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Review Article

CRISPER-CAS9 Utilization for HIV/AIDS Treatment

Chouia Yassine* and Amran Wafa

Department of Medicine, University of Amar Telidji, Algeria

***Corresponding Author:** Chouia Yassine, Department of Medicine, University of Amar Telidji, Algeria.

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Abstract

Human immunodeficiency virus is (HIV) is a disease responsible for HIV/AIDS. The virus attacks immune cells of the host organism which renders them uncapable of assuring their role of defence, !thus leading to the deficiency of the immune system against other pathologies.

While HIV has gained the interest of a lot of scientists and doctors who trying to cure it, none of the treatments has been approved as having a substantial effect that eliminates the virus from the host's body. The current medications only allow the immune system to recover partially while the virus could be under suppression. As technologies develop, new ways of treatment that are more effective and efficient than normal medications are starting to catch the attention of both scientists and doctors alike. One of these fast developing technologies is genetic modification technology, using CRISPR CAS9. This paper concerns itself with the possible applications of CRISPR CAS9 in the treatment of HIV/AIDS.

Keywords: Human Immunodeficiency Virus (HIV); CRISPR CAS9; Immune Cells; Immune System

Plan of work

- Introduction:
  - History of the immunodeficiency virus (HIV).
  - Existing treatments for HIV.
- LT4 cells:
  - What are LT4 cells?
  - Membrane receptors of LT4 cells.
  - Formation of LT4 cells.
  - Roles and functions of LT4 cells.
- HIV:
  - What is HIV?
  - Composition of HIV.
  - Methods of infection.
  - Synthesis of new HIV units.
- CRISPER CAS9:
  - What is CRISPER-CAS9?
  - Composition of CRISPER-CAS9.
  - Method of cell penetration.
  - Use for CRISPR CAS9 technology.
  - CRISPER-CAS9 and HIV.
  - Other implications of CRISPER-CAS9.
  - Limits of CRISPER-CAS9.

History of the immunodeficiency virus (HIV)

The HIV virus was first clinically observed in 1981 in the United states with two distinguishable variants, HIV-1 and HIV-2 which are both believed to have originated in non-human primates in west-central Africa, and are believed to have transferred to humans in the early 20th century. HIV-1 appears to have originated in southern Cameroon through the evolution of Simian immunodeficiency virus (SIVcpz) that infects chimpanzees whereas the closest relative of HIV-2 is SIVsmm, a virus of sooty mangabey.

SIV is a weak virus (mostly suppressed by the immune system within days), and it is thought that several transmissions of the virus within humans may have caused the mutation of the SIV virus to HIV virus. An alternate view (not supported) suggests that unsafe medical practice in Africa during the second world war by unsterile use of single-use instruments and syringes was the initial cause that allowed the virus to adapt to humans.

Existing treatments for HIV [1]:

The treatment for HIV/AIDS is called antiretroviral therapy (ART) and involves taking a combination of HIV medicines every day. Although this method cannot cure HIV/AIDS, it is known to allow people to live longer than with no treatment and also reduces the risk of transmission of HIV amongst humans.

These medicines prevent HIV from multiplying in the human body which reduces the amount of infection, and thus presenting the immune system with the opportunity to recover and produce more LT4 cells which may cause the eradication of the infection in the body, even though there is still some HIV in the body the immune system is at least strong enough to fight off the infection and certain HIV-related cancers.

By reducing the amount of HIV in the body, HIV medicines also reduce the risk of HIV transmission between humans, so the main goal of the treatment is to reduce an infected person's viral load to an undetectable level. An undetectable load is a term for the low level of HIV in the blood, which renders its detection of the virus impossible by the viral load test.

Classes of drugs used in treating HIV:

- **NNRTIs:** Antiviral agent that binds specifically to HIV-1's reverse transcriptase and thus prevents the reverse transcription of the viral RNA to DNA.
- **NRTIs:** The first drug ever to be developed to manage HIV, it mimics the T cell's nucleoside structure, and gets incorporated into the infected DNA, and stop the viral DNA from adding further protein subunits to the chain, without elongation of the DNA chain, the virus cannot progress to the next phase of replication.
- **Pis:** Protease inhibitors that block the activity of protease which results in the production of defective viral proteins and thus defective HIV viruses that are incapable of infecting other T cells.
- **Post Attachment inhibitors:** Post attachment inhibitors are a class of drugs that bind to the CD4 receptor on the host LT4 cell and by its action blocks the attachment of HIV to the CCR5 and CXCR4 co-receptors and thus the viral entry to the targeted host cell. It belongs to the larger group called entry inhibitors
- **FIs:** Fusion inhibitors Block the HIV envelope from merging with the host LT4 cell membrane (prevents fusion) and thus preventing HIV from entering the host cell.
- **CCR5 antagonists:** Antagonists of the co-receptor CCR5 that block the receptors' action and prevents HIV penetration into the cell.
- **INSTIs:** Blocks integrase used by the HIV virus to integrate its DNA into the host cell's DNA and thus preventing the replication of the virus in the body.

LT4 cells

LT4 cells are a specialized type of immune cells involved in the regulation of the body's immune responses. once activated by dendritic cells they proliferate and differentiate into T4 effector cells that regulate the immune response in the body by the intermediation of the cytokines produced such as interleukins (IL).

LT4 cells play an indispensable role in the response of the human system against different infections as by the production of diverse interleukins (such as IL4 and IL6) are responsible cells for activating other immune system cells (LT8 - LB) which lead to the translation of the immune response in the form of antibodies (produced by LB cells) or killer cells (originated from LT8 cells).

Destruction of LT4 cells causes deficiency in the immune system as T8 and B lymphocytes can no longer be activated and thus causing the absence of antibodies and killer cells which play the role of destroying and limiting the spread of infection in the human body.

LT4 cells membrane receptor:

- TCR (T-cell receptor): it is protein complex found on the external portion of the plasma membrane of T lymphocytes and is responsible for the recognition of fragments of antigen as they bound to major histocompatibility complex (MHC) molecules, the binding is of low affinity and degenerate. TCR receptors are consisted of two different protein chains, an alpha (α) chain and a beta (β) chain 95% in humans, whereas the other 5% is composed of gamma (γ) and delta (δ) chains.

- CD4 (Cluster of differentiation 4): a glycoprotein that serves as a co-receptor of T-cell receptors (TCR) found on the surface of immune cells such as T4 lymphocytes, monocytes and macrophages, it is encoded by the CD-4 gene. CD4 is composed of four immunoglobulin domains (D1 to D4) exposed on the extracellular surface of the cell:

    - D1 and D3 resemble immunglobin variable IgV domain.

    - D2 and D4 resemble immunoglobin variable IgC domain.

- CCR5 (C-C chemokine receptor type 5): also known as CD195, encoded by the gene located on the short P arm at position 21 of the chromosome 3. Certain populations have acquired a mutation known as Delta 32 mutation which caused the genetic deletion of the CCR5 receptor and thus immunity against HIV infection. CCR5 is G protein coupled receptor which functions as a chemokine receptor in the CC chemokine group.

- CXCR4 (C-X-C chemokine receptor type 4): also known as fusin or CD184, it is a protein encoded by the CXCR4 gene. CXCR4 is a molecule implied in chemiotactic activity for lymphocytes and one of the known co-receptors that HIV can use to infect T4 lymphocytes. After recognition of the ligand (SDF-1) by CXCR4 receptor, the latter activates specific signaling pathways in the cell that help regulate the cell's growth and division, once the signaling is stimulated the CXCR4 protein is broken down so it can no longer activate these signaling pathways.

Formation of LT4 cells

LT4 cells are first produced in the red bone marrow (in the flat bones such as scapula) after which they get transferred by blood to the thymus gland (primary lymphoid organ situated in the neck of vertebrates) in which they mature and undergo the selection processes which renders these cells capable of assuring their functions in the organism.

The thymus gland is specifically functional at younger ages, its function decreases as the age increases.

After the production of precursor T4 cells they get transported to the thymus gland in which they develop specific receptors (TCR) for different antigens.

Under the influence of factors produced by the thymus gland such as Thymosin, Thymopoetin and Thymic factors the DNA of the T4 cell gets activated to produce Specific types of protein called recombinases (RAG1 and RAG2) responsible for shuffling the DNA of the LT4 cell to produce different forms of TCR (T cell receptor) that are specific for a possible type of antigen. The recombinases proteins assure the diversity of T4 cells and thus the best protection against different forms of antigens.

The remaining cells which are TCR (+), CD4 (+) and CD8 (+) will either mature to become LT4 cells or LT8 cells depending on which type of MHC they recognize. If the cell recognizes MHCI it will upregulate the CD8 receptors which increases their number on the cell membrane and down regulate the CD4 receptors which leads to decreasing their number. Consequently, the cell will differentiate to and LT8 cell as it has more CD8 receptors on its membrane.

If the lymphatic cell recognizes MHCI receptor will upregulate the CD4 receptors which increases their number on the cell membrane and down regulate the CD8 receptors which leads to decreasing their number, consequently the cell will differentiate to and LT4 cell as it has more CD4 receptors on its membrane.

Roles and functions of LT4 cells [2]:

LT4 cells play a very important role in the immune system. They assure the activation of the selected cells (B lymphocytes or T-8

lymphocytes) in case of presence of antigen in the body and thus regulating the body's immune response. The regulation is assured by the intermediate of cytokines such as the previously mentioned Interleukins (IL).

Figure A

HIV

What is HIV? :

- Human immunodeficiency virus (HIV) is a retrovirus (genetic material as ARN reverse transcripted once in the host cell to viral ADN) that attacks the human body's immune system that if not treated will lead to Acquired immunodeficiency syndrome (AIDS).
- HIV mainly attacks T4 lymphatic cells, but can also effect macrophage and other dendritic cells. It is transmitted throughout sexual activity, transfer of blood, pre-ejaculate, semen and vaginal liquid if the viral load is detectable as an undetectable viral load will not cause the transmission of the infection.
- The infection of HIV leads to the destruction of T helper cells as they are used as factories to produce other virions which on the other hand infect other T cells, the diminution in the percentage of T helper cells in the body causes a deficiency in the function of T-8 killer cells and B cells and thus rendering the immune system of the body useless (AIDS) against other infections.

Composition of HIV [3]:

HIV is a roughly spherical virus with a diameter of about 120nm; its genome is composed of two RNA chains containing 9 genes surrounded by a conical capsid composed of about 2000 copies of the viral protein P24. Together they form the nucleocapsid of the virus which is the responsible structure for the protection of HIV genetic material.

The singal stranded RNA is bound to nucleocapsid protein P7 and other enzymes needed for the development of the virus inside its host cell such as reverse transcriptase (responsible for reversing the viral RNA to viral DNA), proteases, ribonuclease, and integrase (responsible for integrating the viral DNA with the host cell's DNA). All these structures are surrounded by the viral protein P17 forming a matrix which insures by its turn the integrity of the virion particle and the viral genome.

All the mentioned structures are surrounded by a viral envelope composed of the lipid bilayer of the last infected cell acquired during the sortie of the virion which houses the glycoproteins (GP120 and GP41) necessary for the recognition of the host cell and the anchoring of the virus to the latter.

The figure below (Figure B) demonstrates the structure of the HIV virus amongst the different proteins/glycoproteins that compose it.

Figure B

Synthesis of new HIV units

The transmission of HIV to the host body can mainly occur by the exposure to different organism fluids. These fluids include:

- Blood, which can be due to sharing drug injection material with an infected person. The unsterile use of sharp objects between people can also cause the transmission

- Semen or vaginal fluids HIV can be acquired through sexual activity by exposure to the seminal liquid of an infected person.
- Rectal fluids, rectal fluids can cause HIV transmission between people through anal sex.
- Breast milk, or from mother to child during pregnancy.

After the virus units enter the host body they recognize the host cells (detailed early in the presentation) in which it aims at reproducing as much units as it could through these precise steps:

- **Binding and fusion:** In this step the virus attaches to the host cell's plasma membrane aiming at entering the host cell. It is due to the interaction of GP160 spike of the virus with the CD4 receptor and CCR5/CXCR4 co-receptors, after this interaction GP120 binds to integrin α4β7 which results the establishment of virological synapses.
- **Penetration:** The bond of the virus to its host cell allows the N-terminal fusion peptide GP41 to penetrate the cell membrane which in itself allows the interaction of HR1 and HR2 repeated sequences in GP41 causing the collapse of the extracellular portion of GP41 into a looped structure bringing the virus and the cell's membrane close together, and thus allowing the fusion of the two membranes.
- **Reverse transcription:** After the viral capsid enters the host cell the enzyme reverse transcriptase allows the transformation of the viral RNA to a viral DNA (complimentary DNA or cDNA) which then binds to the host cell's DNA under the action of an enzyme called integrase and thus incorporating the viral DNA to the cell which it will use to make copies of itself. Reverse transcriptase is extremely prone to errors which results in a number of mutations many of which cause drug resistance.
- **Transcription:** The integrated viral DNA lies dormant in the latent stage of infection, the presence of transcription factors (such as Kappa B) are essential for the virus to actively produce. During the replication phase, the viral DNA is transcribed to messenger RNA (mRNA) that gets exported form the nucleus to the cytoplasm where it gets translated to produce the viral proteins and enzymes necessary for the HIV virus.
- **Assembly:** The final step of the virus' life cycle, this step begins at the plasma membrane of the virus' host cell. The virus' GP41/GP120 get transported from the Golgi apparatus to the plasma membrane in which GP120 gets anchored to it by GP41 with the viral proteins p55 and p160 associating with the inner surface along with the HIV genomic RNA. The cleavage begins that causes the proteins p55 and p160 to mature and transform to the actual matrix. The cleavage step is where the class protease inhibitors assure their role.
- **Release:** The virus is now ready to get released from the host cell. Releasing the virus causes the host cell to lose a portion of its plasma membrane which finally leads to its death along with the excessive use of its resources and tools by the virus.

The released units of HIV virus circulate the blood to infect other host cells which (if not treated well) leads to AIDS (Acquired immunodeficiency syndrome) which is the most advanced stage of HIV infection that signifies the weakening of the immune system and thus the exposure of the organism to other diseases and viruses.

CRISPR-CAS9:

What is CRIPR-CAS9?

- Acronym for "clustered regularly interspaced short palindromic repeats". It is a family of DNA sequences found in the genome of prokaryotic cells such as bacteria and archaea, these sequences are derived from bacteriophages that had previously infected the prokaryotic cell.
- These CRISPR units are used in the prokaryotes to detect and destroy the bacteriophages during infection and thus they play a key role in the prokaryotes' defense system and provide a form acquired immunity to the cell.
- CAS9 (CRISPR associated protein 9) is an enzyme that utilizes CRISPR sequences as a guide to recognize and then cleave specific strands of DNA sequences that are complimentary to the CRISPR sequence.
- CRISPR together with CAS9 form a base for the CRISPR-CAS9 technology that can be used to edit genes within organisms. This technology provides a wide variety of applications including gene editing and removal of viral infections (such as

HIV and the basis of this study) which leads to the treatment of chronic and non-chronic diseases.

- The development of CRISPR-CAS9 technology by Emmanuelle Charpentier and Jennifer Doudna was awarded the 2020 Nobel Prize in chemistry.

Composition of CRISPR-CAS9 [4]:

Crispr-cas9 is composed of two main parts which play an important role in the success of the genome editing technic.

CRISPR: A specialized region of DNA with two distinct characteristics:

- The presence of nucleotide repeaters and spacers (bits of DNA that are interspersed amongst the repeated sequences).
- Repeated sequences of nucleotides distributed throughout a CRISPR region.

CAS9: A protein associated with CRISPR that cuts the foreign DNA (HIV's DNA in this case); it typically binds to two RNA molecules (crRNA and tracrRNA or trans-activating RNA). The two guide CAS9 to the target site where it will make its cuts and thus eliminating the foreign DNA of the cell.

CAS9 uses two separate domains on its structure to cut both strands of the DNA double helix (double stranded break).

Short DNA sequences known as PAMs (protospacer adjacent motifs) serve as tags and sit adjacent to the target DNA sequence. If the CAS9 protein doesn't recognize a PAM next to the DNA sequence it won't cut and thus it doubles as a safety mechanism that prevents errors.

Methods of cell penetration [5]:

- Delivery of nucleic acids into eukaryotic cells is called transfection. The gRNA and CAS9 can be introduced as either DNA, RNA or pre-complexed RNA and a protein called ribonucleoproteins (RNPs).
- Transfection methods can be broadly classified into chemical, physical and viral mediated categories.

Physical transfection

Physical transfection methods create temporary holes in the plasma membrane of the cell throughout which gRNA/CAS9 can pass. Three popular methods are electroporation, nucleofection and microinjection.

Electroporation

It involves suspending cells in a conductive solution and briefly applying high-voltage electrical pulses which lead to the formation of temporary pores in the plasma membrane and the electrical potential across the membrane causes the charged molecules (gRNA/CAS9) to enter the cell through the pores.

Nucleofection

- It is based on electroporation, but utilizes a special machine, called a nucleofector, and has several distinctions from traditional electroporation (view the figure A below).
- Cells are first mixed with an electroporation or a nucleofection agent and then added to CRISPR components (RNP) the cell-RNP mix then into a nucleofector or electroporator. An electrical pulse gets applied to the cells that cause pores in the plasma membrane enabling RNPs to enter the cell.
- Nucleofection also enables nuclear entry of RNPs. Once the electrical pulse is removed the plasma membrane is removed.

These two technics present both advantages and disadvantages:

Advantages:

- Easy and fast.
- Highly efficient.
- Large number of cells can be transfected in a short time.

Disadvantages:

- Requires specialized equipment.
- Cell death may occur from high voltage pulses or incomplete membrane repair.

Figure C

Microinjection

It involves positioning target cell under a microscope and delivering gRNA/CAS9 into the cytoplasmic nucleus trough a glass micropipette. It is a highly technical method that requires specialized equipment (micro injectors and micromanipulators). It is also low-throughput as only one cell can be transfected at a time.

Despite this method's disadvantages it is highly efficient when performed by skilled individuals.

Figure D

Chemical transfection

Several chemical methods can be used to transport molecules into cells, including calcium, phosphate, cationic polymers and cationic amino-acids. One of the most common methods of introducing CRISPR to cells is the method of lipofection.

Lipofection

- Also called lipid-based transfection uses cationic reagent to deliver CRISPR components into cells. It involves constructing lipo-soluble structures called liposomes around the CRISPR components which are transported into the cell through endocytosis.

- This method is easy and economical with minimal toxicity but it is not as high efficient as other methods.

Use cases for CRISPR CAS9 technology

There are three main categories of genetic manipulation which CRISPR CAS9 can perform. These include disruption, deletion and correction/Insertion.

- **Disruption use case:** In a process called non-homologous end joining (that is if a single cut is made) an addition or deletion of base pairs (A,T,C,G) can occur which leads to the disruption of the original DNA sequence and thus causing gene deactivation.

- **Deletion use case:** In which a large fragment of DNA can be deleted by using two guide RNAs that target separate sites. After cleavage at each site, non-homologous joining unites the separate ends causing the deletion of the intervening sequence.

- **Correction/Insertion use case:** Consists of inserting a new DNA template alongside the CRISPR CAS9 machinery allowing the cell to correct a gene or insert a whole new one. This method utilizes a process called homology directed repair.

CRISPR CAS9 and HIV

CRISPR CAS9 is a promising technology that can be implemented in the fight against viral infections such as HIV infection.

Using the CRISPR/Cas9 System to target HIV provirus [6]:

- The CRISPR CAS9 system is able to introduce double-strand breaks in DNA at a site by the guide RNA. Researchers targeted a CRISPR CAS9 system to target the LTR regions (long

terminal repeat which is the center of control for gene expression) of the HIV provirus at positions T5 and T6.

- Cells were transfected with a plasmid containing the humanized CRISPR CAS9 guide RNA genes. Targeting of the LTR by the guide RNA resulted in significantly decreased transcription of the genes under the control of LTR promoter.
- Jurkat cell lines with HIV provirus-like regions that produced GFP instead of the viral proteins were used to determine the effectiveness of the potential Cas9-based therapy.

Figure E

Researchers were able to demonstrate that CRISPR CAS9 can excise the HIV provirus from the host genome itself. After 3 rounds of transfection with CRISPR CAS9, targeting the LTR, scientists measured - on average - the provirus was completely excised from the genome in 31.8% of all cells as demonstrated by the following figure.

Disruption of Co-receptors CCR5 and CXCR4 by CRISPR/Cas9 Technology [7]:

- In addition for targeting HIV-1 genome, CRISPR CAS9 technology can also be used to break the HIV entry in the host cell by editing the receptors that make it possible (mainly CCR5 and CXCR4 co-receptors). But due to the indispensable role of CD4 cells in the immune system disruption of CD4 cells is not an advisable strategy in preventing HIV infection.
- The CRISPR CAS9 approach provides an appropriate method for gene disruption and has been widely used to disrupt CCR5 and CXCR4 expression.
- In 2013 scientists showed that it is possible to achieve CCR5 suppression by CRISPR CAS9 technology in human embryonic kidney (HEK).
- In 2014 an experiment has been conducted using combined CRISPR CAS9 with piggyBac technology to perform homozygous CCR5Δ32 mutation in induced pluripotent cells (iPSCs). These cells could normally differentiate to monocytes/macrophages which present resistance to HIV-1 infection.

Figure F

Other implications of CRISPR CAS9 [8]

CRISPR is a promising technology with implications that far exceed HIV treatment, in this section we will be presenting you with other interesting implications for gene editing with CRISPR:

Figure G

- **Cancer Immunotherapy:** The phase 1 clinical trial in the US opened in 2018 in the intent of using CRISPR CAS9 to edit autologous T cells for cancer immunotherapy against several cancers against several cancers with relapsed tumors and no further curative treatments.

- **Gene Disruption:** Using gene disruption for therapeutic benefit for patients with sickle-cell anemia (SCD) which was tested by both Vertex Pharmaceuticals and CRISPR Therapeutics.

These are merely some examples for the use of CRISPR CAS9 technology, as it can be implemented in other areas of medical science.

Limits of CRISPER-CAS9 [8]:

Although CRISPR is a promising technology, it is as all other technologies limited by several factors, in the next section we will be discussing these limitations:

- It is difficult to deliver the CRISPR CAS material to mature cells in large numbers. This in itself remains a problem for many clinical applications.

- The technology is not 100% efficient as many cells that take in CRISPR CAS material may not have genome editing.

- The technology is not 100% accurate. 'Off-track' edits though very rare may occur and cause severe consequences particularly in clinical applications.

- It is hard to introduce CRISPR CAS9 complex into its host cell, as it can lead to the destruction of the latter.

- Modifying T4 cells can come with great issues as these cells are indispensable for the immune system.

- CRISPR CAS9 is still fairly a new technology and a lot is yet to be developed.

- CRISPR CAS9 is an expensive kind of treatment and might not be available for every patient.

- Editing cells in the human organism comes carrying ethical issues as editing germline could theoretically be used to enhance desirable traits instead of curing diseases.

Conclusion

The results of this study have shown the effectiveness of CRISPR CAS9 technology in the treatment of the human immunodeficiency virus (HIV) as the pathways where the treatment can be utilized differ whether it is targeting HIV itself by genetically deleting or suppressing its genome in the host cell or targeting the host cell by trying to modify the CCR5 or CXCR4 co-receptors.

Although CRISPR CAS9 is still a relatively new technology that has a lot to be developed ranging from cell penetration methods, accuracy or efficiency. It sure is a promising technology that has limitless potential for treating a wide number of diseases especially those that require genetic modification as a basis for therapy (such as SCD or diabetes) and it will soon be (amongst other genetic modification methods) accessible, easy and safe for most patients.

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