Abstract. Chemokines and their receptors play diverse roles in malignant tumor progression, particularly as key mediators of tumor stroma interactions. C-C motif chemokine ligand 2 (CCL2) also called monocyte chemoattractant protein-1 (MCP-1), belongs to the C-C motif chemokine sub-family and is currently believed to mediate its actions through one receptor, C-C motif chemokine receptor 2 (CCR2). CCL2 has been identified as a major chemokine inducing the recruitment of macrophages in human tumors, including those of the bladder, cervix, ovary, lung and breast. In this study of Turkish women, the association of CCL2 A2518G and CCR2 V64I polymorphisms with endometrial cancer was investigated using 50 endometrial cancer patients and 211 controls. In our study, individuals with CCL2 A2518G GG genotype showed a 6.7-fold increased risk for endometrial cancer (p<0.0001) and individuals with CCL2 A2518G A allele had a 7.14-fold lower risk of endometrium cancer (p<0.0001). Individuals carrying the CCR2 64I/64I genotype had a 4.13-fold increased risk for endometrial cancer (p<0.0001). We also found that individuals carrying the CCR2 wt allele had a 4.16-fold increased risk for endometrial cancer (p=0.005). We observed that the CCL2 G: CCR2 64I haplotype frequency was significantly higher in patients compared to controls (p=0.019). In conclusion, we state that there appears to be an association between polymorphism of CCL2 and its receptor CCR2 and endometrial cancer. To the best of our knowledge, this is the first study to show such an association.

Endometrial carcinoma is among the most frequently diagnosed gynecological malignancies in highly developed countries. Research has been conducted for 20 years to define the molecular pathology of this disease and much is already known, but adequate prognostic, diagnostic, and monitoring markers are still lacking (1).

Chemokines and their receptors play diverse roles in malignant tumor progression, particularly as key mediators of tumor-stroma interactions (2, 3). Chemokines are small (8-14 kDa), inducible proinflammatory cytokines which are implicated in many biological processes, such as stimulation of directed migration of leukocytes, embryogenesis, angiogenesis, maintenance of hematopoietic homeostasis, regulation of cell proliferation, tissue morphogenesis and angiogenesis, atherosclerosis, tumor growth and metastasis, and HIV-infection (3-6). Based on the position of the first two cysteines (C) that are adjacent to the amino terminus, they are classified into four conserved groups CXC, CC, C and CX3C, where X is any amino acid (2). Many tumor cell types can express chemokines and chemokine receptors (7, 8). Among more than 50 human chemokines, C-C motif chemokine ligand 2 (CCL2) is of particular importance. CCL2, also called monocyte chemoattractant protein-1 (MCP-1) is a member of the CC chemokine subfamily characterized by the absence of any amino acid between the conserved cysteines in the amino terminal end of the molecule. It is a potent chemoattractant for monocytes, memory T lymphocytes and natural killer cells (9). It is involved in a number of inflammatory conditions associated with monocyte recruitment, including delayed hypersensitivity reactions, bacterial infection, arthritis, and renal disease (9). The A2518G polymorphism in the regulatory region of the CCL2 gene influences CCL2 expression in response to inflammatory...
studies (10). CCL2 has been identified as a major chemokine inducing the recruitment of macrophages in human tumors, including those of the bladder, cervix, ovary, lung and breast (11-19). CCL2 is currently believed to mediate its actions through one receptor, the CC chemokine receptor 2 (CCR2).

CCR2 is a CC chemokine receptor with an affinity for CCL2, CCL7, CCL8 and CCL13 (4). CCR2 is mainly expressed by memory T lymphocytes, monocytes, dendritic cells, B-cells and basophils. The CCR264I mutation (G £ A substitution at position 190 in the CCR2 gene) results in a valine £ isoleucine substitution at position 64 in the CCR2 protein (20, 21).

In this study, we investigated the association between CCL2 A2518G and CCR2 V64I polymorphisms and endometrial cancer in a Turkish population.

Patients and Methods

Study groups. A total of 261 women admitted to the Gynecology Clinic of Istanbul University Istanbul Medical School, Department of Obstetrics and Gynecology, for gynecological evaluation within routine checkups or for abnormal uterine bleeding were included in our study. Endometrial biopsy was performed and on the basis of diagnosis and histological examination, women were divided into two groups: a control group (n=211) and an endometrial cancer group (n=50). The specimens were taken after obtaining informed consent and the study was conducted prospectively. Local Ethical Committee approval was obtained for the study. The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

Polymorphism analysis. Blood samples from all study participants were collected in EDTA-containing tubes. Genomic DNA was extracted from peripheral whole blood according to a salting-out technique (22). Genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism, the procedures of PCR-RFLP are given in Table I (23, 24).

Table I. PCR and RFLP procedures and expected products of CCL2 A2518G and CCR2 V64I genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers (forward and reverse)</th>
<th>PCR conditions</th>
<th>PCR product</th>
<th>Restriction enzyme</th>
<th>Restriction products</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCL2 A2518G</td>
<td>5'-TCT CTC ACG CCA GCA CTG ACC-3'</td>
<td>25 μl of PCR mixture: 1 Mm of each dNTP, 10 pmol/μl of each primer, 25 mM of MgCl₂, 1 U Taq polymerase</td>
<td>234 bp</td>
<td>PvuII</td>
<td>AA: 234 bp, AG: 234 bp, 159 bp, 75 bp, GG: 159 bp, 75 bp,</td>
</tr>
<tr>
<td></td>
<td>5'-GAG TGT TCA CAT AGG CTT CTA-3'</td>
<td>35 cycles: 95˚C 45 s, 52˚C 45 s, 72˚C 45 s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR2 V64I</td>
<td>5'-TTGTTTTGTGGCAACATGATGGG-3'</td>
<td>25 μl of PCR mixture: 1 Mm of each dNTP, 10 pmol/μl of each primer, 25 mM of MgCl₂, 1 U Taq polymerase</td>
<td>173 bp</td>
<td>BsaBI</td>
<td>wt/wt: 173 bp, wt/64I: 173 bp, 149 bp, 24 bp, 64I/64I: 149 bp, 24 bp</td>
</tr>
<tr>
<td></td>
<td>5'-CATGATCCAAAAAGGACCCTC-3'</td>
<td>33 cycles: 94˚C 30 s, 56˚C 30 s</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

Results

Characteristics of patients with endometrial cancer and healthy controls are shown in Table II. There was no statistically significant difference in mean age, menarche age and oral contraceptive use between cases and controls (p>0.05). However, as expected, more smokers were represented in the cases as compared with the controls (p=0.017) (Table II).

Table III shows the frequencies of CCL2 A2518G and CCR2 V64I genotypes and their respective alleles among cases and controls. There was a statistically significant difference between the control and patient groups for CCL2 A2518G (p=0.0019) and CCR2 V64I genotypes (p=0.005).
The CCL2 A2518G AA, GG and AG genotype frequencies for controls and cases were 58.8%, 2.4% and 38.9%, and 52%, 14% and 34%, and respectively. Individuals who had GG genotype showed a 6.7-fold increased risk for endometrial cancer (p<0.0001; χ²=12.46; OR=6.70; 95%CI=2.03-22.12).

The CCL2 2518G allele frequency in the endometrial cancer group was higher than the control group. However the difference was not statistically significant (p=0.38). The CCL2 2518A allele frequency was significantly lower in patients compared to controls (p<0.0001; χ²=12.46; OR:0.14; 95%CI=0.04-0.49). Individuals carrying the A allele had a 7.14-fold lower risk for endometrial cancer (p<0.0001; χ²=12.46; OR=0.14; 95%CI=0.04-0.49).

The CCR2 V64I 64I/64I genotype frequencies for controls and cases were statistically significantly different (p<0.0001; χ²=7.77; OR=4.13; 95%CI=1.42-12.00). Individuals with 64I/64I genotype had a 4.13-fold increased risk for endometrial cancer. CCR2 wt/64I genotype frequency was higher in the control group compared to the endometrial cancer group; however, the difference was not statistically significant (p=0.38). Although the CCR2 V64I wt/wt genotype frequency in the control group was higher than that in the patient group, the difference was not statistically significant (p=0.52).

The CCR2 V64I I allele frequency in the endometrial cancer group was higher than that in the control group. However, the difference was not statistically significant (p=0.52). CCR2 V64I wt allele frequency in the control group was statistically higher than the patient group (p=0.005; χ²=7.77; OR=0.24; 95%CI=0.08-0.70). Individuals carrying the CCR2 wt allele had a 4.16-fold lower risk for endometrial cancer (p=0.005; χ²=7.77; OR=0.24; 95%CI=0.08-0.70).
In addition to single nucleotide polymorphism (SNP) analyses, haplotypes were evaluated for association with endometrial cancer (Table IV). Haplotype analysis confirmed the association of CCL2/CCR2 gene variants with endometrial cancer and revealed that the frequencies of CCL2 A/CCR2 wt and CCL2 G/CCR2 64I haplotypes were significantly lower and higher, respectively, in patients as compared with controls.

Discussion

In this study, we investigated the association between CCL2 A2518G and CCR2 V64I polymorphisms and endometrial cancer in a Turkish women. To the best of our knowledge this is the first study to demonstrate an association between CCL2 A2518G and CCR2 V64I polymorphisms and the development of endometrial cancer.

CCL2 gene is located on chromosome 17 (26). The concentration gradient of CCL2 causes the movement of mononuclear cells, mainly monocytes/macrophages, to sites of inflammation (27, 28). The importance of CCL2 in cancer was manifested by its overexpression in different types of tumors such as glioma, ovarian, esophageal, lung, breast and prostate cancer (9, 29, 30). CCL2 was found to be expressed mainly by tumor cells, and in some tumors by surrounding stromal cells and tumor-infiltrating macrophages (31). A variety of cancer cells, including prostate, breast cancer, and myeloma cells, express CCL2 and its receptor CCR2 (29, 32-35). Prognostic analysis further revealed that high expression of CCL2 and increased serum levels were correlated with advanced tumor stage, lymph node metastasis and early relapse (19, 29, 36-39). Furthermore, CCL2 expression in these tumors was associated with increased angiogenesis, tumor cell proliferation, macrophage infiltration, and matrix metalloproteinase production (31). The A2518G SNP in the regulatory region of CCL2 in man causes increased promoter activity and is associated with elevated circulating levels of CCL2 (40). Homozygous GG individuals produce significantly higher levels of CCL2 than those with the AA genotype (41).

In our study, individuals with CCL2 A2518G GG genotype showed a 6.7-fold increased risk for endometrial cancer. This may be due to the increased promoter activity and elevated circulating levels of CCL2. On the other hand, individuals with the CCL2 A2518G A allele had a 7.14-fold lower risk for endometrial cancer and thus this allele would seem to have a protective effect against endometrial cancer development; this may be due to lower promoter activity and lower CCL2 levels.

CCL2 plays a role as a chemokine by binding to the chemokine C–C motif receptor 2 (CCR2) (41). The CCR2 gene is located in the chemokine receptor gene cluster region on chromosome 3 (3p21) (42). The mostly studied CCR2 SNP is that of 190 G/A located in exon 1. Its mutation leads to the substitution of valine by isoleucine (V64I) in the transmembrane region of the protein (43, 44). Data on the influence of this SNP on the expression of CCR2 are controversial. In the literature there is evidence both for CCR2 V64I function as well as lack of effect on CCR2 expression (45, 46). Nakayama et al. observed up-regulation of CCR2A 64I compared to CCR2A without substitution (45). Although CCR2A mostly localized in the cytoplasm and only a small proportion was observed on the cell surface, a chemotaxis assay showed that cells expressing CCR2A 64I migrated more efficiently than those expressing CCR2A 64V. By contrast, CCR2B expression was not affected by this mutation. Furthermore, pulse–chase experiments have revealed that higher expression of CCR2A 64I was due to increased stability of CCR2A 64I (45).

The CCR2 V64I polymorphism is associated with atherosclerosis, sarcoidosis, multiple sclerosis and HIV infection susceptibility (46-51). Current findings suggest that the CCR2 64I polymorphism might have a protective role against breast cancer development (52).
In this study, we found that individuals carrying CCR2 641/641 genotype had a 4.13-fold increased risk for endometrial cancer. We also found that individuals carrying the CCR2 V641 wt allele had a 4.16-fold lower risk for endometrial cancer. Hence the CCR2 V641 wt allele might have a protective role against endometrial cancer development.

We also analysed the haplotypes and found that CCL2 G/CCR2 641 haplotype is a risk factor for endometrial cancer while CCL2 A/CCR2 wt haplotype has a protective effect. The CCL2 polymorphism may increase transcription of CCL2. It can also act in association with the CCR2 polymorphism which increases the biological activity of the CCR2 receptor. Thus these polymorphisms can increase the biological activity of the CCL2/CCR2 system. Increased activity of this system, in turn, may increase the risk for developing endometrial cancer.

Although the present study has a novel finding, it has some limitations. The major limitation of our study is small sample size. Further larger size samples are required to confirm our results.

References


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248