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The potential of CRISPR-Cas9 in treating HIV: current applications and challenges

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Abstract. Human Immunodeficiency Virus (HIV) and acquired immunodeficiency syndrome (AIDS) it causes are still among the major diseases the affect people throughout the world. Of course, antiretroviral therapies (ARTs) have increased the average life span of patients, but these drugs do not affect latent viral reservoirs, which is why there is a problem of cure. Existing strategies have so far aimed at anti-HIV interventions happening at deoxyribonucleic acid (DNA) level and are currently applicable through CRISPR associated protein 9 (CRISPR-Cas9) gene modification which works towards modifying single genes. CRISPR-Cas9 has relevance in HIV treatment as the deregulate the genomic organization of the virus. However, some limitations include mechanisms through which the virus can evade, consequences on other organisms, and problems in ethical issues concerning gene editing in human beings. This review focuses on such questions alongside analyzing how CRISPR-Cas9 interferes with the HIV genome. In order to provide references to work towards the advancement of using technology in treating diseases such as HIV through gene editing.

Keywords: HIV, AIDS, CRISPR-Cas9.

1. Introduction

Human Immunodeficiency Virus (HIV) is a virus that destroys the body's defense mechanism known as Clusters of differentiation 4 (cells) Where left untreated, HIV may progress to acquired immunodeficiency syndrome (AIDS), a condition whereby the body's antibody preventing system is weakened and which makes the body prone to infections as well as diseases. HIV is a virus, then AIDS refers to the final stages of the HIV disease. While recent treatments for the virus have been developed, millions of people still get infected with HIV, with high incidences across the world. The Joint United Nations Program on HIV/AIDS (UNAIDS) provided the estimates where 38 million people were infected with HIV worldwide in 2022 and 1.5 million new cases were initiated during the year 2022, pointing towards the requirement of useful treatments and potential cures.

At the moment treatment of HIV involves the use of antiretroviral therapies which are commonly referred to as antiretroviral therapies (ARTs) These treatments while managing the virus help in controlling and do not cure it, thus patients are on lifelong antiviral treatment. As such, focus has been geared towards identifying the actual cure and use of gene editing tools including clustered regularly interspaced short palindromic repeats–CRISPR associated protein 9 (CRISPR-Cas9) for HIV cure research.

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Compared to traditional methods, CRISPR-Cas9 provides selectivity in directing action against particular viral sequences within the human deoxyribonucleic acid (DNA). Subsequent to the implementation of HIV into the host genome, CRISPR-Cas9 by a guide ribonucleic acid (RNA) directs the Cas9 enzyme to these specific viral DNA sequences to interfere with viral replication. Current advances reveal that somatic gene therapy can be utilized to remove HIV-infected cells using CRISPR-Cas9, although there are postsurgical complications and questions regarding the morality of such experimentation. This review focuses on such questions alongside analyzing how CRISPR-Cas9 interferes with the HIV genome. However, given that CRISPR-Cas 9 is still under development for other diseases, there is hope that it will be the future approach to the HIV cure and would add a lot of value to the quality of people's lives and medical records worldwide.

2. HIV-1 genome structure and latency mechanism

The different HIV types include HIV-1 which is distributed more in the world mainly due to several genes which are present in HIV important in the life cycle of the HIV virus. Other genes are Gag; this is a gene product that possesses a structural feature that the gene product is used in the construction of the outer layer of the virus. The other gene of HCV is Pol that is involved in replication of the virus through the proteins of reverse transcriptase, integrase and protease. These enzymes assist the virus to become reverse transcribed into the host DNA by assisting the virus to translate its RNA to DNA. Also, from the Env gene, we get the glycopatterns the gp120 and gp41 which gives the HIV the capacity to attach to the host cell. Antigen envelope protein product is gp120 and this interacts with the CD4 in the host cell surfaces in a way that would give the virus a way to attached itself to the cell membrane and merge with it to create an entry point via which the viral RNA could enter the invaded host cell [1].

After entering a human cell or host, HIV-1 unloads its RNA into the host it's planning to infect. This article reveals the enzyme reverse transcriptase converts the RNA of the virus into DNA. The generated viral DNA is then carried to the nucleus of the host cell, and the integrase enzyme inserts the virus DNA into the host DNA called integration. This is the time when the viral DNA becomes integrated with the host DNA and then the virus is called as provirus. Unlike other extra viral particles in the body, the proviral DNA can remain dormant in the tissues of the host, often for long intervals without being easily eliminated by the host immune system. This constant behavior forms a very risky situation because the provirus is capable of generating subclinical viral reservoirs in the different tissues such as the lymphoid tissues, brain and bone marrow. They can rest in reservoirs for years, in fact, it would be incredibly difficult for current treatments to clear the virus entirely from an infected person [1].

The former is even more dangerous because they never manifest in active form and are not affected by ARTs. ARTs have been shown to suppress viral replication in actively replicating cells but are ineffective in suppressing the virus present in these reservoirs. That is why HIV can persist in the body for years, and if ART treatment is stopped, the virus can return to active replication. Of all the mechanisms of latency described here, this type presents the biggest problem to HIV elimination. Hence, identifying ways of how the hidden reservoirs of long-term contributors to the infection can be reached and beaten is important in the search for a preventative solution for HIV [2].

3. How dose CRISPR-Cas9 work

CRISPR-Cas9 is the latest gene-editing tool derived from the bacterial adaptive immune defense machinery. This occurs through the help of a single-guide RNA (sgRNA) that directs the Cas9 endonuclease enzyme to a target DNA sequence of interest and cleaves it. The result of this incision is that the repair mechanism of the cell is triggered, which may cause deletion or changes to be made to the gene of interest [3].

From this discussion, in HIV treatment CRISPR-Cas9 has shown a lot of promise. Speaking only about HIV, it will be feasible to decrease the possibility of its replication by guiding the Cas9 enzyme to remove a part of the virus's DNA sequence [4]. This targeted approach is particularly important because the CRISPR-Cas9 works through targeting HIV sequences without lobbying other regions of DNA. The specificity by which CRISPR-Cas9 treats virus within infected human cells is the basis of

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applying the technology to HIV. This is precise to avoid the probable toxic effects on the normal cells as well as the chances of the virus mutating and becoming a menace to the body.

This specificity is rather advantageous as it may be used to possibly eradication of the original source of infected cells which are not responsive to current ARTs. They remain an issue complicating the fight against the HIV virus since they are carriers of the virus. The ultimate purpose of achieving the goals with the help of application of CRISPR-Cas9 is to eradicate this covert generation, which may contribute to attaining the functional cure [5].

4. Specific applications of CRISPR-Cas9 in HIV treatment

HIV treatments developed back up by the CRISPR-Cas9 system are; the knockout of the C-C chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) which are receptors that facilitate entry of HIV into immune cells. When working with these genes, it is possible to deny the virus the opportunity to enter the cells [6]. A real-life example of this strategy is the "Berlin patient", who was brought back to functional HIV remission after receiving a bone marrow transplant from a donor with a natural CCR5 mutation. While these strategies can lead to profoundly altered cells, CRISPR-Cas9 provides a more minimally invasive approach by modifying the CCR5 gene within the patient's cells.

CRISPR-Cas9's application in modifying CCR5 and CXCR4 receptors has shown promise in blocking viral entry. However, HIV's ability to mutate complicates this approach, as the virus may escape CRISPR targeting through rapid genetic changes. This is because it is hard for the virus to escape multiple targets when multiple single-guide RNAs are used to target the virus. Besides, co-receptor modulation, CRISPR-Cas9 holds other consequences such as editing or eradicating of the viral genome. HIV proviral DNA has been localized and excised by scientists using CRISPR-Cas9. This technique not only inhibits the ability of the virus to proliferate but also provides a means toward functional cure by clearing the virus from the patient's cells [7].

5. Discussion

There are specific advantages and some challenges when it comes to employing CRISPR-Cas9 in HIV treatment. One would always observe a huge problem of viral escape since HIV is a very mutagenic virus. One issue is that the virus can evolve during infection on the sites with vulnerabilities in the CRISPR-Cas9 system. In order to overcome this problem, researchers recommend the use of several single-guide RNAs that cover the entire viral genome so that it is improbable for the virus to mutate and evade the CRISPR/Cas9 system [8].

Another important aspect of the topic is targeted side effects, which is one of the problems with the application of CRISPR-Cas9. It also means that Cas9 can cut through other parts of the genome that are not relevant to the disease and this may cause many complications, risk, to the patients. To manage these risks, scientists are attempting to optimize the CRISPR-Cas9 system through altering single-guide RNA and employing refined Cas9 proteins [9]. These changes are intended to minimize the likelihood of the 'off-target effects,' thereby making it more suitable for medical use.

It is also for some ethical and safety reasons that make it hard to use CRISPR-Cas9 on human beings. There are also latent effects of the gene editing that are still unidentified and patients develop antibodies by components utilized in the editing process [10]. These ethical dilemmas and safety concerns need to be effectively resolved through strict scientific research and various experiments before CRISPR-Cas9 can be implemented in clinical trials.

6. Limitation and outlook

Despite the fact that CRISPR-Cas9 is an innovative approach for HIV therapy, there are several issues which limit the possibility of using this method in practice. One main drawback raised is that HIV undergoes a very frequent mutation thus making it hard for CRISPR-Cas9 targeting since the virus mutates into other strains. This comes with issues of time as the virus may mutate and neutralize the treatment in the long run making it ineffective. Moreover, the issue of off-targeting persists, therefore,

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the Cas9 enzyme can cleave other regions of the genome and cause unwanted damage or genomic instability. This factor of off target mutations further makes it a risky molecule particularly during human trials. This brings an ethical concern of human gene editing especially with respect to the hidden long-term impacts of modifying human DNA. There is also the question with immune responses, as the patients could form oily antibodies against some components of CRISPR-Cas9 system, which would make the problem even worse.

Nonetheless, it is very promising in HIV treatment and other genetic diseases in the future since it has a broader application. Current research area is to optimize the efficiency of the system through selection of the appropriate sgRNA and the definition of Cas9 variants that would have least off-targets. Future enhancement of the vectors used in gene editing in combination with ethical and safety assessment, will shape the basis for the use of gene editing in clinical practice. Its success could help in becoming more than just a HIV cure and treatment but become a multipurpose tool in dealing with viral infections and genetic diseases in this generation.

7. Conclusion

CRISPR-Cas9 has recently gained popularity as a strong candidate for HIV cure, which was earlier attempted by some previous techniques. Another approach and its strength lie in the ability to target and eliminate viral DNA using PCR, as well as eliminate it at the RNA level. Unlike other existing antiretroviral therapies (ART), CRISPR-Cas9 can enter the HIV infected cells and purge the virus out of it and that makes the technology a serious contender for a functional cure. However, some problems involving viral escape off-targeted effects, and ethical points are still significant, preventing its practical application. These concerns should be resolved, to avoid complications where the results are unintended genetic modifications or immune responses. Second, controversy over the morality of human gene editing remains an important issue, therefore all future research should be in conformity to standard ethical protocols.

The advancement made so far with the help of crispr-cas9 indicates that gene editing is likely to alter medicine. Convenience and accuracy are its major advantages, which give hope not only for HIV, but for all other diseases as well. People have pinned their hope on this technology with an expectation that it would lead to marked up improvements in the healthcare systems. CRISPR-Cas9 constantly receives upgrades, thus placing it as one of the potential solutions to the HIV challenge and a solution to enhance health in the world.

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