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REVIEW

Targeting CCR5 for anti-HIV research

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Abstract Highly active antiretroviral therapy (HAART) is the only approach for human immunodeficiency virus (HIV) infection treatment at present. Although HAART is effective in controlling the progression of infection, it is impossible to eradicate the virus from patients. The patients have to live with the virus. Alternative ways for the cure of HIV infection have been investigated. As the major co-receptor for HIV-1 infection, C-C motif chemokine receptor 5 (CCR5) is naturally an ideal target for anti-HIV research. The first CCR5 antagonist, maraviroc, has been approved for the treatment of HIV infection. Several other CCR5 antagonists are in clinical trials. CCR5 delta32 is a natural genotype, conferring resistance to CCR5 using HIV-1 strains. Gene therapy research targeting this mutant has been conducted for HIV infection treatment. A Berlin patient has been cured of HIV infection by the transplantation of stem cells from a CCR5 delta32 genotype donor. The infusion of an engineered zinc finger nuclease (ZFN)-modified autologous cluster of differentiation 4 (CD4) T cells has been proved to be a promising direction recently. In this study, the anti-HIV research targeting CCR5 is summarized, including CCR5 antagonist development, stem cell transplantation, and gene therapy.

Introduction

Human immunodeficiency virus (HIV) attachment to human cells is initiated by the binding of viral gp120 to cluster of differentiation 4 (CD4) molecules on the cell surface. CD4 is the receptor of HIV [1]. To complete the entry, a co-receptor is required for HIV infection. Although many proteins have been identified to act as co-receptors for HIV infection, including C-C motif chemokine receptor 1 (CCR1) [2], CCR2 [3, 4], CCR3 [5], etc., C-X-C motif chemokine receptor (CXCR4) and CCR5 are the two common co-receptors for virus infection to T lymphocytes and macrophages [6–8]. HIV is divided into T-tropic and M-tropic groups according to the common co-receptor usage [9]. The CCR5 gene consists of two introns and four exons, locating in the short arm of chromosome 3 [10]. The CCR5 gene is recognized by two functional promoters. The upstream promoter is called P1. The weak downstream promoter is called P2. The expression product of P1 is two full-length transcript variants. The expression product of P2 is several truncated transcripts which lack exon 1. All the final mature products from P1 and P2 are CCR5 proteins [11, 12]. CCR5 delta32 is a 32-bp deletion in the CCR5 open reading frame, which causes a translation termination with a premature and nonfunctional CCR5 protein [13, 14]. In populations from different ethnic groups, the distribution of CCR5 delta32 is variable. It is usually common in Caucasians but rare in African and Asian people [15, 16]. In European populations, the frequency of CCR5 delta32 is 10–20 % (highest in northern Europe) [17]. The CCR5 receptor has differential expressions according to the polymorphisms in the promoter region [18, 19]. Moreover, a significant difference of allele frequencies is found in different ethnic populations [20–22]. CCR5 is widely expressed in monocytes/macrophages, immature dendritic cells, and memory/effector T cells [23]. CCR5 is a seven transmembrane G protein coupled receptor (GPCR) for three ligands: RANTES (regulated upon activation normal T cell expressed and secreted factor), MIP (macrophage inflammatory protein)-1α, and MIP-1β [24, 25]. Regulating the migration of leukocytes all over the body, CCR5 plays a key role in pathological and physiological activities [26].

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Drug development targeting CCR5

As one of the common co-receptors for HIV, CCR5 is naturally an important target for anti-HIV research. Shortly after the discovery of CCR5 as an HIV co-receptor, anti-HIV research targeting CCR5 was attempted. With chemical modification to RANTES, a derivative was produced. The derivative did not induce chemotaxis. It potently blocked M-tropic HIV infection to diverse cell types, including both macrophages and T cells [27]. Subsequently, a nonpeptide compound with small molecular weight, TAK-779, was found to antagonize the binding of RANTES to CCR5-expressing cells and inhibited the Ca2+ signaling mediated by CCR5. TAK-779 also inhibited the replication of several virus strains, including clinical isolates as well as lab-adopted strains [28]. TAK-779 inhibited the expression of CCR2, CXCR3, and CCR5 mRNAs in mice in another study [29]. TAK-779 was reported to bind to a putative pocket enclosed by transmembrane domains (TMs) 1, 2, 3, and 7 in CCR5. TAK-779 shared Asn252 and Leu255 in TM6 with another CCR5 antagonist, TAK-220 [30, 31]. However, the latter also needed two distinct residues: Gly163 in TM4 and Ile198 in TM5 [32]. TAK-220 showed favorable drug interactions with various antiretroviral agents in vitro, suggesting that further clinical evaluation is required [33]. Five other CC chemokines were found to compete with MIP-1β for binding to CCR5 and were shown to be a weak inhibitor of HIV infection [34]. 4-[(Z)-(4-bromophenyl)-(ethoxyimino)methyl]-1′-[(2,4-dimethyl-3-pyridinyl)carbonyl]-4′-methyl-1,4′-bipiperidine N-oxide (SCH 351125) was reported to be an CCR5 antagonist suitable for HIV infection treatment and orally bioavailable [35–38]. Then, the bioevaluation as well as interconversion studies on rotamers of SCH 351125 as a clinical candidate were conducted [39]. Later, studies identified that human cytochrome P450 enzyme(s) was involved in the metabolism of SCH 351125. Cytochrome P450 3A4 (CYP3A4) mainly mediated the metabolism of SCH 351125 in vitro and cytochrome P450 2C9 (CYP2C9) played a minor role [40]. The nature of benzylic substituent was found to be a critical factor for receptor selectivity [41]. The optimization of the lead benzylic methyl resulted in the production of Sch-417690/Sch-D, which was a potent HIV entry inhibitor. It was tested in clinical trials [41]. Sch-417690/Sch-D (vicriviroc) showed synergistic anti-HIV activity and can be combined with all other classes of approved antiretroviral drugs. Vicriviroc demonstrated higher affinity than SCH-C to CCR5 in competing binding assays [42]. Vicriviroc was metabolized mainly via CYP3A4. CYP2C9, and CYP3A5 (cytochrome P450 3A5) were found to play minor roles in the vicriviroc biotransformation [43]. The safety, pharmacokinetics, and antiviral activity of vicriviroc were studied in a 14-day monotherapy in adults infected with HIV [44] and in healthy volunteers [45]. The vicriviroc was then put into clinical trials for further evaluation [46]. Cyclophilin-18 (C-18) from the protozoan parasite *Toxoplasma gondii* inhibited infectivity of HIV and functioned as a CCR5 antagonist. The structural determinants of anti-HIV activity were approached for further modification of C-18 into an antiviral agent [47]. A spirodiketopiperazine CCR5 antagonist, AK602, showed potent antiviral activity and good tolerance in short-term monotherapy in adults with M-tropic HIV infection [48, 49]. Besides the cases mentioned above, several other CCR5 antagonists have also been reported [50–55]. Maraviroc is the orally administered CCR5 antagonist from Pfizer Inc. and the first promising CCR5 antagonist to be approved for the treatment of HIV infection. A 10-day monotherapy with maraviroc in 63 individuals infected with M-tropic HIV was conducted for the efficacy and safety assessment. The result proved that maraviroc was a viable CCR5 antagonist for the clinical treatment of HIV infection [56]. Maraviroc was finally approved by the Food and Drug Administration (FDA) advisory committee for HIV-1 infection in 2007. Phase II, phase III clinical trials, and post-approval studies were conducted in both treatment-naïve and treatment-experienced patients [57–60]. Reviews of the efficacy, pharmacology, and tolerability of maraviroc in clinical treatments are available [59–65]. As the first approved co-receptor antagonist for HIV infection treatment, maraviroc is effective against most drug-resistant HIV strains when approved. But resistance against maraviroc is developing rapidly [66]. As we mentioned above, several other CCR5 antagonists are in clinical trials or on the way to approval [67]. The drug design targeting CCR5 is a promising direction.

CCR5 delta32/delta32 stem cell transplantation

Populations with the CCR5 delta32 genotype showed lower HIV RNA levels and higher CD4 cell counts when infected by HIV [68, 69]. This indicates resistance to HIV infection [70, 71]. Shortly after the discovery of the resistance of CCR5 delta32 to M-tropic HIV, the mechanism for the generation of the HIV-1-resistant form of CCR5 was approached [72]. An HIV-infected patient was cured after stem cell transplantation, as reported in 2009 [73]. The details are as follows. A 40-year-old man (famously known as the "Berlin patient") with HIV-1 infection for more than 10 years presented to hospital for the treatment of newly diagnosed acute myeloid leukemia. In the last 4 years, the man had been treated with highly active antiretroviral therapy (HAART): 600 mg of efavirenz, 200 mg of emtricitabine, and 300 mg of tenofovir per day [74]. No acquired immunodeficiency syndrome (AIDS)-associated illnesses were observed, with 415/mm^3 CD4 T cell count and an undetectable level of HIV-1 RNA in the patient [74]. A viral rebound of 6.9×10^6 copies of HIV-1 RNA per milliliter occurred when HAART was discontinued for the treatment of the acute myeloid leukemia. HAART was

resumed immediately. HIV-1 RNA reduced to an undetectable level in 3 months [74]. HAART was administered till 1 day before the stem cell transplantation procedure. The man was treated with allogeneic stem cell transplantation [74]. The CD34+ peripheral blood stem cells for transplantation were from a homozygous CCR5 delta32 allele donor with identical human leukocyte antigen (HLA). Engraftment was achieved in 13 days. The man underwent a second transplant with 2.1×10^6 CD34+ cells per kilogram, leading to a complete remission of the acute myeloid leukemia [74]. CCR5-expressing macrophages were detected in the patient 159 days after transplant. Although HAART was discontinued 1 day before the transplant procedure, no active and replicating HIV was detected during a follow-up of 20 months [74]. The CCR5 delta32 allele in the peripheral blood monocytes changed from a heterozygous into a homozygous genotype in the patient [74]. As we know, homozygosity for CCR5 delta32 is significantly resistant to HIV-1, but not completely. The transplant result is remarkable. Although allogeneic stem cell transplantation has been attempted for the control of the HIV-1 infection in other studies, this is the only successful example [74, 75]. The previous transplantation was conducted without regard to the donor's CCR5 delta32 status. The anti-HIV research targeting CCR5 was encouraged by the case.

Gene therapy

Gene therapy usually provides anti-HIV genes to susceptible cells of HIV infection. Anti-HIV genes can be designed for the expression of proteins or RNAs that interrupt the function of cellular or viral proteins or RNAs, thereby disrupting HIV replication [76]. RNA-based gene therapy has been approached for anti-HIV research [76–80]. Gene therapy is a potential treatment to control HIV infection. The cure of HIV infection in the Berlin patient has rekindled interest in genetic engineering strategies to achieve the same result. Significant advances have been achieved in the studies of DNA repair mechanisms and DNA transcription factors interaction in recent years. The cure of AIDS through precise gene editing is now a realistic possibility. Novel gene-editing strategies have been applied for HIV gene therapy [81]. A gene transfer clinical trial was conducted in 74 adults infected with HIV. The patients were treated with a tat-vpr-specific anti-HIV ribozyme in autologous CD34+ cells, indicating that gene transfer via cells is safe enough to be developed as a conventional therapy [82]. Previously studies showed that populations with homozygous CCR5 delta32 deletion are resistant to M-tropic HIV-1 infection [14, 83]. Zinc finger nucleases (ZFNs) were used to generate homozygous CCR5 delta32 genotype de novo. Approximately 50 % of CCR5 alleles of primary CD4+ T cells were disrupted after CCR5 ZFNs transient expression, resulting in a stable, potent, and heritable resistance to HIV-1 infection in vitro and in vivo in an HIV-infected mouse model [84]. Mice that received transplantation of ZFN-modified human hematopoietic stem cells achieved significantly lower HIV-1 levels [85]. A new ZFN that targeted CCR5 delta32 (ZFNCCR5D32) was generated and used for the establishment of cells resistant to M-tropic HIV. The established CCR5 knockout cells were resistant to M-tropic HIV. The new ZFN showed no detectable off-target activity compared with previous studies [86]. Recently, 12 patients received treatment with ZFN-modified autologous CD4 T cells in a single dose. The patients had been treated with HAART for chronic aviremic HIV infection. 11–28 % of the infused 10 billion autologous CD4 T cells were ZFN-modified [87]. A significant increase of the median CD4 T cell count from 448 to 1,517 per cubic millimeter was observed at week 1. CCR5-modified CD4 T cells showed a median concentration of 250 cells per cubic millimeter at 1 week and were estimated to have a mean half-life of 48 weeks [87].

Summary

As the major co-receptor for M-tropic HIV infection, CCR5 has been validated as an ideal target for anti-HIV research. After a long period of development, the first CCR5 antagonist, maraviroc, was finally approved for the clinical treatment of HIV infection in 2007. This stimulates the efforts in looking for new anti-HIV drugs targeting CCR5 and other co-receptors. At the same time, several other CCR5 antagonists are in clinical trials for the assessment of the clinical treatment of AIDS. The populations of CCR5 delta32 naturally confer resistance to CCR5 using HIV-1 strains. This is attractive for anti-HIV research from the very beginning. Multiple approaches have been tried in order to inhibit HIV-1 infection by using the CCR5 delta32 deletion. The Berlin patient is the only case of cure of HIV infection by the transplantation of stem cells from a homozygous CCR5 delta32 donor. Although it is different to conduct this kind of transplantation for HIV infection treatment clinically, this case proves the possibility of the cure of HIV infection via gene therapy by CCR5 gene editing. Recently, two studies were conducted to control HIV infection with ZFN-modified CCR5 delta32 cells. ZFN-modified autologous CD4 T cells were infused into an HIV-infected patient successfully, with exciting results. This greatly encourages the effort in the search for a cure of HIV infection via modified CCR5 delta32 cells infusion in clinical treatment.

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Conflict of interest The authors declare that they have no conflict of interest.

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