Application of Gene Editing Technologies in HIV Infection and Prevention

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Abstract: Human immunodeficiency virus (HIV) causes AIDS by interacting with host receptors like CD4, CCR5, and CXCR4. While antiretroviral therapies suppress viral replication, they don't provide a cure, and drug resistance is a persistent problem. Gene editing technologies, such as CRISPR-Cas9, TALEN, and artificial miRNAs, offer promising solutions for HIV prevention and treatment. By mimicking the CCR5Δ32 mutation, these technologies can block HIV entry into host cells. This review explores HIV infection mechanisms, the role of CCR5 in viral entry, and gene editing strategies to modify CCR5. It also examines animal models for cross-species infection and highlights successful HIV cure cases (the Berlin and London patients). The review provides insights into future gene editing strategies for HIV cure research, focusing on overcoming challenges and advancing treatment possibilities.

1 INTRODUCTION

The human immunodeficiency virus (HIV) is a lentivirus that causes AIDS by interacting with different types of cells in the body and evading the host's immune response to it. HIV is mainly transmitted through blood and reproductive fluids and can be transmitted from an infected mother to a newborn (Levy et al., 1993). The process of infection involves not only the interaction of HIV with CD4 molecules on the cell surface, but also the binding to other cell receptors. Subsequently, the virus fuses with the cell and enters the host cell, where it begins its replication process. After HIV infection, different intracellular mechanisms determine whether the virus triggers productive or latent infection, especially in CD4+ T cells, where HIV replication can lead to syncytial formation and cell death; Other immune cells, such as macrophages, may develop persistent infections, forming a reservoir of the virus.(Moir et al., 2011)Although antiretroviral therapies, such as CCR5 receptor antagonists like maraviroc, effectively inhibit HIV replication and delay disease progression, these treatments do not cure the infection, and drug resistance remains a significant challenge.

In recent years, the rapid development of gene editing technologies such as CRISPR-Cas9, TALEN, and artificial miRNA, has provided new possibilities for the prevention and treatment of HIV. CRISPR-Cas9 technology involves designing a specific guide RNA, which directs the Cas9 protein to the target DNA within the cell. The Cas9 protein, guided by the RNA, then precisely cleaves the DNA, enabling targeted gene modification. TALEN technology consists of two parts: one is the TALE protein, which specifically recognizes and binds to a specific sequence of DNA; The other part is the FokI nuclease, which is able to cleave DNA under the guidance of the TALE protein. Artificial miRNAs specifically inhibit CCR5 expression through RNA interference mechanisms, thereby preventing HIV invasion (Kennedy et al., 2017). The CCR5 receptor plays a crucial role in the infection process of HIV, which enters the host cell by binding to the CCR5 receptor. Therefore, modifying the CCR5 gene to block This pathway is an effective strategy to prevent HIV infection.

The Berlin and London patients are two of the world's most famous cases of being cured of AIDS through mutations in the CCR5 gene. In 2007, the Berlin patient underwent a bone marrow trans-plant with the CCR5Δ32/Δ32 mutation for leukemia, and the HIV virus was completely eliminated after the operation, becoming the first person in the world to

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be cured of AIDS. The London patient received a similar stem cell transplant for Hodgkin lymphoma in 2016 and became the second patient to be cured after the virus was undetected for more than 3 years after the drug was stopped. These two successful cases demonstrate the great potential of CCR5 genetic modification in blocking HIV infection (Shen et al., 2022). With the development of gene editing technology, the modification of CCR5 by means of CRISPR-Cas9 and TALEN has become a research hotspot. The purpose of this review is to deeply explore the application of gene editing technology in the prevention of HIV infection, especially the mechanism of blocking the entry of HIV virus into host cells by modifying the CCR5 receptor, and to discuss its prospects and challenges in practical application, so as to pro-vide a theoretical basis for the development of future HIV prevention strategies.

2 MECHANISM OF HIV INFECTION AND THE ROLE OF CCR5 RECEPTORS

Human immunodeficiency virus (HIV) infection has a unique course characteristic. HIV is mainly transmitted into the human body through blood, semen, vaginal secretions, and mother-to-child transmission. The virus first binds to the CD4 receptor on the surface of CD4+ T cells and then enters the cell via the CCR5 receptor or CXCR4 receptor. In the early stages of infection, the virus spreads less efficiently, but in the acute phase, strong replication occurs and spreads to lymphoid tissues. This is followed by a prolonged chronic phase, which is usually asymptomatic but accompanied by sustained immune activation and viral replication. In advanced stages, CD4+ T cells are significantly depleted, eventually leading to acquired immuno-deficiency syndrome.

The CCR5 receptor plays a crucial role in the invasion of HIV, especially in the acute phase, when HIV enters host cells mainly through CCR5. CCR5 is an important chemokine receptor in the immune system, which is mainly expressed in CD4+ T cells, macrophages and other immune cells. Studies have found that the deletion or mutation of CCR5 receptor (such as CCR5Δ32 mutation) can significantly improve an individual's resistance to HIV, block the binding of HIV to CCR5, and prevent the virus from entering cells, thereby achieving natural immune protection. The CCR5Δ32 mutation causes a loss of function in the CCR5 receptor, and individuals with this mutation are generally less susceptible to HIV infection. Studies have shown that this mutation can effectively block the binding of HIV to immune cells and is an effective natural barrier against HIV, so it has great potential to be applied to mimic this mutation to resist HIV infection through gene editing (Khalili et al., 2017).

3 CRISPR-CAS9 TECHNOLOGY

In nature, bacteria will be attacked by various viruses. Once they survive the attack of the virus, they will record some of the genetic characteristics of the virus and carve it into their own DNA database. If they are attacked by the same virus next time, a large amount of RNA will be transcribed from the virus database. These RNAs contain the genetic characteristics of the virus, which are called guide RNA. Cells also make a protein called CAS. CAS proteins bind to guide RNA to find viruses with this gene signature, and accurately remove the virus's genes to achieve immunity to this virus. Based on the discovery of the bacterial immune system, CRISPR-Cas9 technology artificially designs a piece of guide RNA according to the DNA edited needs, and then introduces the DNA of this piece of guide RNA and CAS protein into the cells. This will produce a lot of CAS proteins and use guide RNA to accurately cleave the DNA that needs to be edited. When DNA breaks, cells look for fragments that are the same as the sequence at the fracture for recombination repair. At this stage, multiple copies of the same DNA fragments at the artificially designed break site are introduced, allowing the cells to use them as a template for recombination and repair, thereby achieving precise gene editing (Jinek et al., 2012).

By manually designing a guide RNA for the CCR5 receptor protein, the DNA of this RNA and CAS protein is introduced into the cell. Then, the CAS protein will accurately cleave the CCR5 receptor protein under the guidance of guide RNA. In this way, the HIV virus cannot recognize helper T cells, thereby achieving immunity to HIV. In Christian L. Boutwell's study, four best gRNAs were tested and found that they were highly editable in T cells, ranging from 52% - 70%, and both effectively reduced CCR5 expression on the surface of CD4+ and CD8+ T cells. The "dual guide" method used to combine two of these gRNAs can effectively edit the CCR5 gene and reduce CCR5 expression. The infection rate of all CCR5-edited CD4+ T cells was

significantly reduced, indicating that CCR5-edited can effectively confer the CD4+ T cells against HIV. Later, mice were used for experiments, and in mice transplanted with CCR5-edited HSPC, CCR5-expressed CD4+ T cells were significantly reduced. The researchers infected mice with high doses of HIV, and the results of CCR5-edited mice showed complete resistance to HIV infection, which strongly demonstrated that high-frequency CCR5 editing can effectively confer protective effects on HIV (Claiborne et al., 2025).

4 TALEN TECHNOLOGY

TALEN technology refers to the use of two parts of DNA recognition domain and endonuclease to form a protein as a gene knockout tool. Scientists have discovered a bacterial protein (TALE), whose dichotomous amino acid corresponds one by one to the four bases. NI recognizes A, NG recognizes T, HD recognizes C, NN recognizes G. Therefore, TALE can be used as a DNA recognition tool to cleave DNA in site-pointed with FokI dimer.(Boch et al., 2014)A study designed two pairs of TALEs for the CCR5 gene, targeting specific regions of the DNA double-stranded, ensuring that the recognition sequence length is 18-20 bp to enhance specificity. It is then combined with FokI to form a protein to accurately knock out CCR5 receptor DNA. The produced protein was injected into CD4+ T cells and hematopoietic stem cells using lentiviral vectors, and the expression level of CCR5 receptors was confirmed through in vitro experiments and the knockout rate was confirmed. Finally, the HIV-1 virus (R5 tropic strain) was introduced into the cells to test their resistance to the virus. The results show that the CCR5 receptor knockout rate exceeds 60%, it is very resistant to HIV-1 virus, the amount of virus is reduced by 90%, and can still maintain stable effects under long-term exposure (Shi et al., 2017).

5 ARTIFICIAL mirNA TECHNOLOGY

Artificial miRNA technology targets and silences the expression of specific genes by introducing specific miRNA sequences. Inside the nucleus, genomic DNA is transcribed into primiRNA and then processed by Drosha enzymes into pre-miRNA, with a length of about 70 - 100 nucleotides. Then, the Dicer enzyme in the cytoplasm cleaves pre-miRNA into double-stranded miR-NA. One strand in a double-stranded miRNA binds to the AGO protein, constituting an RNA-induced silencing complex (RISC) and forming a mature RISC-miRNA complex. RISC-miRNA complex recognizes targets through base complementary pairing of miRNA with target mRNA. After binding to the target mRNA, the RISC-miRNA complex achieves gene silencing in two ways. The first is to inhibit translation, which inhibits protein synthesis by hindering the binding of ribosomes to mRNA or affecting the formation of translation initiation complexes. The second method promotes the degradation of mRNA: attracts nucleases to cleave mRNA, causing it to degrade, resulting in a decrease in the expression level of the target gene (Duchaine et al., 2019). One study used Western blotting to analyze the expression levels of CCR5 protein by transfecting miRNA-103 to CD4+ T lymphocytes, followed by quantitative real-time polymerase chain reaction to detect expression levels of miRNA-103 and CCR5 mRNA, and flow cytometry was used to detect CCR5 expression and HIV-1 infection on the cell surface. Later, through mouse experiments, when miRNA-103 expression was increased, the expression of CCR5 protein was significantly reduced, and when miRNA-103 expression was inhibited, the CCR5 protein would rise. This demonstrates that miRNA-103 can regulate the expression of CCR5 protein. When the CCR5 protein is inhibited, the infection of HIV-1 to cells is significantly reduced (Bellini et al., 2022).

6 RESEARCH PROGRESS OF CCR5 GENE EDITING IN HIV PREVENTION

6.1 Establishment of Animal Models of HIV Infection

Establishing cross-species animal models for HIV infection is essential for advancing our understanding of HIV/AIDS pathogenesis. However, HIV-1 is species-specific and only infects humans and a few non-human primates, making it challenging to study in traditional animal models such as mice. A recent study successfully created a mouse model capable of being infected with HIV-1. By introducing the CD4, CCR5, and CyclinT1 genes into the mouse leukemia cell line L1210 using lentiviral vectors, the researchers enabled these cells to express the

necessary receptors and co-receptors for HIV infection. Fluorescence analysis and sequencing confirmed the significant expression of CD4, CCR5, and CyclinT1 proteins in the transgenic cells, and HIV-1 RNA was detected in the culture medium, indicating successful virus entry and replication. This model provides a critical platform for studying HIV-1 cross-species infection and offers new directions for HIV vaccine development, antiviral drug screening, and further exploration of HIV/AIDS pathogenesis (Karuppusamy et al., 2021).

6.2 Cases of AIDS Cure

The "Berlin Patient," Timothy Ray Brown, became the first person in the world to be cured of AIDS following a bone marrow transplant in 2007. Brown, who was also battling leukemia, received a transplant from a donor with the CCR5Δ32 mutation, which naturally blocks HIV entry into cells. After the procedure, not only was Brown's leukemia successfully treated, but HIV was undetectable in his body, effectively achieving a "double cure." Similarly, the "London Patient," Adam Castillejo, underwent a hematopoietic stem cell transplant for Hodgkin's lymphoma in 2016, receiving cells with the CCR5Δ32 mutation. After discontinuing antiretroviral therapy, HIV remained undetectable in his body for several years, with no recurrence of the infection.

These two groundbreaking cases highlight the potential of CCR5 gene modification for both controlling and potentially curing HIV. However, the procedures involved—particularly bone marrow transplants—are highly complex, risky, and prone to serious complications. Moreover, finding matching donors is exceedingly difficult, limiting the widespread applicability of this treatment. While these cases offer hope and insight for CCR5 gene editing in HIV treatment, they remain exceptional cases and do not yet represent a practical, widely accessible solution. Nonetheless, they provide invaluable direction for ongoing research into CCR5 gene editing and its potential to prevent or cure HIV infection.

7 CONCLUSION

The HIV infection mechanism highlights the crucial role of CCR5 receptors in the virus's ability to enter host cells, making them a key target for potential HIV prevention and treatment strate-gies. Recent advancements in gene editing technologies, such as TALEN, miRNA, and CRISPR/Cas9, have demonstrated the potential to modify or disrupt CCR5, effectively prevent-ing HIV from entering CD4+ T cells. These innovations not only offer a promising approach to blocking HIV infection but also open new possibilities for a functional cure by mimicking the natural CCR5Δ32 mutation that provides resistance to HIV. However, despite these promising developments, several challenges remain in the application of gene editing for HIV prevention and treatment. Issues related to the accuracy of gene editing, off-target effects, and the safety of these technologies must be addressed before they can be widely adopted in clinical practice. Additionally, ethical concerns surrounding gene editing, particularly in human germline modifications, need to be carefully considered and regulated. Ongoing research and clinical trials are essential to refine these technologies, ensure safety, and overcome ethical and legal barriers, ultimately paving the way for gene editing to become a transformative tool in HIV prevention and potential cure strategies.

AUTHORS CONTRIBUTION

All the authors contributed equally and their names were listed in alphabetical order.

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