Investigation of JAM-A (rs790056) and LFA-1 (rs8058823) gene variants in Turkish colorectal cancer patients

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ABSTRACT

Background/Aims: *Lymphocyte function-associated antigen 1 (LFA-1) is a transmembrane glycoprotein expressed on the surface of leukocytes and containing the binding domain for junctional adhesion molecule-A (JAM-A). The aim of the present study was to evaluate the effects of JAM-A and LFA-1 variants on the formation of colorectal cancer and metastasis.*

Materials and Methods: *A total of 82 subjects with colorectal cancer and 67 healthy subjects were studied. DNA was isolated from blood samples, and variations were determined using the polymerase chain reaction and restriction fragment length polymorphism method.*

Results: *JAM-A rs790056 CC genotype and C allele were found to be higher in the colorectal cancer group ($p<0.05$), and approximately 3-fold increased colorectal cancer risk with CC genotype was determined ($p=0.029$). Haplotype analysis showed that GC haplotype (LFA-1 rs8058823G and JAM-A rs790056C) frequency was significantly higher in the patient group ($p=0.041$) than in controls.*

Conclusion: *JAM-A rs790056 variation may be effective in the development of colorectal cancer.*

Keywords: *Colorectal cancer, rs8058823, rs790056, variation*

INTRODUCTION

Colorectal cancer is the one of the most lethal cancer types despite advances in diagnosis, treatment methods, and early screening (1). It can occur as inherited or sporadic. Hereditary cancer is characterized by the presence of specific tumors and defects with family history and young age (2). Sporadic colorectal cancers usually affect the elderly population and emerge without family history (2,3).

Cancer cells pass through multiple-step adhesive reactions before they settle in a distant organ. There are important similarities between the dissemination of tumor cells and the migration and localization of leukocytes into the inflammatory zone (4). Thus, changes in integrin receptors may lead to tumor cell invasion and metastasis (5). Integrins provide a strong adhesion in docking, event of tumor cell adhesion to the endothelial cell, after selectins (6). Lymphocyte function-associated antigen 1 (LFA-1), an integrin from the β2 subgroup, is a transmembrane glycoprotein expressed on the surface of leukocytes (7). It showed the I-domain of LFA-1 containing the binding domain for junctional adhesion molecule-A (JAM-A) (8). During leukocyte migration, these receptors allow leukocytes to pass through the connection via homophilic transendothelial interactions (9). In vivo studies showed that when JAM-A is blocked, inflammation (10) and diapedesis (11) decrease.

Studies on LFA-1 and JAM-A variations are limited in the literature. The effects of LFA-1 and JAM-A variations on breast cancer (12,13), central obesity (14), and Behçet's disease (15) were found in the literature, whereas there was no study investigating these gene variations in patients with colorectal cancer. Since both JAM-A and LFA-1 have potential effects on migration, they might be involved in the development and metastasis of colorectal cancer. Therefore, the aim of the present study was to evaluate the JAM-A and LFA-1 gene variants on the metastasis and development risk of colorectal cancer.

MATERIALS AND METHODS

A total of 82 patients diagnosed with colorectal cancer as the patient group and 67 healthy subjects as the control

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group were studied. Patients with colorectal cancer were selected from patients who were first diagnosed and never used therapeutics. The control group was selected from individuals who did not have colorectal cancer and with no family history of colorectal cancer. This was a prospective case-control study in a single center. The study protocol was approved by the ethical committee of the İstanbul School of Medicine (no. 2016/675). Genomic DNA samples were extracted using QIAzol Lysis Reagent (Qiagen, Crawley, UK) from peripheral blood samples.

Genotyping

Detecting variations of JAM-A gene rs790056 (intron 6) and LFA-1 gene rs8058823 (3′-untranslated region) were performed using the polymerase chain reaction (PCR) and restriction fragment length polymorphism methods. Forward 5′-GCAGTACAAAGGAGAGCCTCT-3′ and reverse 5′-TGGGACACCCATGACTTCA-3′ primer sequences were used for rs790056 variation of JAM-A gene. LFA-1 gene rs8058823 variation primer sequences were forward 5′-CAGCTCACGTCACACTTGGT-3′ and reverse 5′-CCACGCCTGGATTTTGTATT-3′. Thermal cycler (T100™; BioRad, CA, USA) was used for PCR amplifications with a total volume of 25 µl (16). KpnI (Fermentas) and BspHI (Fermentas) enzymes were applied for digesting 314 bp (rs790056) and 437 bp (rs8058823) PCR products, respectively. Agarose gel electrophoresis containing ethidium bromide was used to separate restriction fragments, and after electrophoresis, the gel was visualized under ultraviolet light. The 195 and 119 bp restriction fragments of rs790056 in JAM-A gene were obtained for T allele. The 232 and 205 bp restriction fragments of rs8058823 variation were obtained for A allele.

Statistical analysis

Statistical Package for Social Sciences software for Windows, version 22.0 (IBM Corp.; Armonk, NY, USA) were used for statistical analysis. Chi-square test was applied to evaluate genotypes and haplotypes in the groups. A p value <0.05 was considered as statistically significant.

RESULTS

A total of 82 subjects diagnosed with colorectal cancer were studied as the patient group. The study group consisted of 22 female and 60 male patients. Twenty-two patients were smokers, and 17 patients consumed alcohol. Fourteen patients were diagnosed with type 2 diabetes mellitus, and only one patient was obese. A total of 67 healthy subjects were studied as the control group. The control group consisted of 27 female and 40 male patients. The mean ages of the patient and control groups were 39.2±9.74 years and 62.25±10.69 years, respectively (p<0.001). JAM-A rs790056 was not in the Hardy-Weinberg equilibrium (HWE) (p=0.025), whereas LFA-1 rs8058823 was in the HWE (p>0.05) (Table 1).

JAM-A rs790056 T alleles were 50% and 62.68% in the patient and control groups, respectively. C allele was found in 50% of the patients and 37.31% of the controls. JAM-A rs790056 C allele was higher in the patient group (p=0.028). The mutant CC allele was also found to be higher in the patient group (20.7%, p=0.029). CC genotype carriers were found to have 3-fold increased risk of colorectal cancer (odds ratio (OR) 3.125, 95% confidence interval (CI) 1.102-8.86). LFA-1 rs8058823 A alleles were 59.09% and 64.93% in the patient and control groups, respectively. G allele was found in 40.91% of the patients and 35.07% of the controls (p>0.05). AA, AG, and GG

Table 1. Hardy-Weinberg equilibrium between the groups for rs790056 and rs8058823.

JAM-A rs790056					
Groups	Genotypes	Observed	Expected	Chi-square test	p
Patient	TT	17	23.1	5.008	0.025
	CC+CT	65	58.9		
Control	TT	25	18.9		
	CC+CT	42	48.1		
LFA-1 rs8058823					
Groups	Genotypes	Observed	Expected	Chi-square test	p
Patient	AA	32	35.2	1.149	0.284
	AG+GG	50	46.8		
Control	AA	32	28.8		
	AG+GG	35	38.2		

Table 2. The genotypic distributions of rs790056 and rs8058823.

JAM-A rs790056	Patient	Control	OR (95% CI)	p
T	82 (50%)	84 (62.68%)	Reference	
C	82 (50%)	50 (37.31%)	0.595 (0.374-0.95)	0.028
TT	17 (20.7%)	25 (37.3%)	Reference	
TC	48 (58.5%)	34 (50.7%)	2.08 (0.97-4.425)	0.057
CC	17 (20.7%)	8 (11.9%)	3.125 (1.102-8.86)	0.029
LFA-1 rs8058823	Patient	Control	OR (95% CI)	p
A	91 (59.09%)	87 (64.93%)	Reference	
G	63 (40.91%)	47 (35.07%)	0.78 (0.48-1.26)	0.309
AA	32 (39%)	32 (47.76%)	Reference	
AG	37 (45.1%)	23 (34.33%)	1.609 (0.787-3.288)	0.191
GG	13 (15.9)	12 (17.91%)	1.083 (0.430-2.732)	0.865

Table 3. The distributions of gene variations in the metastasis.

JAM-A rs790056	Metastatic (n=31)	Non-metastatic (n=51)	p
TT	6 (19.35%)	11 (21.57%)	0.725
TC	20 (64.52%)	28 (54.9%)	
CC	5 (16.13%)	12 (23.53%)	
LFA-1 rs8058823	Metastatic (n=31)	Non-metastatic (n=51)	p
AA	11 (35.48%)	21 (41.18%)	0.704
AG	15 (48.39%)	22 (43.14%)	
GG	5 (16.13%)	8 (15.69%)	

Table 4. The frequencies of two allele haplotype sets of JAM-A rs790056 and LFA-1 rs8058823.

Haplotypes	Overall	Patient	Control	Chi-square	p
AC	0.262	0.29	0.228	2.936	0.087
AT	0.369	0.326	0.422	5.767	0.016
GC	0.181	0.21	0.145	4.18	0.041
GT	0.188	0.174	0.205	0.024	0.877

genotypes were not statistically different between the groups (p>0.05) (Table 2). When these gene variations were analyzed in the metastasis, 31 of the 82 patients had metastasis, and no statistical difference was found (p>0.05) (Table 3).

The haplotypes of JAM-A and LFA-1 were also analyzed. The frequency of AT haplotype of LFA-1 rs8058823 A and JAM-A rs790056 T alleles was significantly higher in the control group (p=0.016), whereas the frequency of GC haplotype of LFA-1 rs8058823 G and JAM-A rs790056 C alleles was significantly higher in the patient group (p=0.041). These results are shown in Table 4.

DISCUSSION

The JAM-A rs790056 and LFA-1 rs8058823 genetic variants on the metastasis and development risk of colorectal cancer were studied. JAM-A rs790056 CC genotype carriers were found to have 3-fold increased risk of colorectal cancer (p=0.029), whereas no statistical relationship was found between LFA-1 rs8058823 and colorectal cancer (p>0.05). These two genetic variants were also investigated in the metastatic and non-metastatic colorectal cancer groups, and no statistical difference was found (p>0.05). The frequency of GC haplotype of LFA-1 rs8058823 G and JAM-A rs790056 C alleles was significantly higher in the patient group (p=0.041).

Cell adhesion molecules were studied as targeting agents for cancers and autoimmune diseases (17). JAMs, cell surface adhesion receptors, play a role in inflammation, angiogenesis, and epithelial barrier formation. JAMs interact with integrins, and their cis and trans interactions regulate different processes (18). Since JAMs contribute to cell migration, invasion, and adhesion, they can be essential for tumor development. It is thought that JAM-A contributes to carcinogenesis via different signal pathways and can be evaluated as a prognostic indicator (19).

JAM-A was found to be overexpressed in the kidney, lung, and breast tumor tissues. Furthermore, Goetsch et al. (20) reported that injections of anti-JAM-A antibody inhibit tumor growth of xenograft human tumors in vivo. Some studies found a correlation between the upregula-

tion of JAM-A protein and poor prognosis in breast cancer (21,22). High levels of JAM-A in breast cancer were also found to be associated with developing recurrences within 5 years (21).

The JAM-A gene variation is located at chromosome 1q21.1-21.3, and rs790056 minor C allele in intron 6 was found to be associated with lower systolic blood pressure by Ong et al. (14) they suggested that JAM-A plays a role in blood pressure regulation in both rats and humans. This variation was also found to be associated with significantly increased risks for venous thromboembolism (23). Tokat et al. (16) identified the TT genotype and T allele of JAM-A rs790056 as risk factors for coronary heart disease. JAM-A rs790056T was found to be higher in the breast cancer group than in the control group (13). On the other hand, T allele was found to be higher in the control group than in the patient group (50% and 62.68%, respectively). In our study, C allele was higher in patients with colorectal cancer, and CC genotype was associated with 3-fold increased risk of colorectal cancer (OR 3.125, 95% CI 1.102-8.86). Gene variants and soluble form of JAM-A levels were examined in the study by Ong et al. (24), and when the rs790056 TT/TC+CC genotypes were compared with the levels of soluble JAM-A, no statistically significant difference was observed (p=0.53). In our study, the relationship between genotypes and JAM-A expression was not determined, since soluble JAM-A levels or mRNA and protein expression levels were not measured.

JAM-A is identified as a ligand for LFA-1 (αLβ2 integrin) (18). LFA-1 is not only an adhesion molecule, but it also modulates cell growth, differentiation, and survival. It recognizes a specific tumor antigen and allows cytolytic response to immunogenic tumors. It plays a role in eosinophils anti-tumoral effects on colon cancer (25). LFA-1 rs8058823 in 3'-UTR AA genotype and A allele was identified as a risk factor for coronary heart disease (16). Park et al. (15) found that the A allele of rs8058823 is significantly lower in patients with Behçet's disease and suggested that the major genotype of rs8058823 may play a role in decreasing the susceptibility of Behçet's disease. Fu et al. (12) found that the frequency of AA genotype and A allele of LFA-1 gene rs8058823 variation is lower in breast cancer, and that also AG genotype is significantly higher in cases. However, tumor size and lymph node metastasis were not found to be associated with LFA-1 gene polymorphisms. They suggested that the risk and prognosis of sporadic breast infiltrative duct carcinoma might be predicted with LFA-1 gene polymorphisms. Tokat et al. (13) found that LFA-1 rs8058823A is higher in the breast cancer group than in the control group. However, our results show that LFA-1 rs8058823 A allele was higher in the control group than in the patient group (64.93% and 59.09%, respectively), whereas G allele was found in 40.91% of the patients and 35.07% of the controls (p>0.05). AA, AG, and GG genotypes were not statistically different between the patient and control groups (p>0.05) (Table 2). Our results are compatible with the results by Fu et al. (12), whereas different from the study by Tokat et al. (13) in breast cancer.

Furthermore, the frequency of AT haplotype (LFA-1 rs8058823 A and JAM-A rs790056 T alleles) was found to be higher in the control group (p=0.016). Thus, AT haplotype appears to be reducing colorectal cancer risk. On the other hand, the frequency of GC haplotype was significantly higher in the patient group (p=0.041), and this haplotype was found to be effective to evaluate colorectal cancer risk.

Our study shows for the first time that JAM-A and LFA-1 variations on colorectal cancer and JAM-A rs790056 variation might contribute to colorectal cancer development due to increasing risk. We suggest that this variation can be evaluated as a potential biomarker to predict the risk of colorectal cancer if further analyses with higher number of sample collections conclude the same results.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the İstanbul University İstanbul School of Medicine (Decision Date: February 23, 2018; Decision Number: 2016/675).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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