ARAŞTIRMA MAKALESİ/ORIGINAL RESEARCH

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CD40 -1C>T Genetik Varyantı ve sCD40 Düzeylerinin Kolorektal Kanserde İncelenmesi

Investigation of CD40 -1C>T Genetic Variant and sCD40 Levels in Colorectal Cancer

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ABSTRACT

Introduction: Functional studies have shown that cancer cells inactivate CD40-CD40L costimulators, which contribute to immune escape by inhibiting CD40L expression. CD40 -1C>T (rs1883832) is a 5′ UTR variant and may cause changes in protein level or function in the posttranslational process. In this article, we aimed to determine the importance of CD40 -1C>T variant in terms of histopathological criteria and its effect on sCD40 levels in patients with CRC (colorectal cancer).
Methods: In peripheral blood samples of ninety-three CRC and one hundred sixty-three controls, sCD40 level was detected by ELISA, and CD40-1C>T variant was detected by PCR-RFLP.
Results: 1.48 times higher sCD40 level was found in CRC with CT compared to those with CC (p=0.007). The frequency of CT and TT genotypes was found to be higher in early tumor stage than in advanced tumor stage (p=0.041). It was observed that the frequency of CT genotype was higher in patients with distant organ metastases compared to those without. (p=0.02).
Dsicussion and Conclusion: We do not think that the CD40-1C>T variant is an important part of CRC initiation. On the other hand, our findings in favor of metastasis suggest that CD40 is a factor that may be effective in the progression stages.
Keywords: sCD40, CD40 -1C>T, colorectal cancer

ÖZ

Giriş ve Amaç: Fonksiyonel çalışmalar, kanser hücrelerinin CD40L ekspresyonunu inhibe ederek immün kaçışa katkıda bulunan CD40- CD40L kostimülatörlerini etkisiz hale getirdiğini göstermiştir. CD40 -1C>T (rs1883832) bir 5′ UTR varyantıdır ve translasyon sonrası süreçte protein düzeyinde veya işlevinde değişikliklere neden olabilir. Bu yazıda, KRK (kolorektal kanser) hastalarında CD40 -1C>T varyantının histopatolojik kriterler açısından önemini ve sCD40 düzeylerine etkisini belirlemeyi amaçladık.
Yöntem ve Gereçler: Doksan üç KRK ve Yüz altmış üç kontrole ait periferik kan örneklerinde ELISA ile sCD40 düzeyi ve PCR-RFLP ile CD40-1C>T varyantı saptandı.
Bulgular: KRK'de CT genotipi taşıyanlarda CC'ye göre 1.48 kat daha yüksek sCD40 seviyesi bulundu (p=0.007). CT ve TT genotiplerinin sıklığı, erken tümör evresinde, ileri evre tümör evresine göre daha yüksek olarak saptandı (p=0.041). CT genotipi sıklığı uzak organ metastazı taşıyanlarda taşımayanlara göre yüksek olduğu izlendi (p=0.02).
Tartışma ve Sonuç: CD40-1C>T varyantının CRC başlatmanın önemli bir parçası olduğunu düşünmüyoruz. Öte yandan metastaz lehine bulgularımız CD40'ın progresyon evrelerinde etkili olabilecek bir faktör olduğunu düşündürmektedir.
Anahtar Kelimeler: sCD40, CD40 -1C>T, kolorektal kanser

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INTRODUCTION

Generating a successful adaptive immune response is closely related to multiple molecular signaling processes. The first signaling is the binding of the antigen to an antigen receptor expressed by T and B lymphocytes. Secondary signals include the association of stimulatory molecules expressed by T and B lymphocytes with their ligands. One of thebest characterized of the co-stimulatory molecules is the receptor CD40. CD40 is a member of the TNF-receptor superfamily, such as CD30, OX40, Fas, and CD27. It is a type-1 integral membrane protein, containing 40 amino acids and rich in cysteine. The extracellular domain has two N-linked glycosylated groups. The cytoplasmic domains are small and variable in amino acid sequence, implying different signaling properties. CD40 with soluble forms has a glycoprotein structure of 45 to 50 kDa (1,2). The human CD40 gene is localized at 20q11.2-q13.2 (3). CD40 gene expression has been described in the literature in T-B cells, follicular dendritic cells, thymic epithelium, monocytes, endothelial cells, as well as in some carcinomas (1,4).

Evasion of cancer cells from immune control is a final and critical step in the immune regulation process in cancer biology. Many biochemical and molecular arrangements are required for themanagement of this process. It has been observedthat cancer cells can inhibit CD40L expression and inactivate CD40-CD40L costimulators that contribute to immune escape (5). Based on this mechanism, the antitumoral effects of CD40 induced by monoclonal antibody CP-870,893 and SGN- 40 have been supported by clinical studies (6,7). CD40 -1C>T (rs1883832) is a 5′ UTR variant, and it can cause level or different functional reflections, not sequence changes to the protein in transcription-post-translational processes. In the literature, there are limited studies in cancer for CD40 -1C>T. The frequency of T allele, CT and TT genotypes for CD40 -1C>T in Chinese lung cancer cases were found to be higher than the control group. At the same time, a high frequency of TT genotype was detected in squamous cell carcinomas, while the frequency of CT in young TT in the elderly was found to be higher (8). In a large multi-ethnic cohort, it has been reported that the TT genotype for rs18883832 is more common than the control group in patients with non-hodking lymphoma, and this genotype is associated with increased risk at different rates according to the histological type (9). In a study examining cervical pathologies, CC genotype was not detected in any of the groups with cervicitis, intraepithelial neoplasia and cervical carcinoma, while TT allele carriage was found to be associated with an increased risk in all groups, mostly cervical carcinoma (10).

The high T allele frequency and its relationship with lymph node metastasis have been shown in Chinese breast cancer cases (11). On the other hand, no significant difference was found between the control group in terms of CD40 -1C>T in Turkish breast cancer cases (12). It has been found thatT allele carriage is high in cases with non-small cell carcinoma and CT genotype is associated with poor5-year survival time (13). In acute lymphocytic and chronic lymphoblastic leukemias, tumor remission,reduction in lymph node metastasis and good chemotherapy responses were found in the results ofCD40L encoding gene therapies (14, 15). While CTand TT genotypes were found to be risk factors for the disease in cases with follicular lymphoma, lower sCD40 levels were detected in those with CC geno- type compared to other genotypes (CT/TT). In the control group of this study, CD40 expression was observed to be 2.7 times lower in the TT genotype than in the CC genotype (16).

Limited data were found in our literature review for the relationship between sCD40 levels in solid organ cancers. Although the sCD40 level was found to be higher in patients with pancreatic tumors compared to the control group, the sCD40 levels of the patients could not be associated with clinicopathological criteria (17). On the other hand, sCD40 in pleural effusions and serum of patients with lung cancer was found to be statistically significantly higher and associated with poor prognosis (18). Similarly, data have been obtained that the sCD40 level was found to be extremely high in women with ovarian cancer compared to the control group, and it may be a biomarker candidate (19). In

a group of samples including hematological malignancies, sCD40 level was found to have poor survival, a risk factor, and a stronger expression in youngcases than in old cases (20).

In the data obtained in the case/control and cell culture studies designed by Pang et al. for colorectal cancer, high CD40 level in tumor tissue and decreased proliferation of CD40L after CD40L recombinant plasmid transfection of cell lines were detected (21). It was determined that serum sCD40 levels were associated with liver metastasis in 84 patients with rectal tumors and its level was higher than in the control group (22). Considering these evidences, our study aimed to determine the importance of CD40 -1C>T variant in terms of histopathological criteria and its effect on sCD40 levels in patients with colorectal cancer.

MATERIALS AND METHODS

Patients

Ninety-three patients with colorectal cancer and one hundred sixty-three healthy controls were included in the study after adjusting for gender and agefor two groups in the sample. All patients were selected from the surgery clinic of Istanbul Training and Research Hospital. This study was approved by the Istanbul Training and Research Hospital Clinical Research Ethics Committee (No: 1015). Tumor samples resected from patients were stained with hematoxylin-eosin after pathological tissue processing and evaluation was made according to the tumor-node-metastasis (TNM) staging model AJCC 8th Edition. Patients whose pathological examination was completed were included in the study.

Genotyping

After performing total DNA extraction from peripheral blood samples in accordance with the QIAamp DNA Blood Mini Kit (ID: 51104, US) protocol, the extraction products were measured at 260 nm absorbance in a spectrophotometer. Genotyping for CD40 -1C>T (rs1883832) was performed using the PCR-RFLP method. The primer sequences were F: 5'-ACACAGCAAGATGCGTCCCTAAACT-3'; R: 5'-TCCTTCTCAT TCCCCACTCCCAACT-3'. PCR was carried out in a total volume of 50 µL containing: 200 ng genomic DNA, 25 µL Premix Taq (TaKaRa TaqTM Version 2.0 plus dye) (TaKaRa, Dalian, China), 1 µL of each primer (20 µM), ddH2O were added to bring samples to a final volume of 50 µL. In the thermal heat cycler, the first danuration step was 95˚C for 5 minutes, followed by 30 cycles of 94˚C 45 seconds, 55˚C 45 seconds and 72˚C 45 seconds, and finally 72˚C 5 minutes. The length of PCRproduct was 334 bp. PCR products were detected on a 2% agarose gel using horizontal electrophore- sis and a UV transmitter. For the determination of genotypes from PCR products, enzyme digestionwith Ncol (MBI Fermantas) was carried out at 37˚Cin 5 hours. PCR-RFLP products were evaluated as106 and 228 bp for CC genotype, 334, 106, 228 bp for CT genotype and 334 bp for TT genotype in UV Transmitter.

Elisa

It was centrifuged at 3000 rpm for 5 minutes to separate serum from periferic blood samples. Serum samples were frozen at -20˚C until the ELISA run. sCD40 levels were determined by following the protocol of the Sandwich ELISA kit (Platinum ELISA, Bender MedSystems GmbH, Vienna).

Statistics

Statistical analyzes were performed with Windows version 11.0 and SPSS version. The frequency differences of the CD40 gene polymorphism between the colorectal cancer patient group and the control group and the colorectal cancer subgroups were analyzed by the Chi-square Test. Differences between the serum sCD40 levels of the patient and control subjects were analyzed by the Mann-Whitney U test. For statistical significance, cases where p was less than 0.05 were considered significant.

RESULTS

The genotype and allele distributions in the patient and control groups are shown in Table-1. The frequency of CC genotype was 41.9% (n=39), CT genotype was 39.8% (n=37) and TT genotype was 18.3% (n=17) in the patient group. The frequency of CC genotype was 42.9% (n=70), CT genotype was 42.3% (n=69) and TT genotype was 14.7% (n=24) in the control group. There was no statis-

tically significant difference between the patient and control groups in terms of genotype and allele carriers (p>0.05). The sCD40 level in the patient group was 59.74 ±1.92 ng/L and the control group was 30.24 ±1.66 ng/L, and no statistically significant difference was found between them (p=0.443). The sCD40 levels in the sample according to the genotype carrier are shown in figure-1. The sCD40 level was 37.65±1.26 ng/L in the CCgenotype, 55.92±1.75 ng/L in the CT genotype, and 21.43±1.80 in the TT genotype. Approximately 1.48 times higher sCD40 level was found in patients with CT genotype compared to those with CC genotype (p=0.007).

Table 1: **Genotype and Allele Distribution in Patient and Control Groups**

Genotype	Patient Group	Control Group	p-value
CC	39(41.9%)	70(42.9%)	0.75
CT	37(39.8%)	69(42.3%)	
TT	17(18.3%)	24(14.7%)	
CC	39(41.9%)	70(42.9%)	0.88
T allel	54(58.1%)	93(57.1%)	
C allel	76(81.7%)	139(85.3%)	0.46
TT	17(18.3%)	24(14.7%)	
CC-TT	56(60.2%)	94(57.7%)	0.69
CT	37(39.8%)	69(42.3%)	

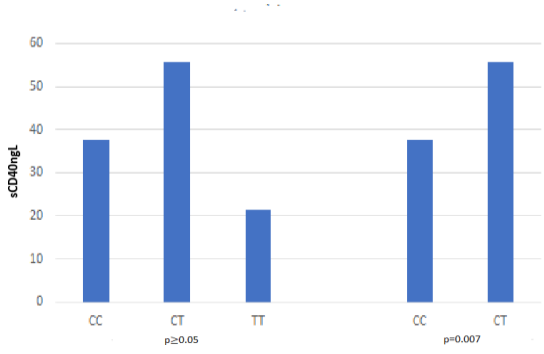


Figure1. Distribution of sCD40ngL according to genotype carriagein patients
Abbreviation: Distribution by three genotypes in the patient group is shown in a. The ones showing statistical significance in pairwise compa- rison combinations of genotypes are shown in b.

Genotype carriers according to histopathological parameters in codominant, dominant, recessive and overdominant models are shown in Table-2. It is observed that 16.1% (n=15) of the patients were in the early tumor stage (T1+T2) and 83.9% (n=78) of them were in the advanced tumor stage (T3+T4). While no lymph node metastasis was detected in 44% (n=41) of the cases (n0), lymph node metastasis was found in 56% (n=52) (n1+n2+n3). Distant organ metastasis (m1) was observed as 16.13% (n=15), while those without distant organ metastases were found to be 83.87% (n=83.87). In the dominant model, the frequencyof CT and TT genotypes (T allele) was found tobe higher in early tumor stage (T1+T2) than in advanced tumor stage (T3+T4) (p=0.041). In the overdominant model, the CT genotype carrier was found to be higher in patients with distant metastasis (m1) than in those without (m0) (p=0.02).

DISCUSSION

It shows that carriage of T allele for CD40 -1C>T variant is associated with the risk of disease in solid organ cancers. Especially in non-small cell lung cancer, the association of heterozygous CT genotype with patients with early onset and poorprognosis has been shown in different samples (8,13). In the axis of hematological cancers, the relationship of TT genotype with disease risk and its histopathological correlations show parallelism with the findings in solid organ cancers. The biggest proof that the similar data obtained do not differ according to gender is that the TT genotype in breast and cervical cancers is significantly higher in patient groups (10,11).

Table 2: Genotype Carriers according to Histopathological Parameters in Codominant, Dominant, Recessive and Overdominant Models

MODEL	CODOMINANT				DOMINANT			RECESSIVE			OVERDOMINANT		
	C/C	C/T	T/T	p	C/C	T Allele	p	C Allele	T/T	p	C/ C-T/T	C/T	p
T													
Early Tumor Stage (T1+T2)	3 (20%)	8 (53.3%)	4 (26.7%)	0.15	3 (20%)	12 (80%)	0.041	11 (73.3%)	4 (26.7%)	0.38	7 (46.7%)	8 (53.3%)	0.25
Advanced Tumor Stage (T3+T4)	36 (46.1%)	29 (37.2%)	13 (16.7%)		36 (46.1%)	42 (53.9%)		65 (83.3%)	13 (16.7%)		49 (62.8%)	29 (37.2%)	
N													
Absence of lymph metastasis (n0)	15 (36.6%)	17 (41.5%)	9 (21.9%)	0.58	15 (36.6%)	26 (63.4%)	0.35	32 (78%)	9 (21.9%)	0.42	24 (60%)	16 (40%)	0.97
Presence of lymph metastasis (n1+n2+n3)	24 (46.1%)	20 (38.5%)	8 (15.4%)		24 (46.1%)	28 (53.9%)		44 (84.6%)	8 (15.4%)		32 (60.4%)	21 (39.6%)	
M													
Absence of distant metastasis (M0)	36 (46.1%)	27 (34.6%)	15 (19.2%)	0.063	36 (46.1%)	42 (53.9%)	0.051	63 (80.8%)	15 (19.2%)	0.58	51 (65.4%)	27 (34.6%)	0.021
Presence of distant metastasis (M1)	3 (20%)	10 (66.7%)	2 (13.3%)		3 (20%)	12 (80%)		13 (86.7%)	2 (13.3%)		5 (33.3%)	10 (66.7%)	

On the other hand, this relationship could not be demonstrated in terms of CD40-1C>T in Turkish breast cancer patients. In immunohistochemical evaluation, CD40 level was found to be statistically high in in situ and ductal carcinomas, while the effect of TT genotype on CD40 expression could not be evaluated statistically due to the low frequency in patients (12). Due to this limitation factor, they could only observe a correlation between CD40 -1C>T and CD40 expression. Similarly, we did not find a significant difference between the patient and control groups in our study on Turkish colorectal cancer cases. These data show parallelism with the data of Dolen et al. On the other hand, we were able to show the relationship between CD40 -1C>T and CD40 in a larger sample of Dolen et al. According to our results, when CD40 -1C>T genotypes were examined in the whole sample, we found approximately 1.48 times higher sCD40 level in those with CT genotype compared to CC genotype. These shows us that CD40 -1C>T, whichis the 5'UTR variant, can change the sCD40 level. Although the 5'UTR region is not translated, possible alterations of this region are known to affect mRNA secondary structure and stability, the stability of translation initiation, or the binding of sequence-specific mRNA-binding proteins.

In terms of sCD40 level, the results in the literature generally show that it is higher in solid organ cancers than in the control group. In addition, it has been found to be associated with poor prognosis in non-small cell lung cancers (18). On the

axis of colorectal cancer, on the other hand, in the study of Meltzer et al., which is the only one in the literature, it was shown that high sCD40 level in patients with rectal cancer is associated with liver metastases. In the epidemiological axis, the data we have defined for the first time in the literature for the CD40-1C>T variant in colorectal cancer shows that the genotypes of this variant are not associated with the disease. T allele carriage, which is often shown to be risky in the literature, was found to be 58.1% in the patients and 57.1% in the control group in our study, but no significant difference was observed. In the sCD40/ CD40-1C>T axis, the relationship between Meltzer and Shuang's results in different samples and metastasis is in line with our results (11, 22).

CONCLUSION

In conclusion, we do not think that the CD40-1C>T variant is an important part of colorectal cancer initiation. On the other hand, our findings in favor of metastasis suggest that CD40 is a factor that may be effective in the progression stages of cancer. The fact that sCD40 level does not show a significant difference in colorectal cancer compared to the control group shows that it can-not be a good biomarker candidate in the colorectal cancer axis. On the other hand, the evidence that we have shown that sCD40 level is affected by the CD40 -1C>T variant can only be evaluated in terms of its pathophysiological importance after being supported by functional studies.

Ethics Committee Approval: İstanbul E.A.H.2017-1015

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