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Interplay between 3'-UTR polymorphisms in the vascular endothelial growth factor (VEGF) gene and metabolic syndrome in determining the risk of colorectal cancer in Koreans

Young Joo Jeon^{1,2†}, Jong Woo Kim^{3†}, Hye Mi Park¹, Hyo Geun Jang^{1,2}, Jung O Kim^{1,2}, Jisu Oh⁴, So Young Chong⁴, Sung Won Kwon³, Eo Jin Kim⁵, Doyeun Oh^{1,4*} and Nam Keun Kim^{1,2*}

Abstract

Background: Polymorphisms in angiogenesis-related genes and metabolic syndrome (MetS) risk factors play important roles in cancer development. Moreover, recent studies have reported associations between a number of 3'-UTR polymorphisms and a variety of cancers. The aim of this study was to investigate the associations of three *VEGF* 3'-UTR polymorphisms (1451C > T [rs3025040], 1612G > A [rs10434], and 1725G > A [rs3025053]) and MetS with colorectal cancer (CRC) susceptibility in Koreans.

Methods: A total of 850 participants (450 CRC patients and 400 controls) were enrolled in the study. The genotyping of *VEGF* polymorphisms was performed by TaqMan allelic discrimination assays. Cancer risks of genetic variations and gene-environment interactions were assessed by adjusted odds ratios (AORs) and 95% confidence intervals (CIs) of multivariate logistic regression analyses.

Results: *VEGF* 1451C > T was significantly associated with rectal cancer risk (Dominant model; AOR = 1.58; 95% CI = 1.09 - 2.28; $p = 0.015$) whereas *VEGF* 1725G > A correlated with MetS risk (Dominant model; AOR = 1.61; 95% CI = 1.06 - 2.46; $p = 0.026$). Of the gene-environment combined effects, the interaction of *VEGF* 1451C > T and MetS contributed to increased rectal cancer risk (AOR = 3.15; 95% CI = 1.74 - 5.70; $p < .001$) whereas the combination of *VEGF* 1725G > A and MetS was involved with elevated colon cancer risk (AOR = 2.68; 95% CI = 1.30 - 1.55; $p = 0.008$).

Conclusions: Our results implicate that *VEGF* 1451C > T and 1725G > A may predispose to CRC susceptibility and the genetic contributions may be varied with the presence of MetS.

Keywords: *VEGF*, 3'-UTR, Polymorphism, Colorectal cancer, Metabolic syndrome

Background

Colorectal cancer (CRC) is the third most common type of cancer and the second leading cause of cancer-related mortality in Western countries [1]. The prognosis of patients with CRC depends on the tumor stage at the time of

diagnosis. However, over 57% of patients have regional or distant spread of tumor cells at the time of diagnosis [2]. The pathogenesis of CRC usually follows a stepwise progression from benign polyp to invasive adenocarcinoma. In colorectal carcinogenesis, the unique molecular and genetic changes that occur within cells result in a specific CRC phenotype. This phenotype is associated with variable tumor behaviors that are relevant to the prognosis and the response to specific therapies. As a result, the term "CRC" no longer refers to a single disease, but rather a heterogeneous group of diseases caused by differential genetic/epigenetic backgrounds. In this respect, many ongoing studies are aimed at assessing biomarkers as potential predictors of

* Correspondence: doh@cha.ac.kr; nkim@cha.ac.kr

†Equal contributors

¹Institute for Clinical Research, School of Medicine, CHA University, 351, Yatap-dong, Bundang-gu, Seongnam 463-712, South Korea

⁴Department of Internal Medicine, School of Medicine, CHA University, 351, Yatap-dong, Bundang-gu, Seongnam 463-712, South Korea

²Department of Biomedical Science, College of Life Science, CHA University, Seongnam 463-712, South Korea

Full list of author information is available at the end of the article

prognosis or response to therapy, which will most likely lead to the individualized management of the disease.

Tumor angiogenesis is important to tumor growth, as evidenced by results showing that tumor growth is dependent on angiogenesis. Furthermore, tumors are able to produce diffusible angiogenic molecules [3], including vascular endothelial growth factor (VEGF). VEGF is a key regulator of angiogenesis with several functions that serve to enhance tumor progression. These functions include enhancing vascular permeability, inducing endothelial cell migration and division, inducing serine protease activity, inhibiting either the apoptosis of endothelial cells or maturation of dendritic cells, and inducing angiogenesis [4,5]. The human *VEGF* gene is located on chromosome 6 and organized into eight exons and seven introns, which encode several different isoforms due to alternative splicing. There are five well-studied *VEGF* polymorphisms that have been linked to CRC: -2578C > A, -1498T > C, -1154G > A, -634G > C, and 936C > T. However, their genetic associations with CRC have been inconsistent [6]. Recent papers have shown some clinical impacts of polymorphisms in the 3'-UTR of certain genes, which may potentially bind to specific miRNAs in various cancers [7-10]. However, variants in the *VEGF* 3'-UTR have not been studied.

The metabolic syndrome (MetS) is a portfolio of metabolic disorders, including abdominal obesity, increased blood pressure (BP), abnormal glucose metabolism, and dyslipidemia [11]. Previous reports show that components of MetS are associated with CRC susceptibility [12,13]. A previous study found a 35% increased CRC risk associated with high BP [14] and there was a similar finding in another prospective epidemiologic study [15]. Adult-onset diabetes mellitus (DM) has generally been associated with a higher risk of CRC [16-20] and elevated blood glucose levels correlated with a significantly elevated CRC risk (Relative risk [RR] = 1.80) [21]. However, gene-environment combined effects of MetS for CRC susceptibility have been infrequently found in previous published database.

In the *VEGF* gene, there are four known 3'-UTR polymorphisms (936C > T [rs3025039], 1451C > T [rs3025040], 1612G > A [rs10434], 1725G > A [rs3025053]). The *VEGF* 1451TT genotype presented a significant log-rank *p* value in non-small-cell lung cancer survival [22]. The *VEGF* 1612A allele was associated with increased gastric cancer risk [23]. The 936C > T polymorphism and its link to CRC susceptibility have been published by our laboratory and others [6,24]. The other three single nucleotide polymorphisms (SNPs) in the *VEGF* 3'-UTR, 1451C > T, 1612G > A, and 1725G > A, are poorly understood in the context of their genetic contributions to CRC susceptibility. The purpose of this study was to investigate whether these polymorphisms of *VEGF* 3'-UTR correlate with CRC susceptibility and the genetic contributions are modified by the presence of MetS.

Methods

Study population

We conducted a case-control study of 850 individuals. Four hundred and fifty patients diagnosed with CRC at CHA Bundang Medical Center (Seongnam, South Korea) were enrolled from June 2004 to January 2009. This study only included CRC patients who had undergone surgical resection with a curative intent and who had histologically confirmed adenocarcinoma. Within the CRC cohort, 264 consecutive patients with colon cancer and 186 consecutive patients with rectal cancer underwent primary surgery. Tumor staging of CRCs was performed according to the sixth edition of the American Joint Committee on Cancer (AJCC) staging manual. The control group consisted of 400 individuals randomly selected following a health screening. This screening excluded patients with a history of cancer. Individuals were diagnosed with MetS if they possessed three or more of the following five risk factors: body mass index (BMI) ≥ 25.0 kg/m²; triglycerides (TG) ≥ 150 mg/dL; high density lipoprotein-cholesterol (HDL-C) < 40 mg/dL for men or < 50 mg/dL for women; BP $\geq 130/85$ mmHg or currently taking anti-hypertension medication; and fasting blood sugar (FBS) ≥ 100 mg/dL or currently taking hypoglycemic medication. All study subjects were ethnic Koreans and provided written informed consent. The study protocol was approved by the Institutional Review Board of CHA Bundang Medical Center, Seongnam, South Korea.

Phenotype measurements

Anthropometric measurements included BMI. Systolic and diastolic BP of subjects were measured in the seated position after 10 min of rest. For measurements of physiological parameters, 3 ml blood was obtained after fasting overnight. The hexokinase method was employed to measure FBS levels; samples were analyzed in duplicate by an automated analyzer (TBA 200FR NEO, Toshiba Medical Systems, Tokyo, Japan). TG and HDL-C levels were determined by enzymatic colorimetric methods using commercial reagent sets (TBA 200FR NEO, Toshiba Medical Systems).

Genotyping

DNA was extracted from leukocytes using a G-DEX™ II Genomic DNA Extraction kit (Intron Biotechnology, Seongnam, Korea) according to the manufacturer's instructions. *VEGF* genotypes were analyzed by TaqMan allelic discrimination analysis. Genotyping of the *VEGF* 1451C > T, *VEGF* 1612G > A, and *VEGF* 1725G > A polymorphisms was determined using real-time polymerase chain reaction (PCR) (RG-6000, Corbett Research, Australia) for allelic discrimination. Primers and TaqMan probes were designed using Primer Express Software

(version 2.0) and synthesized and supplied by Applied Biosystems (Foster City, CA, USA). The reporter dyes used were FAM and JOE. The primer sequences for amplification are as follows: *VEGF* 1451C > T: forward 5'- ACG GAC AGA AAG ACA GAT CAC AG -3' and reverse 5'- CCC AAA GCA CAG CAA TGT C -3'. The selected probes were 5'-FAM- TGA GGA CAC CGG CTC TGA CC -TAMRA-3' (C allele detecting probe) and 5'-JOE-TGA GGA CAC TGG CTC TGA CC -TAMRA-3' (T allele detecting probe). *VEGF* 1612G > A: forward 5'- TTC GCT TAC TCT CAC CTG CTT C -3' and reverse 5'- GCT GTC ATG GGC TGC TTC T -3'. The selected probes were 5'-FAM- CCC AGG AGG CCA CTG GCA -TAMRA-3' (G allele detecting probe) and 5'-JOE- CCC AGG AGA CCA CTG GCA -TAMRA-3' (A allele detecting probe). *VEGF* 1725G > A: forward 5'- CAT GAC AGC TCC CCT TCC T -3' and reverse 5'- TGG TTT CAA TGG TGT GAG GAC -3'. The selected probes were 5'-FAM- CTT CCT GGG GTG CAG CCT AA -TAMRA-3' (G allele detecting probe) and 5'-JOE- CTT CCT GGG ATG CAG CCT AA -TAMRA-3' (A allele detecting probe). For each polymorphism, 30% of the PCR assays were randomly selected and repeated, followed by DNA sequencing, to validate the experimental findings. Sequencing was performed using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The concordance of the quality control samples was 100%.

Quantitative real-time PCR

To perform quantitative real-time PCR (qRT-PCR), total RNA was extracted from 47 tumor and tumor-adjacent tissues from 47 CRC patients by using TRIzol Reagent (Invitrogen, Grand Island, NY, USA) according to the manufacturer's instructions. cDNA was made from total RNA with the SuperScript III First-Strand Synthesis System (Invitrogen, Grand Island, NY, USA). Measurement of the VEGF mRNA was determined using real-time PCR (RG-6000, Corbett Research, Australia). The expression level of VEGF mRNA in 47 tumor and tumor-adjacent tissues was compared by a comparative C_T ($2^{-\Delta\Delta C_T}$) method with housekeeping internal control, 18 s rRNA. The primer sequences for amplification are as follows: 18 s rRNA: forward 5'- AAC TTT CGA TGG TAG TCG CCG -3' and reverse 5'- CCT TGG ATG TGG TAG CCG TTT -3'. VEGF: forward 5'- TGA GCT TCC TAC AGC ACA AC -3' and reverse 5'- ATT TAC ACG TCT GCG GAT CTT -3'.

Statistical analysis

To analyze baseline characteristics, odds ratios (ORs) and 95% confidence intervals (95% CIs) from univariate logistic regression were used to compare patient and control baseline data. Genetic associations of *VEGF* 1451C > T, 1612G > A, and 1725G > A polymorphisms with MetS

and CRC susceptibility were calculated using adjusted odds ratios (AORs) and 95% CIs from multivariate logistic regression. The variables age, gender, and MetS risk factors were selected as adjustment variables. To estimate MetS and CRC risk, we used three genetic susceptibility models: additive, dominant, and recessive. All *VEGF* 3'-UTR genotypes were converted into numeric values for logistic regression according to their genotypes. Wild homozygotes were assigned "0" in all models. Heterozygotes were assigned "1" in additive and dominant models and "0" in the recessive model. Mutant homozygotes were assigned "1" in dominant and recessive models and "2" in the additive model. Gene-environment interaction analysis was performed using the open-source multifactor dimensionality reduction (MDR) software package (v.2.0) available from www.epistasis.org. The comparisons of relative VEGF mRNA expression were analyzed by Mann-Whitney, Kruskal-Wallis, and Wilcoxon signed rank tests. Analyses were performed using GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA) and Medcalc version 12.7.1.0 (Medcalc Software, Mariakerke, Belgium). Haplotypes for multiple loci were estimated using the expectation-maximization algorithm with SNPalyze (Version 5.1; DYNACOM Co, Ltd, Yokohama, Japan).

Results

In this study, we collected data for 450 CRC patients (264 colon cancer [CC] and 186 rectal cancer [RC] patients), including 212 men and 238 women. Both CC and RC groups have higher portion of tumor size ≥ 5 cm and tumor node metastasis (TNM) stage II/III (Table 1). The presence of MetS was associated with CRC susceptibility (CC group: OR = 1.90; 95% CI = 1.35 - 2.66; $p < .001$; RC group: OR = 2.07; 95% CI = 1.43 - 3.01; $p < .001$). Of MetS risk factors, lower HDL-C (<40 mg/dL for men or <50 mg/dL for women) strongly contributed to CC and RC risk (CC group: OR = 3.09; 95% CI = 2.18 - 4.37; $p < .001$; RC group: OR = 3.40; 95% CI = 2.32 - 4.97; $p < .001$). Table 2 shows the distributions of genotypes and haplotypes for *VEGF* 1451C > T, 1612G > A, and 1725G > A polymorphisms stratified by the presence of MetS. The *VEGF* 3'-UTR genotype frequencies of controls were consistent with the Hardy-Weinberg equilibrium. Table 3 presents AOR values for MetS, CC, and RC risk by *VEGF* 3'-UTR polymorphisms. *VEGF* 1451C > T was significantly associated with RC risk (Dominant model: AOR = 1.58; 95% CI = 1.09 - 2.28; $p = 0.015$) whereas *VEGF* 1725G > A correlated with MetS risk (Dominant model: AOR = 1.61; 95% CI = 1.06 - 2.46; $p = 0.026$). As a similar pattern, in haplotype analysis, *VEGF* 1451T/1612G/1725G contributed to RC risk (AOR = 1.40; 95% CI = 1.03 - 1.92; $p = 0.030$) while *VEGF* 1451C/1612A/1725A was involved with MetS risk (AOR = 1.54; 95% CI = 1.02 - 2.34; $p = 0.041$).

Table 1 Baseline characteristics in colorectal cancer patients and control subjects

Characteristics	Control	CC	OR (95% CI)	p	RC	OR (95% CI)	p
N	400	264			186		
Age (mean±SD)	60.89 ± 11.72	61.85 ± 12.85	1.01 (0.99 - 1.02)	0.320	62.33 ± 11.46	1.01 (1.00 - 1.03)	0.165
Gender (male), n (%)	170 (42.5)	124 (47.0)	1.20 (0.88 - 1.64)	0.257	88 (47.3)	1.21 (0.86 - 1.72)	0.275
Metabolic syndrome, n (%)	95 (23.8)	98 (37.1)	1.90 (1.35 - 2.66)	<.001	73 (39.2)	2.07 (1.43 - 3.01)	<.001
Anti-HTN drug or BP ≥ 130/85 mmHg, n (%)	157 (39.3)	159 (60.2)	2.34 (1.71 - 3.22)	<.001	120 (64.5)	2.81 (1.96 - 4.04)	<.001
Anti-DM drug or FBS ≥ 100 mg/dl, n (%)	166 (41.5)	149 (56.4)	1.83 (1.33 - 2.50)	<.001	104 (55.9)	1.79 (1.26 - 2.54)	0.001
TG ≥ 150 mg/dl, n (%)	135 (33.8)	67 (25.4)	0.67 (0.47 - 0.94)	0.022	46 (24.7)	0.65 (0.44 - 0.95)	0.029
BMI ≥ 25 kg/m ² , n (%)	93 (23.3)	65 (24.6)	1.08 (0.75 - 1.55)	0.685	51 (27.4)	1.25 (0.84 - 1.85)	0.276
HDL-C < 40(male)/50(female) mg/dl, n (%)	78 (19.5)	113 (42.8)	3.09 (2.18 - 4.37)	<.001	84 (45.2)	3.40 (2.32 - 4.97)	<.001
Tumor size							
<5 cm	-	94 (35.6)	-	-	87 (46.8)	-	-
≥5 cm	-	170 (64.4)	-	-	99 (53.2)	-	-
TNM stage, n (%)							
I	-	22 (8.3)	-	-	20 (10.8)	-	-
II	-	118 (44.7)	-	-	71 (38.2)	-	-
III	-	94 (35.6)	-	-	79 (42.5)	-	-
IV	-	30 (11.4)	-	-	16 (8.6)	-	-

Colon cancer (CC), Rectal cancer (RC), Odds ratio (OR), Confidence interval (CI), Standard deviation (SD), Blood pressure (BP), Fasting blood sugar (FBS), Hypertension (HTN), Diabetes mellitus (DM), Triglycerides (TG), Body mass index (BMI), High density lipoprotein-cholesterol (HDL-C), Tumor node metastasis (TNM). ORs and p values were calculated by univariate logistic regression.

Table 2 Genotype and haplotype frequencies of VEGF 3'-UTR polymorphisms

Genotype/Haplotype	Without MetS			With MetS		
	Control (n = 305)	CC (n = 166)	RC (n = 113)	Control (n = 95)	CC (n = 98)	RC (n = 73)
VEGF 1451CC	209 (68.5)	108 (65.1)	64 (56.6)	65 (68.4)	72 (73.5)	45 (61.6)
VEGF 1451CT	81 (26.6)	50 (30.1)	43 (38.1)	29 (30.5)	23 (23.5)	27 (37.0)
VEGF 1451TT	15 (4.9)	8 (4.8)	6 (5.3)	1 (1.1)	3 (3.1)	1 (1.4)
HWE-p	0.059	0.483	0.724	0.251	0.493	0.167
VEGF 1612GG	213 (69.8)	127 (76.5)	87 (77.0)	67 (70.5)	68 (69.4)	54 (74.0)
VEGF 1612GA	83 (27.2)	36 (21.7)	21 (18.6)	27 (28.4)	29 (29.6)	18 (24.7)
VEGF 1612AA	9 (3.0)	3 (1.8)	5 (4.4)	1 (1.1)	1 (1.0)	1 (1.4)
HWE-p	0.791	0.809	0.022	0.336	0.271	0.714
VEGF 1725GG	278 (91.1)	147 (88.6)	96 (85.0)	81 (85.3)	79 (80.6)	61 (83.6)
VEGF 1725GA	27 (8.9)	19 (11.4)	17 (15.0)	14 (14.7)	19 (19.4)	12 (16.4)
VEGF 1725AA	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
HWE-p	0.419	0.434	0.387	0.438	0.288	0.444
VEGF 1451C/1612G/1725G	398 (65.2)	221 (65.6)	140 (61.9)	130 (68.4)	134 (68.4)	96 (65.8)
VEGF 1451T/1612G/1725G	111 (18.2)	66 (19.9)	55 (24.3)	31 (16.3)	29 (14.8)	29 (19.9)
VEGF 1451C/1612A/1725G	74 (12.1)	26 (7.8)	14 (6.2)	15 (7.9)	14 (7.1)	9 (6.2)
VEGF 1451C/1612A/1725A	27 (4.4)	16 (4.8)	17 (7.5)	14 (7.4)	17 (8.7)	11 (7.5)
VEGF 1451C/1612G/1725A	0 (0.0)	3 (0.9)	0 (0.0)	0 (0.0)	2 (1.0)	1 (0.7)

Vascular endothelial growth factor (VEGF), Colon cancer (CC), Rectal cancer (RC), Metabolic syndrome (MetS), Hardy-Weinberg equilibrium (HWE).

Table 3 Adjusted odds ratios for metabolic syndrome, colon cancer, and rectal cancer risks according to VEGF 3'-UTR variants

Genotype/Haplotype	Model	Wild type	MetS risk		CC risk		RC risk	
			AOR (95% CI)*	p	AOR (95% CI)**	p	AOR (95% CI)**	p
VEGF 1451C > T	Additive	CC	0.80 (0.61 - 1.05)	0.105	1.05 (0.79 - 1.39)	0.742	1.40 (1.03 - 1.91)	0.031
	Dominant	CC	0.85 (0.62 - 1.16)	0.295	1.04 (0.74 - 1.46)	0.813	1.58 (1.09 - 2.28)	0.015
	Recessive	CC	0.36 (0.14 - 0.96)	0.041	1.16 (0.53 - 2.57)	0.709	1.12 (0.45 - 2.80)	0.804
VEGF 1612G > A	Additive	GG	1.02 (0.76 - 1.36)	0.920	0.82 (0.59 - 1.12)	0.216	0.83 (0.58 - 1.18)	0.304
	Dominant	GG	1.11 (0.80 - 1.54)	0.521	0.81 (0.57 - 1.15)	0.239	0.74 (0.50 - 1.11)	0.149
	Recessive	GG	0.38 (0.11 - 1.33)	0.130	0.68 (0.21 - 2.21)	0.521	1.48 (0.52 - 4.19)	0.458
VEGF 1725G > A	Additive	GG	1.61 (1.06 - 2.46)	0.026	1.34 (0.83 - 2.16)	0.235	1.51 (0.90 - 2.55)	0.118
	Dominant	GG	1.61 (1.06 - 2.46)	0.026	1.34 (0.83 - 2.16)	0.235	1.51 (0.90 - 2.55)	0.118
	Recessive	GG	-	-	-	-	-	-
VEGF 1451C/1612G/1725G		Others	1.14 (0.92 - 1.43)	0.232	1.04 (0.82 - 1.31)	0.762	0.87 (0.67 - 1.12)	0.279
VEGF 1451T/1612G/1725G		Others	0.80 (0.61 - 1.05)	0.101	1.05 (0.79 - 1.41)	0.735	1.40 (1.03 - 1.92)	0.030
VEGF 1451C/1612A/1725G		Others	0.73 (0.50 - 1.07)	0.111	0.69 (0.47 - 1.02)	0.066	0.56 (0.34 - 0.90)	0.017
VEGF 1451C/1612A/1725A		Others	1.54 (1.02 - 2.34)	0.041	1.12 (0.69 - 1.80)	0.652	1.42 (0.86 - 2.36)	0.170

Adjusted odds ratio (AOR), Confidence interval (CI), Metabolic syndrome (MetS), Colon cancer (CC), Rectal cancer (RC), Vascular endothelial growth factor (VEGF). *AORs and p values were adjusted by age and gender. **AORs and p values were adjusted by age, gender, and MetS risk factors.

Because cancer risk is determined by a complex interplay of genetic and environmental factors, we calculated gene-environment combined effects between MetS and VEGF 3'-UTR polymorphisms. Preferentially, we sought to determine whether the contribution of VEGF 3'-UTR genetic variants to CRC susceptibility varied with the presence of MetS (Table 4). The significant involvements of VEGF 1451C > T (Dominant model: AOR = 1.67; 95% CI = 1.07 - 2.61; p = 0.023) and VEGF 1451T/1612G/1725G (AOR = 1.45; 95% CI = 1.00 - 2.09; p = 0.047) with RC risk were found in the absence of MetS. Table 5 displays gene-environment interaction effects between VEGF 3'-UTR polymorphisms and MetS. We examined all possible combinations of gene-environment interactions using MDR methods. The combination of VEGF 1451C > T and MetS was the best model to evaluate RC risk (Cross validation consistency = 9/10), while the combination of VEGF 1725G > A and MetS was the most appropriate model to assess CC risk (Cross validation consistency = 7/10). The interaction of VEGF 1451C > T and MetS contributed to increased RC risk (AOR = 3.15; 95% CI = 1.74 - 5.70; p < .001) whereas the combination of VEGF 1725G > A and MetS was involved with elevated CC risk (AOR = 2.68; 95% CI = 1.30 - 1.55; p = 0.008).

Finally, we quantified expression of VEGF in tissue samples and looked for differences in expression based on the tested haplotypes and genotypes. VEGF expression as a function of each VEGF 3'-UTR genotype or haplotype is presented in Table 6. Relative VEGF mRNA levels in samples with the 1451T/1612G/1725G were significantly decreased relative to the 1451C/1612G/1725G (p < .05). In contrast, relative VEGF mRNA levels

in samples with the 1451C/1612A/1725A were significantly increased from levels in samples with the 1451C/1612G/1725G (p < .05). Table 7 shows VEGF expression between tumor and tumor-adjacent tissues according to studied polymorphisms. Relative VEGF mRNA expression of tumor-tissues is significantly increased in each wild genotype while not in each variant genotype. (Additional file 1: Table S1) displays the frequencies of MetS and VEGF 3'-UTR genotypes according to clinicopathological features of CRC. The frequency of VEGF 1451C > T was different between the CC and RC groups, but this difference was not statistically significant (p = 0.081).

Discussion

In the present study, we investigated whether VEGF 1451C > T, 1612G > A, and 1725G > A are involved with CRC susceptibility. We identified that VEGF 1451C > T was significantly associated with RC risk whereas VEGF 1725G > A correlated with MetS risk. Of the gene-environment combined effects, the interaction of VEGF 1451C > T and MetS contributed to increased RC risk whereas the combination of VEGF 1725G > A and MetS was involved with elevated CC risk. Furthermore, quantitative real-time PCR analysis revealed that relative VEGF mRNA expression in tumor tissues varied with VEGF 1451T/1612G/1725G and 1451C/1612A/1725A haplotypes. To our knowledge, VEGF 1451C > T and 1725G > A may play roles in CRC susceptibility.

Polymorphisms within the VEGF gene are a current topic of interest within the cancer epidemiology field. There are several association studies showing that

Table 4 Stratified effects of metabolic syndrome on colon cancer and rectal cancer risks by VEGF 3'-UTR variants

Genotype/Haplotype	Model	Subgroup	Wild type	CC risk		RC risk	
				AOR (95% CI)	p	AOR (95% CI)	p
VEGF 1451C > T	Additive	Without MetS	CC	1.12 (0.80 - 1.54)	0.513	1.42 (0.99 - 2.03)	0.055
		With MetS	CC	0.89 (0.50 - 1.59)	0.701	1.44 (0.77 - 2.67)	0.255
	Dominant	Without MetS	CC	1.18 (0.79 - 1.77)	0.416	1.67 (1.07 - 2.61)	0.023
		With MetS	CC	0.78 (0.41 - 1.49)	0.456	1.44 (0.74 - 2.78)	0.280
	Recessive	Without MetS	CC	0.99 (0.41 - 2.40)	0.985	1.09 (0.41 - 2.87)	0.868
		With MetS	CC	3.07 (0.30 - 31.00)	0.342	2.14 (0.13 - 35.71)	0.595
VEGF 1612G > A	Additive	Without MetS	GG	0.73 (0.49 - 1.07)	0.105	0.81 (0.53 - 1.24)	0.328
		With MetS	GG	1.10 (0.61 - 2.02)	0.746	0.97 (0.50 - 1.87)	0.928
	Dominant	Without MetS	GG	0.71 (0.46 - 1.09)	0.117	0.69 (0.42 - 1.14)	0.148
		With MetS	GG	1.13 (0.60 - 2.14)	0.704	0.93 (0.46 - 1.89)	0.850
	Recessive	Without MetS	GG	0.58 (0.15 - 2.17)	0.416	1.51 (0.49 - 4.61)	0.469
		With MetS	GG	0.78 (0.05 - 13.40)	0.867	1.72 (0.10 - 28.53)	0.705
VEGF 1725G > A	Additive	Without MetS	GG	1.34 (0.72 - 2.49)	0.360	1.82 (0.95 - 3.48)	0.072
		With MetS	GG	1.37 (0.62 - 2.98)	0.435	1.20 (0.51 - 2.85)	0.672
	Dominant	Without MetS	GG	1.34 (0.72 - 2.49)	0.360	1.82 (0.95 - 3.48)	0.072
		With MetS	GG	1.37 (0.62 - 2.98)	0.435	1.20 (0.51 - 2.85)	0.672
	Recessive	Without MetS	GG	-	-	-	-
		With MetS	GG	-	-	-	-
VEGF 1451C/1612G/1725G	Without MetS	Others	1.06 (0.80 - 1.40)	0.697	0.87 (0.63 - 1.19)	0.375	
	With MetS	Others	0.95 (0.61 - 1.49)	0.840	0.80 (0.50 - 1.28)	0.354	
VEGF 1451T/1612G/1725G	Without MetS	Others	1.13 (0.80 - 1.58)	0.495	1.45 (1.00 - 2.09)	0.047	
	With MetS	Others	0.90 (0.51 - 1.58)	0.705	1.36 (0.77 - 2.42)	0.292	
VEGF 1451C/1612A/1725G	Without MetS	Others	0.61 (0.38 - 0.97)	0.038	0.48 (0.26 - 0.86)	0.015	
	With MetS	Others	1.04 (0.48 - 2.25)	0.930	0.87 (0.37 - 2.08)	0.760	
VEGF 1451C/1612A/1725A	Without MetS	Others	1.09 (0.58 - 2.05)	0.795	1.75 (0.93 - 3.28)	0.081	
	With MetS	Others	1.13 (0.53 - 2.43)	0.745	1.08 (0.47 - 2.51)	0.854	

Adjusted odds ratio (AOR), Confidence interval (CI), Metabolic syndrome (MetS), Colon cancer (CC), Rectal cancer (RC), Vascular endothelial growth factor (VEGF). AORs and p values were adjusted by age and gender.

Table 5 Combined effects of metabolic syndrome and VEGF 3'-UTR variants on colon cancer and rectal cancer risks

Genotype	CC risk				RC risk			
	Without MetS		With MetS		Without MetS		With MetS	
	AOR (95% CI)	p	AOR (95% CI)	p	AOR (95% CI)	p	AOR (95% CI)	p
VEGF 1451CC	1.00 (reference)		2.17 (1.43 - 3.28)	<.001	1.00 (reference)		2.22 (1.38 - 3.57)	0.001
VEGF 1451CT+TT	1.18 (0.79 - 1.77)	0.416	1.76 (0.98 - 3.16)	0.060	1.67 (1.07 - 2.61)	0.023	3.15 (1.74 - 5.70)	<.001
VEGF 1612GG	1.00 (reference)		1.68 (1.12 - 2.53)	0.013	1.00 (reference)		1.94 (1.24 - 3.01)	0.003
VEGF 1612GA+AA	0.71 (0.46 - 1.09)	0.117	1.81 (1.03 - 3.19)	0.040	0.69 (0.42 - 1.14)	0.148	1.66 (0.88 - 3.15)	0.117
VEGF 1725GG	1.00 (reference)		1.88 (1.29 - 2.73)	0.001	1.00 (reference)		2.17 (1.44 - 3.27)	<.001
VEGF 1725GA+AA	1.34 (0.72 - 2.49)	0.360	2.68 (1.30 - 5.55)	0.008	1.82 (0.95 - 3.48)	0.072	2.49 (1.11 - 5.57)	0.027

Adjusted odds ratio (AOR), Confidence interval (CI), Metabolic syndrome (MetS), Colon cancer (CC), Rectal cancer (RC), Vascular endothelial growth factor (VEGF). AORs and p values were adjusted by age and gender.

Table 6 VEGF mRNA expression levels (mean ± SE) according to VEGF 3'-UTR genotypes and haplotypes

	Tumor-adjacent (n = 47)	p	Tumor (n = 47)	p
VEGF 1451CC (n = 32)	1.00 ± 0.45	0.445	1.00 ± 0.47	0.158
VEGF 1451CT+TT (n = 15)	2.54 ± 2.13		0.02 ± 0.01	
VEGF 1612GG (n = 33)	1.00 ± 0.54	0.008	1.00 ± 0.67	0.156
VEGF 1612GA+AA (n = 14)	6.76 ± 4.24		3.67 ± 2.38	
VEGF 1725GG (n = 41)	1.00 ± 0.40	0.038	1.00 ± 0.58	0.011
VEGF 1725GA+AA (n = 6)	5.98 ± 4.30		7.61 ± 5.43	
VEGF 1451C/1612G/1725G (n = 61)	1.00 ± 0.35	0.064	1.00 ± 0.39	0.027
VEGF 1451T/1612G/1725G (n = 18)	2.61 ± 2.19		0.02 ± 0.01	
VEGF 1451C/1612A/1725G (n = 9)	2.72 ± 1.52		0.44 ± 0.42	
VEGF 1451C/1612A/1725A (n = 5)	7.92 ± 6.08		4.59 ± 3.28	

Standard error (SE), Vascular endothelial growth factor (VEGF). p values were calculated by Mann-Whitney and Kruskal-Wallis tests.

VEGF -2578C > A, -1498T > C (-460T > C), -1154G > A, -634G > C (405G > C), and 936C > T correlate with CRC risk [6], and the following alleles: VEGF -2578A, -1498T, -1154A, -634C, and 936T: are associated with reduced VEGF expression [25-27]. Increased CRC incidence seems to occur in genotypes that cause both low (VEGF -2578A and 936T) and high (VEGF -1498C and -634G) VEGF expression [24,28,29].

Angiogenesis under physiological conditions is a strictly regulated process on many levels, including spatial and temporal expression of genes, as well as intensity of the cellular response. Indeed, in the adult body, angiogenesis is constantly suppressed; the levels of anti-angiogenic molecules predominate in every tissue. However, failure of the regulatory processes that inhibit angiogenesis leads to the excessive generation of blood vessels that participate in cancer progression [30]. Higher VEGF expression can increase tumor-related angiogenesis and metastasis [31]. The role of angiogenesis as a prognostic factor of carcinogenesis and cancer progression, however, is still controversial [32,33]. Weidner et al. [3] first reported a direct

correlation between the incidence of metastasis and the number and density of blood vessels in invasive breast carcinomas. Similar studies have made this association in gastrointestinal [34] and colorectal cancers [32,35-39]. An association between elevated angiogenesis and both a high prevalence of metastases and a subsequent decrease in survival has been reported for a vast majority of solid tumors [32,35-39]. Several studies have revealed high angiogenic activity in CRC, which was more likely correlated with aggressive histological and pathological characteristics including parietal invasion, tumor stage, grade of tumor differentiation, metastatic rates, and poor survival rates [32,40,41]. Also, Gurzu et al. [32] reported that augmented levels angiogenesis in CRC were higher during early stages of tumor proliferation, but did not progressively increase as the tumors advanced. For these reasons, anti-angiogenesis is one possible target for cancer prevention and therapy.

However, anti-angiogenesis can induce the metastatic potential of cancer. Inhibition of VEGF/VEGF receptor (VEGFR) signaling causes a decrease in nutrient and

Table 7 VEGF mRNA expression (mean ± SE) between tumor and tumor-adjacent tissues according to VEGF 3'-UTR genotypes and haplotypes

	Tumor-adjacent (n = 47)	Tumor (n = 47)	p
VEGF 1451CC (n = 32)	1.00 ± 0.45	75.37 ± 35.42	0.029
VEGF 1451CT+TT (n = 15)	1.00 ± 0.84	0.59 ± 0.30	0.125
VEGF 1612GG (n = 33)	1.00 ± 0.54	52.24 ± 35.00	0.001
VEGF 1612GA+AA (n = 14)	1.00 ± 0.63	28.36 ± 18.39	0.143
VEGF 1725GG (n = 41)	1.00 ± 0.40	30.65 ± 17.78	0.011
VEGF 1725GA+AA (n = 6)	1.00 ± 0.72	39.00 ± 27.83	0.203
VEGF 1451C/1612G/1725G (n = 61)	1.00 ± 0.35	67.56 ± 26.35	<.001
VEGF 1451T/1612G/1725G (n = 18)	1.00 ± 0.81	0.50 ± 0.25	0.133
VEGF 1451C/1612A/1725G (n = 9)	1.00 ± 0.56	10.93 ± 10.43	0.481
VEGF 1451C/1612A/1725A (n = 5)	1.00 ± 0.77	39.15 ± 27.98	0.210

Standard error (SE), Vascular endothelial growth factor (VEGF). p values were calculated by Wilcoxon signed rank test.

oxygen levels, inducing hypoxia [42]. One of the crucial steps in the cellular response to hypoxia is the stabilization of hypoxia-inducible factor (HIF)-1 in low-oxygen conditions. As a consequence, genes directly regulated by this transcription factor are activated. Because HIF-1 modulates the transcription of genes involved in glycolytic metabolism, oxygen consumption, survival, angiogenesis, migration, and invasion, its stabilization has a dramatic impact on the gene expression profile and ultimately on the behavior of the cells [43]. For example, cancer cells react to the hypoxia caused by anti-angiogenic signals by reprogramming their metabolism, thus increasing the uptake of glucose to sustain energy production through glycolysis. This phenomena is referred to as the Warburg effect [43]. Also, the epithelial-mesenchymal transition when stabilized HIF-1 transactivates the *Met* proto-oncogene, the receptor for hepatocyte growth factor [44]. In addition, recent data suggest that VEGF supplementation may inhibit invasion and epithelial-mesenchymal transition of cancer cells [45]. Therefore, for cancer prevention and therapy, it may be important to keep VEGF expression levels within a normal range. Previous reports showed that polymorphic events affecting the mRNA expression of *VEGF* and *VEGFR* genes could contribute to the survival duration of cancer patients receiving anti-angiogenic treatment [46-49]. The *VEGF* -2578A/-1498C/-634G haplotype showed a shorter survival time in multiple myeloma patients treated with thalidomide [46]. The progression-free survival duration of metastatic renal cell carcinoma patients who received bevacizumab treatment varied between *VEGFR1* rs7993418 alleles [47]. *VEGF* -2578CC, -1498TT, and -634CC were associated with a poorer prognosis and progression-free survival duration in advanced renal cell carcinoma patients receiving first-line sunitinib [48]. *VEGF* -634CC displayed relatively shorter progression free survival duration in advanced castration-resistant prostate cancer patients treated with metronomic cyclophosphamide [49].

We identified an association between lower mRNA expression and RC risk in the *VEGF* 1451T carrier. Moreover, *VEGF* 1451T carrier combined with MetS was linked to increased RC risk, whereas the combination of higher mRNA expression in the *VEGF* 1725A carrier and MetS was linked to elevated CC risk. *VEGF* 1451T carrier could directly contribute to RC risk, but the *VEGF* 1725A allele could not have influence on CC risk alone. In other studies, weakened VEGF/VEGFR signaling was shown to cause a decrease in oxygen levels, and the HIF-1 transcription factor is stabilized in insulin resistance conditions [42,50]. We hypothesize that low oxygen conditions in *VEGF* 1451T carrier and HIF-1 activation in MetS patients may lead to early cancer development. Activated HIF-1 drives the transcription of over

60 genes that are involved in cancer biology, including angiogenesis, cell survival, and glucose metabolism [51]. *VEGF* 1725A carrier may influence a pathological angiogenesis step after persistent HIF-1 activation.

Genetic variation in the 3'-UTR region could affect the stability and translation of the mRNA through altered miRNA binding affinity. Currently, there are no data to directly show altered miRNA binding activity depending on *VEGF* 3'-UTR polymorphisms. Further studies are needed to directly test for miRNA binding activity to *VEGF* 3'-UTR polymorphisms to determine the mechanism by which these polymorphisms may influence cellular proliferation and cancer progression. These studies may have great clinical impact for all diseases related to abnormal angiogenesis and hypoxic conditions.

There are several limitations in this study. First, the mechanism by which 1451C > T, 1612G > A, and 1725G > A polymorphisms in the *VEGF* gene affect development of CRC is still unclear. Further studies of whole *VEGF* sequence variants and their biological functions would uncover the role of these *VEGF* polymorphisms and haplotypes in the development and progression of CRC. Second, the present study lacked information regarding additional environmental risk factors (smoking, alcohol intake, caffeine intake, red meat intake, and multivitamin use) and clinical characteristics (survival time, relapse, death, chemotherapy, and radiotherapy) in the CRC patient cohort. These factors may contribute to overall CRC risk. Lastly, the population of this study was restricted to patients of Korean ethnicity. Because frequencies of genetic polymorphisms often vary between ethnic groups, more studies in diverse ethnic populations are warranted to clarify the association between *VEGF* 3'-UTR polymorphisms and CRC.

Conclusion

We investigated the involvement of *VEGF* polymorphisms 1451C > T, 1612G > A, and 1725G > A with CRC susceptibility in the present study. *VEGF* 1451C > T and 1725G > A could contribute to CRC susceptibility when combined with the presence of MetS. Moreover, VEGF mRNA expression varied in tumor tissues depending on the combination of 3'-UTR polymorphic alleles present. Although results from our study provide the first evidence for *VEGF* 1451C > T, 1612G > A, and 1725G > A as potential biomarkers for CRC prevention, a prospective study on a larger cohort of patients is warranted to validate these findings.

Additional file

Additional file 1: Table S1. The frequencies of MetS and *VEGF* 3'-UTR genotypes according to clinicopathological features of CRC.

Abbreviations

(AJCC): American Joint Committee on Cancer; (AOR): Adjusted odds ratios; (BMI): Body mass index; (BP): Blood pressure; (CC): Colon cancer; (CI): Confidence interval; (CRC): Colorectal cancer; (DM): Diabetes mellitus; (TG): Triglycerides; (FBS): Fasting blood sugar; (HDL-C): High density lipoprotein-cholesterol; (HIF): Hypoxia-inducible factor; (HTN): Hypertension; (HWE): Hardy-Weinberg equilibrium; (MDR): Multifactor dimensionality reduction; (MetS): Metabolic syndrome; (OR): Odds ratio; (PCR): Polymerase chain reaction; (qRT-PCR): Quantitative real-time PCR; (RC): Rectal cancer; (RR): Relative risk; (SD): Standard deviation; (SE): Standard error; (TNM): Tumor node metastasis; (VEGF): Vascular endothelial growth factor; (VEGFR): VEGF receptor.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YJJ conceptualized the study design, analyzed the data, and wrote manuscript. JWK conceptualized the study design, recruited participants, and wrote manuscript. HMP conceptualized the research study and analyzed the data. HGJ conceptualized the research study and analyzed the data. JOK conceptualized the research study and analyzed the data. JO recruited participants and collected data. SYC recruited participants and collected data. EJK conceptualized the research study and analyzed the data. SWK recruited participants and collected data. DO recruited participants, conceptualized the study design, analyzed the data, and wrote manuscript. NKK obtained funding for the project, conceptualized the study design, analyzed the data, and wrote manuscript. All authors read and approved the final manuscript.

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Author details

¹Institute for Clinical Research, School of Medicine, CHA University, 351, Yatap-dong, Bundang-gu, Seongnam 463-712, South Korea. ²Department of Biomedical Science, College of Life Science, CHA University, Seongnam 463-712, South Korea. ³Department of Surgery, School of Medicine, CHA University, Seongnam 463-712, South Korea. ⁴Department of Internal Medicine, School of Medicine, CHA University, 351, Yatap-dong Bundang-gu, Seongnam 463-712, South Korea. ⁵Department of Medicine, College of Medicine, Chung-Ang University, Seoul 456-756, South Korea.

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