Molecular investigation of the stromal cell-derived factor-1 chemokine in lymphoid leukemia and lymphoma patients from Brazil

Gabriela Gonçalves de Oliveira Cavassin, a Fernando Luiz De Lucca, b Nayara Delgado André, a Dimas Tadeu Covas, b,c Maria Helena Pelegrinelli Fungaro, d Júlio César Voltarelli, b,c and Maria Angelica Ehara Watanabe a,*

a Department of Pathological Sciences, Londrina State University, Londrina, PR, Brazil
b School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
c Ribeirão Preto Blood Center, Ribeirão Preto, SP, Brazil
d Department of Biology, Londrina State University, Londrina, PR, Brazil

Submitted 26 January 2004; revised 21 April 2004
(Communicated by M. Lichtman, M.D., 22 April 2004)
Available online 28 May 2004

Abstract

The stromal cell-derived factor-1 (SDF-1) gene contains a common polymorphism, termed SDF1-3′V, in an evolutionarily conserved segment of the 3′-untranslated region (3′-UTR). We compared SDF-1 genotypes in patients diagnosed with lymphoid leukemias and lymphomas. Since the SDF1-3′V variant deletes the MspI restriction site, PCR-restriction fragment length polymorphism (RFLP) analysis was used for identification of genotypes. We identified the heterozygous genotype (3′VA/wt) in 38.8% (24/62) of lymphoma patients and in 26.2% (11/42) of lymphoid leukemias. The percentage of 3′VA carriers was significantly higher in lymphomas (43.5%) than in lymphoid leukemias (26.2%; P < 0.05). Our study indicates that lymphoma patients from Brazil are more likely to carry the 3′VA gene than patients with lymphoid leukemias, suggesting that this polymorphism may be a differential determinant of lymphomas and lymphoid leukemia.

© 2004 Elsevier Inc. All rights reserved.

Keywords: SDF-1 chemokine; Lymphoma; Lymphoid leukemia

Introduction

The development of a large solid tumor requires not only engagement of blood vessels, but also infiltrating cells that comprise a stroma which promotes tumor growth. Ultimately, tumors spread through the body by metastases, sometimes to a preferred location depending upon their histological origin [1]. Human chemokines are a large family of molecules, characterized by structural homologies based on conserved cystein residues, as well as binding capacities to particular G protein-coupled receptors [2,3]. Four families of chemokines have been described, based on the relative position of the conserved cystein residues—CC, CXC, XC and CX3C—with the CC and CXC groups being by far the largest. Stromal cell-derived factors 1 alpha and 1 beta (SDF1α and SDF1β) are small cytokines belonging to the intercrine CXC subfamily originally isolated from a murine marrow stroma cell line. cDNA and genomic clones of human SDF1α and SDF1β have been isolated and characterized and show that cDNAs encode proteins of 89 and 93 amino acids, respectively. SDF1α and SDF1β sequences are more than 92% identical to their human counterparts. The genomic structure of the SDF1 gene shows that the α and β isoforms arise by alternative splicing of a single gene, and that they are encoded by 3 and 4 exons, respectively [4]. Sequence analysis of the human SDF gene (GenBank accession number L36033) revealed a common variant containing a G→A transition in an evolutionarily conserved segment of the 3′-untranslated region (3′-UTR). This polymorphism designated SDF-1-3′A, is present in the SDF-1β transcripts. Because this variant eliminates the MspI restric-
tion enzyme site, PCR-restriction fragment length polymorphism (RFLP) analysis can be used for rapid identification of genotypes. This variant SDF1 gene allele may play an important regulatory role by increasing production of SDF-1 which bound to SDF chemokine receptor CXCR4 from T cells [5].

The mRNA 3'-untranslated region (3'-UTR) of many genes has been identified as an important regulator of the mRNA transcript itself, as well as the translated product [6].

The present report focuses on distribution of the SDF-1 in cancer patients attending the Cancer Hospital, Londrina, Brazil. Genotyping was performed using RFLP analysis with MspI restriction enzyme mapping.

**Subjects and methods**

**Study population**

Following approval from the Human Ethics Committee of the Londrina State University, peripheral blood was collected from 62 patients with a clinical and histopathological diagnosis of Hodgkin’s lymphoma (HL-18) or non-Hodgkin’s lymphoma (NHL-44), and in 42 patients with a clinical and hematological diagnosis of acute lymphoid leukemia (ALL-31) or chronic lymphoid leukemia (CLL-11).

**Polymerase chain reaction**

Genomic DNA was isolated from peripheral blood cells by a method described by Miller et al. [7] and 100 ng of DNA were analysed by PCR using primers for SDF 3'-UTR-F1 (sense, 5'-CAGTCAACCTGGGCAAAGCC-3') and SDF 3'-UTR-R2 (antisense, 5'-CCTGAGAGTCCTTTGCGGG-3'). Samples were amplified using the kit buffer plus 1.5 mmol/l taq polymerase (Invitrogen, Life Technologies, USA). PCR conditions were 5 min denaturation at 94°C, 35 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C, and 10 min elongation at 72°C in a thermocycler (PCR-Sprint Hybaid). PCR products of 293 base pairs were analysed by electrophoresis in a 2% agarose gel and visualized using UV fluorescence after staining with ethidium bromide.

**SDF-1 genotyping**

PCR products were subjected to restriction digestion by incubating with MspI (Invitrogen, Life Technologies, USA) for 3 h at 37°C and then subjected to electrophoresis in 2% agarose gels. SDF-1 wild-type alleles (SDF1-wt) yielded 100 and 193 base-pair products, while SDF1-3'A alleles yielded a 293 base-pair product.

**Statistical analyses**

Molecular data were analyzed statistically by the Chi-square test ($\chi^2$).

**Results**

In the present study we used PCR-RFLP techniques to examine SDF-1 genotypes in patients diagnosed with lymphoma or with lymphoid leukemia. Demographic data are summarized in Table 1. There were no group differences in age, or gender (Table 1).

![Fig. 1. SDF1-3'A polymorphism in the lymphoma and lymphoid leukemia patients. SDF1 genotyping was performed in blood cell DNA by a PCR-RFLP technique able to detect G-to-A mutation in the terminal region of the SDF-1 3' gene. The rare 3'A homozygotes were mixed with heterozygotes in a group referred to as 3'A carriers. Percentage of 3'A carriers in lymphomas (43.5%) were significantly higher than in lymphoid leukemias (26.2%; chi-square test).](image-url)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic characteristics of patients in lymphomas and lymphoid leukemias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALL (31)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>6–10</td>
<td>8</td>
</tr>
<tr>
<td>11–20</td>
<td>12</td>
</tr>
<tr>
<td>21–30</td>
<td>3</td>
</tr>
<tr>
<td>&gt;30</td>
<td>8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
</tr>
</tbody>
</table>

ALL, acute lymphoid leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin lymphoma; HL, Hodgkin lymphoma.
We found that the 3'A/wt heterozygous genotype was present in 38.8% (24/62) of lymphoma patients, and in 26.2% (11/42) of lymphoid leukemia subjects (Table 2). There were no statistical differences between acute and chronic lymphoid leukemias or between Hodgkin's and non-Hodgkin’s lymphomas. This difference for 3'A carriers from lymphoma patients was found to be statistically significant by the $\chi^2$ test ($P < 0.05$, Fig. 1). For all samples the genotype did not differ from the theoretical distribution given by Hardy-Weinberg equilibrium.

Discussion

Acute lymphoid leukemia, also called lymphoblastic leukemia (ALL), accounts for about 75–80% of the childhood leukemias. In this form of the disease, the lymphocyte cell line is affected, causing overproduction of immature lymphoblasts and crowding out other blood cells in the bone marrow and lymphoid organs. This proliferation interferes with normal function of hematopoietic and immune systems. Chronic lymphocytic leukemia (CLL), the most prevalent adult leukemia in the Western world is a neoplastic disease of advancing age, characterized by a progressive accumulation of functionally incompetent, long-lived small mature monoclonal B lymphocytes.

Non-Hodgkin’s lymphomas comprises many histologically and perhaps biologically distinct lymphoid malignancies, each one with poorly understood but putatively distinct etiologies. The majority of lymphoma cases arise in lymph nodes, but primary extranodal disease accounts for 20–30% of cases [8,9].

Hodgkin lymphoma is a malignancy of unknown pathogenesis. The malignant Hodgkin and Reed/Sternberg cells present a heterogeneous and largely uncharacterized phenotype. The hallmark of Hodgkin lymphoma is the presence of large, mononucleated Hodgkin and multinucleated Reed/Sternberg cells. These cells represent the tumor cells, but usually comprise less than 1% of the cellular infiltrate in the lymphoma tissue [10].

SDF-1 was initially identified as a bone marrow stromal cell-derived factor, and as a pre-B-cell stimulatory factor [11]. SDF-1 is also a highly efficient lymphocyte chemotactic [12] and plays an important role in B-lymphopoiesis and homing of hematopoietic stem cells to the bone marrow [13]. The SDF-1 gene variant which has a G→A transition in the 3'-untranslated region may have an important regulatory function increasing the production of SDF-1 [5]. SDF-1 mRNA is abundantly expressed in stromal cells isolated from the lymph nodes of patients with malignant lymphoma [14].

In this context, 3'A gene carriers individuals may be good candidates for presenting proliferation of neoplastic cells in the lymph nodes, because this mutation can be associated to an increase production of SDF. By contrast, lymphoid leukemia patients carrying the 3'A gene may harbor immature blood cells (blasts) in the marrow but not an overpopulation in the blood. The mRNA 3'-untranslated region (3'-UTR) of many genes has been identified as an important regulator of the mRNA transcript, as well as the translated product [6]. In most cases, translational control mechanisms result from the interaction of RNA-binding proteins with 5'- or 3'-UTRs. A multispecies analysis has shown that in most vertebrates, 3'-UTRs are substantially longer than their 5' counterparts, indicating a significant potential for regulation. In addition, the average length of 3'-UTR sequences has increased during evolution, suggesting that their utilization might contribute to organism complexity [15].

Normal B-lymphocyte maturation and proliferation are regulated by chemokines, and genetic polymorphisms in chemokines and chemokine receptors modify progression of human immunodeficiency virus-1 infection. In the acquired immunodeficiency syndrome, the SDF1-3'A chemokine variant was associated with approximate doubling of the risk in heterozygotes and roughly a fourfold increase in homozygotes [16].

SDF-1 substantially enhances migration of follicular center lymphoma B cells, but not migration of freshly purified germinal center B cells. This difference may be related to the extended survival of follicular center lymphoma versus germinal center B cells. SDF-1 produced in follicular center lymphoma lymph nodes may play a role in the local dissemination of tumor [17].

In our study, the percentage of 3'A gene carriers were significantly different between the two groups of patients studied: 43.5% for lymphomas and 26.2% for lymphoid leukemias. The variant SDF-1 gene allele that results from a 3'UTR substitution may play an important regulatory function by increasing production of SDF-1 by lymph node stromal cells. The present work suggests that this polymorphism may be responsible for different clinical and biological behaviour of lymphomas and lymphoid leukemias.

Acknowledgments

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq, Coordenação de Aperfeicoamento de Pessoal de Nível Superior-CAPES and Coordenadoria de Pós-Graduação-Londrina State University-CPG-UEL.

References


