Proceedings of the 2nd International Conference on Biological Engineering and Medical Science (ICBioMed 2022), Part II
DOI: 10.54254/2753-8818/4/20220662

The Impact of CRISPR and Cas9 on AIDS

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Abstract. AIDS is a chronic infectious disease caused by the human immunodeficiency virus (HIV), and the current treatment for the disease is a combination of highly effective antiretroviral therapy, consisting of a combination of at least three of these drugs to combat HIV infection. However, this therapy only inhibits HIV replication, but does not completely eliminate the virus. Once antiretroviral treatment is interrupted, the patient's disease is prone to relapse. Therefore we need gene editing, which is the process of modifying, adding, deleting and replacing information in the body's DNA in order to modify diseases caused by genetic mutations, which can only be controlled by drugs. It is hoped that in the near future, gene editing technology will revolutionise the treatment of cancer and genetic diseases, thanks to the work of scientists.

Keywords: AIDS, gene editing, antiretroviral therapy, CRISPR, Cas9

1. Introduction

The treatment of AIDS has been a scientific challenge for several years. Gene editing technology is an emerging treatment that can be used to transport the body's normal genes or specific therapeutic genes through a vector to a designated location in the body for treatment at the genetic level, which is not only more effective than traditional methods, but can also achieve complete eradication of the virus in the body. This is because conventional treatments for HIV have produced many adverse effects in most patients and patients are prone to relapse once antiretroviral therapy is discontinued, as antiretroviral therapy is what inhibits HIV replication but does not completely eradicate the virus. This article reviews the impact of CRISPR/Cas9 on HIV, as well as the causes of HIV and the traditional treatment of HIV, antiretroviral therapy. Currently, CRISPR technology has been applied to a range of scientific studies, including precise genome editing and transcriptional regulation, which allows scientists to manipulate genetic sequences "at will". Existing treatments for tumors can be enhanced and improved by gene editing.

2. Introduction to AIDS

AIDS is an acronym for acquired immunodeficiency syndrome (AIDS), a chronic infectious disease caused by the human immunodeficiency virus (HIV), which is transmitted through the exchange of bodily fluids, with unprotected sex and contaminated needles being the main means of transmission. Fortunately, HIV cannot be transmitted by air, water or contact. HIV can infect people of any age, sexual orientation, gender and ethnicity, and it mainly attacks and destroys CD4+ T-lymphocytes, leading to impairment or deficiency of immune cells and/or functions, and eventually to serious opportunistic

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DOI: 10.54254/2753-8818/4/20220662

infections and tumours. In recent years, the HIV epidemic in Asia has been growing rapidly. [1] UNAIDS estimates that by the end of 2020, 37.7 million people will be living with HIV/AIDS, 1.5 million will be newly infected with HIV, and 27.5 million will be receiving antiretroviral therapy (ART) [2].

3. Causes of AIDS

HIV is an enveloped virus with two identical strands of single-stranded RNA, reverse transcriptase and proteins. when HIV enters the body, it does not attack all cells, the proteins on the surface of HIV act as keys that open the doors of only a few cells. But the doors made with the CD4 protein are the main ones that the HIV virus unlocks, and the cells selected are the CD4+ T cells that recognise the antigen and direct the immune system to fight the pathogen. The RNA, which carries the genetic information, uses raw material from its own cells and undergoes reverse transcription. With the action of enzymes, it synthesises its own double-stranded DNA, which is then integrated into the genome of the cell, allowing the entire cellular factory to operate for it, replicating in large numbers and eventually causing its own immune system to become paralysed. If one is infected with HIV, it can damage one's own immune system and, over time, lead to AIDS. Flu-like symptoms usually appear 2-4 weeks after infection and after the initial symptoms have disappeared, most people have no symptoms even without treatment or occasionally have only mild symptoms, an interval of few or no symptoms that can last from 2-15 years. HIV can develop AIDS... associated with opportunistic infections. If the condition is severe it can induce cancer and eventually lead to death.

4. Current treatments for AIDS

The current treatments for AIDS are mainly anti-retroviral therapy, immunomodulatory therapy and drug therapy [3]. We already have effective drugs that can control HIV levels and prevent low T-cell counts from developing into AIDS. With antiretroviral therapy, most HIV-positive patients are able to continue living normal lives and will also be less likely to infect others.

4.1. Antiretroviral therapy (cocktail therapy)

On 19 March 1987, the first drug on the market to treat AIDS, zidovudine, was introduced. When activated, it has a structure similar to deoxyribonucleoside triphosphate, and reverse transcriptase mistakes it for raw material and integrates it into DNA, and the whole process of reverse transcription gets stuck, so he is also known as a nucleoside reverse transcriptase inhibitor, after which non-nucleoside reverse-entry enzyme inhibitors, protease inhibitors, integrase strand transfer inhibitors, membrane fusion inhibitors, each step, each target was gradually developed as an anti-AIDS drug. Although these drugs could only control the number of viruses and did not cure AIDS completely, they did improve the life expectancy of patients. But then it was discovered that the HIV virus becomes resistant in the human body and several of the potent drugs born in the early years lost their effect on the HIV virus. But then a scientist of Chinese descent, Da-Yi Ho, pioneered highly effective antiretroviral treatment, commonly known as cocktail therapy. The combination of three or more antiretroviral drugs is used to target different parts of the HIV reproductive cycle, thereby suppressing or killing the HIV virus. Initially, due to the lack of effective treatment, the mortality rate of HIV patients was extremely high until 2016 when the US FDA approved a total of about 30 drugs for use against HIV infection. therapy (HAA-RT), or cocktail therapy[4].

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4.2. Highly active anti-retroviral combination therapy for clinical use

4.2.1. Safety of HARRT treatment. Twenty-seven patients with advanced HIV I-1 who underwent free HAART, i.e. drug treatment with tenofovir + lamivudine + efavirenz, from May 2011 to May 2014 at Gaoyao People's Hospital in Zhaoqing City, Guangdong Province, were selected for a duration of 1 to 2 years, with an average of 18 months. This was followed up for 6 months to 2 years with regular monitoring of HIV-RNA viral load, CTL (HIV-specific cytotoxic T lymphocyte) response, cD4+ T lymphocyte count, routine biochemical indicators and clinical observations[5].

Table 1. Statistics on adverse reactions of patients after taking the drug [1].

Adverse reactions	Gastrointestinal reactions	Abnormal fat metabolism	Skin lesions	Abnormalities of the blood system	Liver injury and hyperlactate blood cancer
Number of examples	17	3	11	3	18
Percentage%	62.96(17/27)	11.11(3/27)	40.74(11/27)	7.41(2/27)	66.67(18/27)

The majority of patients experienced a number of adverse reactions during cocktail therapy, the main ones being gastrointestinal reactions and liver damage and hyperlactatemia. The main adverse effects were: gastrointestinal reactions and liver damage and hyperlactatemia, accounting for 62.96% and 66.67% of the total.

4.2.2. Efficacy of HARRT treatment. The changes in HIV-1 viral load before and after 6, 12, 18 and 24 months of treatment and the changes in CD4+ T cell count before and after 6, 12, 18 and 24 months of treatment were compared in these 27 patients.

Table 2. Change in plasma HIV-1 viral load of patients after treatment (n/%).

Treatment time	6 months	12 months	18 months	24 months	Control group
Proportion of HIV viral load <50 copies/mL 1%	3/27 (11.10)	7/27 (25.90)	11/27 (40.70)	19/27 (70.40)	1/27 (3.70)
X^2	1.08	5.28	10.71	25.73	−
p	> 0.05	< 0.05	< 0.05	< 0.05	−

Table 3. Changes in the number of CD4+ T cells in the beneficiaries after treatment (n=27,$\bar{x} \pm S$).

Treatment time	6 months	12 months	18 months	24 months	Control group
CD4+ T cell count($amount/\mu L$)	182±74	205±92	243±117	276±129	143±35
t	2.48	3.27	4.25	5.17	−
p	< 0.05	< 0.05	< 0.05	< 0.05	−

The results showed that: As the cocktail therapy progressed, the HIV-1 viral load in the plasma of 27 patients gradually decreased, and the difference in the data after 12 months was statistically significant ($P < 0.05$), and the CD4+ T cells in the patients gradually increased, and the difference in the data from

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6 to 24 months was statistically significant ($P < 0.05$), because CD4+ T cells are an important type of immune cells in the human immune cells, and since it is the CD4+ T cells that the HIV virus attacks, the results of their testing play an important role in determining the efficacy of AIDS treatment. Dr Jeremy Luban, Professor of Molecular Medicine at the University of Massachusetts Medical School, said: "The cells that HIV infects are permanent crops of the viral genome. If a patient stops taking antiretrovirals, at any point they are a ticking time bomb that can relapse." [6] So antiretroviral therapy can inhibit HIV replication, but it does not completely remove the virus, and the HIV-1 virus persists in the host cells, creating a latent HIV reservoir. Therefore, once antiretroviral therapy is interrupted, the patient's disease is prone to relapse.

5. Disadvantages of the combination of highly active anti-retroviral therapy approach

There are two difficulties with this approach at present. Firstly, HIV-positive patients must remain on medication for the rest of their lives and if they stop, HIV will deteriorate. Those clinical drugs most commonly used stop the virus from genetically replicating and entering the host cell DNA, and others stop the virus from mutating and assembling so that the HIV virus cannot infect other cells in the body, but the HIV virus can hide in places where the current drugs cannot reach, such as in the DNA of healthy T cells, most of which die rapidly after being infected by HIV, but a very small percentage is used as a site for building more HIV virus and will remain dormant, sometimes for years. Thus, even if we were able to remove every single HIV virus that infects the body, one such T cell could be activated and retransmit the virus. Another difficulty is that not everyone in the world has access to drugs that could save their lives.

HIV-1 DNA persists in the host cell and is integrated into the host cell genome, forming a latent HIV viral reservoir. Once antiretroviral therapy is interrupted, the patient's condition is prone to relapse.

It is quite effective in early-stage AIDS patients but is of little help in patients with intermediate to advanced disease, whose immune systems have been irreversibly destroyed by HIV. It is also expensive and not affordable for the general public. The Chinese scientist Ho Tai-yi agrees that drug treatment is very difficult, not only is it complicated to take and has side effects, but it is also expensive and since the cocktail is a mixture of various drugs, the size of the side effects depends on the specific drugs the patient is taking. For example: adverse gastrointestinal reactions, abnormal fat metabolism, and skin lesions. However, if the medication is stopped, the virus can return even if there is 0.001% of the virus left.

6. Why gene editing is needed

Gene editing is the process of modifying, adding, deleting and replacing information in the human DNA in order to modify diseases caused by genetic mutations that can only be controlled by drugs. Gene editing can be done at the source, by finding the wrong DNA fragment, cutting it out with a pair of molecular scissors and either eliminating the wrong gene or filling in the gap with the correct DNA fragment. Back in the 1990s, scientists discovered a number of proteins that could cut through DNA, each with its own specific structure that could only be combined with a specific DNA fragment to precisely cut through the target gene, but if scientists wanted to cut through another DNA, even with a small change in sequence, it would take a long time to redesign and reassemble the new protein. This is a complex and time-consuming technique. It was not until 2021 that Jennifer Doudna and Feng Zhang found miracle scissors, cas9, which do not require the design of new protein scissors to cut a gene, like the protein scissors of the past, crisper from start to finish, it only needs universal protein scissors, cas9, plus a guide RNA, to be able to cut all the DNA. If the target gene is changed, it is only necessary to order a new RNA, not to redesign a complex protein. This lowers the technical and price barrier considerably.

7. How CRISPR/Cas9 works

CRISPR/Cas9 is a defence system in bacteria that can deal with viruses that invade the bacteria and inject their own DNA into the bacteria; the Crisper is the equivalent of a database that stores the virus'

DOI: 10.54254/2753-8818/4/20220662

information, its DNA fragments, and the Cas is the equivalent of a weapons factory that produces various proteins, think of them as different types of scissors. Whenever a new virus attacks, the protein scissors run out and cut its DNA and take a small piece of it back to the library (the crisper) for storage. They then cut off a small piece of DNA from the virus and combine it with their own DNA, and then insert a piece of DNA that is different from their own in order to recognise it, but for immunity against the virus. They then add a repeated sequence before and after it, and in between these two repeated sequences is the sequence of the virus, indicating that they have recognised the virus. The DNA is then transcribed into a guide RNA, for example, if the virus has a T base then the RNA is A and if the DNA is G then the RNA is C. The guide RNA will use this complementary relationship to compare the new virus with this fragment, and if it is complementary, then the new virus is the same virus. There is a protein cas9 that can cut the DNA and it will catch this segment of RNA, so that when the same virus attacks again, they can quickly match the enemy and then scissor the DNA of the other side to destroy the infestation. This bacterial immune mechanism is called CRISPR/Cas9, but this gene editing process is error-prone.

With the advent of CRISPR/Cas9 gene editing technology, scientists have gained the powerful ability to modify the genome and can use it to make cancer models with various gene mutations to study the mechanism of cancer development and screen therapeutic drugs; they can also use it directly to edit oncogenes or oncogenic viruses to treat cancer; and they can use it to edit immune cells to treat cancer through immune cells[7] .

Its ability to act not only in the treatment of AIDS itself, but also through the unique mutations of various types of tumours, triggering the body's immune system to recognise them, thus effectively preventing the occurrence of various cancers [8].

8. Current problems with gene editing technology

8.1. Off-target effects

This is because CRISPR/Cas9 technology itself has a high tolerance for errors. If there is a discrepancy between the guide RNA and the DNA of the target gene, it is likely that the Cas9 protein will continue its shearing work and cut out other genes in the body, as the DNA will be repaired after being cut, but this process is prone to errors, such as the loss of some accessories, which will lead to the original gene to fail, which is also known as an off-target phenomenon[9].

The current application method has found a large number of off-targets in mice. The genomic sequence in the normal human body is very long, and when the enzyme is targeted to find AIDS-related gene fragments, it will erroneously find some human normal fragments and cut them off, which is actually very dangerous because the human normal sequence it has a certain role in human physiological functions, so that when the AIDS virus is cut off When the HIV virus is cut out, if some unknown genes are cut out, you may not notice anything different at the moment, but afterwards you may cause some irreparable damage to your own body.

8.2. Ethical issues

Because of the unique nature of CRISPR/Cas9 gene editing technology, which can permanently alter traits through gene editing, this gene editing technology has challenged human ethical concepts throughout its development. The Associated Press reported on 26 November 2018 that Chinese scientist He Jiankui's modified CRISPR/Cas9 gene editing twin babies had been born that month, news that immediately sparked national and worldwide attention and discussion. At present, most scientists believe that the technology is still inadequate to be used to modify the human genome, and that it is not only unscientific to attempt high-risk genetic modification experiments directly on human individuals, but also a violation of the ethical rules that scientists are supposed to follow. [10] Because once gene editing can be used to modify the genes of future generations, it could divide us into two groups: decision-makers who decide how genes are modified, and instrumental beings who are forced to look like them. Then humanity would become a commodity.

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9. Conclusion

Gene therapy is an emerging therapeutic tool that can be used to transport normal genes or specific therapeutic genes through a vector to a designated location in the body for treatment at the genetic level, which is not only more effective than traditional methods, but can also achieve complete eradication of viruses in the body. In recent years, gene therapy has been used in the treatment of genetic diseases, cardiovascular diseases, infectious diseases and malignancies. Although there is currently little clinical experience with gene therapy in the treatment of AIDS, some scholars have found through research that CD4+ T lymphocytes that have been genetically modified are more stable than the original, and that gene therapy has a solid theoretical basis in the treatment of tumours, which shows that gene therapy is effective and bright for both the HIV virus in AIDS itself and for malignant tumours complicated by AIDS. This suggests that gene therapy has good utility and a bright future, both for HIV itself and for HIV-associated malignancies. [Since its development as a genome editing tool, CRISPR/Cas9 gene editing has revolutionised biology by providing a simple and versatile way to manipulate the genome, transcriptome and epigenome. The potential of CRISPR/Cas9 gene editing technologies in the field of basic and translational medical research on tumour mechanisms, drug target screening and clinical therapy is beginning to emerge [12].

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