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Relationship between the Promoter-Methylation of Suppressor Gene-*SFRP2*, *HPP1* and Some Clinical Pathologic Indices in Rectal Cancer

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Abstract We analyzed the relationship between methylation status in promoter region of tumor suppressor genes *SFRP2* (secreted frizzled-related protein 2), *HPP1* (hyperplastic polyposis protein 1) in tissues, stools of 68 rectal cancer patients and 60 healthy men, 49 FFPET (Formalin fixed and paraffin embedded tissues), and their clinical pathologic signs. Genomic DNA was isolated from the tissues by phenol/chloroform method and modified by sodium bisulfate, and then it was purified using 1% agarose gel. Using purified DNA as template, we analyzed the methylation status of promoter CpG Island of tumor suppressor genes-*SFRP2* and *HPP1* by applying MS-PCR (methylation specific-PCR). The promoter methylation rates of *SFRP2* were 3.3, 0% in tissues and stools of normal, whereas they were 94.1, 89.7% in patients with rectal cancer. The promoter methylation rates of *HPP1* were 0% in both of tissues and stools of normal, whereas they were 92.3, 86.8% in patients with rectal cancer.

Key words promoter methylation, *SFRP2, HPP1*, rectal cancer

Introduction

The great leader Comrade **Kim Jong Il** said.

"**Drawing on the successes already achieved, medical science must open the fields of genetic engineering, immunology and molecular biology and stimulate research to adopt widely in curative and preventive services the latest scientific and technological achievements, including electronics and laser engineering.**"("ON THE FURTHER IMPROVEMENT OF THE HEALTH SERVICE" P. 18)

Hypermethylation of the promoter regions of genes are connected with many kinds of carcinogenesis including rectal cancer, hepatocarcinoma, lung cancer, gastric cancer and breast cancer.

Epigenetic silencing of tumor suppressor genes by methylation of discrete regions of the CpG islands is a major mechanism underlying tumor genesis [2, 4].

In recent years, hypermethylation of the promoter regions of genes are increasing more and more in cancer genesis. Many cellular pathways such as DNA repair (*hMLH1, MGMT* and *BRCA1*), cell cycle (*p16INK4a, p15INK4b, p14ARF,* and *Cyclin D2*), hormone and receptor-mediated cell signaling (*ER, RARβ2* and *THRβ*), transcriptional regulation (*HOXA5*), apoptosis (*RASSF1A, Twist* and *HIN1*) are inactivated by these epigenetic events [8, 9]. However, not a gene is methylated in kinds of every tumor; it was obvious that strong specificity is apparent with respect to the original tissue [3, 5, 6].

Rectal cancer tends to appear increasingly in many countries in the world, particularly; there are quite differences of incidences according to regions and countries. In addition, in many cases, it probably has close relations with heredity and environmental factors [1, 7].

We are going to analyze the methylation status of promoter regions of tumor suppressor gene-*SFRP2* and *HPP1* in the patients with rectal cancer, and clarify the relationship between the methylation of suppressor gene and its clinical pathologic signs.

1. Material and Method

1.1. Material

49 FFPET (Formalin fixed and paraffin embedded tissues), biopsy tissues, stools from 60 normal people and 68 patients with rectal carcinoma who were path-histologically confirmed in Tumor Institute of Medicine Academy and hospital of Pyongyang Medical College of **Kim Il Sung** University are used.

1.2. Method

After separating genomic DNA from different samples (tissue, stool), genomic DNA was repaired using bisulfite sodium, and then purified in 1% agarose gel. AS this purified genomic DNA, we carried out MS-PCR and observed the results using 8% polyacrylamide gel electrophoresis.

10μL of DNA solution (1μg DNA) was denatured with 1μL of 3mol/L NaOH for 15min at 37℃. 6μL of 10 mmol/L hydroquinone and 104μL of 3mol/L sodium bisulfite (pH 5.0) were added, followed by incubation at 50℃ for 16h.

The modified DNA was purified using 1% agarose gel. The purified DNA was desulphonated with NaOH and precipitated with absolute ethanol in the presence of glycogen and ammonium acetate [6, 7, 10].

MS-PCR was carried out using 50～100 ng of bisulfite treated DNA in a PCR mixture containing 1×PCR buffer, 2mmol/L $MgCl_2$, dNTPs each at 1.25 mmol/L and primers each 1.6μmol/L and 0.7U of Taq polymerase in a 15μL reaction. PCR product was confirmed by 8% polyacrylamide gel electrophoresis. The primer sequence is same as table 1.

Table 1. MS-PCR primer sequence

Gene	Primer sequence (5′−3′)	Annealing temperature/℃	Product size /bp
SFRP2	MF: GGGTCGGAGTTTTTCGGAGTTGCGC MR: CCGCTCTCTTCGCTAAATACGACTCG	62	138
	UF: TTTTGGGTTGGAGTTTTTTGGAGTTG UR:AACCCACTCTCTTCACTAAATACAACTCA	58	145
HPP1	MF: TTTAGCGGACGATTTTTTCGTTTCG MR: AACGACGACGATAACAATAA	57	122
	UF: TTTAGTGGATGATTTTTTTGTTTTG UR: AACAACAACAATAACAATAA	57	122

2. Result and Analysis

2.1. Methylation rate of CpG Island of suppressor gene promoter in rectal cancer

First, we analyzed the methylation rate of promoter of *SFRP2* and *HPP1* in rectal carcinoma.

Fig. 1 shows the result of progressing MS-PCR using methylated, non-methylated specific primer to *SFRP2* of patients with rectal cancer and amplified PCR products were 138, 145bp.

Fig. 2 shows the result of progressing MS-PCR using methylated, non-methylated specific primer to *HPP1* of patients with rectal cancer and amplified PCR products were 122, 122bp.

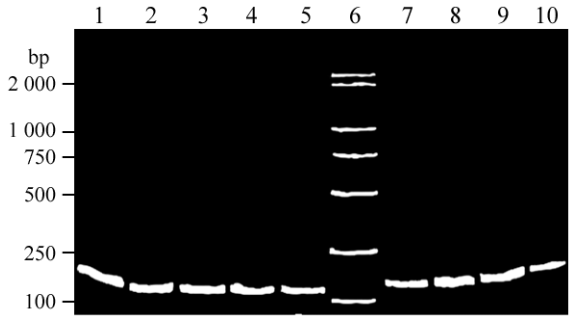

Fig. 1. Polyacrylamide gel electrophoresis of MS-PCR product of *SFRP2*
1－5－methylated specific primer, 6－DNA marker, 7－10－non-methylated specific primer

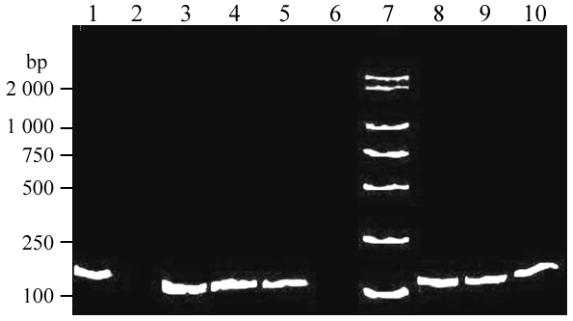

Fig. 2. Polyacrylamide gel electrophoresis of MS-PCR product of *HPP1*
1－6－methylated specific primer, 7－DNA marker, 8－10－non-methylated specific primer

Next, we examined the methylation rate of *SFRP2* and *HPP1* from tissues and stools of normal person and patient with rectal cancer.

Table 2 shows that methylation rate of tissues and stools of *SFRP2* were 3.3, 0% in normal, and 94.1 and 89.7% in rectal cancer. The methylation rate of patient with rectal cancer was clearly high when compared with the normal, but it was no significant differenence between the tissues and stools.

Table 3 shows that methylation rate of tissues and stools of *HPP1* were 0, 0% in normal, and 88.2 and 86.8% in rectal cancer. The methylation rate of patient with rectal cancer was clearly high to compare with normal, but it was no significant difference between the tissues and stools.

Table 2. Methylation rate in promoter region of *SFRP2* in tissues and stools

Sample	Case	Tissues	Stools
Normal	60	2(3.3)	0(0)
Rectal cancer	68	64*(94.1%)	61*(89.7%)

* $p<0.01$(to compare with normal)

Table 3. Methylation in promoter region of *HPP1* in tissues and stools

Sample	Case	Tissues	Stools
Normal	60	0(0%)	0(0%)
Rectal cancer	68	60*(88.2%)	59*(86.8%)

* $p<0.01$(to compare with normal)

2.2. Relationship between the rates of promoter methylation of suppressor gene and clinical pathologic indices in rectal cancer patients

We analyzed the clinical significance by showing the relationship between promoter methylation of *SFRP2*, *HPP1* and clinical pathologic indices such as age, history, stage, grading and differentiation grade of the patients with rectal cancer (table 4).

Table 4. Relationship between promoter methylation of *SFRP2*, *HPP1* and clinical pathologic indices of patients with rectal cancer

Clinical indices		Case	Genotype	
			SFRP2	*HPP1*
Age	20－40	19	17(89.5%)	17(89.5%)
	41－60	71	69(97.2%)	67(94.4%)
	61≤	27	25(92.6%)	24(88.9%)
History	Positive	16	16(100%)	16(100%)
	Negative	101	95(94.1%)	92(91.1%)
Grading	Proper lamina	2	2(100%)	2(100%)
	Sub mucous layer	19	18(94.7%)	17(89.5%)
	Muscular tunic	67	64(95.5%)	63(94%)
	Ambient tissue	29	27(93.1%)	26(89.7%)
Stage	T1-T3a	60	56(93.3%)	55(91.7%)
	T3b-T4	57	55(96.5%)	53(93%)
Differentiation grade	Poor	6	6(100%)	6(100%)
	Medium	49	47(95.9%)	46(93.8%)
	High	62	58(93.5%)	56(90.3%)

As shown in table 4, there was no clear correlation between the promoter methylation of *SFRP2*, *HPP1* and clinical pathologic indices of patients with rectal cancer.

Conclusion

We analyzed the methylation rate of *SFRP2* and *HPP1* from tissues and stools of normal person and patient in rectal cancer.

The methylation rate of patient with rectal cancer was clearly high when compared with the normal, but it was no significant difference between the tissues and stools.

The promoter methylation rate of *SFRP2* were 3.3, 0% in tissues and stools of normal were 94.1, 89.7% in patients with rectal cancer.

The promoter methylation rate of *HPP1* were 0, 0% in tissues and stools of normal, and 92.3, 86.8% in patients with rectal cancer.

We analyzed the clinical significance by showing the relationship between promoter methylation of *SFRP2*, *HPP1* and clinical pathologic indices such as age, history, stage, grading and differentiation grade of the patients with rectal cancer.

There was no clear correlation between the promoter methylation of *SFRP2, HPP1* and clinical pathologic indices of patients with rectal cancer.

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