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CCR5 as a Novel Cell and Gene Therapy Strategies Based on Induction of Resistance to HIV

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Cc-chemokine receptor-5 (CCR5) is known as a main co-receptor in human immunodeficiency virus-1 (HIV-1) infection. So, it could be a target for inhibition of HIV-1 entry into CD 4$^+$ immune cells. Many studies showed homozygote individual with 32bp deletion in *CCR5* gene had nature resistance to HIV-1. In this manner, recent treatments are focused on inducing this resistance to HIV-1 infected patients with *CCR5*. Berlin and Boston patients transplanted with allogeneic hematopoietic stem cell (HSC) and demonstrated effective cure for HIV-1 infection. In addition, zinc finger nuclease (ZFN) eliminated some problems of Berlin and Boston patients by site-specific *CCR5* gene modification. These recent strategies declined highly-active anti-retroviral therapy (HAART) restrictions such as toxicity, low safety, the side effects following long-term consuming and virus reloading immediately after cut the drugs off. In this review, in addition of introductory biologic and immune-genetic roles of CCR5, we consider novel treatment strategies for HIV-1 infected patient by *CCR5* gene targeted therapy.

Key word: CCR5, HIV-1, hematopoietic stem cell therapy, gene therapy, gene modification, nuclease.

It is demonstrated that a cc-chemokine receptor (CCR)-based targeted therapy can play critical role in more effective cure of patients infected by human immune-deficiency virus-1 (HIV-1)[1-4]. CCRs are a group of G-protein coupled family of receptors (GPCR) and approximately 800 genes have been introduced that encoded functional GPCR and made up about %1 of human genome[5, 6]. It is demonstrated that about half of clinical drugs design for GPCRs, with blocking their ligands or increasing ligands accessibility. So their roles in our bodies are very important[7].

Human GPCRs consist of six families whose major groups are A, B and C and their largest group is A family. One of the most popular subfamilies of class A is CCR5 which for many years has attracted scientists. The vast majority of investigations have focused to provide the novel approaches to the treatment of HIV based on targeted therapy. We have previously suggested induction of resistance to HIV by *CCR5* gene therapy and stem cell transplantation[8, 9]. Here we present most recently discovered approaches to the treatment of HIV, based on CCR5, which may define the better treatment, especially target therapy.

CCR5 genetic basis, structure and molecular signaling

CCR5 is encoded by *CMKBR5* gene located on p21.31 region of human chromosome 3[10]. Protein product of this gene includes 3 sections[11, 12]: 1) A seven helical trans-membrane domain which provide 3 extra-cellular and 3 intra-cellular hydrophilic loops. 2) C-terminal residue that

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regulates receptor by serine-threonine phosphorylation. 3) N-terminal residue that is bound to ligand. MIP 1α, β (also known as CCL3, CCL4), RANTES (also known as CCL5) and the other β-chemokines are ligands that are bound to NT site in CCR5[12, 13]. After ligand binding to NT or 3 extracellular loops, a molecular mechanism activate that cause receptor conformation change and the dissociation of G-protein subunits. Gα-GTP and Gβγ subunits are freed and regulate enzymes activity such as adenylate cyclase, phospholipase C isoforms and ion channels. These enzymes regulate protein kinase function, finally increase intracellular ca^{2+} ions and stimulate chemotaxis[14]. Pharmacologist have named GPCR as "spare receptor" because full biologic response happen after 5% occupancy[15]. Thus GPCRs associate with cell metabolism, growth, migration, differentiation and death of multiple cell type (apoptosis)[16, 17].

Resistance to HIV-1 infection by *CCR5*

Any change in the sequence of gene variants causes alternation in HIV infection or AIDS process, named as AIDS Restricted Gene (ARG). Some of these variants are detected in special group population which causes natural selection and/or genetic drift different ARG polymorphism/ mutation. To this date, more than 35 ARGs have been identified. First of them is *CCR5 Δ32* and[18-22]. This 32bp deletion (*CCR5Δ32*) in the single-coding exon provides resistance mechanism to HIV/AIDS infection[12, 23]. *CCR5Δ32* is caused by replicative

Table 1. CCR5 polymorphisms that affected HIV-1infection

Type of polymorphism	HeterozygoteOr Homozygote	Effect on HIV/AIDS
CCR5*Δ32/Δ32	Homozygote	Create resistance to HIV-1 infection.
CCR5Δ32/WT	Heterozygote	Infected by HIV-1 but delay to AIDS for 2-4 years.
CCR5Δ32/**m303	Heterozygote	Create resistance to HIV-1 infection.

*CCR5Δ32: 32bp deletion in exon 1 *CCR5* gene that cause second extracellular loop be defected and truncate protein created.
**CCR5 m303 (C101X): Transversion of T to A in 303 nucleotide open reading frame that created nonsense mutation in 101 amino-acid at first extracellular loop CCR5.

Table 2. Some of the current drugs for HIV treatment

Drug(s)	Function	Reference
Maraviroc	HIV entry inhibitor (CCR5 antagonist)	[40]
Enfuviritude	HIV entry inhibitor (fusion inhibitor)	[40]
Abacavir, Didanosine, Emtricitabine, Lamivudine, Stavudine, Tenofovir disoproxil fumarate (DF), Zalcitabine, Zidovudine	Nucleotide reverse transcriptase (RT) inhibitors	[75]
Rilpivirine, Nevirapine, Etravirine, Efavirenz, Delavirdine	Non-nucleotide reverse transcriptase (RT) inhibitors	[75]
Raltegravir and Elvitegravir	Integrase inhibitor	[75]
Vorinostat	Histone deacetylase inhibitor	[76]
Atazanavir, Darunavir, Fosamprenavir, Indinavir, Lopinavir/ritonavir, Nelfinavir, Ritonavir, Saquinavir, Tipranavir	Protease inhibitor	[75]
Elvitegravir/Cobicistat/Emtricitabine/Tenofovir	combinations of reverse transcriptase and integrase inhibitors	[75]
Abacavir/Lamivudine	Reverse transcriptase inhibitors (fixed-dose combinations of nucleotide analogues)	[75]
Abacavir/Lamivudine/Zidovudine		
Emtricitabine/Tenofovir DF		
Lamivudine/Zidovudine		
Efavirenz/Emtricitabine/Tenofovir DF	Reverse transcriptase inhibitors (fixed-dose combinations of both types of inhibitors)	[75]
Emtricitabine/Rilpivirine/Tenofovir DF		

slippage by RNA pol due to the presence of a direct repeat flanking the deleted region which leads to elimination of second extracellular loop on receptor. Therefore, HIV-1 enters to bloodstream but could not infect $CD4^+$ T cell and macrophage[12]. A number of studies have shown that individual with homozygote *CCR5Δ32* allele exhibits a natural resistance to HIV and Acquired immunodeficiency syndrome (AIDS)[12, 23, 24]. It is also worth mentioning that heterozygote individual after exposing to human immunodeficiency virus display slower progression to AIDS than homozygote individual for the wild type allele[25, 26]. In Northern Europe, Caucasian population indicate the highest *CCR5Δ32*, 20% of whom are heterozygote and 1% homozygote for this mutation, respectively[27]. Because of these remarkable detections, CCR5 is known as a major co-receptor, even when immune-cells present CD4 on their surface. If CCR5 is not presented on cell surface (following knock-out it or when using its inhibitors) HIV-1could not contaminate cells[28, 29].

CCR5Δ32 is highly resistant to HIV-1 infection, but not completely. Since CXCR4 is another co-receptor for HIV-1 entries. So CCR5 is not the only co-receptor for HIV-1 infection and one of the reasons that HIV/AIDS have epidemiological heterogeneity is genetic variants in host receptor and co-receptors (CCR5 or CXCR4) that HIV can switch itself to per variant[30].

Resistance to HIV-1 infection or delay to AIDS process by Another polymorphisms

In addition to *CCR5Δ32,* Some other mutations/polymorphisms have been discovered which provide the resistance mechanism, such as *CCR5/m303* **(table 1)**[31], *IDH1C*[32]. But although some of the others like *CCR5Δ32* heterozygote are susceptible to HIV infection, they delay the AIDS progression, such as *CCR2 64I*[33], *SDF1 3'A*[25], *HLA B*57* [18], *HLA B27*[34], *KIR 3DS1*[35, 36], *PROX1 Hap-CGT*[37], *ACSM4 A*[38].

Thus, the variant of host genotype may change the function or alternate gene expression after HIV-1 contamination, known as HIV-dependency factors (HDFs). HDFs are needed for HIV-1 infection process, transmission, viral loading and challenge with immune-system[39]. However, host genetic background is a part of factors that affect HIV-1infection. The others are HIV acquisition, immune-system condition and HAART (highly-active-anti-retroviral therapy) results.

Current therapeutic methods: benefits and limitations

To date, several drugs have been introduced for inhibition of AIDS progression, like enfuviritude (T20) and maraviroc. Maraviroc is a co-receptor antagonist blocks interaction between CCR5 and envelope (*env*) protein coating HIV-1 surface[40]. Enfuviritudes act as fusion inhibitor disrupting conformation change in glycoprotein-41 (gp41)[40]. In addition to, highly active anti-retroviral therapy (HAART) is emerging which targets virus enzymes **(Table 2)**. It is showed that ART have significant positive effect on the reduction of latent reservoir virus in immune system at an early stage or immediately after informing of HIV-1 infection. For example, when an infant was burn from a mother that infected by HIV-1, after 30 hours of birth undergone ART and continued until 18 months. In the months 30, no pro-viral DNA or plasma RNA of HIV was detected in peripheral mononuclear cells[41].

One of the problems in HIV-1 treatment by ART is "drugs resistance". This phenomenon mainly caused by new mutation patterns in HIV-1 genes that virus need for its essential proteins like protease[42]. Hence, HAART could not eradicate virus, although reduce its replication significance. In addition, they should be used long time and maybe discontinue of them in any time cause RNA and DNA virus rebounding from latent reservoirs. Moreover, HAART cannot act in different individuals by the same efficiency. High cost and some side effects following long-term therapy are another restriction the use of HAART[43, 44].

Recent treatment strategies are focused on gene therapy especially against HIV and other refractory disease, we present some study in this field previously[8, 9, 45-49]. Here, we present most recently discovered approaches to the treatment of HIV, based on CCR5, which may define the better treatment, especially target therapy.

Novel therapeutics approaches: stem cell transplantation and gene-modification

Novel treatment strategies with stem cell transplantation (SCT) overcome to some problems that observed in ART[8, 9]. Successful allogeneic SCT have performed for acute myeloid leukemia (AML) in a patient co-infected by HIV and HCV and had

been undergone HAART in 2002[50]. Following this, Hutter *et al*. in 2009 transplanted allogeneic (*CCR5* Δ*32*/Δ*32*) stem cell as a treatment for "Berlin patient" who was suffering from AML and HIV infection[1]. The patient was undergoing HAART for 10 years and discontinued them after SCT[1]. Rebounding of HIV may be observed if HAART discontinued because of another CXCR4 co-receptor existence[51], but in this patient after 20 months not observed signs of virus activating or replicating[1]. Hutter *et al*. sequenced CXCR4 patient and checked its variants, but not detected any trail of CXCR4 and concluded HIV-1 in this patient was not binding with CXCR4 as a co-receptor. Number of CD 4^+ T-cells after HSCT for *CCR5* Δ*32*/Δ*32* increased such as normal range in health people and HIV DNA or RNA was undetectable gradually[1]. Result of this treatment showed no existence of RNA or DNA virus after 3.5 years, even when HAART had been discontinued[2, 52]. Likewise, it was reported Berlin patient body remained free of HIV-1 after 5.5 years[53].

In 2012, Henrich *et al*. performed HSCT for two patients, Boston patients, who have heterozygote genotype for *CCR5* (*CCR5 WT/CCR5*Δ*32*) and transplanted with WT CCR5 cells. Result of this study suggested that replication of DNA or RNA virus had been suppressed and HIV-1 reservoir reduced after transplantation[3].

Although results of Hutter *et al*. and Henrich *et al*. made a revolution in HIV-1 treatment, but there were problems such as low frequency of homozygote *CCR5*Δ*32* in population and founding a suitable HLA match donor with target patient. These problems encouraged Duarte *et al*. in 2015 to transplanted hematopoietic stem cell of umbilical cord blood (CB) to a patient infected by HIV-1 from CCR5 $^{-/-}$ donor that have AML[54-56]. HCT from CB not needed to stringent HLA matching like as HSCT with bone marrow. This study showed peripheral mononuclear cells were resistance to HIV-1 infection.

As describe above, artificial disruption methods with "*CCR5* gene modification" were exerted widely and eliminated some problems of allogeneic HSCT[57]. Gene modification is permanent, inheritable and transmitted HIV-1 resistant cells to next generation.

For example for site-specific nuclease, zinc finger protein (ZFP) surveyed extensive. ZFP is a transcription factor that binding to Fok I restriction enzyme domains with their zinc finger motifs and provide zinc finger nuclease (ZFN) [58, 59]. Theoretically, following DSB in DNA by Fok I, repair DNA mechanism applied error prone non-homologous end joining (NHEJ) [60] or homology directly repair by homologous recombination (HR)[61]. Then, small nucleotide deletion or addition observed and result in disruption of reading frame and gene expression. It could engineer with nucleases artificially. Predominant repair with ZFN is error-prone NHEJ.

First in 2005, alternation of *CCR5* with ZFN in *in vitro* condition was showed[62]. Then *CCR5* disruption by ZFN in mouse model was applied and exhibited specify and sufficient *in vivo* functions for induction of resistance to HIV[63]. In this manner, genetic modification with ZFNs utilized in various living organism and cell lines; Including primary T cell, HSC and humanize mice[64-67]. Afterwards, *CCR5* was modified by ZFN with adenovirus vector on *ex vivo* condition and performance was efficiently in healthy and HIV infected $CD4^+$ T cell in 2013[68]. Knock out of *CCR5* by ZFP modification artificially and infusion of autologous $CD4^+$ T cell transplantation showed safety and immune reconstitution with increasing in $CD4^+$ T cells, In 2014[4]. In this study DNA virus level decreased in most patients and RNA virus level were undetectable in one patient and modified T cells for *CCR5* were stable[4].

Another kind of site-specific nuclease is TALEN (Transcription Activator-like Effectors Nuclease). In comparison to ZFN, TALEN has lower cytotoxicity and reduce off-target activity in CCR5 locus. But both of them could disrupt genes about 45% [69]. In comparison to ZFN more delivery problems observed, due to large TALEN protein size. But had been showed that TALEN expressed by adenoviral vector[70].

In addition to ZFN and TALEN, CRISPR/Cas9 is another targeted gene disruption in HIV therapy. Clustered regularly interspaced palindromic repeats (CRISPRs) are short direct repeat (21-47 nt) with vary intervening spacer sequence that surround by CRISPR associated gene (Cas9) in bacteria. When CRISPR is transcribed, pre-crRNA converts to crRNA by RNase III and associate with trans-acting RNA (tracRNA) that diagnose target DNA. Then, this

RNA duplex bind to Cas9 and the crRNA guide complex to target DNA that is complementary to spacer sequence. This ribonucleotide complex cleave target DNA with Cas9 and cause DSB[71]. Some studies suggested CRISPR/Cas9 could efficiently ablate the viral genome from latently HIV-1 infected cells[72, 73]. *CCR5* in primary human $CD4^+$ T cells and $CD34^+$ hematopoietic is targeted progenitor and stem cells and demonstrated ablate viral genes with minimal off-target mutagenesis by CRISPR/Cas9, In 2014[74].

T cell recovery and suppression of HIV-1 are achieved by gene modification and/or cell therapy. Recent approaches provide effective cure of HIV-1 but there are some challenges in *CCR5* candidate for HIV treatment and need to more investigations.

CONCLUSION

Results of many studies have shown that *CCR5* can be a therapeutic target for treatment of HIV-1. A new horizon of stem cell therapy (such as cord blood stem cell) is shifted to obtain more effective and easier methods that can apply for many people with no HLA-matching problems and donor finding restrictions. We suggest identification of another unknown polymorphism to apply more effective treatment of HIV by find out more genetic factors that promote or restrict HIV replication. We think also it would be interesting to study the therapeutic effect of autologous embryonic stem cell transplantation that modify by human artificial chromosome (HAC) and *ZFN* gene. It can cleavages *CCR5* gene specifically and induces the resistance to HIV-1 infection.

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