CRISPR/Cas9
The Revolutionary Genome Editing Mechanism for HIV Curation

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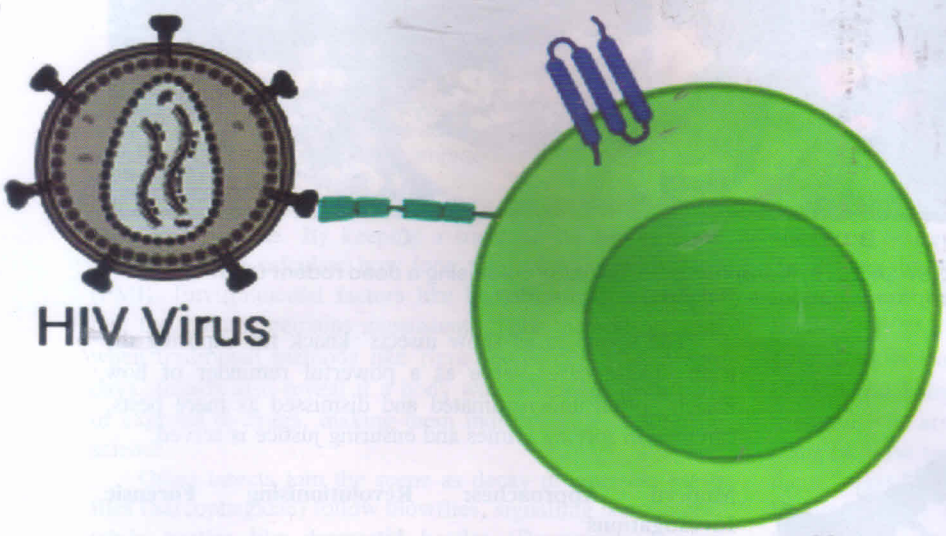

HIV attaches with CD4+ T cells for entry

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has shown a promising approach for the treatment of HIV-1 (Human Immunodeficiency Virus). With its unparalleled capacity to induce precise genetic alterations, the CRISPR/Cas9 system — first identified as an adaptive immune defence mechanism in bacteria and archaea — has quickly revolutionised the field of genome editing itself. The basic idea of CRISPR arrays is to store bits of viral DNA that the prokaryotic host comes into contact with. These bits are subsequently converted into short RNAs. Through the use of complementary sequences in invasive nucleic acids, Cas9 endonuclease works as molecular scissors to cause specific Double-strand Breaks (DSBs) in DNA. Through a single guide RNA (sgRNA), Cas9 is guided to a particular genomic locus that has the Protospacer Adjacent Motif (PAM) sequence. It is a site which helps in the determination of the target for CRISPR/Cas9. Upon recognition, the Cas9 nuclease generates a DSB near the PAM site. This targeted cleavage forms the molecular basis for editing genomes in a highly specific and efficient manner.

Despite developments in antiretroviral treatments, HIV continues to pose a serious threat to global health. By successfully inhibiting viral replication, Highly Active Antiretroviral Therapy (HAART) has changed HIV infection from a deadly illness to a chronic, treatable condition. These anti-retroviral therapies can cause several side effects in patients; however, there is a need for other clinical approaches for the treatment of HIV.

The use of CRISPR/Cas9 in HIV therapy is justified by the capability of the system to mediate accurate, effective, and long-lasting genetic modifications. By creating guide RNAs that are complementary to conserved segments of the HIV genome, especially in Long Terminal Repeat (LTR) sections or crucial regulatory genes, the Cas9 endonuclease can generate Double-strand Breaks (DSBs) that cause the viral genome to be disrupted, mutated, or removed entirely. Apart from eliminating proviral sequences, CRISPR/Cas9 can also be used to modify host cell genes that are essential for HIV infection, like the co-receptors CCR5 and CXCR4, which facilitate viral entrance into CD4+ T cells (immune cells in the human body).

The genetic disruption of host components essential for viral entry and replication is another crucial strategy in CRISPR-based HIV treatment. These include the well-characterised HIV infection facilitators CCR5 and CXCR4, which are co-receptors expressed on CD4+ T cells. Through these receptors, HIV enters the body and infects the host. HIV resistance can be transferred to host cells by deletion or modification of these receptors with the help of CRISPR. Specifically, most of the famous deletion is the delta 32 mutation, in which the 32 base pairs were deleted/mutated, which makes the person vulnerable to HIV. To expand the resistance spectrum, CRISPR/Cas9 CXCR4 modification has also been attempted.

Designing guide RNAs that target conserved viral genomic regions, including the LTRs, and regulating genes which are essential for viral replication and transcription, is a key method in CRISPR/Cas9 HIV treatment. These guide RNAs allow the Cas9 nuclease to cause double-strand breaks in viral DNA that is integrated into the host genome. By causing insertions or deletions that deactivate the virus and stop it from expressing and spreading, the DNA breaks set off mutagenesis. Multiplexing, which involves combining many sgRNAs that target distinct loci, is used to reduce viral escape by concurrently interrupting several crucial viral genes because of HIV's rapid rate of mutation and quasispecies nature. This combinatorial approach decreases the possibility of resistant mutants developing while enhancing the strength and persistence of proviral inactivation.

Using latently infected cell lines with integrated HIV genomes, CRISPR/Cas9 has undergone extensive *in vitro* testing. These investigations showed that the introduction