Associations between Single Nucleotide Polymorphisms of *MMP2*, *VEGF*, and *HIF1A* Genes and the Risk of Developing Colorectal Cancer

MIN-JUNG KANG, SUNG-AE JUNG, JI MIN JUNG, SEONG-EUN KIM, HYE-KYUNG JUNG, TAE-HUN KIM, KI-NAM SHIM, SUN YOUNG YI, KWON YOO and IL HWAN MOON

Department of Internal Medicine, Ewha Medical Research Institute, Ewha Womans University School of Medicine, Seoul, Korea

Abstract. *The aim of this study was to investigate the association between the risk for colorectal cancer and single nucleotide polymorphisms (SNP) of matrix metalloproteinase-2 (MMP2) -1306C/T, vascular endothelial growth factor (VEGF) 936C/T and hypoxia inducible factor-1α (HIF1A) 1772C/T. Patients and Methods: A total 50 colorectal cancer patients (46% women, mean age 68±11 years) were enrolled. Healthy controls without evidence of cancer history or family cancer predispositions were frequency-matched to the cases by sex and age (±5 years). Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and the genotype distribution and risk estimate were analyzed. The correlation between the genotypes and clinicopathological parameters (Dukes stage, phenotype, location, differentiation and size) among colorectal cancer patients were investigated. Results: There was a significant association between colorectal cancer and T allele-bearing genotype distribution of HIF1A 1772C/T polymorphism (Odds ratio, OR=3.63, 95% confidence interval, CI=1.08-12.18, p=0.03 for CT and TT genotypes relative to CC genotype). In addition, when stratified by age, the association remained in patients older than 60 years old (OR=13.60, 95% CI=1.63-113.24, p=0.01). However, there was no association between the genotypes of the MMP2, VEGF and HIF1A SNP and clinicopathological parameters of colorectal cancer. Conclusion: There is a significant association between the HIF1A 1772C/T SNP and the risk of developing colorectal cancer, especially in individuals older than 60 years.*

Correspondence to: Sung-Ae Jung, MD, Department of Internal Medicine, Ewha Medical Research Institute Ewha Womans University School of Medicine, Ewha Womans University Mokdong Hospital 911-1 Mokdong Yangcheon-Ku, Seoul, Korea. Tel: +82 226502680, Fax: +82 226550984, e-mail: jassa@ewha.ac.kr

Key Words: Polymorphism, colorectal cancer, MMP2, VEGF, HIF1A, SNP.

Colorectal cancer is one of the major causes of mortality worldwide. It is the most common malignancy of the gastrointestinal tract and its occurrence is rapidly increasing in Korea (1). The adenoma-carcinoma sequence has been accepted as the main pathway for colorectal cancer development, involving numerous molecular events, such as the activation of oncogenes and the inactivation of suppressor genes (2). Recently, with the completion of the Human Genomic Project, single nucleotide polymorphisms (SNPs) have been the focus of biomedical research (3).

Polymorphisms are naturally occurring DNA sequence variations, which differ from gene mutations. They occur in the 'normal' healthy population and have a frequency of at least 1% (4). Approximately 90% of DNA polymorphisms are SNPs due to their single base substitutions, while others include insertion, deletion, minisatellite, and microsatellite polymorphisms (5). Although most polymorphisms are functionally neutral, some have effects on the regulation of gene expression or on the function of the coded protein. These functional polymorphisms could contribute to the difference between individuals according to the susceptibility and severity of diseases (5). Polymorphisms alone or in combination with environmental factors may affect the angiogenic pathway, and thereby the susceptibility and severity of cancer (4). SNPs in several genes have been suggested to be implicated in the pathogenesis of colorectal cancer (6).

MMPs function as proteolytic enzymes degrading the extracellular matrix and the basement membrane (5). The overexpression of MMPs is known to be associated with tumor invasion, metastasis, and a worse prognosis (7). Of all the MMPs, *MMP2* (gelatinase A), after being processed into its active form as type IV collagenase, primarily hydrolyzes type IV collagen, which is the major structural component of the basement membrane. Several polymorphisms within the *MMP2* promoter regions have been reported in cases with oncogenesis and tumor progression, especially in colorectal carcinogenesis (8). Recently, the SNP C→T transition at -

1306 is reported to disrupt the Sp1-type promoter site (CCACC box), displaying a strikingly lower promoter activity and also reducing the transcriptional activity (9). Sp1 is a ubiquitously expressed transcription factor that binds to GC/GT-rich elements to regulate a variety of genes. The CCACC box has been shown to be essential for Sp1 binding and promoter function in several genes by invariably activating transcription (10, 11). The *MMP2* –1306C/T polymorphism that abolishes Sp1 binding has the potential to affect the level and specificity of gene transcription. This has been demonstrated by *in vitro* experiments using the transient transfection method (12). Previous studies have demonstrated that the –1306CC genotype can double or quadruple the risk factors for maliganacies, such as lung, stomach, colorectal, oral cavity and breast cancer (9). But these findings are not always in accordance with other results and the association of *MMP2* –1306C/T polymorphism in colorectal cancer is still in investigation.

VEGF is one of the most important activators of tumor angiogenesis, stimulating new blood vessel formation from existing vasculature, increasing the tumor oxygenation, and ultimately, leading to tumor growth (13). Cells under hypoxic conditions consistently produce pro-angiogenic factors, mostly VEGF which triggers multiple signaling pathways that result in endothelial cell survival, mitogenesis, migration, differentiation, and also increased vascular permeability and mobilization of endothelial progenitor cells (14). The overexpression of *VEGF* mRNA and its protein has been associated with tumor progression and the poor prognosis of various malignancies, including melanoma, ovarian carcinoma, prostate carcinoma, and colon cancer (15). Moreover, the overexpression of VEGF was clearly demonstrated in metastatic compared to non-metastatic colon cancer, and known to be directly correlated with increased vessel counts, the extent of neovascularization and the degree of proliferation (15). DNA sequence variations in the *VEGF* gene seem to affect the VEGF level and/or its activity, thereby causing differences between patients in lymphangiogenesis and lymphatic tumor spread. In particular, variations occurres in the 3'-untranslated region (UTR) are known to be correlated with key regulatory elements that are sensitive to hypoxia and, therefore, contribute to high variability in VEGF level among tissues (16). The SNP C→T transition especially at 936 within the 3'-UTR region of the *VEGF* gene has recently been associated with colorectal cancer (17).

HIF1A is a key transcription factor that regulates the cellular response to hypoxia (18). Under hypoxic conditions, degradation of HIF1A is suppressed and its intracellular accumulation activates VEGF, which induces the tumor growth *via* angiogenesis (19-20). An increased HIF1A level, therefore, plays a critical role in the ability of cancer cells to survive and metastasize in the hostile hypoxic environment. Recent studies have demonstrated the existence of SNPs in the human HIF1A gene that lead to amino acid substitutions within the oxygen-dependent degradation domain (ODD) (21). Mutations in this critical regulatory domain may lead to the overexpression of this protein and also the subsequent changes in the expression of downstream target genes, thus contributing to the development of cancer cell resistance. SNP in *HIF1A*, C→T transition at 1772 (which may result in an amino acid change from proline 582 to serine) was shown to have a higher transactivating capability *in vitro* compared to the wild-type allele (21), and the genotype distribution and functional significance of the *HIF1A* SNP in human malignancy, especially in colorectal cancer, is still under investigation.

In our previous study, we confirmed the up-regulated mRNA expression of *MMP2, VEGF*, and *HIF1A* in colorectal cancer tissues compared with the noncancerous tissue (22). Here, we investigated the risk of colorectal cancer and the clinical manifestations of the SNPs, including genes for MMP2 (C→T transition at –1306), VEGF (C→T transition especially at 936 within the 3'-UTR), and HIF1A (C→T transition at 1772) in 50 colorectal cancer patients and healthy controls in Korea.

Patients and Methods

Patients and samples. A total of 50 colorectal cancer patients (46% women, mean age of 68±11 years) were enrolled from January to December 2008 at Ewha Womans University Mokdong Hospital. Clinicopathological data were reviewed. Healthy controls without any evidence of cancer history or family cancer predisposition were frequency-matched to the cases by sex and age (±5 years). DNA of each sample was amplified by polymerase chain reaction for *MMP2, VEGF*, and *HIF1A* SNP. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and the genotype distribution and risk estimate were also analyzed. The tumor stage was described according to the Dukes staging system. The correlation between genotypes and clinicopathological parameters such as the tumor location, differentiation, size, lymphatic invasion, lymph node metastasis, and phenotype among colorectal cancer patients were investigated. This study was approved by the Institutional Review Board (IRB number ECT 216-1) of Ewha Womans University, and the written consent was obtained from each participant.

Genomic DNA extraction. Genomic DNA samples were prepared by proteinase K digestion and extracted with LaboPass™ Tissue Mini (COSMO GENETECH, Seoul, Korea) and Genomic Blood DNA Extraction Mini Kit (iNtRON Biotechnology, SungNam, Korea) from surgically resected cancer tissues and whole blood samples of controls respectively. The concentration of each genomic DNA was measured by the absorbance at 260 nm and samples were frozen at –20˚C until further analysis.

Genotyping of SNP MMP2, VEGF, and HIF1A by PCR-RFLP. Genotypes were determined by the PCR-RFLP method. The PCR reaction was performed in a 20 μL sample volume containing 100 ng of DNA template, 2 μL of 10× PCR buffer, 0.5 U of Taq DNA polymerase (Takara Bio Inc., Shiga, Japan), 200 μM of each dNTPs,

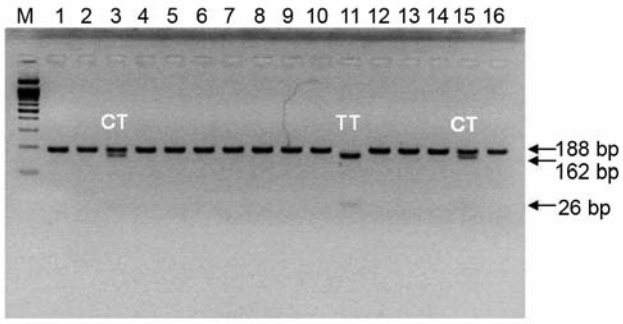

Figure 1. *Electrophoresis patterns for the genotypes of MMP2 -1306C/T polymorphism analyzed by PCR-RFLP based assay. Lane M shows DNA marker. Lane 1, 2, 4-10, 12-14 and 16 (188-bp) show individuals with homozygous polymorphism (MMP2 –1306CC). Lane 3 and 15 (26-, 162- and 188-bp) show those with heterozygous polymorphism (MMP2 -1306CT). Lane 11 (26- and 162-bp) shows those with mutant type (MMP2 -1306TT).*

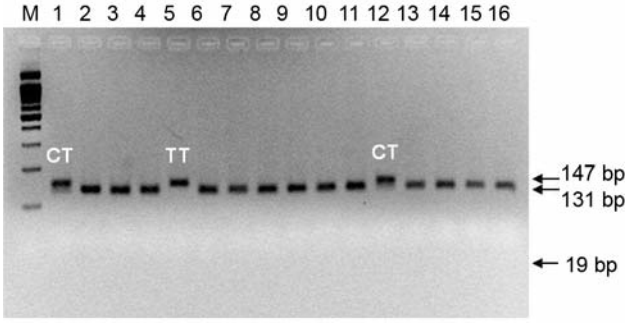

Figure 3. *Electrophoresis patterns for the genotypes of HIF1A 1772C/T polymorphism analyzed by PCR-RFLP based assay. Lane M shows DNA marker. Lane 2-4, 6-11 and 13-16 (131- and 19-bp) show individuals with homozygous polymorphism (HIF1A 1772CC). Lane 1 and 12 (19, 131- and 147-bp) show those with heterozygous polymorphism (HIF1A 1772CT). Lane 5 (147-bp) shows those with mutant type (HIF1A 1772TT).*

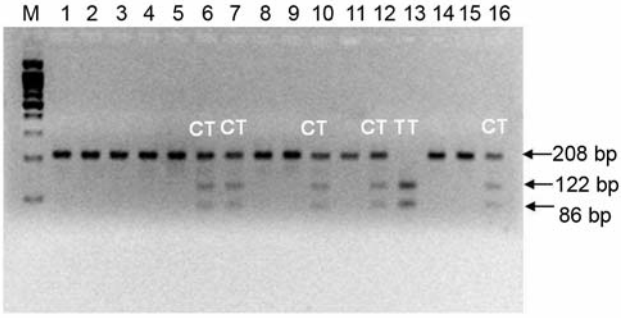

Figure 2. *Electrophoresis patterns for the genotypes of VEGF 936C/T polymorphism analyzed by PCR-RFLP based assay. Lane M shows DNA marker. Lane 1-5, 8, 9, 11, 14 and 15 (208-bp) show individuals with homozygous polymorphism (VEGF 936CC). Lane 6, 7, 10, 12 and 16 (86-, 122- and 208-bp) show those with heterozygous polymorphism (VEGF 936CT). Lane 13 (122- and 208-bp) shows those with mutant type (VEGF 936TT).*

and 0.5 μM of each primer. All PCR reactions were performed by a thermal cycler GeneAmp PCR system 9600 (Perkin-Elmer Corp., Norwalk, CT, USA). The PCR products were separated by running on 3% agarose gel stained with ethidium bromide and detected by UV transillumination. The GelDoc 2000 system (BioRad, Hercules, CA, USA) was used to detect each band.

Genotyping of MMP2 polymorphism. A 188 base pairs (bps) fragment was amplified from genomic DNA using the following primers: 5'-CTT CCT AGG CTG GTC CTT ACT GA-3(forward) and 5'-CTG AGA CCT GAA GAG CTA AAG AGC T-3(reverse). The PCR cycling conditions were 5 minutes at 94˚C followed by 35 cycles of 45 seconds at 94˚C, 45 seconds at 58˚C and 45 seconds at 72˚C, with a final step at 72˚C for 5 minutes to allow the complete extension of all PCR fragments. The PCR products were digested at 37˚C overnight with 5 U of restriction enzyme, Xsp I (Takara Bio Inc.).

On electrophoresis of PCR products, CC homozygotes showed a single band at 188 bp, and CT heterozygotes showed bands at 188, 162, and 26 bp, while TT homozygotes showed double bands at 162 and 26 bp (Figure 1).

Genotyping of VEGF polymorphism. The PCR primers used to detect the *VEGF* 936C/T polymorphism were 5'-AAG GAA GAG GAG ACT CTG CGC AGA GC-3(forward) and 5'-TAA ATG TAT GTA TGT GGG TGG GTG TGT CTA CAG-3(reverse). Thermal conditions included a denaturation step at 94˚ for 5 minutes, followed by 35 cycles at 94˚C for 40 second, annealing at 64˚C for 1 minute, and extension at 72˚ for 40 seconds with a final extension step at 72˚ for 5 minutes. The PCR product was digested overnight with 5 U of restriction enzyme, N1a III (New England Biolabs, Ipswich, MA, USA). On electrophoresis of PCR products, CC homozygotes showed a single band at 208 bp, CT heterozygotes showed bands at 208, 122, and 86 bp, while TT homozygotes showed double bands at 86 bp and 122 bp (Figure 2).

Genotyping of HIF1A polymorphism. The primer sequences to detect the 1772C/T polymorphism were 5'-TGT GGC CAT TGT AAA AAC TCA-3(forward) and 5'-CTT GCG GAA CTG CCT TCT AA-3(reverse). The PCR temperature profile was 1 cycle of 94˚C for 5 minute, followed by 35 cycles of 94˚C for 1 minute, 55˚C for 1 min, and 1 min at 72˚C with the final extension step at 72˚C for 5 min. The 147 bp PCR fragment was incubated overnight with 10 U of restriction enzyme, Bsl I (New England Biolabs), at 37˚C. On electrophoresis of PCR products, CC homozygotes showed double bands at 131 and 19 bp, CT heterozygotes showed bands at 147, 131, and 19 bp, while TT homozygotes showed a single band at 147 bp only (Figure 3). The details of primer sequence and reaction conditions used for RFLP typing are summarized in Table I.

Statistical analysis. All data were analyzed using SPSS version 13.0 for Windows (SPSS Inc, Chicago, IL, USA). Independent *t*-test was used for continuous variables such as age, and the Pearson chi-square test was for genotype distribution and risk estimate for

Table I. *Primers and reaction conditions used for RFLP typing.*

	Primers	PCR product size (bp)/ Annealing temp. (˚C)	Restriction enzyme	DNA fragment size (bp)
MMP2 (-1306C/T)	F: 5'-CTT CCT AGG CTG GTC CTT ACT GA-3' R: 5'-CTG AGA CCT GAA GAG CTA AAG AGC T-3'	188/58	Xsp I	188 162 26
VEGF (936C/T)	F: 5'-AAG GAA GAG GAG ACT CTG CGC AGA GC-3' R: 5'-TAA ATG TAT GTA TGT GGG TGG GTG TGT CTA CAG-3'	208/64	Nla III	208 122 86
HIF1A (1772C/T)	F: 5'-TGT GGC CAT TGT AAA AAC TCA-3' R: 5'-CTT GCG GAA CTG CCT TCT AA-3'	147/55	Bsl I	147 131 19

F, Forward primer; R, reverse primer.

MMP2, VEGF, and *HIF1A* SNP. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated by logistic regression analysis. Clinicopathological parameters such as the tumor location, differentiation, size, and phenotype among colorectal cancer patients were investigated using the Pearson Chi-square test or the Fischer's exact test according to the genotype. *P*-values less than 0.05 were regarded as statistically significant.

Results

Patient demographics. The baseline characteristics of colorectal cancer patients and the control group are shown in Table II. Of the 50 patients with colorectal cancer at the time of diagnosis, the majority were found to have tumor located at the colon (n=36, 72.0%). The size of the tumor mass was found to be larger than 4 cm in the majority of patients (n=37, 74.0%). Ulcerative tumor was found in most patients (n=41, 82.0%) and most tumors were moderately differentiated (n=43, 91.4%).

MMP2 –1306 C/T polymorphism. The genotype distribution and risk estimate among the patient and control groups were analyzed (Table III). No significant association was found between the risk of developing colorectal cancer and genotype distribution of *MMP2* –1306 C/T polymorphism (OR=0.675, 95% CI=0.246-1.854, p=0.446). Nor was any significant association observed in the genotype distribution and risk estimate when stratified by gender (male, OR=1.307, 95% CI=0.310-5.509, p=0.715; female, OR=1.676, 95% CI=0.403-6.966, p=0.447) (Table IV) or age (>60 years old, OR=0.517, 95% CI=0.165-1.625, p=0.259; ≤60 years old, OR=2.154, 95% CI=0.174-25.672, p=0.550) (Table V). Finally, no association was found between the genotype distribution of the *MMP2* polymorphism and clinicopathological parameters (localization, differentiation, size, lymph node invasion, lymph node metastasis, ulcerative/polypoid phenotype and Dukes' stage) of colorectal cancer (Table VI).

VEGF 936 C/T polymorphism. No significant association was found between the risk of developing colorectal cancer and genotype distribution of *VEGF* 936 C/T polymorphism (OR=1.179, 95% CI=0.532-2.610, p=0.685). No significant association was observed in genotype distribution and risk estimate when stratified by gender (male, OR=1.169, 95% CI=0.391-3.494, p=0.780; female, OR=1.192, 95% CI=0.373-3.807, p=0.767) (Table IV) or age (>60 years old, OR=1.263, 95% CI=0.489-3.261, p=0.629; ≤60 years old, OR=1.000, 95% CI=0.232-4.310, p=1.000) (Table V). Finally, no association was found between the genotype distribution of the *VEGF* polymorphism and clinicopathological parameters of colorectal cancer (Table VII).

HIF1A 1772 C/T polymorphism. There was a significant association found in the risk of developing colorectal cancer and genotype distribution of *HIF1A* 1772 C/T polymorphism (OR=3.632, 95% CI=1.082-12.183, p=0.037) for CT and TT genotypes relative to CC genotype. Moreover, when stratified by age, significant correlation was found in patients older than 60 years (OR=13.600, 95% CI=1.633-113.246, p=0.016) for C/T and T/T genotypes relative to CC genotype (Table V). However, no significant association was observed in the genotype distribution and risk estimate when stratified by gender (male, OR=3.250, 95% CI=0.361-33.409, p=0.321; female, OR=4.286, 95% CI=0.981-18.721, p=0.053) (Table IV). Finally, no association was found in the genotype distribution of the *HIF1A* polymorphism with clinicopathological parameters of colorectal cancer (Table VIII).

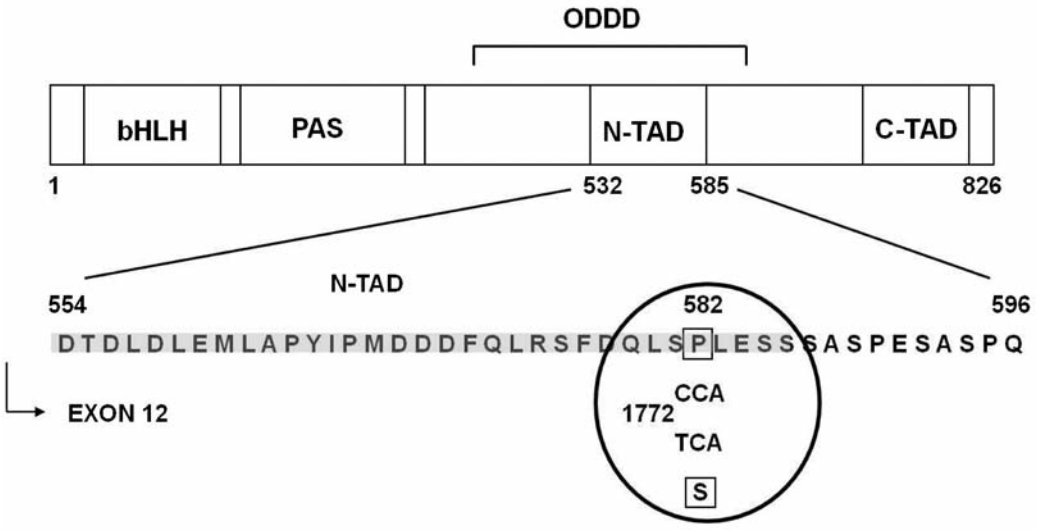

Figure 4. *Structure of human HIF1A presenting the position of 1772C/T single nucleotide polymorphism. (Bottom panel) Amino acid sequence encoded in exon 12 of N-TAD. Open boxes show positions of amino acid substitutions caused by single nucleotide polymorphism. Numbers indicate positions of nucleotides or amino acids, respectively. bHLH, Basic helix-loop-helix domain; PAS, Per-Arnt-Sim domain; ODDD, oxygen-dependent degradation domain; N- and C-TAD, N- and C-terminal transactivation domains.*

Table II. *Clinicopathological characteristics of study participants.*

	Controls (n=50) (%)	Cases (n=50) (%)
Age (years) (mean±SD)	68±12	68±11
Male/Female	27 (54.0)/23 (46.0)	27 (54.0)/23 (46.0)
Location of tumor		
Colon	-	36 (72.0)
Rectum	-	14 (28.0)
Differentiation		
Well-differentiated	-	2 (4.3)
Moderately differentiated	-	43 (91.4)
Poorly differentiated	-	2 (4.3)
Tumor size		
<4 cm	-	13 (26.0)
≥4 cm	-	37 (74.0)
Lymphatic invasion		
Yes	-	28 (56.0)
No	-	22 (44.0)
Lymph node metastasis		
Yes	-	26 (52.0)
No	-	24 (48.0)
Phenotype		
Polypoid	-	9 (18.0)
Ulcerative	-	41 (82.0)
Modified Dukes' stage		
A	-	1 (2.0)
B	-	19 (38.0)
C	-	17 (34.0)
D	-	13 (26.0)

SD, Standard deviation.

Table III. *Distribution of genotypes and risk estimate.*

	Controls (%)	Cases (%)	OR (95% CI)	*P*-value
Overall				
MMP2				0.446
CC	39 (78.0)	42 (84.0)	1.0	
CT+TT	11 (22.0)	8 (16.0)	0.675 (0.246-1.854)	
VEGF				0.685
CC	30 (60.0)	28 (56.0)	1.0	
CT+TT	20 (40.0)	22 (44.0)	1.179 (0.532-2.610)	
HIF1A				0.037
CC	46 (92.0)	38 (76.0)	1.0	
CT+TT	4 (8.0)	12 (24.0)	3.632 (1.082-12.183)	

OR, Odds ratio; CI, confidence interval.

Discussion

The role of genetic polymorphisms which function as the important factors determining endogenous causes of cancer, especially in the risk of colorectal cancer, has attracted increasing interest due to advances in DNA analyzing technologies and also the knowledge of the human genome. To date, however, very little about this matter is proven and known.

MMPs are zinc metalloproteases that degrade collagens of the extracellular matrix that are important for tissue remodeling and repair, during the development and inflammation. MMPs are also involved in controlling cell

Table IV. *Distribution of genotypes and risk estimate according to gender.*

	Control (%)	Case (%)	OR (95% CI)	*P*-value
Male				
MMP2				0.715
CC	5 (18.5)	3 (11.1)	1.0	
CT+TT	22 (81.5)	23 (85.2)	1.307 (0.310-5.509)	
VEGF				0.780
CC	17 (63.0)	16 (59.3)	1.0	
CT+TT	10 (37.0)	11 (40.7)	1.169 (0.391-3.494)	
HIF1A				0.321
CC	26 (96.3)	24 (88.9)	1.0	
CT+TT	1 (3.7)	3 (11.1)	3.250 (0.316-33.409)	
Female				
MMP2				0.477
CC	6 (26.1)	4 (17.4)	1.0	
CT+TT	17 (73.9)	19 (82.6)	1.676 (0.403-6.966)	
VEGF				0.767
CC	13 (56.5)	12 (52.2)	1.0	
CT+TT	10 (43.5)	11 (47.8)	1.192 (0.373-3.807)	
HIF1A				0.053
CC	20 (87.0)	14 (60.9)	1.0	
CT+TT	3 (13.0)	9 (39.1)	4.286 (0.981-18.721)	

OR, Odds ratio; CI, confidence interval.

Table V. *Distribution of genotypes and risk estimate according to age.*

	Controls (%)	Cases (%)	OR (95% CI)	*P*-value
>60 years old				
MMP2				0.259
CC	25 (71.4)	29 (82.9)	1.0	
CT+TT	10 (28.6)	6 (17.1)	0.517 (0.165-1.625)	
VEGF				0.629
CC	21 (60.0)	19 (54.3)	1.0	
CT+TT	14 (40.0)	16 (45.7)	1.263 (0.489-3.261)	
HIF1A				0.016
CC	34 (97.1)	25 (71.4)	1.0	
CT+TT	1 (2.9)	10 (28.6)	13.600 (1.633-113.246)	
≤60 years old				
MMP-2				0.550
CC	14 (93.3)	13 (13.3)	1.0	
CT+TT	1 (6.7)	2 (13.3)	2.154 (0.174-25.672)	
VEGF				1.000
CC	9 (60.0)	9 (60.0)	1.0	
CT+TT	6 (40.0)	6 (40.0)	1.000 (0.232-4.310)	
HIF1A				0.626
CC	12 (8.0)	13 (86.7)	1.0	
CT+TT	3 (20.0)	2 (13.3)	0.615 (0.087-4.341)	

OR, Odds ratio; CI, confidence interval.

cycle checkpoints, genomic instability, and cell adhesion (23). Excessive or inappropriate expression of MMPs may contribute to the pathogenesis of cancer by facilitating tissue degradation. Currently, more than 20 MMPs have been identified, which can be categorized by their substrate specificity (7, 23). Accumulating evidence has shown that MMP promoter SNPs affecting the gene transcription are associated with an enhanced susceptibility to developing malignant diseases, including colorectal cancer, significantly affecting survival and prognosis.

The *MMP2* promoter contains sequences for the binding of AP-2, p53, Sp1, and Sp3 (11). Price *et al*. (12) identified the -1306C/T polymorphism in the *MMP2* promoter and showed a strikingly lower promoter activity with the T allele *via in vitro* transient transfection experiment. The resulting base transition occurs in the CCACC box of the Sp1 binding site and eliminates promoter activity. It is likely that the -1306CC genotype may be associated with a high transcription level and enzyme activity of *MMP-2*, and eventually, it might affect individual susceptibilities to neoplasms. Xu *et al*. (9) found some evidence that individuals with CC genotypes had a higher risk (OR=1.959, 95% CI=1.06-3.64) of developing colorectal cancer compared with those with CT or TT genotypes, and that patients with CC genotype more often had tumors with extended invasion into the serosa or adventitia layer. Langers *et al*. (24) found significant association between *MMP2* -1306C/T SNP, tumor stage, and the survival of the patient. Hettiaratchi *et al*. (25) and Elander *et al*. (26), however, showed no significant association between the highly active C allele of *MMP2* -1306 C/T polymorphism and clinicopatholgical parameters or susceptibility of colorectal cancer. In our study, there was no significant association between genotype distribution of the *MMP2* -1306 C/T polymorphism and colorectal cancer. Moreover, no significant difference was noted in *MMP2* -1306 C/T polymorphism and clinicopathological parameters.

Evidence from preclinical and clinical studies have shown that VEGF functions as a predominant angiogenic factor in human colorectal cancer, which is associated with the metastases and poor prognosis (13). *VEGF* gene, which contains eight exons and seven introns, is known to be located on the chromosomal subband 6p21.3. At least 30 SNPs in this gene region have been described in published studies (27, 28). In particular, the 936C/T SNP in the 3'-UTR is of particular note. The 3'-UTR of the *VEGF* gene has been proven to increase the stability of mRNA and to be associated with the hypoxic induction of VEGF (29-32). Recently, genes designed as Hu family have been identified and their products have been shown to bind the AU-rich element of 3'-UTR of several genes, including the *VEGF* mRNA (29, 33-35). It is suggested that the proteins of the

Table VI. *Genotype of MMP2 and clinicopathological characteristics in 50 patients with colorectal cancer.*

Parameter	Genotype		*P*-value
	CC (%)	CT+TT (%)	
Location of tumor			0.287
Colon	29 (69.0)	7 (87.5)	
Rectum	13 (31.0)	1 (12.5)	
Differentiation			0.066
Well-differentiated	1 (2.4)	1 (16.7)	
Moderately differentiated	39 (95.1)	4 (66.7)	
Poorly differentiated	1 (2.4)	1 (16.7)	
Tumor size			0.418
<4 cm	10 (23.8)	3 (37.5)	
≥4 cm	32 (76.2)	5 (16.0)	
Lymphatic invasion			0.709
Yes	18 (42.9)	4 (50.0)	
No	24 (57.1)	4 (50.0)	
Lymph node metastasis			0.517
Yes	21 (50.0)	3 (37.5)	
No	21 (50.0)	5 (62.5)	
Phenotype			0.695
Polypoid	8 (19.0)	1 (12.5)	
Ulcerative	34 (81.0)	7 (87.5)	
Modified Dukes' stage			0.345
A/B	18 (42.9)	2 (25)	
C/D	24 (57.1)	6 (75)	

Table VIII. *Genotype of HIF1A and clinicopathological characteristics in 50 patients with colorectal cancer.*

Parameter	Genotype		*P*-value
	CC (%)	CT+TT (%)	
Location of tumor			0.791
Colon	27 (71.1)	9 (75.0)	
Rectum	11 (28.9)	3 (25.0)	
Differentiation			0.417
Well-differentiated	0 (0.0)	2 (18.2)	
Moderately differentiated	34 (94.4)	9 (81.8)	
Poorly differentiated	2 (5.6)	0 (0.0)	
Tumor size			0.928
<4 cm	10 (26.3)	3 (25.0)	
≥4 cm	28 (73.7)	9 (75.0)	
Lymphatic invasion			0.393
Yes	18 (47.4)	4 (33.3)	
No	20 (52.6)	8 (66.7)	
Lymph node metastasis			0.614
Yes	19 (50.0)	5 (41.7)	
No	19 (50.0)	7 (58.3)	
Phenotype			0.469
Polypoid	6 (15.8)	3 (25.0)	
Ulcerative	32 (84.2)	9 (75.0)	
Modified Dukes' stage			0.224
A/B	17 (44.7)	3 (25.0)	
C/D	21 (55.3)	9 (75.0)	

Table VII. *Genotype of VEGF and clinicopathological characteristics in 50 patients with colorectal cancer.*

Parameter	Genotype		*P*-value
	CC (%)	CT+TT (%)	
Location of tumor			0.594
Colon	21 (75.0)	15 (68.2)	
Rectum	7 (25.0)	7 (31.8)	
Differentiation			0.991
Well-differentiated	1 (4.0)	1 (4.5)	
Moderately differentiated	23 (92.0)	20 (90.9)	
Poorly differentiated	1 (4.0)	1 (4.5)	
Tumor size			0.640
<4 cm	8 (28.6)	5 (22.7)	
≥4 cm	20 (71.4)	17 (77.3)	
Lymphatic invasion			0.124
Yes	15 (53.6)	7 (31.8)	
No	13 (46.4)	15 (68.2)	
Lymph node metastasis			0.802
Yes	13 (46.4)	11 (50.0)	
No	15 (53.6)	11 (50.0)	
Phenotype			0.477
Polypoid	6 (21.4)	3 (13.6)	
Ulcerative	22 (78.6)	19 (86.4)	
Modified Dukes' stage			0.485
A/B	10 (35.7)	10 (45.5)	
C/D	18 (64.3)	12 (54.5)	

Hu family change the *VEGF* mRNA conformation so that the mRNA is not attacked by RNAase. Alterations in a few nucleotides in the 3'-UTR such as SNPs have been shown to be associated with the deregulation of affected genes (36). Therefore, it is suggested that nucleotide polymorphism in the 3'-UTR may alter the mRNA conformational integrity, resulting in genetic variation of *VEGF* gene expression.

Several studies have reported the significant association of the 936T allele of *VEGF* with the risk of developing cancer such as oral cancer, gastric cancer and colon cancer (37), whereas in other studies on breast and lung cancer (38), protective effects of the variant were found. Concerning colorectal cancer, Yamamori *et al*. (28) comparatively analyzed different *VEGF* gene polymorphisms located in the regions of the promoter, the 5'-and the 3'-UTR from the tumor tissues of 18 Japanese patients, and their findings indicate an association between reduced risk of colorectal cancer with certain SNPs in the *VEGF* gene. Bae *et al*. (17) found a significant gender difference by investigating the 936T allele-bearing genotype with the risk of colon cancer. Recently, some investigators found that T allele-bearing genotypes in *VEGF* 936C/T SNP seem to be related to a lower overall survival, and that *VEGF* polymorphism could be an independent prognostic marker and genetic determinent for colorectal patients (17, 27, 39). However,

Hofmann *et al*. (40) have recently reported that there was no association between the 936T allele and the risk of colon cancer, and also, no correlation was found between genotype of 936C/T SNP and tumor characteristics such as size, histological grading, positive regional lymph node metastases or tumor stage. They also found that common SNPs including 936 C/T polymorphism in *VEGF* were not associated with an individual's susceptibility to CRC. In the present study, we did not find any association with either the genotype distribution or the other clinicopathological parameters. A reason for these inconsistant findings could be a linkage disequilibrium with other, perhaps not yet discovered, functional SNPs in the *VEGF* gene or unknown SNPs in angiogenesis-linked signal pathways (37).

SNP C→T transition at 1772 of *HIF1A* was shown to cause amino acid substitutions within or near the N-terminal transactivation domain (N-TAD) within the ODD of *HIF1A* (41) (Figure 4). Tanimoto *et al*. (21) showed the elevated transactivation capacity of variant forms of HIF1A, by assessing the transcription activity in co-transfection experiment with a hypoxia-response element (HRE)-driven luciferase reporter gene in COS7 cells, which implied a role of *HIF1A* polymorphisms in generating individually different tumor progression potential. They demonstrated that the presence of these polymorphic variants seems to cause a significantly higher transcriptional activity than the activity of the wild-type (21), and that these polymorphic variants were associated with higher microvessel density and higher disease stage in patients with head and neck squamous cell carcinoma (21). A significant difference in genotype distribution of the 1772C/T polymorphism between patients and the control group was seen in androgen-independent prostate cancer (42). Konac *et al*. (43) showed a statistically significant increase in the percentage of carriers of the T allele (CT, TT and CT+TT genotypes) in cervical and endometrial cancer, which indicates that carrying a T allele would be a risk factor in the development of those cancers. The polymorphic allele of 1772C/T polymorphism was also shown to be associated with increased risk of developing the breast cancer (44), but the association was not seen in some cases (45, 46).

To date, only few SNP studies concerning colorectal cancer have been published. In 2004, Kuwai *et al*. (47) claimed that HIF1A 1772C/T polymorphism is not involved in progression or metastasis of colorectal carcinoma. However, two years later, Fransen *et al*. (48) showed a significantly higher risk for the development of colorectal cancers with more severe ulcerative growth pattern in heterozygous (CT) patients, compared to the patients who were homozygous (CC) wild-type. In our results, we did not find any association between *HIF1A* polymorphism and the phenotype (polypoid vs. ulcerative) of tumor (p=0.465). But we did find a higher risk for the development of colorectal cancer in T-allele bearing genotypes of the *HIF1A* gene and this was much more evident in patients of age older than 60 years. Previous studies have suggested that molecular and pathophysiological changes occurring throughout life progressively modify molecular homeostasis of colonic epithelial cells and lead to neoplasia (49). DNA damage is certainly increased in older rodents, suggesting frequent stochastic cellular insults (50-52). Aging was also proving to increase epithelial proliferation in rodent (53) and human (54) colon. In spite of several studies on SNPs, the mechanism which results in the replacement with serine (T-allele) at amino acid 582, and therefore affect HIF1A structure and function, is unclear in the elderly group. Although any further attempt to explain the mechanism behind this interaction would be imprudent, these findings clearly warrant further investigations into the role of the *HIF1A* 1772C/T polymorphism, particularly with advancing age.

During the last few years, in spite of previous attempts on clarifying the association of SNP with colorectal cancer in order to determine the higher risk groups, its prognostic effect, and eventually help early diagnosis and cure, clinical relevance has not yet been shown. To our best knowledge, this is the first study to investigate the genotype distribution, clinicopathological impact, and susceptibility to colorectal cancer of three genes, *MMP2, VEGF* and *HIF1A* SNPs in the same study population. Moreover, we demonstrated significant association between 1772C/T polymorphism of *HIF1A* gene and colorectal cancer in this Korean population. Given the ethnically homogenous background of Korean patients, any potential confounding effect due to ethnicity is likely to be small in the present study.

In conclusion, our findings indicate that the *HIF1A* C/T polymorphism may be a useful indicator of the susceptibility to colorectal cancer and that this may be useful in detecting early carcinogenesis and progression of colorectal cancer. Furthermore, larger scaled genetic studies of *MMP2, VEGF* and *HIF1A* SNPs are needed for their functional relevance to be elucidated.

References

1 Yoon YS, Yu CS, Jung SH, Choi PW, Han KR, Kim HC and Kim JC: Characteristics of Colorectal Cancer Detected at the Health Promotion Center. J Korean Soc Coloproctol *23*: 321-326, 2007.

2 Chung DC: The genetic basis of colorectal cancer: insights into critical pathways of tumorigenesis. Gastroenterology *119*: 854-865, 2000.

3 Hood L and Galas D: The digital code of DNA. Nature *421*: 444-448, 2003.

4 Balasubramanian SP, Brown NJ and Reed MW: Role of genetic polymorphisms in tumour angiogenesis. Br J Cancer *87*: 1057-1065, 2002.

5 Ye S: Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. Matrix Biol *19*: 623-629, 2000.

6 Erichsen HC and Chanock SJ: SNPs in cancer research and treatment. Br J Cancer *90*: 747-751, 2004.

7 Egeblad M and Werb Z: New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer *2*: 161-174, 2002.

8 Decock J, Paridaens R and Ye S: Genetic polymorphisms of matrix metalloproteinases in lung, breast and colorectal cancer. Clin Genet *73*: 197-211, 2008.

9 Xu E, Lai M, Lv B, Xing X, Huang Q and Xia X: A single nucleotide polymorphism in the matrix metalloproteinase-2 promoter is associated with colorectal cancer. Biochem Biophys Res Commun *324*: 999-1003, 2004.

10 Kang SH, Brown DA, Kitajima I, Xu X, Heidenreich O, Gryaznov S and Nerenberg M: Binding and functional effects of transcriptional factor Sp1 on the murine interleukin-6 promotor. J Biol Chem *271*: 7330-7335, 1996.

11 Qin H, Sun Y and Benveniste EN: The transcription factors Sp1, Sp3, and AP-2 are required for constitutive matrix metalloproteinase-2 gene expression in astroglioma cells. J Biol Chem *274*: 29130-29137, 1999.

12 Price SJ, Greaves DR and Watkins H: Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. J Biol Chem *276*: 7549-7558, 2001.

13 Guba M, Seeliger H, Kleespies A, Jauch KW and Bruns C: Vascular endothelial growth factor in colorectal cancer. Int J Colorectal Dis *19*: 510-517, 2004.

14 Dvorak HF: Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol *20*: 4368-4380, 2002.

15 Takahashi Y, Kitadai Y, Bucana CD, Cleary KR and Ellis LM: Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res *55*: 3964-3968, 1995.

16 Jain L, Vargo CA, Danesi R, Sissung TM, Price DK, Venzon D and Figg WD: The role of vascular endothelial growth factor SNPs as predictive and prognostic markers for major solid tumors. Mol Cancer Ther *8*: 2496-2508, 2009.

17 Bae SJ, Kim JW, Kang H, Hwang SG, Oh D and Kim NK: Gender-specific association between polymorphism of vascular endothelial growth factor (VEGF 936 C>T) gene and colon cancer in Korea. Anticancer Res *28*: 1271-1276, 2008.

18 Smith TG, Robbins PA and Ratcliffe PJ: The human side of hypoxia-inducible factor. Br J Haematol *141*: 325-334, 2008.

19 Lee JW, Bae SH, Jeong JW, Kim SH and Kim KW: Hypoxia-inducible factor (HIF-1)alpha: its protein stability and biological functions. Exp Mol Med *36*: 1-12, 2004.

20 Kuwai T, Kitadai Y, Tanaka S, Onogawa S, Matsutani N, Kaio E, Ito M and Chayama K: Expression of hypoxia-inducible factor-1alpha is associated with tumor vascularization in human colorectal carcinoma. Int J Cancer *105*: 176-181, 2003.

21 Tanimoto K, Yoshiga K, Eguchi H, Kaneyasu M, Ukon K, Kumazaki T, Oue N, Yasui W, Imai K, Nakachi K, Poellinger L and Nishiyama M: Hypoxia-inducible factor-1alpha polymorphisms associated with enhanced transactivation capacity, implying clinical significance. Carcinogenesis *24*: 1779-1783, 2003.

22 Shin JE, Jung SA, Kim SE, Joo YH, Shim KN, Kim TH, Yoo K and Moon IH: Expression of MMP-2, HIF-1alpha and VEGF in colon adenoma and colon cancer. Korean J Gastroenterol *50*: 9-18, 2007.

23 Dos Reis ST, Pontes J, Jr., Villanova FE, Borra PM, Antunes AA, Dall'oglio MF, Srougi M and Leite KR: Genetic polymorphisms of matrix metalloproteinases: susceptibility and prognostic implications for prostate cancer. J Urol *181*: 2320-2325, 2009.

24 Langers AM, Sier CF, Hawinkels LJ, Kubben FJ, van Duijn W, van der Reijden JJ, Lamers CB, Hommes DW and Verspaget HW: MMP-2 geno-phenotype is prognostic for colorectal cancer survival, whereas MMP-9 is not. Br J Cancer *98*: 1820-1823, 2008.

25 Hettiaratchi A, Hawkins NJ, McKenzie G, Ward RL, Hunt JE, Wakefield D and Di Girolamo N: The collagenase-1 (*MMP-1*) gene promoter polymorphism - 1607/2G is associated with favourable prognosis in patients with colorectal cancer. Br J Cancer *96*: 783-792, 2007.

26 Elander N, Soderkvist P and Fransen K: Matrix metalloproteinase (MMP) -1, -2, -3 and -9 promoter polymorphisms in colorectal cancer. Anticancer Res *26*: 791-795, 2006.

27 Dassoulas K, Gazouli M, Rizos S, Theodoropoulos G, Christoni Z, Nikiteas N and Karakitsos P: Common polymorphisms in the vascular endothelial growth factor gene and colorectal cancer development, prognosis, and survival. Mol Carcinog *48*: 563-569, 2009.

28 Yamamori M, Sakaeda T, Nakamura T, Okamura N, Tamura T, Aoyama N, Kamigaki T, Ohno M, Shirakawa T, Gotoh A, Kuroda Y, Matsuo M, Kasuga M and Okumura K: Association of VEGF genotype with mRNA level in colorectal adenocarcinomas. Biochem Biophys Res Commun *325*: 144-150, 2004.

29 Awata T, Inoue K, Kurihara S, Ohkubo T, Watanabe M, Inukai K, Inoue I and Katayama S: A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes *51*: 1635-1639, 2002.

30 Dibbens JA, Miller DL, Damert A, Risau W, Vadas MA and Goodall GJ: Hypoxic regulation of vascular endothelial growth factor mRNA stability requires the cooperation of multiple RNA elements. Mol Biol Cell *10*: 907-919, 1999.

31 Greener MJ and Sewry CA, Muntoni F and Roberts RG: The 3'-untranslated region of the dystrophin gene - conservation and consequences of loss. Eur J Hum Genet *10*: 413-420, 2002.

32 Iida K, Kawakami Y, Sone H, Suzuki H, Yatoh S, Isobe K, Takekoshi K and Yamada N: Vascular endothelial growth factor gene expression in a retinal pigmented cell is up-regulated by glucose deprivation through 3' UTR. Life Sci *71*: 1607-1614, 2002.

33 Ma WJ, Cheng S, Campbell C, Wright A and Furneaux H: Cloning and characterization of HuR, a ubiquitously expressed Elav-like protein. J Biol Chem *271*: 8144-8151, 1996.

34 Nabors LB, Gillespie GY, Harkins L and King PH: HuR, a RNA stability factor, is expressed in malignant brain tumors and binds to adenine- and uridine-rich elements within the 3' untranslated regions of cytokine and angiogenic factor mRNAs. Cancer Res *61*: 2154-2161, 2001.

35 Goldberg-Cohen I, Furneauxb H and Levy AP: A 40-bp RNA element that mediates stabilization of vascular endothelial growth factor mRNA by HuR. J Biol Chem *277*: 13635-13640, 2002.

36 Ramamoorti N, Kumarvelu J, Shanmugasundaram GK, Rani K and Banerjea AC: High frequency of G to A transition mutation in the stromal cell derived factor-1 gene in India, a chemokine that blocks HIV-1 (X4) infection: multiple proteins bind to 3'-untranslated region of SDF-1 RNA. Genes Immun *2*: 408-410, 2001.

37 Chae YS, Kim JG, Sohn SK, Cho YY, Ahn BM, Moon JH, Jeon SW, Park JY, Lee IT, Choi GS and Jun SH: Association of vascular endothelial growth factor gene polymorphisms with susceptibility and clinicopathologic characteristics of colorectal cancer. J Korean Med Sci *23*: 421-427, 2008.

38 Lee SJ, Lee SY, Jeon HS, Park SH, Jang JS, Lee GY, Son JW, Kim CH, Lee WK, Kam S, Park RW, Park TI, Kang YM, Kim IS, Jung TH and Park JY: Vascular endothelial growth factor gene polymorphisms and risk of primary lung cancer. Cancer Epidemiol Biomarkers Prev *14*: 571-575, 2005.

39 Kim JG, Chae YS, Sohn SK, Cho YY, Moon JH, Park JY, Jeon SW, Lee IT, Choi GS and Jun SH: Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with colorectal cancer. Clin Cancer Res *14*: 62-66, 2008.

40 Hofmann G, Langsenlehner U, Renner W, Langsenlehner T, Yazdani-Biuki B, Clar H, Gerger A, Wehrschuetz M, Samonigg H and Krippl P: Common single nucleotide polymorphisms in the vascular endothelial growth factor gene and colorectal cancer risk. J Cancer Res Clin Oncol *134*: 591-595, 2008.

41 Bardos JI and Ashcroft M: Hypoxia-inducible factor-1 and oncogenic signalling. Bioessays *26*: 262-269, 2004.

42 Chau CH, Permenter MG, Steinberg SM, Retter AS, Dahut WL, Price DK and Figg WD: Polymorphism in the hypoxia-inducible factor 1alpha gene may confer susceptibility to androgen-independent prostate cancer. Cancer Biol Ther *4*: 1222-1225, 2005.

43 Konac E, Onen HI, Metindir J, Alp E, Biri AA and Ekmekci A: An investigation of relationships between hypoxia-inducible factor-1 alpha gene polymorphisms and ovarian, cervical and endometrial cancers. Cancer Detect Prev *31*: 102-109, 2007.

44 Kim HO, Jo YH, Lee J, Lee SS and Yoon KS: The C1772T genetic polymorphism in human HIF-1alpha gene associates with expression of HIF-1alpha protein in breast cancer. Oncol Rep *20*: 1181-1187, 2008.

45 Apaydin I, Konac E, Onen HI, Akbaba M, Tekin E and Ekmekci A: Single nucleotide polymorphisms in the hypoxia-inducible factor-1alpha (HIF-1alpha) gene in human sporadic breast cancer. Arch Med Res *39*: 338-345, 2008.

46 Konac E, Dogan I, Onen HI, Yurdakul AS, Ozturk C, Varol A and Ekmecki A: Genetic variations in the hypoxia-inducible factor-1alpha gene and lung cancer. Exp Biol Med (Maywood) *234*: 1109-1116, 2009.

47 Kuwai T, Kitadai Y, Tanaka S, Kuroda T, Ochiumi T, Matsumura S, Oue N, Yasui W, Kaneyasu M, Tanimoto K, Nishiyama M and Chayama K: Single nucleotide polymorphism in the hypoxia-inducible factor-1alpha gene in colorectal carcinoma. Oncol Rep *12*: 1033-1037, 2004.

48 Fransen K, Fenech M, Fredrikson M, Dabrosin C and Soderkvist P: Association between ulcerative growth and hypoxia inducible factor-1alpha polymorphisms in colorectal cancer patients. Mol Carcinog *45*: 833-840, 2006.

49 Ono T, Uehara Y, Saito Y and Ikehata H: Mutation theory of aging, assessed in transgenic mice and knockout mice. Mech Ageing Dev *123*: 1543-1552, 2002.

50 Taylor RW, Barron MJ, Borthwick GM, Gospel A, Chinnery PF, Samuels DC, Taylor GA, Plusa SM, Needham SJ, Greaves LC, Kirkwood TB and Turnbull DM: Mitochondrial DNA mutations in human colonic crypt stem cells. J Clin Invest *112*: 1351-1360, 2003.

51 Ono T, Ikehata H, Pithani VP, Uehara Y, Chen Y, Kinouchi Y Shimosegawa T and Hosoi Y: Spontaneous mutations in digestive tract of old mice show tissue-specific patterns of genomic instability. Cancer Res *64*: 6919-6923, 2004.

52 Dolle ME, Snyder WK, Gossen JA, Lohman PH and Vijg J: Distinct spectra of somatic mutations accumulated with age in mouse heart and small intestine. Proc Natl Acad Sci USA *97*: 8403-8408, 2000.

53 Holt PR and Yeh KY: Colonic proliferation is increased in senescent rats. Gastroenterology *95*: 1556-1563, 1988.

54 Roncucci L, Ponz de Leon M, Scalmati A, Malagoli G, Pratissoli S, Perini M and Chahin NJ: The influence of age on colonic epithelial cell proliferation. Cancer *62*: 2373-2377, 1988.

Received November 26, 2010
Revised January 11, 2011
Accepted January 12, 2011