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Applications of CRISPR-Cas9 Technology to Treat CCR5 Tropic HIV-1

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) is most notable for its role in directly infecting and killing CD4 T cells in the immune system, which can lead to the acquisition of Acquired Immunodeficiency Syndrome (AIDS). Currently, there are no cures for HIV-1, leaving 37 million people with HIV-1 infections and no definitive treatment. Researchers are working towards developing long-term treatments for HIV-1 using CRISPR-Cas9. These therapies include genetically engineering antiviral resistance into vulnerable cell types and cutting out viral genomes from infected cells. This article will explain the HIV-1 life cycle in detail, give an overview of CRISPR-Cas9 technology, and then go into detail about the potential applications of CRISPR-Cas9 for the treatment of CCR5 Tropic HIV-1.

Introduction

Human immunodeficiency virus type 1 (HIV-1) is a virus that is responsible for attacking the body's immune system. HIV-1 primarily infects and kills CD4 T lymphocyte cells (T cells), which are essential in directing the immune response against numerous infections.[1] As HIV-1 infection progresses and CD4 cells die off, infections can transition to Acquired Immunodeficiency Syndrome (AIDS). This leaves patients vulnerable to opportunistic infections that can be fatal.[2] Over the past decades, more than 75 million people have been diagnosed with HIV-1 and there are currently over 37 million individuals living with HIV-1 infection.[3] Antiretroviral Therapy (ART) is the primary method used to treat HIV,-1 which consists of a range of drugs that inhibits HIV-1 infection at various stages of the virus' life cycle.[2] When multiple ART drugs are taken, the treatment becomes more effective by preventing the development of resistant strains.[2] Patients are capable of living a normal lifespan while taking ART, but in no means is ART able to cure HIV-1.[2,4] This is due to the fact that HIV-1 integrates into the genome of long living CD4 T cells, creating a lasting viral reservoir that ART is not able to remove.[1,2] If ART is no longer taken, HIV-1 can rebound from these reservoirs and renew progression towards AIDS.[1,2]

To this day, there are two people who have been cured of CCR5 tropic HIV-1, which are strains of HIV-1 that require the CCR5 cell surface protein to enter cells.[5,6] Timothy Ray Brown, more commonly known as the Berlin Patient, was initially diagnosed with HIV-1 and then later acute myeloid leukemia cancer.[5] Similarly, an individual known as the London Patient was initially diagnosed with HIV-1 then later was diagnosed with Hodgkin's lymphoma.[6] Treatment for their cancers required a hematopoietic stem cell (blood forming stem cell) transplant that was considered dangerous and a last resort treatment. This is due to the harsh conditioning (chemotherapy) that is required to prepare them for the transplant, as well as the risk of developing Graft Versus Host Disease, which can be fatal.[7] Still, each patient received hematopoietic stem cell transplants from people with a mutated CCR5 gene, which is uncommon but results in the loss of CCR5 protein in all immune cells, including CD4 T cells. Following the transplant, their new blood cells contained the mutant CCR5 gene, which prevented CCR5 tropic HIV-1 from binding to CCR5 coreceptors and infecting their T cells. Therefore, when the patients stopped taking ART, they did not experience HIV-1 rebound due to their new T cells being HIV-1

resistant, and they were considered cured. This procedure is extremely rare and is not applicable for the majority of patients due to the following reasons: 1) the stem cell transplant procedure itself is considered dangerous for the average HIV-1 patient, 2) this treatment only works for patients with CCR5 tropic HIV-1 strains, and 3) it is difficult to find a matched stem cell donor due to the rarity of the CCR5 mutant gene.[8]

Discussion

HIV Life Cycle

There are 7 major steps in the HIV-1 life cycle (Figure 1).[9] This is the process in which HIV-1 infects a target cell and then replicates in order to infect other CD4 T cells. The 1st step is binding, where HIV-1 binds to a CD4 receptor on a CD4 T cell, then attaches to either of two cell surface coreceptors, CCR5 or CXCR4. HIV-1 that uses the CXCR4 coreceptor is known as CXCR4 tropic HIV-1. The 2nd stage is fusion, where the HIV-1 envelope and CD4 cell fuse together, allowing the HIV-1 capsid to pass into the cell. The capsid contains HIV-1's genomic RNA and the reverse transcriptase and integrase proteins needed for production of new viruses. Next, reverse transcription occurs, where the enzyme reverse transcriptase converts the genomic RNA into DNA. When the RNA is converted to DNA, this creates many mutations that result in production of numerous mutant viruses, making it difficult to treat HIV-1 with any single treatment. The viral DNA is then brought into the nucleus of the cell. In step 4, integration, the enzyme integrase inserts the viral DNA into the genomic DNA of the CD4 cell. The integration of HIV-1 DNA into the DNA of CD4 cells prevents it from being completely treated by any ART treatment and establishes a long-term viral reservoir. Step 5 is replication: cellular machinery is used to create viral genomes and proteins based on the integrated HIV-1 genome. In step 6, HIV-1 particles move to the surface of the cell and come together to create new viruses in a process called assembly. From there, step 7, budding, occurs: newly formed viruses "bud off" of the cell membrane and can go on to infect other cells.[9] Without treatment, HIV-1 is able to generate more than 1 billion new infectious particles per day.[1]

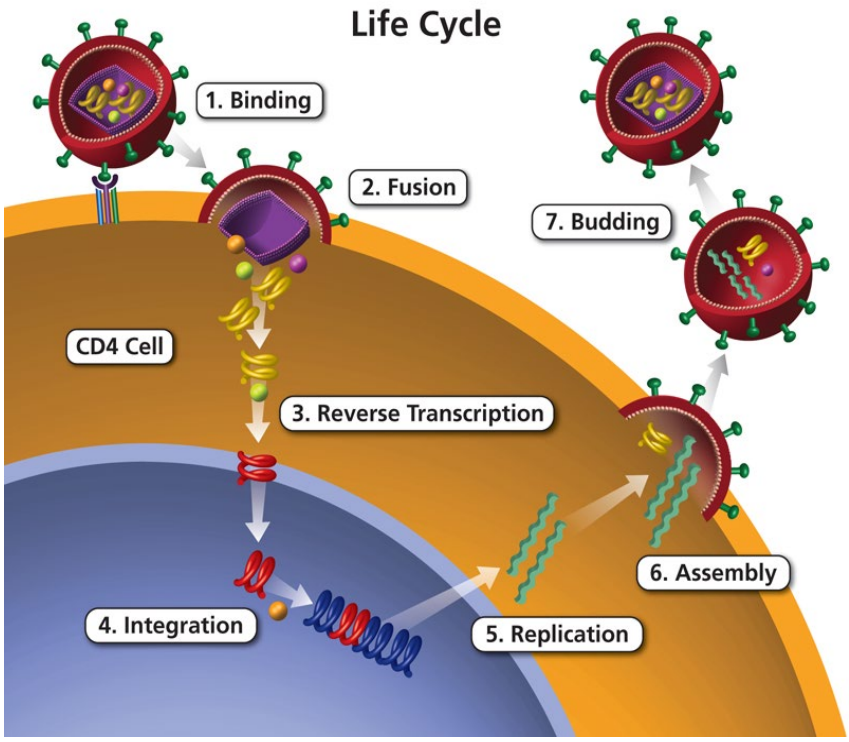

Figure 1. HIV-1 Life Cycle[10]

Overview of CRISPR-Cas9 Technology

CRISPR-Cas9 is a gene editing tool that allows scientists to change an organism's DNA by either removing, adding, or altering nucleotides in the DNA's sequence. CRISPR-Cas9 is made up of a guide RNA that guides the system to bind the target sequences of DNA and Cas9 protein that creates a double stranded break in the target sequence.[11] The cell then repairs the break through either non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Figure 2). NHEJ brings together the two ends of the double stranded break in an error prone process that can result in small insertions or deletions (indels). Researchers can take advantage of indel formation to knockout genes of interest by creating frameshift mutations at precise locations. HDR uses a donor template, which is a DNA sequence that matches the area of either side of the cut site, to repair the cut strand and insert any sequence provided in the middle of the homologous region. This allows research to alter genes by providing a custom donor template that is used to repair the double stranded break. Taking advantage of HDR allows for more precise control to introduce specific insertions or deletions ranging from a single nucleotide to entire genes. In this way, researchers can both knockout genes and knock in custom sequences that allow for the expression of specific proteins.[12]

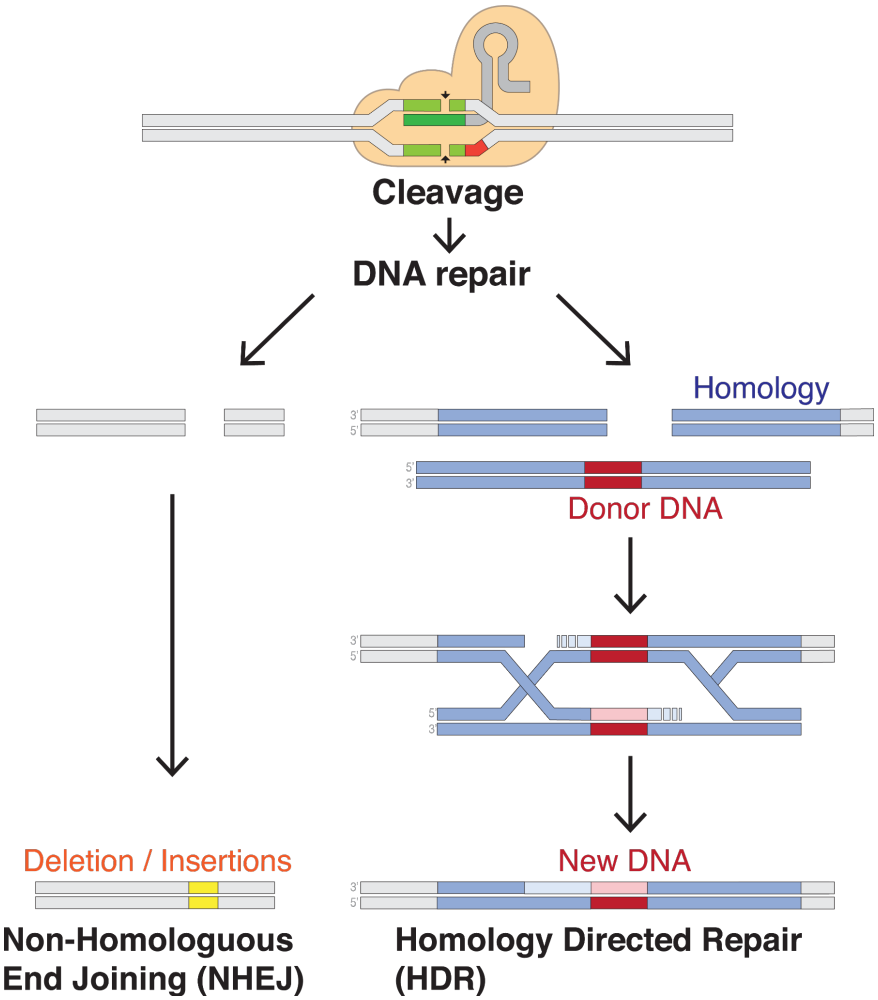

Figure 2. NHEJ vs HDR[13]

Current Applications of CRISPR-Cas9 Technology for Treating HIV-1

CRISPR-Cas9 is currently being used to develop therapies to treat HIV-1. One example of this is using CRISPR to directly knockout the CCR5 gene, mirroring the successful treatment of the London and Berlin patients.[14-18] Researchers are using CRISPR-Cas9 to create cuts within the CCR5 gene, which form indels that can create frameshift mutations to knockout the gene.[11,12] This prevents HIV-1 from infecting CD4 T cells via the CCR5 co-receptor. [1,9] One approach researchers are taking to apply this strategy is directly editing CD4 T cells to make them resistant to CCR5 tropic HIV-1.[14-16] Transplant of these resistant cells would allow the patient to maintain healthy CD4 T cells and prevent the onset of AIDS. However, the transplanted T cells have a limited lifespan and would need to be replaced frequently, limiting the viability of this treatment. An approach researchers are taking to solves this problem is to edit the hematopoietic stem cells directly, so new CD4 T cells that are produced from the long-living stem cells have CCR5 knocked out.[17, 18] This strategy is similar to that used to cure the London and Berlin patients, but instead uses gene editing in order to knockout the CCR5 gene in a patient's own cells, whereas the London and Berlin patients received cells with the CCR5 knockout gene from a donor.[5, 6] This approach solves the need to find a matched donor and diminishes the risk of the transplant by preventing the possibility of the patient developing Graft Versus Host Disease. However, the approach of knocking out CCR5 only treats CCR5 tropic HIV-1, not CXCR4 tropic HIV-1.[19]

To make therapies that are more broadly applicable, researchers have developed strategies to combat both CCR5 and CXCR4 tropic HIV-1. For example, one approach researchers are taking is to knockout both CCR5 and CXCR4 in the same T cells.[20, 21] This is achieved by treating the T cells with Cas9 and two guide RNAs, one specific for CCR5 and another for CXCR4. From this treatment, indels are created in both genes, resulting in frameshift mutations that knockout both proteins.[11,12] This prevents both CCR5 and CXCR4 tropic HIV-1 from entering from the cells.[1,9] This strategy allows the patient to maintain a healthy population of T cells to prevent the onset of AIDS. However, as discussed above, this is not a permanent solution because the body will continue to produce new T cells that contain the wild type CXCR4 and CCR5 genes, and the edited cells will fall out of the population. Over time, the edited cells will therefore need to be replenished for the therapy to continue working.

Another strategy that researchers are using to target the long lasting viral reservoirs is by using CRISPR-Cas9 to directly cut the viral genomes in infected cells.[22-25] To do this, researchers have designed guide RNAs that can target Cas9 to specifically cleave viral genomes within infected cells. This can create mutations that prevent the production of new viruses.[11, 12] Researchers are hoping to use this system to remove all viral genomes in a patient's cells, which can cure the patient of their infection by completely removing the viral reservoir. Therefore, the patient would not have viral rebound after stopping ART. However, current technology is not capable of editing all the infected cells in the human body, so this leaves viral reservoirs that are capable of producing new HIV-1 and continuing the infection.[25]

Conclusion

Despite ART and other treatments that have been developed to combat HIV-1, it is still a widespread problem in the world.[2,3] Those living with HIV-1 have no readily available cures, and will have to continue taking ART for their lifetime to maintain viral suppression.[2,4] However, the Berlin and London patients prove that curing HIV-1 is possible by eliminating the CCR5 gene in hematopoietic stem cells, which is a non-essential gene. [5,6] They received hematopoietic stem cell transplants from donors who carry a knockout mutation within the CCR5 gene, making it so that their HIV-1 could no longer infect their new cells. This has given inspiration to researchers using an emerging piece of technology, CRISPR-Cas9, which works by allowing researchers to make breaks in the genome by cutting the gene at a specific location.[12, 14] These breaks can then be repaired by the cell to

introduce mutations or insertions. One possible treatment is using CRISPR-Cas9 to edit a patient's T cells to make them HIV-1 resistant.[14-16, 21] A more effective treatment may be editing the hematopoietic stem cells directly for CCR5 knockout to mirror the Berlin and London patients, which would represent a lifetime therapy.[17,18] Another way CRISPR-Cas9 is being used is to cut viral reservoirs within already infected cells, though this strategy will need significant technological advancements before it is clinically applicable.[22-25] The next generation of treatments using CRISPR-Cas9 for HIV-1 will likely build off of prior efforts with earlier gene editing technologies to move beyond just knocking out CCR5, CXCR4, or viral reservoirs. This can be achieved by taking advantage of HDR to knock in anti-HIV factors, as was done with earlier studies that used lentivirus and zinc finger nucleases to insert protective genes.[26-29] Thus, future works could build on these studies by using CRISPR-Cas9 to generate precise knockouts with knockins to simultaneously eliminate coreceptor expression and introduce anti-HIV genes, fighting HIV-1 at multiple stages of its life cycle. Though we are a long way from a universal cure for HIV-1, as CRISPR-Cas9 technology advances the promise for a widespread cure is becoming more obtainable.

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