, methylation was found to be present in 66.66% (20/30) cases whereas no methylation was found in 33.33% (10/30) of the cases. Out of 20 CRC cases with hypermethylated *SFRP2* gene promoters, 20% (4/20) showed both methylated and unmethylated bands whereas 80% (16/20) showed only methylated bands.

In the adjacent normal tissues, only 10% (3/30) showed the presence of methylation. While the remaining normal tissues showed no methylation.

Further *SFRP2* gene promoter methylation pattern was correlated with several clinicopathological characteristics such as age, gender, tumor location, tumor grading, tumor staging, smoking status and family history. The connection between the *SFRP2* promoter methylation status and other clinicopathological characteristics is summarized in Table 3.

Characteristics	Methylation present	Methylation absent	Odds ratio (95% CI)	P-value	Chi^2
Age					
>50	3 (50)	3 (50)			
≤ 50	17 (29.17)	7 (70.83)	0.41 (0.06-2.55)	0.333	0.9375
Gender					
Male	10 (62.50)	6 (37.50)	0.66 (0.14-3.10)	0.605	0.2679
Female	10 (71.43)	4 (28.57)			
Dwelling					
Rural	9 (64.29)	5 (35.71)			
Urban	11 (68.75)	5 (31.25)	0.81 (0.17-3.74)	0.796	0.0670
Social class					
Low	7 (58.33)	5 (41.67)			
Middle	13 (66.67)	5 (33.33)	0.53 (0.11-2.51)	0.429	0.6250
Family history					
Yes	5 (62.50)	3 (37.50)			
No	15 (68.18)	7 (31.82)	0.77 (0.14-4.21)	0.770	0.0852
Smoking status					
Smoker	8 (66.67)	4 (33.33)			
Non-smoker	12 (66.67)	6 (33.33)	1 (0.21-4.70)	1.000	0.000
Lifestyle					
Active	14 (63.64)	8 (36.36)			
Sedentary	6 (75)	2 (25)	0.58 (0.09-3.60)	0.559	0.3409
Body mass					
Normal	10 (55.56)	8 (44.44)			
Obese	9 (90)	1 (10)	0.138 (0.01-1.33)	0.157	3.7
Underweight	1(50)	1(50)	9 (0.28-285.5)		
Red meat consumption					
Yes	9 (64.29)	5 (35.71)	0.81 (0.17-3.74)	0.796	0.0670
No	11 (68.75)	5 (31.25)			
Sundried vegetables					
Yes	17 (65.38)	9 (34.62)	5.6 (0.51-62.65)	0.704	0.1442
No	3 (75)	1 (25)			
Junk food consumption					
Yes	4 (100)	0 (0)			
No	16 (61.54)	10 (38.46)	-	0.129	2.3077
Pesticide exposure					

Yes	5 (83.33)	1 (16.67)			
No	15 (62.50)	9 (37.50)	3 (0.30-29.94)	0.333	0.9375
Site of tumor					
Colon	7 (87.50)	1 (12.50)	4.84 (0.50-46.49)	0.144	2.1307
Rectum	13 (59.09)	9 (40.91)			
Tumor differentiation					
Well	9 (75)	3 (25)			
Moderate	7 (70)	3 (30)	1.28 (0.19-8.43)	0.490	1.4250
Poor	4 (50)	4 (50)	2.33 (0.33-16.18)		
TNM stage					
T1	9 (75)	3 (25)			
T2	8 (80)	2 (20)			
T3	2 (33.33)	4 (66.67)	5.6 (0.99-32.4)		
T4	1 (50)	1(50)		0.126	8.6011
T1+T2	17 (77.27)	5 (22.73)	0.75 (0.09-5.6)		
T3+T4	3 (37.50)	5 (62.50)			
Stage					
I+II	16 (72.73)	6 (27.27)			
III+IV	4 (50)	4 (50)	2.66 (0.50-14.21)	0.243	1.3636
Tumour grade					
1	9 (75)	3 (25)	1.28 (0.19-8.43)		
2	7 (70)	3 (30)	2.33 (0.33-16.18)	0.490	1.4250
3	4 (50)	4 (50)			

Table 3: Correlation between the *SFRP2* gene's methylation pattern and clinicopathological traits in tissue samples from CRC patients

We found that the *SFRP2* gene promoter methylation pattern did not significantly correlate with any of the examined clinicopathological characteristics.

Methylation status of *SFRP2* in blood samples

Figure 2 shows representative outcomes for MSP analysis seen in blood samples of subjects.

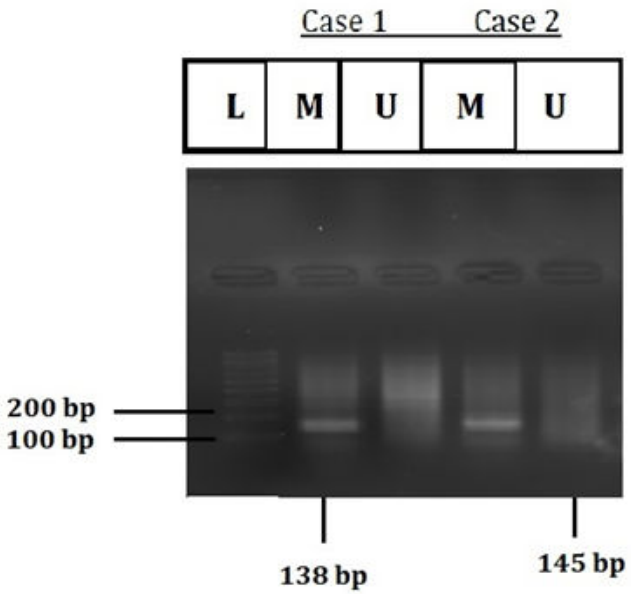

Figure 2: Representative image of *SFRP2* gene promoter methylation pattern of blood samples of subjects by MSP.

L: 100 bp unmethylated SFRP2 is indicated by the DNA marker U: (145 bp); while methylated *SFRP2* is indicated by M: (138 bp).

In blood, the *SFRP2* gene promoter methylation pattern was assessed in 20 CRC cases and also 20 controls taken from healthy individuals, using MSP. In CRC, methylation was found to be present in 60% (12/20) cases whereas 40% (8/20) cases showed the absence of methylation. Out of 12 cases in which methylation were present only 8.3% (1/12) showed both methylated and unmethylated bands whereas 91.6% (11/12) showed only methylated bands. In the normal blood samples, no methylation was found.

Correlation between the *SFRP2* gene's methylation pattern and clinicopathological traits in blood samples from CRC patients.

SFRP2 gene promoter methylation pattern was correlated with several clinicopathological characteristics such as age, gender, tumor location, tumor grading, tumor staging, smoking status and family history. The correlation between the *SFRP2* promoter methylation status and several clinicopathological characteristics is summarized in Table 4.

Characteristics	Methylation present	Methylation absent	Odds ratio (95% CI)	P-value	Chi2
Age					
>50	5 (55.56)	4 (44.44)	0.714 (0.11-4.31)	0.714	0.1347
≤ 50	7 (63.64)	4 (36.36)			
Gender					
Male	9 (69.23)	4 (30.77)	3 (0.446-20.153)	0.251	1.3187
Female	3 (42.86)	4 (57.14)			
Dwelling					
Rural	9 (60)	6 40)	1 (0.126-7.893)	1.000	0.000
Urban	3 (60)	2 (40)			
Social class					
Low	2 (66.67)	1 (33.33)	1.4 (0.105-18.61)	0.798	0.0654
Middle	10 (58.82)	7 (41.18)			
Family history					
Yes	4 (100)	0 (0)	----------	0.068	3.33
No	8 (50)	8 (50)			
Smoking status					
Smoker	7 (87.50)	1 (12.50)	9.8 (0.89-106.8)	0.04	5.20
Non-smoker	5 (41.67)	7 (58.33)			
Lifestyle					

Active	9 (56.25)	7 (43.75)	0.4 (0.03-5.06)	0.494	0.468
Sedentary	3 (75)	1 (25)			
Body mass					
Normal	4 (36.36)	7 (63.64)	----------	0.05	5.92
Obese	3 (100)	0 (0)			
Underweight	5 (83.33)	1 (16.67)			
Salt tea intake					
Yes	11 (61.11)	7 (38.89)	1.57 (0.08-29.4)	0.761	0.092
No	1 (50)	1 (50)			
Red meat consumption					
Yes	9 (64.29)	5 (35.41)	1.8 (0.25-12.5)	0.550	0.357
No	3 (50)	3 (50)			
Sundried vegetables					
Yes	9 (60)	6 (40)	1 (0.126-7.893)	1	0.0000
No	3 (60)	2 (40)			
Junk food consumption					
Yes	1 (33.33)	2 (66.67)	0.27 (0.02-3.66)	0.306	1.045
No	11 (64.71)	6 (35.29)			
Pesticide exposure					
Yes	2 (100)	0 (0)	----------	0.224	1.48
No	10 (55.56)	8 (44.44)			
Site of tumour					
Colon	7 (77.78)	2 (22.22)	0.77 (0.08-6.98)	0.0822	0.0505
Rectum	9 (81.82)	2 (18.18)			
Tumour differentiation					
Well	3 (75.0)	1 (25.0)	0.66 (0.04-10.25)	0.232	2.922
Moderate	9 (81.82)	2 (18.18)			
Poor	2 (40)	3 (60)	6.75 (0.64-71.17)		
TNM stage					
T1	2 (66.67)	1 (33.33)	0.28 (0.11-6.91) 0.56 (0.04-7.44)	0.926	1.379
T2	7 (87.50)	1 (12.50)			
T3	6 (85.71)	1 (14.29)			
T4	2 (100)	0 (0)			
T1+T2	9 (81.82)	2 (18.18)			
T3+T4	8 (88.89)	1 (11.11)			
Stage					

I+II	12 (85.71)	2 (14.29)	----------	0.329	0.9524
III+IV	6 (100)	0 (0)			
Tumour grade					
1	3 (75.0)	1 (25.0)			
2	9 (81.82)	2 (18.18)	0.66 (0.04-10.25	0.232	2.922
3	2 (40)	3 (60)	6.75 (0.64-71.17)		

Table 4: Hypermethylation of the *SFRP2* gene in colorectal cancer blood and clinicopathological factors are correlated.

We observed that smoking status showed a significant association with *SFRP2* gene promoter methylation (p-value <0.05).

DISCUSSION

CRC is a frequent malignancy that develops from benign neoplasms and progresses from adenomas or hyperplastic polyps/serrated adenomas into adenocarcinomas in a stepwise histological progression sequence. Genetic changes have been linked to particular stages in this adenoma-carcinoma sequence and it is thought that these changes are what propel CRC histopathological advancement [13,14]. The presence of epigenetic changes, particularly DNA methylation (hyper or hypomethylation), in colorectal polyps, adenomas and CRCs has been demonstrated. The onset and progression of colorectal polyps and adenomas to CRC appear to be influenced by aberrant gene methylation in conjunction with genetic changes. Another epigenetic method used by colorectal cancer to silence tumor suppressor genes is aberrant gene methylation. Changes in DNA methylation have been identified as a major mechanism of colorectal cancer development [15]. It has been discovered that the main mechanism in the inactivation of a number of tumor suppressor genes is aberrant hypermethylation of CpG islands in gene promoters. The presence of gene silence caused by hypermethylation is a crucial aspect of colorectal tumors, though [16].

A family of secreted glycoproteins known as Secreted Frizzled Related Proteins (*SFRP*s). In the N-terminal half of the proteins, *SFRPs* have a distinctive Cysteine Rich Domain (CRD) that is homologous to the CRD of the Frizzled (Fz) receptor of Wnt. As a result, *SFRPs* may inhibit Wnt signaling by creating inactive complexes with Fz or by interacting with Wnt proteins to prevent them from binding to Fz proteins. It has been noted that *SFRP*s may be able to block the complete canonical Wnt pathway in colon cancer cells, even in the presence of mutations that activate APC or β-catenin downstream of the Fz receptor. Therefore, it may be necessary for the abnormal activation of the Wnt canonical pathway in the development of colorectal tumors for the *SFRP* genes to be silenced [17]. One *SFRP* gene family member involved in the wingless/Wnt signaling pathway is *SFRP2*. The genes are crucial for cell proliferation, apoptosis and the control of cell differentiation. In human malignancies such as prostate, hepatocellular and CRC, it is frequently methylated. Tumor development and Wnt signaling activity are tightly related to its downregulation. A growing body of evidence suggests that *SFRP2*, a crucial member of the *SFRP* family, inhibits the oncogenic Wnt pathway by competing with frizzled membrane bound receptors. Epigenetic inactivation of *SFRP2* by promoter hypermethylation is typically found in a wide variety of cancers, including CRC. Numerous malignancies, including colorectal, ovarian, breast, gastric and liver cancers, have regularly shown loss of *SFRP2* expression due to promoter hypermethylation [18].

In the present study, we have explored the methylation pattern of the *SFRP2* gene promoter region in colorectal cancers by MSP as a possible mechanism for the downregulation of *SFRP2* and also correlated results with several clinico pathological characteristics.

When colorectal tumor tissues were analyzed it was observed that 20 out of 30 (66.66%) CRC tissues had methylation present *i.e.* were hypermethylated and 10 out of 30 (33.33%) had no methylation present. In contrast, in colorectal cancer blood samples, it was observed that 12 out of 20 (60%) CRC blood samples had methylation present *i.e.* were also hypermethylated and 8 out of 20 (40%) had no methylation present. In our study majority of colorectal cancer tissues, 66.66% and 60% of CRC blood samples were hypermethylated. The hypermethylation in CRC tissues and CRC blood might be a mechanism underlying decreased expression of the *SFRP2* gene in colorectal cancers which may be an important event in colon carcinogenesis. Similar results have also been observed by Tang, et al., where they found *SFRP2* gene promoters to be hypermethylated in colon cancers [19]. Another study revealed that *SFRP2* mRNA is considerably downregulated in melanoma and this downregulation of *SFRP2* may be caused by promoter methylation. When the *SFRP2* gene was demethylated by 5-aza-dCyd in melanoma cell lines, *SFRP2* expression was restored at both the mRNA and protein levels, which prevented cell invasion [20]. Canonical Wnt signaling controls a number of processes involved in embryogenesis and adult homeostasis, according to research by, Bian et al. At the base of the crypt in the adult intestine, the Wnt pathway is turned on to preserve intestinal stem cell compartments. But the disruption of the Wnt pathway is primarily responsible for colon cancer cells' expansion, invasion, and survival [21]. According to Sui, et al., in CRC, DNA hypermethylation of the *SFRP2* gene has been found in a variety of studies using tissue, feces and blood detection. *SFRP2* methylation occurred at the same time as the CRC's histological evolution phase.

All of these findings lend credence to our study's finding that the *SFRP2* gene promoter hypermethylation is a key element in the development of colon cancer. These findings imply that *SFRP2* methylation is intricately connected to the beginning and development of CRC carcinogenesis. In order to identify CRC before it manifests itself fully, it may be helpful to check the *SFRP2* methylation status. In our study, we observed that smoking status showed a significant association with *SFRP2* gene promoter methylation (p value<0.05) in CRC blood samples, *SFRP2* methylation status, however, did not correlate with any of the other factors that were investigated, such as age, gender, tumor size, lymph node metastasis or TNM stage.

In CRC tumor samples, there was no discernible relationship between *SFRP2* gene promoter hypermethylation and any clinicopathological factor such as age, tumor differentiation or stage. However, the study's sample size was extremely tiny, which might account for the lack of statistical significance.

No significant study in the past has correlated hypermethylation of the *SFRP2* gene with clinicopathological characteristics whereas Li, et al., correlated the expression of the *SFRP2* gene with several clinicopathological characteristics and found a statistically significant association with early stage disease.

Conclusion

We investigated the significance of *SFRP2* gene hypermethylation in colorectal cancer, as a possible underlying mechanism affecting its expression. Our study is an attempt to accentuate the diagnostic and prognostic importance of *SFRP2* gene hypermethylation in CRC. Hypermethylation was evident in both CRC tissues and blood samples. This research encourages further investigation into the diagnostic and prognostic value of the methylation status of the Secreted Frizzled Related Protein 2 (*SFRP2*) in Colorectal Cancer (CRC).

References

1. Testa U, Castelli G, Pelosi E (2020) Genetic alterations of metastatic colorectal cancer. Biomedicines 8: 414.
2. Peluso G, Incollingo P, Calogero A, Tammaro V, Rupealta N, et al. (2017) Current tissue molecular markers in colorectal cancer: A literature review. Biomed Res Int 2017: 1-8.
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, et al. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249.
4. Pehlivan S, Artac M, Sever T, Bozcuk H, Kilincarslan C, Pehlivan M. Gene methylation of *SFRP2*, *P16*, *DAPK1*, *HIC1*, and *MGMT* and *KRAS* mutations in sporadic colorectal cancer. Cancer Genetics Cytogenet 201: 128-132.
5. Huang Z, Li L, Wang J (2007) Hypermethylation of *SFRP2* as a potential marker for stool based detection of colorectal cancer and precancerous lesions. Dig Dis Sci 52: 2287-2291.
6. Jung G, Hernandez-Illan E, Moreira L, Balaguer F, Goel A (2020) Epigenetics of colorectal cancer: Biomarker and therapeutic potential. Nat Rev Gastroenterol Hepatol 17: 111-130.
7. Ng C, Li H, Wu WK, Wong SH, Yu J (2019) Genomics and metagenomics of colorectal cancer. J Gastrointest Oncol 10: 1164-1170.
8. Qi J (2006) Hypermethylation and expression regulation of secreted frizzled related protein genes in colorectal tumor. WJG 12: 7113-7117.
9. Tariq K, Ghias K. Colorectal cancer carcinogenesis: A review of mechanisms. Cancer Biol Med 13: 120.
10. Marley AR, Nan H (2016) Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet 7: 105-114.
11. Liu X, Fu J, Bi H, Ge A, Xia T, et al. (2019) DNA methylation of *SFRP1, SFRP2,* and *WIF1* and prognosis of postoperative colorectal cancer patients. BMC Cancer 19: 1212.
12. Jass JR (2004) Hyperplastic polyps and colorectal cancer: Is there a link?. Clin Gastroenterol Hepatol 2: 1-8.
13. Souglakos J (2007) Genetic alterations in sporadic and hereditary colorectal cancer: Implementations for screening and follow-up. Dig Dis 25: 9-19.
14. Wang DR, Tang D (2008) Hypermethylated *SFRP2* gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. World J Gastroenterol 14: 524-531.
15. Qi J, Zhu YQ, Luo J, Tao WH, Zhang JM (2007) Hypermethylation and regulation of expression of secreted frizzled related protein genes in colorectal tumor. Zhonghua Zhong Liu Za Zhi 29: 842-845.
16. Li H, Wang Z, Zhao G, Ma Y, Chen Y, et al. (2019) Performance of a methylight assay for methylated SFRP2 DNA detection in colorectal cancer tissue and serum. Int J Biol Markers 34: 54-59.
17. Takagi H, Sasaki S, Suzuki H, Toyota M, Maruyama R, et al. (2008) Frequent epigenetic inactivation of *SFRP* genes in hepatocellular carcinoma. J Gastroenterol 43: 378-389.
18. Tang D, Liu J, Wang D, Yu H, Li Y, et al. (2011) Diagnostic and prognostic value of the methylation status of secreted frizzled related protein 2 in colorectal cancer. Clin Invest Med 34: 88-95.
19. Luo X, Wei B, Chen A, Zhao H, Huang K, et al. (2016) Methylation mediated loss of SFRP2 enhances melanoma cell invasion *via* Wnt signaling. Am J Transl Res 8: 1502.
20. Bian J, Dannappel M, Wan C, Firestein R (2020) Transcriptional regulation of Wnt/β-catenin pathway in colorectal cancer. Cells 9: 2125.
21. Sui C, Ma J, Chen Q, Yang Y (2016) The variation trends of *SFRP2* methylation of tissue, feces and blood detection in colorectal cancer development. Eur J Cancer Prev 25: 288-298.