

A Systematic Review of Gene Editing Therapy for Acquired Immunodeficiency Syndrome

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Abstract. Acquired immunodeficiency syndrome (AIDS) is a deadly immunological disease that affects the immune system and becomes more common in recent years, which is brought on by the inflection of human immunodeficiency (HIV). The treatment of AIDS has always been a major challenge for the medical community to overcome because the existing treatment methods are not universal. At present, the scientific community is focusing on gene editing therapy, and the feasible tools are zinc finger nucleases (ZFNs), transcription activator-like nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9). Since gene editing technology has advanced so quickly, gene editing therapy is now thought to be one of the most promising AIDS treatment. Though these systems are powerful and efficient, there are still several serious challenges that scientists should face. The clinical safety issues have always existed and been controversial, especially after He Jiankui's case that editing AIDS-resistance human embryos coming to the light. And the legal regulations were strongly criticized due to gaps in the bottom line of scientific research. This review summarizes existing applications of gene editing in AIDS with the safety and ethical issues of clinical application, searching for the relevant solutions to the current challenges.

Keywords: HIV/AIDS, ZFNs, TALENs, CRISPR/Cas9, gene editing, ethical issue.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is a deadly viral infectious disease that affects humans and the causative agent for it is human immunodeficiency virus (HIV). It has been over three decades since AIDS was found and HIV was identified. This disease has been suggested horizontal transmission by sexual contact, blood contamination, drug injection, or from mother to infant. HIV transfers through body fluids and attacks the T lymphocytes of the immune system. Many people will not develop any illnesses at the first exposed to HIV virus, while some of whom got infected may have flu-like symptoms such as fever, headache, malaise and enlarged lymph node within several months after infection [1]. In recent years, AIDS has shown a global trend toward a younger population. According to the data extracted from the Global Burden of Disease and the UNAIDS in 2019, over 0.5% of the population is infected. 5,000 new infections per day, 10% of them are children [2].

Currently, no treatment has been found to eradicate HIV from the patients. The most widely used treatment for it is antiretroviral therapy (ART), which combining several medicines to limit the amount of virus in bodies [1]. Unfortunately, it can only suppress virus replication but cannot completely cure the disease. The 10 years survival rate in AIDS patients receiving ART were 61%; yet the survival rates in patients who did not receive ART were 18%. Although ART has reduced the fatality rate of AIDS, access to therapy is not general, the development of a cure and an effective vaccine remains uncertain, and long-term use of ART has a range of side effects [2]. Because of these disadvantages, ART is in fact no longer the preferred choice of treating AIDS. Finding alternative treatments has become a pressing need.

Recently, precision-targeted therapies utilizing gene editing has emerged as an alternative to overcome the limitations of traditional treatments. And the efficiency of gene editing and its potential to enable a wide treatment has attracted the attention of the scientific community. Insertion or removal of target protein genes in specific genomes can provide more control over combating viral invasion. Nuclease-mediated gene editing tools, including zinc finger nucleases (ZFNs), transcription activator-

like nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) have been widely invested in treatment researches of AIDS [3]. This review will illustrate the different gene editing platforms developed so far and the applications that these techniques utilized to treat AIDS, discussing the challenges and outlooks of gene editing applying in routine clinical application.

2. Gene Editing Tools for Therapeutic Use

2.1. ZFNs

Targeted genome editing is made possible by ZFNs (Zinc finger nucleases), which is a class of DNA-binding proteins that trigger DNA double-strand breaks at specific user-specified regions. ZFNs mediated a human chemokine (C-C motif) receptor 5 gene (CCR5), which encoded a coreceptor to entry HIV, modifying in CD4 T cells for protecting the viral infection. The success of the clinical trial proved that ZFN-CCR5 works [4].

In 2008, the first human clinical trial for using ZFNs to modify protein CCR5 in CD4 T cells for curing HIV/AIDS was conducted by Perez et al.. The primary co-receptor for HIV-1 entrance is CCR5, which has a seven-transmembrane chemokine receptor, and also conferring protection to HIV-1 infection is the homozygous 32 deletion in CCR5 [5]. After using engineered ZFNs to disrupt the $\Delta 32$ in endogenous CCR5, the phenocopy of the $\Delta 32$ CCR5 null genotype can also prevent the entrance of HIV-1. As a result, CCR5 has been proven to be a primary target for HIV treatment [6]. Perez et al. claimed the experimental design that generating primary human CD4 T cells which contain the $\Delta 32$ CCR5 null genotype for preventing HIV invasion. Through the preclinical studies, they found that ZFN-disrupted CCR5 cell enrichment by almost three times, comparing with cells which weren't modified by ZFNs in the HIV-infected test (27.5% versus 8.5%) [5].

After Perez's experiment, Tebas et al. successfully demonstrated that CCR5-modified CD4 T cells by ZFNs can efficiently function in response to mitogens, which avoided the HIV infection, and reduced RNA levels in preclinical tests. The estimated even half-life of modified cells is 48 weeks. Study showed it was the viability that was higher of CD4 T cells which the CCR5 protein modified than that of untreated cells. One of the four patients that underwent evaluation showed a result that the HIV RNA in blood became undetectable and in the majority of the patients, the total amount of DNA of HIV was shown dropped. One of the 12 participants in the clinical trial occurred serious adverse event, but the symptoms were attributed to a transfusion reaction associated with experimental drug [4]. It was concluded that the infusion of CD4 T cells whose dysfunction results from treatment of CCR5 receptors by ZFNs is usually safe.

2.2. TALENs

TALENs (transcription activator-like nucleases) are similar with the principle of ZFNs, which are also composed of DNA binding and restriction domain [7]. Nevertheless, a study reported that TALE is more potential than ZFNs because TALE repeats can be assembled and reconstructed basing on targeting 18 740 human protein-coding genes. Scientists intended to use TALENs to disrupt the interaction between integrase and LEDGF/p75, hence interrupting integration [8].

Like CCR5, a therapeutic target for this gene is LEDGF/p75 with PSIP1 in human CD4 T cells [8]. Altered integrase inhibitors (ALLINIs) were identified as a kind of drugs capable of disrupting the relationship between LEDGF and HIV-1 in vitro, thereby impairing the intracellular integration. TALENs were employed by Fadel et al. to eliminate LEDGF/p75 expression by editing specific parts of the human cells PSIP1 gene or exons that code for integrase binding domains. The replication for HIV-1 invasion in these cells was greatly hampered by PSIP1 deletion. They conducted an experiment that showed the mechanism of ALLINI is independent of LEDGF/p75 and that focused deletion of the PSIP1 gene has therapeutic promise for treating HIV-1 illness. Targeted removal of proteins is difficult, but can be achieved with TALENs, which proved its potential in the field of HIV therapy. As Fadel et al. claimed that very few cellular LEDGF is sufficient to interrupt the infection of HIV

[8]. Although LEDGF/p75 showed promising data in stopping viral infections in initial experiments, no further clinical trials proved the feasibility of this design and further studies were in need.

Other experiments are also in progress. In addition to modifying the proviral integration protein LEDGF/p75, researchers have looked at TALEN-mediated editing of host cell genes such the CCR5 and CXCR4 HIV entry receptors. Currently, Romito et al. enhanced the TALEN design and combined it with new RNA transfer methods to very specificity delete CCR5 in CD4 T cells. They showed the stability of CCR5 knockout cells that the reduction of CCR5 was remained stable for 21 days, with 50% reducing of the susceptibility of HIV. However, the clinical test is still lacking [8].

2.3. CRISPR/Cas9

CRISPR (clustered regularly interspaced short palindromic repeat)/Cas9 (CRISPR-associated protein 9) is one of the most promising gene editing techniques. CRISPR/Cas9 is a specific and multifunctional gene-editing system, which can harness to modify and mediate gene editing effectively. The CRISPR-Cas9 accurately cuts DNA, then leaving it to the DNA's own system to recover. The Cas9 helicase can combining with RNA, which is transcribed from host DNA palindromic repeats, and cleaving incursive DNA linked with RNA that is a transcript from host DNA short lengths obtained from additional chromosomal elements [3].

CRISPR/Cas9 was first tested in AIDS treatment in 2013, when Ebina et al. successfully used this technology to target HIV-1 LTR for eradicating the expression of HIV-1 genes in Jurkat cell lines. They measured the capability of CRISPR/Cas9 technology for preventing vital expression through editing the internal DNA of the virus. Transfection of Cas9 and gRNA targeting HIV-1 LTR significantly inhibits Tat protein regulates LTR-driven expression, which is TAR (trans activation response) binding. Basing on their experiment, CRISPR/Cas9 editing LTR-specific components made interference in HIV-1 expression. Two targets were chosen to construct the gRNA-expressing plasmids, and consequently, both of which were shown effective disruption of HIV. And the more effective one decreased the average proportion from 45.6% to 20.0% of the tested positive cells. Essentially, not only did this disruption limit transcriptionally active proviruses, but it also blocks expression of latent integrating proviruses [9].

CRISPR/Cas9 technology was also researching in editing CCR5 genes to block HIV entry into host cells. Comparing with ZFNs, the CRISPR/Cas9 approach is simple in design and plasmid construction and can provide suitable target. Recently, Xu et al. discovered a CRISPR/Cas9 gene editing method with excellent cleavage efficiency and negligible off-target impact on hematopoietic stem/progenitor cells (HSPCs). Additionally, they obtained in vivo that firm CCR5 destruction in animals that were given long-term recombinant and secondary transplants showed notable antiviral effects. Biweekly post-infection, a considerable drop in the HIV-1 RNA levels in mouse blood plasma was seen [10]. Xiao et al. reported that the provides a fundament of using CCR5-modified cells as a clinically effective HIV-1/AIDS therapy and concluded that CRISPR/Cas9 has extreme potential in treating AIDS [3].

CRISPR/Cas9 technology was applied in researching of controlling LEDGF/p75 expression by editing specific parts of the PSIP1 gene. Moreover, comparing to TALENs, CRISPR/Cas9 system has merit in size [9]. Therefore, Lampi et al. used site-specific editing of the PSIP1 gene in HEK293T cells through CRISPR/Cas9 to impair the interaction with HIV while maintaining LEDGF/p75 cellular activity. They tested the LEDGF D366N cells to evident whether HIV virus can replicate. Experimentally, it was demonstrated that cells carrying the D366N mutation gene showed resistance for HIV attack and survived without intracellular viral replication after infection with HIV strains. However, there is still a lack of clinical trial data. While the current process of CRISPR integration of knocked-in genes is extremely less efficient than the knocking out, this problem will be solved with advances in technology.

3. Current deficiencies of gene editing tools

3.1. The ethicla controversy

With the advancement of gene editing technologies, particularly the rapid advancement of CRISPR/Cas9, more incredible breakthroughs in genetic therapy field contribute to the challenges of legal and moral ethics because of laking of regulation. In November 2018, He Jiankui, who was an associate professor at the Southern University of Science and Technology in Shenzhen, claimed that a pair of gene edited twins babies have been born in China, with their CCR5 gene in both modified to naturally defend against AIDS [11]. In this experiment, the team used CRISPR/Cas9 gene editing technology, editing genome with precision, even possible to modify only a single base of the genome. He's team applied this technology to modify the early embryonic genes by injecting the Cas9 protein and specific boot sequence into a fertilized egg to achieve the effect of combating AIDS. However, in 2015, it was identified that gene editing technology will cause many unpredictable and uncontrollable genetic mutations in human embryo [12]. Disruption of unrelated normal genes is likely to lead to a range of serious genetic disorders that can even be passed on to future generations. This may even leaded immeasurable potential risks and hazards to human being.

He's experiment violated a set of ethical principles. In 2003, China has issued a ban on the implantation of embryos for research purposes, He has also violated China's official ethical norms on embryo research. According to the First International Summit on Human Gene Editing in 2015 in Washington, DC in 2015, it is reckless to do human experiment without resolving safety issues and societal consensus. He did not report any animal test and did not emphasize the risks of gene editing as well as off-targeting effects [13]. This violation of the scientific consensus, not only did not reduce the risks of embryo editing, but also infringed on the subject's right to know and the human rights of the twins, which is highly inappropriate on both technical and ethical levels.

Ethicists were also concerned about whether this technology once widely used, could result in artificially designed babies that alter a person's height, intelligence, and appearance, bringing unknown fears and exacerbating social tensions [11].

3.2. Law-making and technology regulation challenges

He Jiankui's case is a reminder to improve the legal regulation of gene-editing technology. Firstly, international community should hold rational attitude towards law and permit genetic research on a case-by-case basis. Preclinical experiments and gene editing of somatic cells are allowed; yet editing of human embryo is not mature, needing to develop more definite regulation methods according to the current process of technology. Additionally, gene encoding technology of legal norm should be analysed in terms of civil, administrative and criminal liability [14].

Secondly, although in China, there were a set of regulations about technology innovation management, real-time tracking was hard to realize due to many limitations due to time, labour, and knowledge aspects, which gave rise to the possibility of the gene-editing babies [15]. Besides providing for gene editing in specialized laws and regulations, a strict legal regulatory mechanism for gene encoding should be established. Government regulation of gene editing techniques should be carried out jointly by health authorities and judicial department. Only perfecting the legislation and legal supervision can ensure the correct usage of gene editing based on law [14].

4. Discussion

In recent years, gene editing technology has broken down many technical barriers, achieved industrial upgrading, and broken through the limitations of current clinical treatments, which are especially an wide range of complex and deadly monogenic illnesses. For HIV/AIDS curing, gene editing technologies provided new therapeutic directions. ZFNs made progresses in targeted encoding the receptor CCR5 that is especially recognized by HIV; TALENs was putting into the research that can disrupt the interaction of integrase-LEDGF/p75 and interrupt virus integration with the research

of CCR5 was also being carried out simultaneously; CRISPR/Cas9 had been used in modifying CCR5 as well as LEDGF/p75-HIV connecting interruption. Moreover, inhibiting HIV-1 LTR expressing by editing TAR was experimented by using CRISPR/Cas9 tools.

Though the essential contribution of genetic engineering technology in the field of AIDS treatment, using gene editing for human cells still faces many challenges. There are several reasons why such promising treatments have never been deployed in large numbers. ZFNs and TALENs are still challenging to design, develop, and empirically test in a cellular environment. That means both development and production cost a lot of money. It is vital that TALENs techniques are still not been used in human test, which reduces people's trust. Thankfully, the CRISPR/Cas9 method has a size advantage over TALENs, and comparing with ZFNs, the CRISPR/Cas9 is also simple in design, plasmid construction as well as it can provide suitable target. As a result, lentivirus vectors have the ability to deliver the CRISPR system, whereas TALENs and ZFNs cannot due to their huge size and repeat sequences [13]. This technology holds promise for reducing costs. the residual off-target effects still bring great resistance to widespread treatment, is still necessary to be addressed through further studies.

Scientific community is still hesitating that the using of gene editing treatments may cause unpredictable hazards due to related ethical and moral issues. Current regulations and laws are not complete, which give rise to some immoral scientific or industries the opportunities to exploit loopholes of laws. There is no possibility of putting gene editing therapy into service without solving the regulation issues. Lacking clinical safety and efficient delivery targeting or off-targeting system, and most essentially, ethical issues have greatly hindered the extension of gene technology in clinical applications of AIDS treatment [3]. Although above questions are remained, it is true that using gene editing technology to treat AIDS is one of the most promising and efficient methods. And how to solve the ethical problems of gene editing of human embryonic cells and eliminate the pressure of public bias on human genetic engineering is also known as the challenge that the scientific community must face.

5. Conclusion

AIDS is a deadly immune disease that tends to be common, which is caused by the inflection of HIV, leading to flu-like symptoms within several months after infection and eventually died of infection due to complete loss of immunity. Current treatments such as ART are either low efficient or not universal as well as high costing. The treatment of HIV is in urgent need of change. The progress of science and technology has brought new hope to the treatment of severe and difficult diseases.

Gene editing therapy, including ZFNs, TALENs, and CRISPR/Cas9, are recognized by scientists as a technology of great research value for the treatment of HIV/AIDS. However, there are several drawbacks of gene editing tools. They are not cheap and had not done the final experiments. The most critical consequence is off-targeting, which can trigger a series of unforeseen and serious consequences. The pressure of public opinion on gene editing technology has never abated. Ethical concerning had reached a new climax when He Jianku's artificial man project was thoroughly criticized and questioned the bottom line of science and put forward higher requirements for the regulation of the law regulations.

However, gene editing technologies still have great hope in AIDS therapy, especially CRISPR/Cas9 system, which brings a complete innovation when it comes to gene editing, greatly eases the burden of cutting genes, and also reduces the possibility of off-target effects to the lowest level so far. Through further research, it can be believed that gene editing tools led by CRISPR/Cas9 can provide better treatment effects for AIDS and other complicated diseases, and find a balance between ethics and scientific research.

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