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ORIGINAL ARTICLE

Variation in Turkish Patients with Colorectal Cancer

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Abstract

Introduction: Leukocyte function-associated antigen 1 (LFA-1) is expressed on leukocyte surfaces and interacts with the intercellular adhesion molecules. Since LFA-1 may have roles in tumor survival and growth, so we aimed to investigate the effects of rs2230433 variation on.

Methods: Sixty-seven healthy subjects without any cancer history as control group and 100 subjects diagnosed with colorectal cancer as patient group were included in our study. DNA isolated from the blood samples and polymerase chain reaction-restriction fragment length polymorphism methods were performed. Statistical analysis was performed using SPSS software for Windows, version 22.0 and $p<0.05$ was considered as statistically significant.

Results: LFA-1 rs2230433 genotypes and alleles were found similar between patient and control groups. No significant difference was observed between genotype and allele comparisons for disease risk assessment ($p>0.05$).

Discussion and Conclusion: Our results showed that LFA-1 rs2230433 is not associated with colorectal cancer.

Keywords: Colorectal cancer; leukocyte function-associated antigen; rs2230433.

Integrins are membrane cell adhesion molecules consisting of two subunits (α/β) linked together by non-covalent interactions[1]. β2 integrins, a member of the integrin family, involve in the migration of leukocytes to the inflammation site and killing target cells by the NK (natural killer) and cytotoxic T-lymphocytes[2,3]. Among all β2 integrins (CD11/CD18)[4] expressed on all leukocyte surfaces, CD11a/CD18 (the other name: Leukocyte function-associated antigen 1, LFA-1) is the most commonly expressed. LFA-1 interacts with the intercellular adhesion molecules (ICAMs) 1, 2, and 3 taking part in the intercellular adhesion of leukocytes[5]. Myeloid cells may contribute to tumor development or limit tumor growth depending on tumor content. In particular, hematopoietic tumors express LFA-1 and may be targeted by antibodies[6]. In addition, the presence of Treg cells in the microenvironment of solid tumors and the necessity of LFA-1 expression for the function of these cells indicate that LFA-1 may be effective in tumor survival and growth[6]. It was reported that LFA-1 expression in the breast cancer cell line causes transendothelial migration of breast cancer cells[7] and may pose a risk for cancer because of the association with adhesion molecules such as ICAM[8,9]. Arginine-threonine (Arg766Thr, rs2230433, G2372C) substitution in the αL-subunit (CD11a) of the αLβ2integrin (LFA-1) which is encoded by the integrin αL gene (ITGAL)[10]. The rs2230433 G allele frequency was found low in breast cancer pa-

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tients (p<0.05). Fu et al.[11] reported that the variation of rs2230433 may affect the function and expression of LFA-1 in the development of sporadic infiltrative breast cancer. However, none of the studies of rs2230433 variation in the literature were conducted with colorectal cancer cases, so we aimed to investigate the effects of rs2230433 variation on colorectal cancer.

Materials and Methods

Study Population

Two sample groups were used in the study. The control group consisted of DNA samples obtained from blood samples of 67 healthy individuals without any cancer history. The patient group consisted of DNA samples isolated from blood samples taken from 100 patients diagnosed with colorectal cancer. The study protocol was approved by the Ethical Committee of the Kartal Kosuyolu Yüksek İhtisas Training and Research Hospital (No: 2018/6/59).

Genotyping

DNA was extracted with QIAzol Lysis Reagent (QIAGEN) from peripheral blood samples. Detecting of rs2230433 (in exon 21) was performed with polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) methods. Forward 5′-GATATTCCCCACCCTGATCC-3′ and reverse 5′-CACCTTCAGCATCTCCACCT-3′ primer sequences were used. PCR amplifications were performed in a thermal cycler (T100™, BioRad) with a total volume of 25 µl containing 1 µl of genomic DNA (100 ng), 1.5 µl of 10×Taq buffer (Fermentas), 1.5 µl of 25 mM MgCl2 (Fermentas), 5 µl of 1 mM dNTPs (Fermentas), 1 µl of 50 pmol/µl of each primer, 0.3 µl of 5U Taq DNA polymerase (Fermentas), and 14.7 µl of distilled water. Thermal conditions for amplification consisting of 5 min at 94°C, followed by 30 cycles of 94°C for 45 s, 59°C for 45 s, and 72°C for 45 s with a final extension step for 5 min at 72°C. 5.2 µl reaction mixture (0.25 µl BanII enzyme [10 u/µl], 0.5 µl ×10 Tango buffer, and 4.5 µl distilled water) and 5 µl PCR product were incubated at 37°C for 16 h. Agarose gel electrophoresis containing ethidium bromide was used to separate restriction fragments and visualized under ultraviolet light. Using BanII restriction enzyme, 135 and 65 bp for normal G allele (Arg707) and 200 bp for mutant C allele (Thr707) were obtained and genotyping was performed accordingly.

Statistical Analysis

Statistical analysis was performed using SPSS software for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). The rs2230433 variation genotypes in the patient and control groups were evaluated with Chi-square test. Spearman's rho test was used for correlation. P<0.05 was considered as statistically significant.

Results

Sixty-one were male and 39 were female of the patient group, while control group consisted of 31 males and 36 females. Sixty-two tumors were localized in the colon, 34 in the rectum, and four in the cecum. About 41% of the patients were smoking and 20% were drinking alcohol. Two patients had type 1 diabetes mellitus, 14 had type 2 diabetes mellitus, and 14 had inflammatory bowel disease. About 15% of the patients had polyps and 31% had family history in the first or second degree relatives, especially colon, leukemia, and breast cancers. The mean age of the patient group was 61.6±11.4 years, while the mean age of the control group was 39.2±9.7 years (p<0.01) (data not shown).

LFA-1 rs2230433 variation was found in Hardy–Weinberg equilibrium (p>0.05) (Table 1). LFA-1 rs2230433 genotypes and alleles were found similar between patient and control groups (Table 2). No significant difference was observed between genotype and allele comparisons for disease risk assessment (p>0.05) (Table 3). There was a correlation between polyp history and CC and CG genotypes (p=0.05) (data not shown).

Discussion

LFA-1 rs2230433 GG genotype was found 5% in patient group, while 3% in control group. The ratio of mutant CC genotype was 18% in patient and 20.9% in control groups. However, the ratio of GC genotype was also similar in pa-

Table 1. Hardy-Weinberg equilibrium for rs2230433

Groups	Genotypes	Observed	Expected	Chi-square	p
Patient	GG	5	4.2	0.406	0.524
	GC+CC	95	95.8		
Control	GG	2	2.8		
	GC+CC	65	64.2		

Table 2. Distribution relationship between patient and control groups

LFA-1 rs2230433	Groups (%)		Total (%)	p
	Patient	Control		
GG	5 (5)	2 (3)	7 (4.2)	0.75
CC	18 (18)	14 (20.9)	32 (19.2)	
GC	77 (77)	51 (76.1)	128 (76.6)	
G	87 (43.5)	55 (41)	142 (42.5)	0.65
C	113 (56.5)	79 (59)	192 (57.5)	

LFA-1: Leukocyte function-associated antigen 1.

Table 3. Relative risk estimation for LFA-1 rs2230433

LFA-1	Patient	Control	OR (%95CI)	p
G	87	55	Reference	
C	113	79	1.12 (0.71-1.72)	0.66
GG	5	2	Reference	
CC	18	14	1.94 (0.33-11.56)	0.46
GC	77	51	1.66 (0.31-8.86)	0.55
GC+CC	95	65	1.71 (0.32-9.08)	0.52

LFA-1: Leukocyte function-associated antigen 1; CI: Confidence interval.

tients (77%) and controls (76.1%). No significant difference was observed between genotype and allele comparisons for risk assessment of colorectal cancer (p>0.05).

The combination of serum carcinoembryonic antigen/soluble ICAM-1 concentrations is identified as a novel risk score for poor survival in colorectal cancer[12]. ICAM-1 is a ligand of LFA-1 which is expressed in natural killer (NK) cells[13]. Barber et al.[14] showed that NK cells receive early activation signals directly through LFA-1. Thus, leukocytes can bind to metastatic cancer cells through LFA-1/ICAM-1 interaction[15]. This function makes LFA-1 important for cancer therapies and a variation in LFA-1 gene can affect LFA-1/ICAM-1 interaction.

LFA-1 rs2230433 variation in the exon 21 causes the amino acid substitution (arginine-threonine)[11]. LFA-1 rs2230433 variation was found associated with high anti-surface antigen of hepatitis B virus antibody serum levels[16]. Furthermore, rs2230433 was also found correlated with allergic disease in mice[17]. However, the genotypes and allele frequencies of rs2230433 were not found significantly different between Behçet's disease and control groups[18]. Yang et al.[19] found that the mutant allele of rs2230433 tended to protect Graves' disease from evolving into Graves' ophthalmopathy. Using Cox proportional hazards regression analysis, Shang et al.[20] found that rs2230433 was not associated with hepatitis B virus-related hepatocellular carcinoma. Lenci et al.[21] found that the frequencies of the genotypes of rs2230433 were similar in malignant melanoma patients and controls in the German population (p>0.05). The GG genotype and G allele were found significantly lower in sporadic infiltrative duct breast carcinoma (SIDBC) (p<0.05), while GC genotype was higher in Chinese patients with SIDBC. However, GG genotype and G allele were found higher in estrogen receptor positive cases (p<0.05)[11]. On the other hand, GG genotype frequency was found higher in the Turkish patient with breast cancer. The frequency of the LFA-1 rs2230433 G allele was found 43.52% in breast cancer patients and 37.3% in controls[22]. We found that GG genotype was higher in colorectal cancer, but that was not statistically significant. The frequency of G allele was also similar in the groups (patients: 43.5%, control: 41%, p>0.05). However, the frequency of G allele in colorectal cancer was compatible in breast cancer which study was conducted by Tokat et al.[22].

Conclusion

Our results showed that LFA-1 rs2230433 is not associated with colorectal cancer. The limitations of our study were the low number of samples and the fact that we did not show our findings as gene and protein expression. In addition, we evaluated our study as colorectal cancer, but we could analyze our findings as colon or rectal cancer compared to tumor location if the number of samples was high.

Ethics Committee Approval: Study was approved by the Kartal Kosuyolu Yüksek İhtisas Training and Research Hospital Clinical Research Ethics Committee (date: 15/11/2018, number: 2018/6/59).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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