Acta Scientific Biotechnology

Volume 1 Issue 10 October 2020

Mini Review

CRISPR Technology: A Potential Cure for HIV

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Received: September 04, 2020

Published: September 30,2020

Abstract

HIV is a virus that effects the immune system of the body which in turn increases the risk of attaining an infection and diseases. It has no cure till now however some treatment can help like antiretroviral drugs and an individual can lead a normal life not a healthy reproductive life.

However, there are some cells which have a mutation CCR5-delta 32 which are immune to HIV virus. It was found in the bone marrow of a donor which was then transplanted in a patient known as "Berlin patient". These cells show a great potential of curing HIV but still there are some rare cases in which the individual were infected by HIV virus although they had two copies of CCR5 mutant cells form each of patients. So, researchers used CRISPR technology to gene edit normal cells into CCR5 mutant cells and inserted in the patient body to cure HIV.

Keywords: CRISPR Technology; HIV; CCR5 Mutant Cells; CCR5-Delta 32

HIV

Human immunodeficiency virus (HIV) is a retrovirus that attacks CD4 cells also known as helper T cells to take over the host machinery and make new virus particles. It initially attacks a cell that has a CD4 receptor which is found on the immune cells such as helper T cells that helps in activating the immune system to fight infections and eradicate the foreign particles. Initially the virus was present in a chimpanzee in Central Africa as the source of HIV infection in humans but it was a different kind of virus which was called as "simian immunodeficiency virus".

Structure

HIV virus is encapsulated within a lipid membrane which is derived from the host cell membranes. There are two proteins present in the lipid membrane:

1. Transmembrane protein-GP41: It is a glycoprotein which helps in fusion of virus lipid membrane with the host cell lipid membrane.
2. GP120: It is a glycoprotein which binds to the CD4+ receptor of the target cells. It helps in primary attachment of the virus and the target cell.

However, the virus undergoes series of action after entering into the helper T cells to take over the host machinery. For infection CD4+ receptor is the primary receptor that HIV needs to infect the helper cells (T-cells) but there is also a co-receptor (CXCR4 and CCR5) present on the membrane of the host cell to either of which the HIV virus binds. CCR5 is preferred as a co-receptor. When GP120 binds with CD4+ receptor then it induces a conformational change in the CD4+ receptor and allows the co-receptor (CCR5 or CXCR4) to grab a hold to the viral membrane and fuses this membrane and T- cell membrane together. This fusion allows HIV capsid that contains its genetic material (RNA) to enter the CD4 cells.

Then the protease present in the host cell interacts with the capsid protein P24 due to which the viral DNA becomes free and by using the enzyme reverse transcriptase the genetic material of virus (i.e. RNA) converts to DNA and inserts its viral DNA into cell's DNA to make "New viral DNA" with the help of integrase enzyme which is too released by HIV virus. The new viral DNA is also called as "Proviral DNA". Once integrated the HIV virus uses the machinery of CD4 cells to replicate its protein to make viral proteins.

Now the newly formed RNA proteins move to the cell surface and a new immature HIV is formed. Then finally the virus is released with the help of viral protease which cleaves the long protein chains in the immature virus and form mature virus which spreads the virus [1].

Detection

HIV can be detected by ELISA (Enzyme linked absorbent assay) which detects the HIV virus in the bloodstream of an individual. But there is no cure currently available for HIV or AIDS. An individual diagnosed with HIV virus at initial stages he/she can lead to a normal life by "Antiretroviral Therapy".

In antiretroviral therapy the "antiretroviral drugs" are used which doesn't actively kill the HIV virus but it blocks it targets and blocks different stages of virus life cycle.

Over the years the detection of HIV has become more accurate. But initially it wasn't the case. Initially, there were 1st generation HIV antibody tests in which the virus infected tissues were used to detect IgG antibody to HIV-1 only. The antibiotics negative window was up to 12 weeks. Then there were 2nd and 3rd generation HIV tests which produced more accurate results in comparison to 1st generation.

In 2nd generation the recombinant antigens were added to which HIV-1 and HIV-2 antibodies were detection. The addition IgM detection resulted in 3rd generation HIV tests which reduces the antibody -ve window to approximately 3 weeks post infection. But most advanced tests are 4th and 5th generation HIV test which is made by adding up p24 antigen these tests reduce the number of false positive results [2].

CCR5 gene

CCR5 gene that encodes the CCR5 protein is located on the short (p) arm at position 21 on chromosome 3. CCR5 is a type of chemokine. The first chemokine which was discovered in 1977. On the basis of the structure and number of cysteine residues.

Chemokines are classified into C, CC, CXC, CX3C and we write 'R' along with the chemokines which shows the indicator. There are total seven transmembrane domains and CCR5 is a C-C chemokine receptor type 5. It is homologous to CCR2 but both have different ligands and show different effects.

Along with CCR5 other chemokine receptors work together to regulate T- cells functions. In 1996 it was discovered that CCR5 gene play a vital role during HIV replication. It behaves as a co-receptor using which HIV virus enters and degrades the level of healthy helper T-cells.

It is present on the white blood cells (WBC). But the mutation which is called CCR5-delta 32 mutation in the CCR5 gene tends the CCR5 receptor which is present on the WBC's hampers the ability of HIV virus to enter the cell. If an individual has only one copy form the parent (heterozygous condition) reduces the HIV infection to some extent and delays the progress of AIDS. If an individual inherits a copy of mutated CCR5 gene from both of the parents (homozygous condition) prevents immunity against HIV virus.

However, another chemokine receptor which behaves as a co-receptor for HIV virus is CXCR4 (C-X-C chemokine receptor type 4).But HIV virus enters into the cells by using different receptors rather than CCR5 receptor so an individual even containing two copies of mutated CCR5 gene are infected with HIV [3].

CRISPR technology

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. This technology is used to target specific stretches of genetic code to edit the DNA at specific locations, drug development, agriculture, mutagenic animals, treating humans with genetic disorders etc. This system is found in bacteria which prevents itself from invading viruses.

It serves as an adaptive immunity for a bacterium against pathogens like bacteriophage. When a virus invades the bacteria in normal conditions the bacteriophage is able to kill the bacteria but due to the tendency of bacterial chromosome to fuse with the viral genome prevents the death of the bacteria. Now the bacterial chromosome has a component of the viral genome due to which the bacteria is able to produce immunity against the bacteriophage. Bacteria produces Cr RNA (CRISPR RNA) along with cas proteins and they from a complex to carry out this process. Cr RNA binds with the specific 20 nucleotide sequence of the target DNA with the help of guide RNA also called as spacer that is complementary to the target DNA. Cas 9 is an endonuclease which acts as a molecular scissor that cuts the target sequence where PAM (protospacer adjacent motifs) sites are present. These sites are NGG sequences, where "N" stands for any nucleotide and they are found 3 - 4

nucleotides downstream from the cut site. Cas 9 is a multidomain endonuclease that contains two distinct sites-an HNH-like nuclease domain that cleaves target strand and an RuvC-like nuclease domain which cleaves the complementary strand (non-target strand).

On the basis of Cas protein used types of CRISPR Cas systems are of six types (I-VI) each of them uses different Cas protein complexes or a single cas protein. Among them type II is the efficient one in which tracr RNA (trans activating CRISPR RNA) is also present that stabilizes the complex. It forms a loop by interlinking with the Cr RNA and when the target site is recognized by the Cr RNA then it gets cleaved by RNase III. Once the complex is in this nucleus then it binds with the PAM (Protospacer adjacent motif) which is found 3 - 4 nucleotides downstream from the cut site. It helps Cas9 to cut the DNA. Initially after binding the Cas9 unzips the DNA and match the sequence. If the match is complete then Cas9 use two molecular scissors to cut the DNA. At the end double strand breaks (DBS) are formed and the host DNA repair mechanism try to repair it by using Nonhomologous end joining (NHEJ) that causes Frameshift mutations and Homology directed repair (HDR) that causes Precise point mutations because this process is error prone which leads to random mutations.

This can be done in stem cells which can give rise to many different cell types and also be done in fertilized eggs to make targeted mutations.

However, CRISPR system can also work with Cpf1 enzyme forming CRISPR-Cpf1.

Cpf1 is different from Cas9 in many ways like:

- It requires only a single RNA for making the complex.
- It is smaller than Cas9 that is easier to deliver into cells and tissues.
- It cuts the DNA in different manner unlike Cas9 it cuts the both the strands at the same place.
- It has different PAM sites however, it also requires a PAM site initially and the target site is chosen adjacent to the PAM site [4,5].

Using gene edited cells to treat HIV

In 2007, Timothy Roy Brown a patient who was diagnosed with leukaemia needed a bone marrow transplantation to cure its leukaemia. The donor who gave him bone marrow had a mutated CCR5 gene which was immune to HIV virus. However, this man was also a HIV +ve patient and the doctors were confident that after replacing its bone marrow its HIV cells will be replaced by the immune cells due to presence of mutated CCR5 gene.

Researchers tend to use CRISPR technology to engineer HIV resistant blood stem cells so that it can be used more widely. So, they experimented on a 27-year-old man in China who had leukaemia and HIV and required a bone marrow replacement. They took the bone marrow cells and used CRISPR-Cas9 to change them into mutated CCR5-delta32 cells.

Initially, researchers found it difficult to transform CCR5 mutants. They were able to only edit 17.8% of donor stem cells. But for safety purposes they mixed edited and non-edited cells so, that the leukaemia could also be treated.

The researchers should be cautious while using CRISPR technology because it causes mutations in cells which could be proven disastrous for the patient.

Then the patient was diagnosed and it was seen that immune cells were only 5 - 8% of the patient's total stem cells. Which meant that more than half of the CCR5 mutant cells died in the patient's body. However, the patient's leukaemia was in remission but he was still infected with HIV. It proved that 5% of CCR5 mutant cells were not enough to lower the viral load. Positives of this trial was that the patient didn't suffer any side effects. This was believed as a potential cure for HIV patients but only after further research in this area [6].

However, there were some researchers who found out that these CCR5 mutant genes when inserted reduces the life expectancy. An individual carrying two copies of disabled CCR5 are more likely to die before 76 comparing to people having only one disabled CCR5 gene. The researchers said that these people were more prone to having diseases like influenza, West Nile virus etc.

The CCR5 gene which allows the HIV virus to enter CD4 cells they controls the longevity of an individual and when it is mutated they leads to promoting some diseases like Crohn's disease and Type 1 diabetes. But they also promoted some factors like time required for a person after undergoing a stroke which was decreased, improvement in the memory of a person was also seen. But the data was not enough which could give a fool proof about this theory [7].

So, CCR5 mutant is a potential candidate for curing HIV and after progressed researching it can be implemented more efficiently and hopefully we can save many lives in future.

However, the problem that arises is the presence of dormant virus which can re-emerge and begin the whole infection again. It resides within the host genome and it is called as latent reservoir.

So, researchers used the CRISPR/Cas9 technique to edit the HIV-1 genome due to which its function would be disrupted and the latent provirus is not able to infect the host body. The CRISPR components are directly transferred to the latent reservoir and inducible helper-T cells which cleaved at the specific sites and mutated it. This mutation hindered the genome. After performing sequence analysis it was seen that the hinderance caused lead to the loss of the activity of latent reservoir cells [8].

Else some researches suggested that we can use CRISPR/Cas9 as an intracellular defense against HIV. It provides an adaptive immune resistance against the HIV virus by creating site specific DNA double stranded breaks. Here, they used type 2 CRISPR-Sp Cas9 protein from streptococcus pyogenes with guided RNA so that target genome editing can be achieved. In this technique they basically disrupt host- cell interactions [9].

But using single guided RNA (gRNA) the virus can escape from this due to some specific mutations at the target sites where CRISPR acts. So, to prevent it we can use 2 single guided RNA which completely inactivates the genome [10].

Recently researchers discovered that the combination of LASERT ART (antiretroviral therapy) and CRISPR tool is effective against the latent reservoir cells of HIV which are difficult to kill and possess a threat for the reemergence of HIV. It was practised on mice models. LASER ART (antiretroviral therapy) is better than ART because the ART drugs gets quickly excreted out of the body because of its water soluble nature. However, LASER ART drugs remains for a longer period of time in the body and has a slow effective release. It consists of rilpivirine, dolutegravir, lamivudine, and abacavir and they are packaged inside a small fat-soluble particles. When they treated a HIV-1 infected mice by either one of the techniques then there was no effect but when they used a combination of both then the latent reservoir of HIV virus got eradicated [11].

So, CRISPR is a potential candidate for curing HIV and after progressed researching it can be implemented more efficiently and hopefully we can save many lives in future.

Year	
2007	A patient got cured due to bone marrow transplantation due to presence of CCR5 cells.
2013	Using CRISPR/Cas9 along with single gRNA.
2015	CRISPR-Sp Cas9 protein from streptococcus pyogenes as an intracellular defense against HIV.
2019	Using CRISPR/Cas9 for editing bone marrow cells into CCR5 mutant cells.
2020	Using CRISPR/Cas9 along with two gRNA. Using a combination of LASER ART and CRISPR technique.

Table: Development in CRISPR technology.

Conclusion

This article depicts that CRISPR technology has a potential of being a to cure to HIV. This gene editing tool is used to is used to cause edit genomes and causing mutations in the viral genome due to which the infection does not proceeds further in the host. But latent reservoir of viral pathogen which are in a dormant stage present in the host cells for lifetime possess a threat of becoming active and causing HIV. So, eliminating the reservoir cells is the key to cure HIV. There have been development in CRISPR technology along the years which increases the efficiency of CRISPR technology in curing HIV. But these tests are done using mice models and these techniques require a lot of improvement until we conduct trials on humans.

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