Review

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Genome editing-based HIV therapies

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Genome editing (GE)-based HIV therapy is achieved by modification of infection-related genes to produce HIV-resistant cells followed by reinfusion of the modified cells into patients. The ultimate goal is to achieve a functional or actual cure for HIV infection. Despite multiple potential targets for GE-based HIV therapies, *CCR5* is the most feasible owing to the naturally existing *CCR5* δ32 genotype which confers resistance to HIV. A recent clinical trial of infusion of modified autologous CD4$^+$ T cells proved safety and efficacy within the limits of the studies. However, long-term evaluation of the safety and efficacy is required before GE-based HIV therapy is ready for clinical implementation.

A therapy beyond highly-active antiretroviral therapy is needed

Acquired immunodeficiency syndrome (AIDS; see Glossary) is a serious infectious disease characterized by the systemic collapse of the immune system caused by a retrovirus designated HIV-1. The pathogen was identified 2 years after the first report of the disease in 1981 [1]. The HIV-1 genome is composed of two identical copies of single-stranded RNA. The high mutation rate during the replication cycle results in a great likelihood for the development of drug resistance of the virus.

More than 30 drugs have been developed for the clinical treatment of AIDS, and a substantial number of drug candidates are in clinical or preclinical trials, but there is no effective treatment to eliminate the virus from patients [2]. Although a preventive vaccine for HIV would be ideal, developing a successful preventive vaccine for HIV has been a long and complicated process. The only effective therapy used in clinical treatment for HIV infection is highly-active antiretroviral therapy (HAART), a combination of antiviral drugs targeting different steps in the virus life cycle [3]. However, an increasing number of patients have to face the problem that, due to the serious drug resistance, there are no suitable drugs to choose from for use in HAART for HIV infection.

Scientists must develop alternative methods to combat the resistance of the virus to current therapies. HIV attacks mainly human CD4$^+$ T lymphocytes, peripheral blood mononuclear cells (PBMC), monocytes, dendritic cells, and macrophages (Box 1). HIV infection is a complex interaction between virus and host. A substantial number of viral and host proteins are involved in this interaction. Multiple proteins involved in the HIV life cycle have been validated for antiviral drug development. Theoretically, each step of HIV replication could be targeted for anti-HIV therapy (Box 1). The HIV-1 genome consists of nine genes, which are also important targets for anti-HIV research (Box 1). Developing molecular therapeutics for these targets could be viable, but GE-based therapies stand out as a possible alternative to HAART for the treatment of HIV infection.

Numerous studies have been conducted to foster the development of successful GE-based therapies against HIV infection. In 2009, a patient was functionally cured of HIV infection by transplantation of allogeneic stem cells from a donor homozygous for the *CCR5* δ32 allele [4,5]. In the search for a 10/10 allele human leukocyte antigen (HLA)-match donor for stem cell transplants, in most cases, more than one donor and occasionally more than 100 donors are available and the frequency of homozygosity for the *CCR5* δ32 allele is around 1% in Caucasians. There is thus a small, but reasonable chance of finding an HLA-matched donor homozygous for the *CCR5* δ32 allele [6].

Mimicking the natural homozygous *CCR5* δ32 by using GE technologies to induce the mutation is a viable choice.

Glossary

Acquired immunodeficiency syndrome (AIDS): a serious infectious disease characterized by the systemic collapse of the immune system caused by human immunodeficiency virus.

Clustered regularly interspaced palindromic repeats (CRISPR): DNA loci contained in bacterial genomes. CRISPR consists of an array composed of 21–47 nucleotide (32 on average) short direct repeats with various intervening spacers. CRISPR is one component of the pathway used by bacteria to destroy foreign invaders.

Highly-active antiretroviral therapy (HAART): the only effective treatment for controlling AIDS progression. It is a combination of drugs targeting different steps of virus infection.

Hematopoietic stem and progenitor cells (HSPC): blood cells that can differentiate into all other blood cells, including myeloid and lymphoid lineages.

Human induced pluripotent stem cells (hiPSCs): a type of pluripotent stem cells generated from adult cells by introducing specific genes.

Human embryonic stem cells (hESCs): a type of pluripotent stem cells originating from the inner cell mass of blastocysts in the early preimplantation embryo.

Transcription activator-like effectors (TALE): a class of DNA-binding proteins originating from *Xanthomonas*, a plant bacterial pathogen. The N and C termini of TALE proteins are responsible for localization and activation, while the central domain is responsible for binding to specific DNA sequences.

Transcription activator-like effector nucleases (TALENs): a pair of DNA-binding domains from TALE is linked to the *Fok*I endonuclease for binding to the target DNA at opposite sides. TALEs consist of multiple 33–35 amino acid repeats in which each repeat recognizes only one nucleotide. TALENs result in targeted double-stranded breaks which activate DNA damage response pathways. TALENs are widely used in various cell types and multiple organisms including humans.

Zinc-finger nucleases (ZFNs): these nucleases comprise ZF motifs from ZF proteins linked to the nuclease domain of the restriction endonuclease *Fok*1. Each ZF module (30 amino acids) recognizes three sequence-specific nucleotides. Each ZF module array containing 3–6 ZF modules thus recognizes 9–18 bp. ZFNs are widely used in various cell types and multiple organisms including humans.

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The success of the stem cell transplant has encouraged the continued study of GE-based HIV therapies. Based on the findings of DNA repair studies [7], further studies on GE-based therapies against HIV infection have made significant progress in recent years. In this paper I outline why GE-based therapies are promising alternative treatments for HIV infection, and critically evaluate their limitations. The general concerns of GE-based HIV therapies include target selection, the choice of GE technology, and evaluation of safety and efficacy. Although there are many problems to be solved, GE-based therapies have the potential to be applied in clinical treatment in the future.

Advances in GE-based therapies

The technology that underpins GE is crucial for successful therapy, and will make future therapeutic advancements possible. Transcription activator-like effector nucleases (TALENs) [8–14], zinc-finger nucleases (ZFNs) [15–17], and the clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) systems have all been used in GE-based therapies (Table 1) [18–23]. The ZFN system has been the most widely used in GE studies targeting HIV infection [24]. Although there are only a few reports of GE-based HIV therapies using the TALEN and CRISPR/Cas 9 systems, these two GE tools may have great potential.

The major strategy for GE-based HIV therapies is to produce engineered immune cells that are resistant to HIV infection or replication. The possible cell types for modification range from hematopoietic stem cells to dendritic cells and $CD4^+$ T cells [25,26]. $CD4^+$ T cells and $CD34^+$ stem cells are the two groups of cells used most frequently for GE-based HIV therapies. The most frequently used method consists of two steps: modifying the cells *in vitro* then reinfusing the modified cells into patients (Figure 1) [27,28].

Multiple targets have been selected for GE-based therapies (Table 2). The current approaches are mainly classified into two categories: therapies targeting host genes involved in virus infection and replication, such as *CCR*5, and therapies introducing genes that interfere with HIV replication, such as host restriction factors and fusion inhibitors [28–30]. The first category plays a key role. Because CD4, the major receptor for HIV, is very important for the human immune system, deletion of the *CD4* gene is lethal. Several clinical trials involving *CD4* modification for HIV therapies have been conducted, but these studies are no longer being investigated for clinical treatment [31–33]. Although the CD4 receptor may not be an appropriate target, the co-receptors for viral entry are ideal targets for disruption [34].

CCR5

A naturally existing 32 bp deletion in the *CCR5* gene leads to a non-functional receptor, which confers resistance to HIV strains that use CCR5. Cells that are naturally resistant to HIV are usually from donors with the *CCR5* δ32 genotype [35]. Consequently, multiple methods have been developed to construct engineered cells with non-functional CCR5. Since the first modification of *CCR5* with ZFN, many studies have been carried out in this area [36,37]. ZFN-mediated modification of *CCR5* in human induced pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) demonstrated the potential for treatment of HIV infection [38]. Similar studies showed that mice treated with *CCR5*-disrupted hematopoietic stem or progenitor cells (HSPCs) achieved resistance to HIV infection [39,40]. Recently, an engineered *CCR5* mutation near the δ32 region was introduced by ZFNs into TZM-bl cells, which then exhibited resistance to multiple CCR5-tropic HIV strains [41]. Another study provides evidence for the safety of the permanent dysfunction of *CCR5* by modification with ZFN (http://www.clinicaltrials.gov, Phase I by University of Pennsylvania, NCT00842634). In this study, 12 patients with chronic aviremic HIV infection received a single dose of autologous CD4 T cells modified by ZFN. Immune reconstitution and HIV resistance were evaluated by CD4 T cell count and viral RNA or DNA detection [42]. A significant increase in the CD4 T cell count was observed. Blood HIV DNA levels decreased in most patients. Viral RNA was undetectable in one patient (of four patients evaluated) [42].

Sangamo Biosciences has initiated a similar clinical trial (NCT01252641 and NCT01044654) [43]. Another clinical trial with modified *CCR5* in autologous $CD4^+$ T cells is ongoing (NCT01543152) [44]. Similarly, ZFN-modified *CCR5* has been introduced into autologous hematopoietic stem cells for clinical trial [45]. *CCR5* gene disruption by

Box 1. The HIV infection cycle and the HIV genome present multiple targets for anti-HIV therapies

Co-receptor usage can shift in different stages of infection progression (Figure IA). T-tropic HIV uses CXCR4 as the co-receptor for infection of T lymphocytes at later stages of HIV infection, while M-tropic HIV uses CCR5 as the co-receptor for infection of macrophages, monocytes, peripheral blood mononuclear cells (PBMC), and T lymphocytes in early infection and throughout all stages of the infection [76,77]. M-tropic viruses are predominant in the early stages of infection. T-tropic viruses emerge when stable infection is established. Co-receptor usage may therefore shift from one to the other following therapies targeting one co-receptor.

The HIV replication cycle (Figure IB) can be divided into the following stages: attachment and entry, reverse transcription, integration, protein translation, assembly, and release [78]. HIV infection begins with attachment to human cells. Initially, the viral gp120 binds to the CD4 receptor on the plasma membrane. The binding of gp120 to the CD4 molecule results in a conformational change in the viral gp120 that exposes the co-receptor interaction domain. The subsequent interaction between gp120 and the co-receptor leads to fusion of the virus with the cytoplasm membrane [79], the final stage of virus entry into human cells. After entry, the HIV RNA is reverse transcribed into DNA to generate the provirus which is transported to the cell nucleus for integration. Provirus transport is a complex process that involves many host factors [80]. The integration of the provirus into the host genome is also a crucial step for HIV proliferation. The HIV genes located in the provirus are transcribed into mRNAs for protein translation, and the structural proteins that form the virus particle are translated in the cytoplasm [81]. The plasma membrane is the location of virus assembly, and newly assembled viruses are released through the plasma membrane [78]. The HIV-1 genome (Figure IC) consists of nine genes (*Gag*, *Pol*, *Env*, *Vif*, *Vpr*, *Vpu*, *Tat*, *Rev*, and *Nef*). A LTR (long terminal repeat) region is located at both extremities of the integrated HIV-1 genome [82].