Lack of Correlation Between the CCR5-Δ32 Mutation and Acute Myeloid Leukemia in Iranian Patients

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Received: 25 February 2013 / Accepted: 16 May 2014 / Published online: 19 June 2014
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Abstract Chemokines and their receptors are crucially important in the pathogenesis of acute myeloblastic leukemia (AML). The CC chemokine receptor 5 (CCR5) is a specific chemokine receptor for CC chemokine ligand 3 (CCL3), CCL4 and CCL5 which all play key roles in identifying cancer properties and localization of leukemia cells. It has been demonstrated that the known mutation in CCR5 gene (CCR5-Δ32) leads to mal-expression of the receptor and affect its function. The aim of this study was to determine the rate of CCR5-Δ32 mutation within Iranian AML patients. In this study, blood samples were obtained from 60 AML patients and 300 healthy controls. The CCR5-Δ32 mutation was evaluated using Gap-PCR technique. Our results showed that CCR5-Δ32 mutation was not found in the patients, while three out of the controls had heterozygotic form of this mutation. The rest of studied samples had the wild form of the gene. According to these

findings, it can probably be concluded that the CCR5-Δ32 is not associated with susceptibility to AML in Iranian patients.

Keywords CCR5-Δ32 mutation · Acute myeloblastic leukemia · Chemokine

Introduction

Iran is reported as a region with low prevalence for AML [1, 2]. Several studies proposed that differences in host genetic and epigenetic settings between AML patients and healthy controls could be responsible for the development of this cancer occurrence [3]. Chemokines are a subgroup of cytokines with several biological effects such as recruitment of leukocytes to the sites of injury/infection, angiogenesis, and angiostatic properties [4, 5]. The CC the CCR5 is a specific chemokine receptor for CC chemokine ligands CCL3, CCL4 and CCL5, all play key part in localization of infiltrated leukemia cells [6]. Therefore, it is plausible to hypothesize that down-regulation of this receptor may lead to low proliferation and infiltration leucocyte progenitor cells in AML. It is now well established that deletion of 32 nucleotides from the exon 1 of CCR5 (known as Δ32 mutation) causes decreased expression and in turn dysfunction of CCR5 receptor [7–9]. More recent evidences revealed that this polymorphic variant is different in populations with various ethnic groups [10, 11]. Thus, based on the possible mechanisms that have been postulated in which down-regulation of CCR5 may results in prevention of leukemic cells proliferation and metastasis. Therefore, the aim of the present study was to determine the CCR5-Δ32 mutation in the Iranian patients suffering from with AML compared to healthy individuals.

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Materials and Methods

Subjects

Peripheral blood specimens were collected from 60 AML patients 300 healthy controls from 2008 to 2012, in Rafsanjan city (southeastern part of Iran), in 5.5 mL EDTA pre-coated tubes. Patients were recruited for the study based on the assessment of their previous clinical history. AML patients and controls were matched for age, sex and socio-economical status. Socio-economic conditions of participants were assessed based on the levels of education and monthly income. The anti-coagulant treated specimens were subjected to genomic DNA extraction immediately after arrival at the laboratory. This study was approved by the ethical committee of the Rafsanjan University of Medical Sciences and written informed consent was obtained from all of participants, either patient or control subject, prior to sample collection.

Genomic DNA Extraction and Polymorphism Analysis

The whole genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using a commercial kit purchased from Bioneer Company, South-Korea, and then the resultant DNA was stored at -20°C for further use. The CCR5- $\Delta 32$ mutation was determined using Gap-PCR technique with the primers which were designed in a manner that could cover complete deleted region. 188 bp fragment showed a wild type gene without CCR5- $\Delta 32$ mutation, while, the presence of a PCR product with 156 bp confirms CCR5- $\Delta 32$ mutation in the CCR5 gene. Furthermore PCR condition, the sequences of forward and reverse primers and gel electrophoresis were described in our previous studies [11–13].

Data Analysis and Statistical Methods

Results were analyzed by T test statistical method and a *P* value less than 0.05 was considered as significant.

Results

Results of this study showed that the CCR5- $\Delta 32$ mutation was not found in AML patients, while only three cases (3 %) of healthy controls exhibited heterozygotic form of this mutation. These results also did not show any significant difference between both groups ($P > 0.1$).

Discussion

Unlimited proliferation of myeloid progenitors is the main feature of AML and, hence, it appears that chemokine/

receptor axes play important role in this phenomenon. The crucial role played by CCR5 is important in recruitment, activation and proliferation of leukocytes, including T lymphocytes and macrophages [12]. Previous studies evidenced prominent roles for CCR5 ligands (CCL3, 4 and 5) in the pathogenesis of AML [14]. Therefore, genetical and epigenetical factors affecting the expression of both CCR5 and its ligands are probably involved in pathogenesis of AML. Our results demonstrated that CCR5- $\Delta 32$ mutation was not associated with susceptibility to AML in a sample of Iranian patients, hence, it can be concluded that this mutation does not contribute to the AML occurrence and pathogenesis in Iranian patients. To the best of our knowledge, present study is the first world report which addressed the CCR5- $\Delta 32$ mutation in AML patients but previous in vitro and in vivo studies demonstrated that, CCL3 (one of CCR5 ligands) inhibited the proliferation of immature hematopoietic progenitors [15]. CCL3 also reported to inhibit osteoblastic function in myeloma in an animal model [16]. The inhibitory effects of CCL3 on proliferation of AML cells were also reported by Owen-Lynch et al., [17]. Compelling evidences have also emphasized that, the expression of CCR5 is disrupted in leukocytes obtained from AML patients leukocytes [14, 18, 19], hence, it seems that CCR5/ligands interaction could be considered as a predominant mechanism which is disrupted in AML and probably is involved in the disease pathogenesis. Again, based on our results, it is likely that CCR5- $\Delta 32$ mutation probably does not affect the CCR5 expression in our study AML patients and other genetical and epigenetical factors such as polymorphisms, methylation and acetylation of CCR5 gene as well as inhibitory effects of micro-RNA can be considered for future investigation.

Approximately all of the investigations have evaluated CCR5- $\Delta 32$ statuses in Caucasoid ethnic groups. However, these investigations were carried out on people living in different countries but with the same ethnicity. Thus, there might be differences with regard to populations, in various parameters such as genetic background or environmental factors. To the best of our knowledge there is not a comparable study in literature either Iranian population or more closed nationalities in neighboring countries including Afghanistan and/or the Eurasian part of the former Soviet Union that explored this polymorphism. In summary, here in we are reporting that we found that CCR5- $\Delta 32$ was not correlated with AML. Moreover, considering these findings at least partially we are only able to conclude that CCR5- $\Delta 32$ does probably not play predominant roles in the susceptibility to AML in this sample of Iranian patients. Further experimental based studies are also deserved to examine and address the presence of this polymorphism in AML patients in either different Iranian ethnic groups or patients in neighboring countries to more

precisely find if there is a relation between CCR5-Δ32 and the disease.

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