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Autologous Hematopoietic Stem Cells transplantation and genetic modification of CCR5 m303/m303 mutant patient for HIV/AIDS

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Abstract

HIV and AIDS is one of the biggest challenges all over the world. There are approximately 34 million people living with the virus, and a large number of them become infected each year. Although there are some antiviral drugs for HIV viral load reduction, they are not sufficient. There is no cure for AIDS. Nowadays natural resistance or immunity has absorbed attentions. Because in some HIV positive patients progression trend is slow or even they indicate resistance to AIDS. One of the most interesting approaches in this category is CCR5 gene. CCR5 is a main cc-chemokine co-receptor that facilitates HIV-1 entry to macrophage and CD4+ T cells. To now, many polymorphisms have been known by CCR5 gene that produces a truncated protein with no function. So, HIV-1 could not entry to immune-cells and the body resistant to HIV/AIDS. Δ32/Δ32 and m303/m303 homozygotes are example of mutations that could create this resistance mechanism. There is a new treatment, such as hematopoietic stem cell transplantation (HSCT) in Berlin and Boston patients for Δ32/Δ32 mutation. It could eliminate co-receptor antagonist and highly-active-anti retroviral therapy (HAART) drugs problems such as toxicity, low safety and side-effects. Now there, the aim of this hypothesis will be evaluation of a new mutation CCR5 m303/m303 as autologous HSCT. This novel hypothesis indicates that autologous HSCT for m303/m303 could be effective treatment for anyone HIV/AIDS affected patient worldwide.

Keywords
hematopoietic stem cell transplantation, autologous, gene therapy, CCR5, m303/m303 mutation, HIV-1, AIDS.
Introduction

Acquired Immunodeficiency Syndrome (AIDS) is a disease of human immune system that CD4+ T cells count have been below 200 cell per µl and caused by infection with Human Immunodeficiency Virus (HIV) [1]. Cc-chemokine receptor 5 (CCR5) is one of the important co-receptors that facilitate HIV entry to the immuno-cells, such as macrophage and CD 4+ T cells [2, 3]. CCR5 belongs to class A subfamily of G-protein coupled family of receptors (GPCRs) that is located on p21.31 region of human chromosome 3 and encoded by the CMKBR5 gene within a cluster including most of the other cc-chemokine receptor genes [4]. Production of this gene is a trans-membrane protein, including 3 section [5, 6]: 1) intra cellular C-terminus containing Serine-threonine residues that regulate receptor with phosphorylation. 2) Seven helical trans-membrane domains with three intracellular (ICL) and three extracellular (ECL) hydrophilic loops. 3) N-terminal end that act as a binding ligand section. MIP 1α,β (also known as CCL3, CCL4), RANTES (also known as CCL5) and other β-chemokine are ligands that binding to NT site in CCR5[6, 7]. After ligand binding to CCR5, cellular signal transduction activated and intra cellular calcium (ca2+) ions increased, causing a process known as chemo-taxis [6]. Despite above introduced ligands, HIV-1 virus enable to binding NT CCR5 at CD4+ T cells and macrophage surfaces [2, 3]. This binding occurs while gp120, an extra envelope virus glycoprotein, binds to CD4, main receptor for attachment, then CCR5 as a main co-receptor, binding M tropism with 218 amino acid in its NT domain with gp120 [8-10]. Then, some signal and factors will be activated that cause gp41, a trans-membrane viral glycoprotein, changed self-formation and fused macrophage and HIV-1 membrane in initial stage [8].

But if a mutation occurred in any part of this receptor gene, may be changed immunoresponse body. For example, one of the prominent mutation in CCR5 is deletion of 32bp (CCR5Δ32) that could be create resistance to infection by HIV-1 in homozygote [6, 11, 12]. The
mechanism of this resistance is follow 32bp deletion in gene segment encoding the second extracellular loop of CCR5. So, 32bp deletion occurred between 794-825 nucleotide sequence of cDNA and caused frame shift mutation after amino acid 174 [6, 11]. Afterward, new 7 amino acids are created and premature termination occurred in codon 182 and protein produced could not present in CD4+ T cells and remain at reticulum endoplasmic and rapidly degrade by proteasome [6, 11]. Therefore, CCR5Δ32 prevents from fusion CCR5 with glycoprotein in HIV-1 surface.

But in heterozygote individuals, HIV stage in slower progression exhibits AIDS [13, 14]. Despite the function of CCR5 in the immune system, it has been associated a number of pathologies including autoimmune disorders, pulmonary disease, transplant rejection, cancer and vascular disease [15]. For this reasons, it would be obvious that CCR5 has an important role in the body system.

Up to date, over 70 mutations have been described in the CCR5 gene [16, 17]. One of the rare mutations which has protective role when exposing to HIV-1 virus is m303/m303 [18]. This mutation exhibit highly resistance to HIV-1 Such as Δ32/Δ32. The T → A point mutation at nucleotide 303 caused an early stop codon in the open reading frame of the CCR5 gene and final product of the CCR5 m303 mutant allele was a truncated protein without any co-receptor function, because first extracellular loop structure was impaired [18]. Therefore, treatment to immune system function improvement will be required for HIV/AIDS patients.

Antiretroviral drugs called co-receptor antagonist, blocking interaction between CCR5 or CXCR4 co-receptors and virus glycoprotein that would protect the host cell from viral entry in spite of prevention of viral replication, transmission and pathogenesis in other cells [19]. Some co-receptor antagonists including modified chemokines (RANTES, ZNF, intrabody, RNAi), monoclonal antibodies, peptides, small molecules (maraviroc, vicriviroc, aplaviroc) have been found mild to severe side-effects [19]. Then this restriction does not allow us to
give them easily to patients. In the other hand, according to WHO announcement since the beginning of the epidemic, almost 75 million people have been infected with the HIV and about 36 million people have died of HIV. Although it is known how to prevent HIV/AIDS, few people have access to prevention tools such as HIV education, condoms, clean needles and programs mother-to-child transmission. In some countries, access remains inadequate for many people. So, more apply treatment/cure for HIV/AIDS patient with safer, lower side-effect and less toxic have been needed [19].

To date, new gene therapy [20-22] and cell therapy [23] approached for some diseases related to immune system. One of the successful treatments for HIV/AIDS patients is both gene and cell therapy with Hematopoietic Stem Cells. Hematopoietic Stem Cell transplantation (HSCT) is the transplantation of multi-potent Hematopoietic Stem Cell, usually derived from bone marrow, peripheral blood or umbilical cord blood. The combination of genetic modification and HSCT may provide the necessary means to develop an alternative treatment option to conventional antiretroviral therapy [24, 25]. In 2009, Hutter et al. described a functional cure for a patient who suffering from acute myeloid lymphoma (AML) and received allogeneic Hematopoietic Stem Cell transplantation from a CCR5 Δ32/Δ32 donor [26]. After four years following transplantation in this Berlin patient, no detectable HIV-1 viral replication was observed in any of the lymphoid tissues [27-29]. In 2010, two HIV-1 patients, known as Boston patients, received allogeneic transplantation from a homozygous for full length CCR5 donor that has been heterozygous [30]. These patient who kept on HAART throughout the transplantation procedure, have been virus free nearly 4 years following HSCT [30]. These findings demonstrate that HSCT could be efficient than co-receptor antagonist drugs.
The hypothesis

Therefore, beside the side-effects of antagonist co-receptor drugs, there is need for a safe and more effective treatment for HIV/AIDS patients. New HSCT treatment (Berlin [26], Boston patients [30]) have shown that stem cell therapy can be reliable method in treatment [24]. Having attention to allogeneic transplantation problems and m303/m303 mutant advantages, the goal of this hypothesis is to evaluate this mutation that can resist to HIV-1 infection and prevent replication virus, CD4+ impairment. With autologous Hematopoietic Stem Cells transplantation and genetic modification CCR5 m303/m303 mutant for HIV/AIDS patient, it is expected that new treatment for this mutation such as Δ32/Δ32 could be efficient and reduce current drugs problems and susceptibility to HIV/AIDS infection.

Evaluation of the hypothesis

To perform the hypothesis the following experiments are proposed:

(1) HSCs will obtain from a patient who has been affected for HIV/AIDS and is a candidate for treatment.

(2) An adenoassociated virus construct (vector) is provided which included our target mutant gene (m303/m303). AAV has combine some advantage of both adenovirus and retrovirus; first ambling to entry divide and non-divide cells, second could integrate to the host genome and producing stable transduction of the target cell [31].

(3) Transduction of vector to HSC patient on ex vivo condition.

(4) Evaluation of mRNA expression with Quantitative-Real time PCR and m303/m303 homozygoute point mutation with amplification-refractory mutation system (ARMS) PCR before transfusion HSCs to patient.

(5) Transfusion of her/his modified gene HSC to own blood stream.
Consequences of the hypothesis and discussion

Since CCR5 is a major co-receptor for the macrophage-tropic strain of HIV-1, a mutation such as Δ32/Δ32 homozygote could be resistant to HIV-1 infection; so, immune response could be changed in the body. With paying attention to incomplete success in co-receptor antagonist drugs, Hematopoietic Stem Cell Transplantation (HSCT) results indicated this novel treatment approach for HIV/AIDS affected could be more effective than co-receptor antagonist drugs; because side-effects, toxicity and another problems that drugs custumers have, would not be saw in Berlin and Boston patients after years. Award this success HSCT treatment, because of resistance mechanism in CCR5 Δ32/Δ32 individuals; we can say another mutation type (m303/m303) could be treatment with autologous HSCT.

In the other hand, allogeneic HSCT need for match donors and maybe required %100 donor chimerism and greater chance of morbidity/mortality due to GVHD and immunosuppressive therapy. Following consume immunosuppressive drugs, tumorgenesis saw in allogeneic HSCT. Therefore, in allogeneic HSCT one of the problems is founding two individuals that their HLA mapping is 100% the same with target mutation such as CCR5 Δ32/Δ32 (like Berlin patient) or m303/m303. But in autolougous HSCT did not see this challenges [32]. In this hypothesis we will hope that autologous HSCT could show effective response for treatment; and m303/m303 will be one of the gene and cell therapy targets for HIV/AIDS cure.

Additionally, distribution of CCR5 m303/m303 homozygote frequency with low rate saw about 1.4% in Europeans population [18]; but did not a document base on prevalence of m303/m303 in Iranian population to found two person for allogeneic HSCT, we thought that autologous HSCT for m303/m303 would be good option. It is also considerable that maximum epidemiologic distribution of CCR5 Δ32/m303 heterozygote mutation has been
only detected in %5.56 (1/18) of France and %2.86 (1/35) of African-American population [18, 33].

For this reason autologous HSCT will be applied for everyone in Iran or other worldwide countries and will not be mandatory to search for HLA-matching and immune response problems.

Conflict of interest statement
The authors have no conflicts of interest to declare.

References


The authors declare no conflict of interest.