Pathol. Oncol. Res. (2010) 16:223–227
DOI 10.1007/s12253-009-9214-5

SDF1-3'A Gene Polymorphism is Associated with Laryngeal Cancer

Łukasz Kruszyna · Margarita Lianeri · Małgorzata Rydzanicz · Krzysztof Szyfter · Paweł P. Jagodziński

Received: 30 July 2009 / Accepted: 24 September 2009 / Published online: 16 October 2009

Abstract The *SDF1-3'* G801A (rs 1801157) polymorphism is associated with increased risk of various types of cancers, including those of the neck and head. Using PCR-RFLPs, we investigated the distribution of *SDF1-3'* G801A genotypes in patients with laryngeal cancer (n=118) and controls (n=250) in Poland. We found that patients with *SDF1-3'* A/A and G/A genotypes exhibit a 1.863-fold increased risk of laryngeal cancer (95% CI=1.177–2.949, p=0.0086). However, there was no significant increase in risk for the homozygous *SDF1-3'* A/A genotype OR= 3.235 (95% CI=0.5330−19.633, p=0.3329). We also did not observe a significant association between tumor characteristics and prevalence of alleles or genotypes for the *SDF1-3'* G801A polymorphism. Our findings suggest that the *SDF1-3'A* variant may be associated with an increased risk of laryngeal cancer.

Keywords SDF1-3' G801A · Polymorphism · Laryngeal cancer · CXCR4 · PCR-RFLP

Ł. Kruszyna · M. Lianeri · P. P. Jagodziński (✉)
Department of Biochemistry and Molecular Biology,
Poznań University of Medical Sciences,
6 Święcickiego St.,
60-781 Poznań, Poland
e-mail: pjagodzi@am.poznan.pl

M. Rydzanicz · K. Szyfter
Department of Environmental Mutagenesis,
Institute of Human Genetics, Polish Academy of Sciences,
Poznań, Poland

K. Szyfter
Department of Otolaryngology and Laryngeal Oncology,
Poznań University of Medical Sciences,
Poznań, Poland

Introduction

Cancer of the larynx constitutes 1–2% of malignancies recognized worldwide and develops most often in older men and middle-aged men [1–3]. Cigarette smoking and alcohol abuse are considered major risk factors for laryngeal cancer [3]. An increased risk of laryngeal cancer is also associated with epigenetic and genetic factors. These factors alter the expression of oncogenes, tumor suppressor genes, and genes encoding enzymes involved in carcinogen inactivation [1, 4, 5]. The precise etiology and the molecular biology of laryngeal cancer progression have not yet been elucidated.

Chemokines include a group of low molecular weight (8–14 kDa) chemotactic cytokines, which interact with specific G-protein–coupled plasma cell membrane receptors [6]. They recruit hematopoietic and immune cells to sites of differentiation and inflammation, respectively [6–8]. Their biosynthesis is up-regulated during viral and bacterial infections, and tissue repair [6–8]. However, chemokines also contribute to pathologic processes, encompassing metastasis and proliferation of tumor cells [9].

CXCL12, also called stromal cell-derived factor-1 (SDF1), is a chemokine responsible for chemoattraction of T cells and monocytes. This chemokine is also involved in organogenesis, lymphopoiesis, and myelopoiesis [10, 11]. The significance of the SDF1/CXCR4 system has been documented in breast, colon, melanoma, pancreatic, ovarian, prostate, lung, gastric and oral carcinoma progression [12–20].

SDF1 has demonstrated a G801A transition at position 801 in the 3′-untranslated region of the transcript, known as *SDF1-3'A* (rs 1801157) [21, 22]. It has been suggested that this polymorphism may have a significant regulatory function by increasing biosynthesis of the SDF1 protein

[21, 22]. However, the effect of the *SDF1-3′* G801A polymorphism on SDF1 production has not been clearly documented [23]. The *SDF1-3′* G801A polymorphism has been associated with increased risk of various cancer types, including neck and head cancers [24–29]. However, little is known on how the *SDF1-3′* G801A polymorphism affects laryngeal cancer. Therefore, we investigated the distribution of *SDF1-3′* G801A genotypes in patients with laryngeal cancer (n=118) and controls (n=250) in a Polish population.

Materials and Methods

Patients and Controls

The patients include 118 men with histologically confirmed squamous cell carcinoma of the larynx, diagnosed between April 1999 and May 2001 in the Clinic of Otolaryngology and Laryngological Oncology, Poznań University of Medical Sciences in Poznań, Poland (Table 1). Approximately 97% of these laryngeal cancer patients were long-term and current cigarette smokers. The control group was composed of 250 unrelated healthy male blood donors selected randomly and healthy volunteers. Controls were matched by age to the patients (Table 1). All individuals were Caucasian and from the same region of Poland. Written informed consent was obtained from all participating individuals. The procedures of the study were approved by the Local Ethical Committee of Poznań University of Medical Sciences.

Table 1 Clinical characteristics of patients and controls

Characteristic	Patients (n=118, men only)	Controls (n=250, men only)
Mean age±SD	59.1±9.1	52.7±4.4
Histological grade		
G1	44 (37.3%)	
G2	49 (41.5%)	
G3	4 (3.4%)	
Gx	21 (17.8%)	
Tumour stage		
T1	15 (12.7%)	
T2	30 (25.4%)	
T3	33 (28.0%)	
T4	40 (33.9%)	
Lymph nodes		
N0	64 (54.2%)	
N1	39 (33.1%)	
N2	13 (11.0%)	
N3	2 (1.7%)	
Clinical stage		
I	14 (11.9%)	
II	25 (21.2%)	
III	32 (27.1%)	
IV	47 (39.8%)	

Genotyping

DNA was isolated from peripheral blood lymphocytes by salt extraction. Polymorphic variants of *SDF1-3′* G801A (rs 1801157) were identified using PCR with the primer pair 5′ TTATTGTACTTGCCTTATTAGAG3′ and 5′GTAGTT CACCCCAAAGGACC3′; enzyme digestion followed the identification process. The PCR-amplified fragments of the *SDF1* that were 732 bp in length were isolated and subjected to digestion with *MspI* (C/CGG). The *SDF1-3′G* allele was cleaved into 456 bp and 276 bp fragments, whereas the *SDF1-3′A* allele remained uncut at 732 bp. DNA fragments were separated by electrophoresis on 3% agarose gel and visualized by ethidium bromide staining. Confirmation of polymorphism was performed by commercial sequencing analysis.

Statistical Analysis

The distribution of genotypes in all groups was tested for deviation from Hardy-Weinberg equilibrium (HWE). The Fisher's exact test was applied to examine differences in genotypic and allelic distribution between patients and controls. Moreover, the Odds Ratio and 95% Confidence Intervals (95% CI) were calculated. A p value <0.05 was considered statistically significant.

Results

Prevalence of SDF1-3′ G801A Alleles and Genotypes in Patients and Controls

Genotype analysis of all investigated polymorphisms revealed no significant deviation from Hardy-Weinberg equilibrium in any group.

There was an association of the *SDF1-3′A* allele with development of laryngeal cancer. The frequency of the *SDF1-3′* A/A genotypes in patients with laryngeal cancer and controls amounted to 3% and 1%, respectively (Table 2). Distribution of the heterozygous *SDF1-3′* G/A genotype in patients was approximately 1.4-fold higher than in controls and reached 39% and 27%, respectively (Table 2). The prevalence of the *SDF1-3′A* allele was also higher in patients than in controls, and amounted to 22% and 14% in these groups, respectively (Table 2).

Table 2 Distribution of the *SDF 1* G801A polymorphism in patients and controls

	n	Genotype distribution absolute number (frequency%)			Allele distribution absolute number (frequency%)		Odds ratio (95% CI)	P[c]
		G/G	G/A	A/A	G	A		
SDF1 G801A	Controls							
(rs 1801157)	Total 250	181 (72)	67 (27)	2 (1)	429 (86)	71 (14)		
	Cancer						3.235 (0.5330−19.633)[a]	0.3329
	Total 118	69 (58)	46 (39)	3 (3)	184 (78)	52 (22)	1.863 (1.177−2.949)[b]	0.0086

The Odds ratio was calculated for patients [a] A/A vs G/G or G/A; [b] A/A or G/A vs G/G. [c] Fisher exact test

We found that patients with the *SDF1-3'* A/A and A/G genotypes exhibited a 1.863-fold increased risk of laryngeal cancer (95% CI=1.177−2.949, p=0.0086). However, there was no significant risk with the homozygous *SDF1-3'* A/A genotype OR=3.235 (95% CI=0.5330−19.633, p=0.3329) (Table 2). We also did not observe a significant association between tumor characteristics and prevalence of alleles or genotypes for the *SDF1-3'* G801A polymorphism (Table 3). The latter finding could be interpreted as an association of *SDF1-3'* G801A polymorphism with incidence but not progression of laryngeal cancer.

Discussion

The human genome project has brought to light more than ten million single nucleotide polymorphisms. However, the importance of most of them in either health or disease states is still unclear [30].

The *SDF1* human gene is located at 10q11.1 and its transcription produces alpha and beta alternative splice variants [31]. The SDF1 protein binds to the CXCR4 receptor and triggers several downstream effectors, which promote migration, invasion, adhesion, proliferation, and angiogenesis of cancer cells [9, 32, 33].

We observed an association of the *SDF1-3'A* gene variant with laryngeal cancer incidence. To date, the *SDF1-3'A* gene variant has been considered a factor of increased susceptibility to oral cancer in Taiwanese patients [27]. Khademi et al. (2008) indicated that the *SDF1-3'A* variant may be associated with squamous cell carcinomas (SCC) of the head and neck, but not with salivary gland tumors [28]. In contrast, Vairaktaris et al. (2008) detected that *SDF1-3'A* allele frequency was significantly lower in patients with advanced cancer compared with controls [29]. However, the *SDF1-3'A* gene variant contributed to an increased risk of lymphoma and breast and lung cancers [24–26].

CXCR4 is also considered a co-receptor for entry of the human immumodeficiency virus (HIV) [34]. Winkler et al. (1998), based on a genetic association analysis of 2857 patients, indicated that homozygous *SDF1-3 'A/'A* individuals had delayed onset of AIDS [21]. Since SDF1 competes with HIV entry to target cells, this may suggest that *SDF1-3'A* variants are responsible for higher production of SDF1 [21].

These observations may support our and other findings that demonstrate a significant increase in *SDF1-3'A* variant distribution in different types of cancers compared with controls [24–28]. Abundant expression of the SDF1 protein from the *SDF1-3'A* variant can be responsible for an increase in cancer cell proliferation and anti-apoptotic effects, leading to cancer development [35, 36]. The SDF1/CXCR4 signaling system triggers phosphatidylinositol-3-kinase activating

Table 3 Prevalence of SDF1 genotypes between patients age, tumor size, lymph node metastases and histological grade

	Mean Age±SD Years	Tumor size				Regional lymph node metastases				Histological grade			
		T1	T2	T3	T4	N0	N1	N2	N3	G1	G2	G3	GX
SDF1 G>A													
GG(n=69)	58.9±9.1	5	18	17	29	36	25	6	2	31	24	1	12
GA(n=46)	58.9±9.2	10	11	14	11	26	13	7	0	13	23	3	8
AA(n=3)	65.7±4.5	0	1	2	0	2	1	0	0	0	2	0	1
		p=0.0956				p=0.7387				p=0.2559			

Data are presented as number, p-values represent significance of genotype distribution between tumor characteristics and were determined by Chi-square test

protein kinase (AKT) and mitogen-activated protein (MAP) kinase. Active AKT inhibits apoptosis and increases malignant cell proliferation, supports the development of various carcinomas [35]. MAP kinase controls the expression of genes encoding proteins involved in the proliferation and survival of malignant cells [36].

To date, the increase in SDF1 and CXCR4 expression was associated with epithelial-mesenchymal transition in human oral SCC and has been used as a marker of highly-invasive cancer [20]. Oliveira-Neto et al. (2008) suggested that the SDF1/CXCR4 system promotes neoplastic transformation and the spread of oral tumor cells to lymph nodes [19]. Fibroblastic SDF1 production may also have an impact on local progression of oral cancers [37]. Uchida et al. (2007) demonstrated that, in oral SCC, the paracrine SDF1/CXCR4 system increases lymph node metastasis, but distant metastases could require autocrine interaction of SDF1/CXCR4 [38].

These observations suggest that higher production of SDF1, which can be supported by *SDF1-3'A* variant may increase in neoplastic transformation and spread of oral tumor cells.

However, to determine more precisely the significance of the *SDF1* G801A polymorphism in laryngeal cancer development, further investigation of the distribution of these variants in other populations is needed.

Acknowledgements Supported by a grant No 502-01-01124182-07474 Poznań University of Medical Sciences.

References

1. Sadri M, McMahon J, Parker A (2006) Management of laryngeal dysplasia. Eur Arch Otorhinolaryngol 263:843–852
2. Sapkota A, Hsu CC, Zaridze D et al (2008) Dietary risk factors for squamous cell carcinoma of the upper aerodigestive tract in central and eastern Europe. Cancer Causes Control 19:1161–1170
3. Freedman ND, Abnet CC, Leitzmann MF, Hollenbeck AR et al (2007) Prospective investigation of the cigarette smoking-head and neck cancer association by sex. Cancer 110:1593–1601
4. Szyfter K, Szmeja Z, Szyfter W et al (1999) Molecular and cellular alterations in tobacco smoke-associated larynx cancer. Mutat Res 445:259–274
5. Bradford CR (1999) Predictive factors in head and neck cancer. Hematol Oncol Clin North Am 13:777–785
6. Moser B, Loetscher P (2001) Lymphocyte traffic control by chemokines. Nat Immunol 2:123–128
7. Zlotnik A, Yoshie O (2000) Chemokines: a new classification system and their role in immunity. Immunity 12:121–127
8. Campbell JJ, Butcher E (2000) Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. Curr Opin Immunol 12:336–341
9. Balkwill F (2003) Chemokine biology in cancer. Sem Immunol 15:49–55
10. Zou YR, Kottmann AH, Kuroda M et al (1998) Function of the chemokine receptor CXCR4 in a heamatopoiesis and in cerebellar development. Nature 393:595–599
11. Onai N, Zhang Y, Yoneyama H et al (2008) Impairment of lymphopoiesis and myelopoiesis in mice reconstituted with bone marrow-hematopoietic progenitor cells expressing SDF-1-intrakine. Blood 96:2074–2080
12. Yasuoka H, Tsujimoto M, Yoshidome K et al (2008) Cytoplasmic CXCR4 expression in breast cancer: induction by nitric oxide and correlation with lymph node metastasis and poor prognosis. BMC Cancer 8:340
13. Kim J, Mori T, Chen SL et al (2006) Chemokine receptor CXCR4 expression in patients with melanoma and colorectal cancer liver metastases and the association with disease outcome. Ann Surg 244:113–120
14. Maréchal R, Demetter P, Nagy N et al (2009) High expression of CXCR4 may predict poor survival in resected pancreatic adenocarcinoma. Br J Cancer 100:1444–1451
15. Kajiyama H, Shibata K, Terauchi M et al (2008) Involvement of SDF-1alpha/CXCR4 axis in the enhanced peritoneal metastasis of epithelial ovarian carcinoma. Int J Cancer 122:91–99
16. Akashi T, Koizumi K, Tsuneyama K et al (2008) Chemokine receptor CXCR4 expression and prognosis in patients with metastatic prostate cancer. Cancer Sci 99:539–542
17. Kijima T, Maulik G, Ma PC et al (2002) Regulation of cellular proliferation, cytoskeletal function, and signal transduction through CXCR4 and c-Kit in small cell lung cancer cells. Cancer Res 62:6304–6311
18. Lee HJ, Kim SW, Kim HY et al (2009) Chemokine receptor CXCR4 expression, function, and clinical implications in gastric cancer. Int J Oncol 34:473–480
19. Oliveira-Neto HH, Silva ET, Leles CR et al (2008) Involvement of CXCL12 and CXCR4 in lymph node metastases and development of oral squamous cell carcinomas. Tumour Biol 29:262–271
20. Taki M, Higashikawa K, Yoneda S et al (2008) Up-regulation of stromal cell-derived factor-1alpha and its receptor CXCR4 expression accompanied with epithelial-mesenchymal transition in human oral squamous cell carcinoma. Oncol Rep 19:993–998
21. Winkler C, Modi W, Smith MW et al (1998) Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. Science 279:389–393
22. Watanabe MA, de Oliveira Cavassin GG, Orellana MD et al (2003) SDF-1 gene polymorphisms and syncytia induction in Brazilian HIV-1 infected individuals. Microb Pathog 35:31–34
23. Kimura R, Nishioka T, Ishida T (2003) The SDF1–G801A polymorphism is not associated with SDF1 gene expression in Epstein–Barr virus-transformed lymphoblastoid cells. Genes Immun 4:356–361
24. de Oliveira Cavassin GG, De Lucca FL, Delgado Andre N et al (2004) Molecular investigation of the stromal cell-derived factor-1 chemokine in lymphoid leukemia and lymphoma patients from Brazil. Blood Cells Mol Diseases 33:90–93
25. Razmkhah M, Talei AR, Doroudchi M et al (2004) Stromal cell-derived factor-1 (SDF-1) alleles and susceptibility to breast carcinoma. Cancer Lett 225:261–266
26. Razmkhah M, Doroudchi M, Ghayumi SM et al (2005) Stromal cell-derived factor-1 (SDF-1) gene and susceptibility of Iranian patients with lung cancer. Lung Cancer 49:311–315
27. Teng YH, Liu TH, Tseng HC et al (2009) Contribution of genetic polymorphisms of stromal cell-derived factor-1 and its receptor, CXCR4, to the susceptibility and clinicopathologic development of oral cancer. Head Neck. doi:10.1002/hed.21094

28. Khademi B, Razmkhah M, Erfani N et al (2008) SDF-1 and CCR5 genes polymorphism in patients with head and neck cancer. Pathol Oncol Res 14:45–50
29. Vairaktaris E, Vylliotis A, Spyridonodou S et al (2008) A DNA polymorphism of stromal-derived factor-1 is associated with advanced stages of oral cancer. Anticancer Res 28:271–275
30. Frazer KA, Ballinger DG, Cox DR et al (2007) A second generation human haplotype map of over 3.1 million SNPs. Nature 449:851–861
31. Shirozu M, Nakano T, Inazawa J et al (1995) Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. Genomics 28:495–500
32. Zlotnik A (2006) Chemokines and cancer. Int J Cancer 119:2026–2029
33. Zlotnik A (2008) New insights on the role of CXCR4 in cancer metastasis. J Pathol 215:211–213
34. Bleul CC, Farzan M, Choe H et al (1996) The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature 382:829–833
35. Vlahakis SR, Villasis-Keever A, Gomez T et al (2002) G protein-coupled chemokine receptors induce both survival and apoptotic signaling pathways. J Immunol 169:5546–5554
36. Chang L, Karin M (2001) Mammalian MAP kinase signalling cascades. Nature 410:37–40
37. Daly AJ, McIlreavey L, Irwin CR (2008) Regulation of HGF and SDF-1 expression by oral fibroblasts–implications for invasion of oral cancer. Oral Oncol 44:646–651
38. Uchida D, Onoue T, Tomizuka Y et al (2007) Involvement of an autocrine stromal cell derived factor-1/CXCR4 system on the distant metastasis of human oral squamous cell carcinoma. Mol Cancer Res 5:685–694