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ORIGINAL PAPER

SDF-1 and CCR5 Genes Polymorphism in Patients with Head and Neck Cancer

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Abstract The frequency of SDF1–3′A and CCR5Δ32 in patients with head and neck cancer were determined in this study. The frequencies of alleles and genotypes of SDF-1 and CCR5 were assessed by PCR method in 156 patients with malignant head and neck cancer, 125 (80.1%) cases with squamous cell carcinoma and 31 (19.9%) cases with salivary gland tumors and compared with 262 age–sex matched healthy control individuals. SDF-1 genotypes in patients with SCC of head and neck, but not with salivary gland tumors, showed a statistically significant difference compared to the normal group ($P<0.005$ for SCC and $P=0.3$ for salivary gland tumors). There were no significant differences in the frequencies of SDF1–3′A allele, CCR5 genotypes and alleles between patients and controls. Based on the present study SDF1–3́A may be associated with the susceptibility of patients to SCC of head and neck cancer.

Keywords Head and neck cancer · SDF-1 · CCR5 · Polymorphism · Metastasis

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Introduction

Head and neck cancer is still a morbid and fatal disease and despite progress in molecular and immunological aspects, there is no distinct marker for early diagnosis, screening and progression of head and neck tumors. [1] However, leukocyte infiltration in tumor mediated by chemokine and chemokine receptors is regarded as one of the major factors in tumor invasion and progression. [2, 3] In this case, SDF-1, and its receptor, CXCR4 (known as CD184), [4] also RANTES and its receptor CCR5 have markedly been considered in this process. [5]

SDF-1 is a chemoattractant for resting T lymphocytes, dendritic cells (DCs), monocytes, and CD34 + progenitor cells. [6, 7] This chemokine demonstrated to have key roles in tumor growth, progression and metastasis of different types of tumors including osteosarcoma, [8] lung, prostate, brain and breast cancers. [4, 9]

In addition, it has been indicated that CXCR4 [10] and SDF-1 [11] are overexpressed in breast cancer cells compared to normal mammary epithelial cells. Moreover, upregulation of CXCR4 in breast cancer cells represents as essential mechanism of tumor growth and progression. [4] SDF-1 is constitutively expressed on common sites of metastatic breast cancer such as liver, lymph nodes, bone marrow and adrenal glands. [4] Interestingly, SDF-1/CXCR4 axis is highly involved in lymph node metastasis in oral squamous cell carcinoma (SCC), [3] through the increasing of HNSCC cell adhesion and metalloproteinase-9 (MMP-9) secretion. [12] Thus, CXCR4 may be an important regulator of oral SCC metastasis. [3, 12, 13] Moreover, Almofti et al. demonstrated that CXCR4 expression is strongly associated with poor prognosis in oral SCC. [14] The role of autocrine SDF-1α on local invasion of anaplastic thyroid carcinomas (ATC) has also

been reported. [15] Thus, by considering the important role of SDF-1/CXCR4 axis in tumor development, anti-CXCR4 antagonists such as TN14003 [16] and T134 peptide [8] can effectively prevent the metastasis of tumor cells in various malignancies. Additionally, anti-CXCR4 antibody has previously been suggested as a therapeutic agent for prostate cancer cell metastasis [17] and colon cancer. [18]

SDF-1 has a G→A mutation at position 801 in 3′-untranslated region (SDF-1-G801A) in its β transcript, known as SDF1–3′A. [19] This polymorphism may have an important regulatory role by increasing the production of SDF-1 protein. [20] However, the relationship between SDF-1-G801A polymorphism and SDF-1 production is still not documented. [21] The single nucleotide polymorphism of SDF-1 gene has been investigated in type 1 diabetes, [22] HIV-1 infection [23] and is shown to be associated with an increased risk of lymphoma, [24] breast [25] and lung [26] cancers.

CCR5, which contributes to breast cancer progression by recruiting macrophages, [27] has a 32-bp deletion in the open reading frame of its gene, called CCR5Δ32. This polymorphism leads to a premature stop codon and results in a foreshortened non-functional protein, which is failed to reach the cell surface. [28] This mutation has been reported as a protective factor in Rheumatoid Arthritis (RA) [29] and type 1 diabetes. [30]

The present study focuses on SDF-1 and CCR5 genes polymorphism in patients with head and neck cancer and a healthy control group.

Materials and Methods

Study Population

In this study alleles and genotypes frequencies of SDF-1 and CCR5 were assessed in 156 patients, of whom 122 (78.2%) were men and 34 (21.8%) were women. Patients were recruited from Shiraz Khalili hospital, with surgical and pathological diagnosis of head and neck cancer. Our patient group was consisted of 125 (80.1%) cases with SCC (101 males and 24 females) and 31 (19.9%) cases with salivary gland tumors (21 males and 10 females). The mean and median age of patients with SCC were 57.7 and 60± 13 years and for patients with salivary gland tumors were 45.1 and 44±19, respectively. The frequency of different types of head and neck cancer between our patients and different stages are shown in Table 1. Results of whole patients were compared to 262 age–sex matched healthy individuals who had no evidence of malignancy or autoimmune disease. They were selected from our DNA bank as the control group.

Table 1 Different types and stages of head and neck cancer in studied population

Tumor type	Frequency (%)
[a]Squamous cell carcinoma	125 (80.1)
[b]Salivary gland tumors	31 (19.9)
Total	156 (100)
Stages	
1	27 (17.4)
2	30 (19.2)
3	59 (37.8)
4	39 (25)
Missing	1 (0.6)
Total	156 (100)

[a] Larynx = 64 (48 glottic, 16 supraglottic), Pharynx = 19 (nasopharynx = 12, tonsil = 3, hypopharynx = 4), Tongue = 24, Parotid = 4, Skin = 10, Maxillary sinus = 4.
[b] Mucoepidermoid carcinoma = 15 (high grade = 2, low grade = 13, all in parotid gland), Adenoidcystic carcinoma = 15 (oral cavity = 7, parotid gland = 3, submandibular gland = 1 paranasal sinuses = 4) and Acinicell carcinoma = 1 (parotid gland).
Notice: For 4 cases of adenoidcystic carcinoma of paranasal sinuses: ethmoid sinus = 3, maxillary sinus = 1.

DNA Preparation

Peripheral blood samples were collected in a volume of 10 ml and genomic DNA was extracted by salting out method as described previously. [31]

Genotyping

SDF-1 genotypes were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method using the following primers: [32] 5′-CAG TCA ACC TGG GCA AAG CC-3′ (F) and 5′-AGC TTT GGT CCT GAG AGT CC-3′ (R).

For PCR amplification, 0.3 μg DNA was amplified in each 25 μl PCR reaction containing 16 μl distilled water, 2.5 μl of 10x PCR buffer, 0.75 μl of 10 μM dNTPs, 0.75 μl of 50 μM $MgCl_2$, 1 μ of each primer and 2U of Taq DNA polymerase. Touch down method was done with the following program: an initial denaturation at 94°C for 3 min and two loops of amplification. Loop 1 included 7 cycles with this program: 94°C for 20 s, 67°C for 45 s, 72°C for 80 s and loop 2 included 28 cycles with the following program: 94°C for 20 s, 60°C for 30 s, 72°C for 1 min and a 5 min final extension step at 72°C. RFLP was carried out in a total volume of 20 μl including 10 μl DNA products, 7.4 μl distilled water, 2 μl buffer and 0.6 μl (6u/reaction) of MSPI restriction enzyme (Fermentas, Lithvania). The mixture was incubated at 37°C for 16 hours. Electrophoresis of digested PCR products on 2.5% agarose gel revealed a 302-bp band (undigested) for AA genotype, 302, 202 and 110-bp fragments for AG genotype.

GG genotype was emerged with two 202 and 110-bp bands (Fig. 1).

CCR5 gene polymorphism was performed by PCR with the appropriate PCR primers [33] that flanking the 32-bp deletion without using restriction endonuclease. The primer set: 5′-AGG TCT TCA TTA CAC CTG CAG C-3′ and 5′ CTT CTC ATT TCG ACA CCG AAG C-3′ amplified a fragment of 169-bp for wild type allele and 137-bp for mutant allele (Fig. 2). CCR5Δ32 variant was detected by electrophoresis on 2.5% agarose gel and using ethidium bromide staining. The PCR mix was prepared similar to that described for SDF-1. PCR was done for 35 cycles, consisting of a 94°C for 3 min as initial denaturation, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and further extension at 72°C for 5 min.

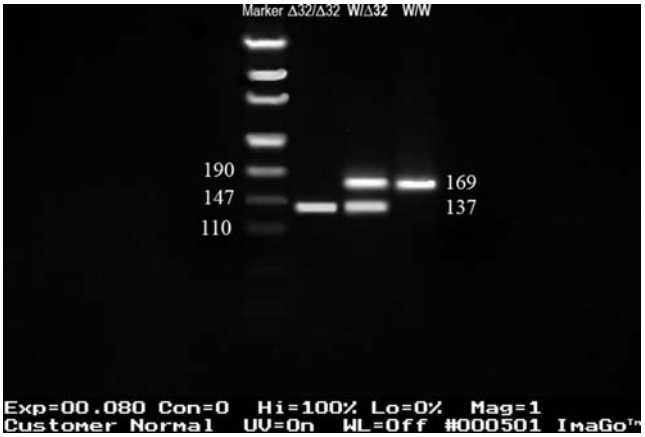

Fig. 2 PCR results of CCR5 gene on 2.5% agarose gel. One single 169 bp band in wild type homozygotes (W/W), one single 137 bp band in mutant homozygotes (Δ32/Δ32) and two bands in heterozygotes (W/Δ32) will be visible

Statistical Analysis

The analysis of data was determined by χ^2-test using EPI-Info 2002 and SPSS software, version 11.5. Results were considered significant at *P*-value <0.05.

Results

The analysis of SDF-1 genotypes of patients with head and neck cancer and controls showed a statistically significant difference ($P<0.004$, Table 2).

The genotypes of SDF-1 were also analyzed for SCC and salivary gland tumors, separately. As a result, SDF-1 genotypes in patients with SCC of head and neck, but not with salivary gland tumors, showed a statistically significant difference compared with the normal group ($P<0.005$ for SCC and $P=0.3$ for salivary gland tumors, Tables 3 and 4, respectively). Genotype frequencies distribution was in accordance with Hardy–Weinberg Equilibrium (HWE) in both groups.

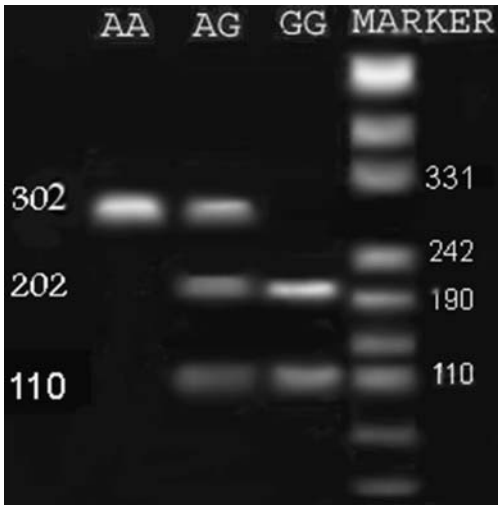

Fig. 1 Electrophoresis of digested PCR products of SDF-1: A 302-bp band (undigested) for AA genotype and 302, 202 and 110-bp fragments for AG genotype and two 202 and 110-bp bands for GG genotype

SDF-1 genotypes in patients with SCC were distributed as shown in Table 3. As a result, GG genotype was significantly more distributed between controls than patients ($P=0.005$). The analysis of allelic frequencies in SCC patients and controls was also determined in Table 3. It was revealed that patients had more A allele compared to the controls; though this difference was not statistically significant ($P=0.09$, Table 3).

On the other hand, the analysis of genotypes and alleles of CCR5 gene showed no statistically significant difference between patients and controls (Table 5). Genotype frequency distributions were in agreement with the HWE in both groups.

CCR5 genotypes and alleles were also analyzed for SCC and salivary gland tumors separately; there were no statistically significant difference when compared with the normal group.

Table 2 The frequency of different SDF-1 genotypes and alleles in patients with head and neck cancer (SCC and salivary gland tumors) and controls

Genotypes	Patients (%)	Control (%)	*P* value
GG	64 (41)	145 (55.3)	0.004
AG	84 (53.8)	97 (37)	
AA	8 (5.2)	20 (7.7)	
Total	156 (100)	262 (100)	
Alleles	Patients (%)	Control (%)	0.07
G	212 (67.9)	387 (73.9)	
A	100 (32.1%)	137 (26.1%)	

Table 3 The frequency of different SDF-1 genotypes and alleles in patients with SCC of head and neck and controls

Genotypes	Patients (%)	Controls (%)	*P* value
GG	51 (40.8)	145 (55.3)	0.005
AG	68 (54.4)	97 (37)	
AA	6 (4.8)	20 (7.7)	
Total	125 (100)	262 (100)	
Alleles	Patients (%)	Controls (%)	0.09
G	170 (68)	387 (73.9)	
A	80 (32)	137 (26.1)	

Table 5 Different CCR5 genotype and allele frequencies in patients with head and neck cancer and controls

Genotypes	Patients (%)	Control (%)	*P* value
W/W	149 (95.5)	246 (93.9)	0.5
W/Δ32	7 (4.5)	16 (6.1)	
Δ32/Δ32	0	0	
Total	156 (100)	262 (100)	
Alleles	Patients (%)	Control (%)	0.5
W	305 (97.8)	508 (96.9)	
Δ32	7 (2.2)	16 (3.1)	

The genotype frequencies of both genes were compared to the age, sex, disease stages and different pathologic types of head and neck cancer. No statistical significant correlation was observed.

Discussion

The current study has focused on the SDF-1 and CCR5 genes polymorphism in patients with head and neck cancer, SCC and salivary gland tumors, in comparison with a control group.

Based on the present study, SDF1–3′A may be associated with the susceptibility to head and neck cancer, most probably to SCC but not to salivary gland tumors. It is important to note that the low number of patients with salivary gland tumors in our patient group may be the reason of the lack of significant difference compared with controls. Thus for most reliable results a large number of patients with this type of tumor need to be genotyped. In the case of CCR5, there was no significant difference in the frequency of Δ32 allele between patients and controls.

SDF-1 is a CXC chemokine that mediates the intracellular stress fiber formation, chemotactic activity, invasion and cell growth in vitro. [34] CXCR4/SDF-1 axis has been shown to be involved in the susceptibility and metastasis of a number of important malignancies. [4] For instance, CXCR4 expression has been reported to be significantly higher in invasive breast cancer and be highly correlated with lymph node metastasis. [4, 35] Thus, CXCR4 expression is a poor prognostic factor in breast cancer patients and high levels of this receptor decreases the survival of these patients. [4] Similarly, this receptor is overexpressed in oral SCC and malignant epithelia of prostate cancer. [3, 36] SDF-1 expression has been reported to be about two-fold higher in breast epithelial cancer cells compared to normal epithelial cells. [11] In addition, it has been proposed that SDF-1 and CXCR4 are constitutively produced in different tissues [37] such as brain [38] and bone marrow [39] and have been reported to be involved in brain development, astrocytes [38, 40, 41] and ovarian cell proliferation. [42] SDF-1/CXCR4 signaling pathway has been suggested to cause glioma cell invasion [43] and deregulation of this pathway contribute to uncontrolled cellular proliferation and subsequently to glioblastoma tumors. [38] On the other hand, SDF1-3′A variant may play an important regulatory role by increasing the production of SDF-1 protein. [20]

We conclude that, this polymorphism may trigger the SDF-1 induced cell cycle dysfunction by increasing the SDF-1 production, cellular transformation and finally tumor formation in allele "A" carrying individuals. Interestingly, SDF1–3′A has been reported to be associated with AIDS related non-Hodgkin's lymphoma (NHL) with double risk in heterozygotes and four fold in homozygotes. [44] Moreover, consistent with this study, we have previously shown a significant difference in SDF-1 alleles and genotypes frequencies in breast [25] and lung [26] cancers.

In addition, based on our study CCR5Δ32 is probably not associated with the susceptibility of head and neck cancer. This finding is consistent with a previously report in a Turkish population that showed no relationship between Δ32 allele and certain tumors such as breast, laryngeal, thyroid and brain carcinomas. [45]

By considering the importance of SDF-1/CXCR4 axis in tumor progression, more investigations on its functional role and gene expression in different types of cancers in our population remain to be determined.

Table 4 The frequency of different SDF-1 genotypes and alleles in patients with salivary gland tumors and controls

Genotypes	Patients (%)	Controls (%)	P value
GG	13 (41.9)	145 (55.3)	0.3
AG	16 (51.6)	97 (37)	
AA	2 (6.5)	20 (7.7)	
Total	31 (100)	262 (100)	
Alleles	Patients (%)	Controls (%)	0.3
G	42 (67.7)	387 (73.9)	
A	20 (32.3)	137 (26.1)	

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