Stromal cell-derived factor-1 G801A polymorphism and the risk factors for cervical cancer

ANDRZEJ ROSZAK1,2, MATTHEW MISZTAL3, ANNA SOWIŃSKA4 and Paweł P. JAGODZIŃSKI3

1Department of Radiotherapy and Gynecological Oncology, Greater Poland Cancer Center, Poznań 61-866; Departments of 2Electroradiology, 3Biochemistry and Molecular Biology, and 4Computer Science and Statistics, Poznań University of Medical Sciences, Poznań 60-781, Poland

Received March 21, 2014; Accepted January 2, 2015

DOI: 10.3892/mmr.2015.3315

Abstract. Although certain studies have demonstrated no association between the stromal cell-derived factor-1 (SDF1-3') G801A single nucleotide polymorphism (SNP) and cervical carcinoma, the interactions between the SDF1-3' G801A SNP and contraceptive use, menopausal status, parity and tobacco smoking remain to be fully elucidated. Using polymerase chain reaction-restriction fragment length polymorphism, the distribution of SDF1-3' G801A genotypes in patients with cervical cancer (n=462) against control groups (n=497) was investigated. Logistic regression analysis, adjusting for age, pregnancy, oral contraceptive use, tobacco smoking and menopausal status, did not identify the SDF1-3' G801A polymorphism as a genetic risk factor for cervical cancer. The adjusted odds ratio (OR) for patients with the A/G, vs. G/G genotype was 1.203, with a 95% confidence interval (CI) of 0.909-1.591 (P=0.196). The adjusted OR for the A/A, vs. G/G genotype was 1.296 (95% CI=0.930-1.807; P=0.125) and for the A/A or A/G, vs. G/G genotype was 1.262 (95% CI=0.964-1.653; P=0.090)]. The P-value of the χ² test of the trend observed for the SDF1-3' G801A polymorphism was at the borderline of being statistically significant (Pₚₐₓₚ=0.0484). Stratified analyses between the distribution of the SDF1-3' G801A genotypes and cervical cancer risks demonstrated that this polymorphism may be a risk factor for patients with a positive history of tobacco smoking (1.778; 95% CI=1.078-2.934; P=0.0235). These findings suggested that the SDF1-3' G801A polymorphism may be a genetic risk factor for cervical cancer in patients with a positive history of tobacco smoking.

Introduction

Cervical tumors are the most common type of gynecological malignancy worldwide and constitute the tenth most frequent type of cancer occurring in females in developed countries (1,2). The number of young females affected by cervical cancer has been increasing (1-3). Cervical carcinogenesis encompasses the transformation of normal cervical epithelium to cervical intraepithelial neoplasia (CIN), which may develop into an invasive cervical tumor (4,5). There are several risk factors for cervical cancer, including human papillomavirus, impairment of the immune system, expression of tumor suppressor genes and gain of function mutations in proto-oncogenes (4,5). In addition, contraceptive use, tobacco consumption, age and environmental exposures are also considered possible causative factors in cervical carcinogenesis (6). C-X-C motif chemokine 12 is a chemokine, also termed stromal cell-derived factor-1 (SDF1), which binds to the CXCR4/CXCR7 receptors (7).

The human SDF1 gene is expressed as α and β alternative splice variants (8). SDF1 is involved in lymphopoiesis and myelopoiesis and attracts lymphocytes, megakaryocytes, endothelial cells and stem cells (9-11). In addition, the interaction of SDF1 with CXCR4 controls the embryonic growth of vascular, cardiac, neuronal and craniofacial systems (12). However, the binding of SDF1 to CXCR4 contributes to the progression of cancer of the colon, pancreas, ovaries, prostate, lung, stomach, mouth, breast and skin, in addition to cervical cancer (13-21).

SDF1 is present in common genetic variants due to a G801A transition in the 3'-untranslated region (rs 1801157) (22). The possible role of the SDF1-3' A variant in the increased levels of transcription and protein has been reported (22). Certain studies have demonstrated no association between the SDF1-3' G801A single nucleotide polymorphism (SNP) and cervical carcinoma (23,24), however, the interaction between the SDF1-3' G801A SNP with other known risk factors of cervical cancer remain to be fully elucidated. In the present study, the SDF1-3' G801A genotype and allele frequencies were investigated in patients with cervical cancer (n=462) and healthy controls (n=497) in the Polish population, stratified based on contraceptive use, menopausal status, parity and history of tobacco smoking.

Correspondence to: Dr Paweł P. Jagodzinski, Department of Biochemistry and Molecular Biology, Poznań University of Medical Sciences, 6 Święcickiego, Poznań 60-781, Poland E-mail: pjagodzi@am.poznan.pl

Key words: cervical carcinoma, stromal cell-derived factor-1, polymorphisms
Patients and methods

Patients and controls. The patients consisted of 462 females with histologically-determined cervical carcinoma, according to the International Federation of Gynecology and Obstetrics. All the females were enrolled between April 2007 and January 2014 at the Department of Radiotherapy, Greater Poland Cancer Center (Poznań, Poland; Table I). The controls included 497 unrelated healthy female volunteers, who were matched by age to the patients (Table I). Data regarding pregnancy, oral contraceptive use, tobacco smoking and menopausal status were obtained during clinical interviews. All individuals were Caucasian and were enrolled from the Wielkopolska (Greater Poland) area of Poland. The patients and controls provided written informed consent and the study was approved by the Local Ethical Committee of Poznań University of Medical Sciences (Poznań, Poland).

Genotyping. DNA was isolated from peripheral blood leukocytes using a salting-out procedure, in which 10 ml peripheral blood were obtained using BD Vacutainer® (Becton Dickinson, Franklin Lakes, NJ USA). The presence of the SDF1-3′G801A (rs1801157) transition was determined by polymerase chain reaction (PCR) using Dream Taq DNA Polymerase (Thermo Scientific,
W. Ilili, Lithuania) and a PTC-200 DNA Engine Thermocycler (MJ Research Inc, St. Bruno, QC, Canada). The primer sequence was as follows, 5'-TTATTTGACTGACCTATAGG-3' and 5'-GTTACCTACCCAAAAGGACC-3'. The PCR was followed by digestion with MspI (C/CGG; Thermo Scientific) according to manufacturer's instructions. The SDF1-3' A allele remained uncut at 732 bp, whereas the SDF1-3' G allele was cleaved into 456 bp and 276 bp fragments. The DNA fragments were separated by electrophoresis on a 3% agarose gel and visualized with ethidium bromide staining (Sigma-Aldrich, St. Louis, MO). The presence of the SDF1-3' G801A transition was confirmed by Sanger sequencing of G801A (rs 1801157) polymorphism and cervical cancer.

The frequency of the G801A polymorphism was at the 15% of the samples, which are highlighted in bold font. CI, confidence interval.

### Results

**Distribution of the SDF1-3' G801A polymorphism in females with cervical cancer.** The prevalence of the SDF1-3' G801A genotypes did not exhibit a significant divergence from the Hardy-Weinberg (HW) equilibrium was evaluated using a $\chi^2$ test. The polymorphism was assessed for association with cervical cancer incidence using a $\chi^2$ test for trend ($p_{\text{trend}}$), odds ratio (OR) and 95% confidence intervals (CI). Unconditional logistic regression analysis was used to adjust for the effect of confounders, including age, pregnancy, oral contraceptive use, tobacco smoking and menopausal status. $p<0.05$ was considered to indicate a statistically significant difference.

**Stratified analysis between the SDF1-3' G801A genotypes and cervical cancer risks.** The age adjusted analysis of the SDF1-3' G801A genotypes and cervical cancer risk, stratified by pregnancy, oral contraceptive use, tobacco smoking, and menopausal status is presented in Table III. An increase in cervical cancer risk was observed only among patients with a positive history of tobacco smoking for the adjusted OR with the A/G, vs. G/G genotype at 1.778 (95% CI=1.078-2.934; $p=0.00235$). However, no significant association was observed between SDF1-3' G801A and smoking for the A/G, vs. G/G genotype at 1.189 (95% CI=0.731-1.905; $p=0.4975$) or A/A or A/G, vs. A/A genotype (1.352; 95% CI=0.862-2.122; $p=0.1877$). Furthermore, no significant association was observed between SDF1-3' G801A and pregnancy, oral contraceptive use or menopausal status (Table III). No association was observed between the SDF1-3' G801A polymorphism and tumor stage, histological grade or type of tumor (data not shown) on stratification of the patients based on clinical characteristics.

**Discussion**

The SDF1-3' A gene variant has been suggested as a factor that upregulates SDF1α levels, and the SDF1/CXCR4/CXCR7 axis is considered to contribute significantly to the biology and metastasis of several types of cancer (22,25). In addition, the SDF1/CXCR4 interaction has been demonstrated to be important in the progression of cervical cancer (26-30). Wei et al (26) suggested that the progression of cervical tumors is accompanied with an increased production of SDF1α. In addition to these findings, Huang et al (27) demonstrated an increase in the co-expression levels of SDF1/CXCR4 in CIN and cervical carcinoma as a durative process in cervical carcinogenesis. The SDF1-CXCR4 axis initiates invasiveness via changes to the adhesion and secretion of matrix metalloproteinase-2. (OMIM '120360) and promotes the metastasis of tumor cells.

### Table II. Association between the stromal cell-derived factor-1-3' G801A (rs 1801157) polymorphism and cervical cancer.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patient (frequency)</th>
<th>Control (frequency)</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
<th>Adjusted odds ratio (95% CI)</th>
<th>P-value</th>
<th>$p_{\text{trend}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>289 (0.63)</td>
<td>337 (0.68)</td>
<td>Referent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>149 (0.32)</td>
<td>144 (0.29)</td>
<td>1.207 (0.914-1.593)</td>
<td>0.1849</td>
<td>1.203 (0.909-1.591)</td>
<td>0.196</td>
<td>0.0484</td>
</tr>
<tr>
<td>A/A</td>
<td>24 (0.05)</td>
<td>16 (0.03)</td>
<td>1.749 (0.911-3.57)</td>
<td>0.0892</td>
<td>1.296 (0.930-1.807)</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>A/G+A/A</td>
<td>173 (0.37)</td>
<td>160 (0.32)</td>
<td>1.261 (0.966-1.646)</td>
<td>0.0878</td>
<td>1.262 (0.964-1.653)</td>
<td>0.090</td>
<td></td>
</tr>
<tr>
<td>Minor allele frequency</td>
<td>0.21</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2$ analysis. Odds ratios were adjusted by age, pregnancy, oral contraceptive use, tobacco smoking and menopausal status. Significant results are highlighted in bold font. CI, confidence interval.
toward lymph nodes and the pelvic cavity in patients with cervical cancer (29,30). SDF1α also provokes significant signal transduction events, including chemotaxis and rescue from apoptosis in cervical cancer cells (21,30). In addition to these findings Majka et al. (21) demonstrated that SDF1α augments cervical cancer cell scattering and supported the nuclear localization of the β-catenin gene and also increased its target gene expression, cyclin D1. In addition, it was observed that SDF1α interacts with CXCR4 and leads to the activation of numerous downstream cytoplasmic signaling pathways, which support the invasiveness of cervical cancer (21).

Genetic variants of SDF1 may have an impact on cervical cancer development and its clinicopathological variables. In the present study, the SDF1-3′ G801A SNP was not identified as a risk factor for cervical cancer. However, in the present study, the P-value assessment of the trend observed for the SDF1-3′ G801A polymorphism was on the borderline of statistical significance. In addition, the present study revealed that the SDF1-3′ A/A genotype may be a risk for cervical cancer in females with a positive history of tobacco smoking. This is consistent with previous reports suggesting the possible causative role of tobacco consumption in cervical carcinogenesis (6,31,32). However, no other confounding variables, including contraceptive use, menopausal status or parity affected the SDF1-3′ G801A polymorphism as a risk factor for cervical cancer.

The SDF1-3′ G801A polymorphism has been reported as a risk factor in the development of breast, laryngeal, oral, lung, prostate and hepatocellular carcinoma, as well as lymphoma (33-39). The effect of the SDF1-3′ G801A SNP on SDF1α biosynthesis has been based mainly on the analysis of subjects infected with human immunodeficiency (HIV) (22). The SDF1-3′ A variant has been suggested as a genetic variant,
which increases the production of SDF1α (22). These findings were consistent with a study by Chang et al (40) who observed that fibroblasts from patients with colon cancer and the SDF1-3' GA or AA genotypes biosynthesized three times more SDF1α transcript compared with fibroblasts with the GG genotype. In addition, Garcia-Moruja et al (41) demonstrated that the SDF1-3' A transcript variant exhibited a two-fold longer half-life than the SDF1-3' G transcript variant. By contrast, a study by Kimura et al (42), using Epstein-Barr virus-transformed lymphoblastoid cell lines, did not observe any effects of the SDF1-3' G801A SNP on the SDF1α mRNA levels. In addition to these findings, Watanabe et al (43), using the syncytiotum model, also observed no correlation between the SDF1-3' G801A SNP gene variant and syncytium-inducing HIV (43).

In conclusion, the present genetic study is the first, to the best of our knowledge, to demonstrate that the SDF1-3' A gene variant may be a risk factor for cervical carcinoma in patients with a positive history of tobacco smoking; therefore this evaluation should be replicated in other independent ethnicities.

Acknowledgements

The present study was supported by Poznań University of Medical Sciences (grant no. 502-01-0112482-07474). The technical assistance of Ms. Alicja Pinczewska is gratefully acknowledged.

References