

A REVIEW ON THERAPEUTIC APPLICATION OF CRISPR/Cas9 FOR HIV

ABSTRACT

Despite the significant progress that has been made in the battle against HIV-1, the virus remains a major threat to global health. Since it can only be suppressed with the utilization of highly active antiretroviral therapy, it remains a challenge to prevent the flow of the infection. According to UNAIDS, only 70% of individuals with HIV are diagnosed and only 53% are treated. This means that 44% of the tainted people have undetectable viral load. Despite the efforts made by various governments and private organizations, the battle against HIV infection remains a challenge that requires a comprehensive approach. The fast rise and evolution of technologies that enable the modification of qualities at the DNA level has raised hopes for an end to the epidemic. Through the employment of endonucleases, we've been able to build exact changes in DNA of being cells. The capacity to alter the outflow of the HIV gene by replicating the LTR promoter played vital role. If the promoter is edited, then the impact of viral transcription might be suppressed. Using a more limited form of the nuclease Cas9, the evacuation of crucial parts of the infective agent ordering was with success performed in HIV transgenic mice. The outcomes showed that the decrease of HIV-RNA was significant in all examined tissues and circulating lymphocytes. (Kaminski *et al.* Gene Ther 2016; 23:690-5). This review objective is to summarise the work of eminent researcher on the utilization of CRISPR-Cas' squality treatment for the treatment of HIV-1.

KEY-WORDS: HIV, Co-receptors, CRIPSR-Cas

INTRODUCTION

Human immunodeficiency virus-1 is an enveloped lenti virus made of two single-stranded RNA molecules, enfold with capsid and a string of genes known as gag, env, tat, vif, vpr and vpu. It has an extended terminal rehash (LTR) settled at each finish of the virus. The virus can transmit diseases through various routes such as sexual contact, blood circulation, and vertical transmission. The WHO released a data in 2017 that revealed around 36.9 million individuals around the globe were reportedly having HIV contamination. The most severe symptoms of HIV contamination are cauterization by other opportunistic diseases such as acquired immunodeficiency syndrome (AIDS). Early start of the illness is also common among individuals with HIV infection.

Various gene editing devices have been broadly utilized in the research of HIV-1 and AIDS. These tools include zinc finger nucleases, transcription activator-like Nucleases, and CRISPR-associated nuclease 9 (Tebase *et al.*, Yin *et al.*, 2018; Yu *et al.*, 2018). ZFN-mediated modification of C-C chemokine receptors type five (CCR5) has shown promising results in humanized CD4 T cells which reveal ZFNs-CCR5 modification to be safe and effective in human AIDS infection. (Tebase *et al.*, 2014). The first application of the CRISPR-Cas9 framework in the anticipation of HIV disease was acted in 2013. This method was successful in suppressing latent HIV-1 provirus. (Ebina *et al.*, 2013).

The CRISPR-Cas9 framework is a widely utilized anti-HIV strategy. This is a bacterial system that goes about as a versatile safe framework by detecting and suppressing the foreign nucleic acids of invading virus. (Makarova *et al.*, 2011; Gasiunas *et al.*, 2012; Wiedenheft *et al.*, 2012) The endonuclease Cas9 from *Streptococcus pyogenes* was utilized to build up an amazing genome altering apparatus that can separate DNA double-stranded. Its sequence specificity is mediated by the endonuclease's 20 nucleotides at the 5-prime end

of the guide RNA (gRNA) that manage complementary sequence in the objective DNA. Only complementary arrangements of the protospacer neighbouring theme are cleaved by Cas9. These sequences can be directed to a novel site by a simple plan of a gRNA with a 5' sequence.

The CRISPR-Cas9 framework is a unique and robust nuclease platform that has been generally utilized in genome altering. Its high specificity and flexibility make it an excellent platform for developing new applications. Cas9 consists of two a chemically idle sites initiate double standard brake. When inactivated, these sites create a single-stranded break in the DNA of the target. (Gasiunas *et al.*, 2012; Jinek *et al.*, 2012). Both sites of Cas9 were cleaved to form a catalytically inactive version called dCas9. This variant is equipped for stifling the statement of specific genes by interfering with their DNA-binding affinity (Qi *et al.*, 2013). Through the utilization of dCas9, transcription repressors and activator domains had the option to produce gRNA-coordinated record factors that that are essential for cell division and transcriptome modulation. Frameworks are generally utilized in the preparation of genome adjustment and infection control. (Gilbert *et al.*, 2013). The CRISPR-Cas9 framework is designed to enable the focusing of novel sites with minimal changes of the host cell genome. Its standard style makes it simple to switch one site with lowest interference. This review focus on the significance of CRISPR-Cas-based quality treatment of HIV-1 infection. It describes how the technology can focus all the steps of the viral contamination cycle.

HIV env Gene and Coreceptors

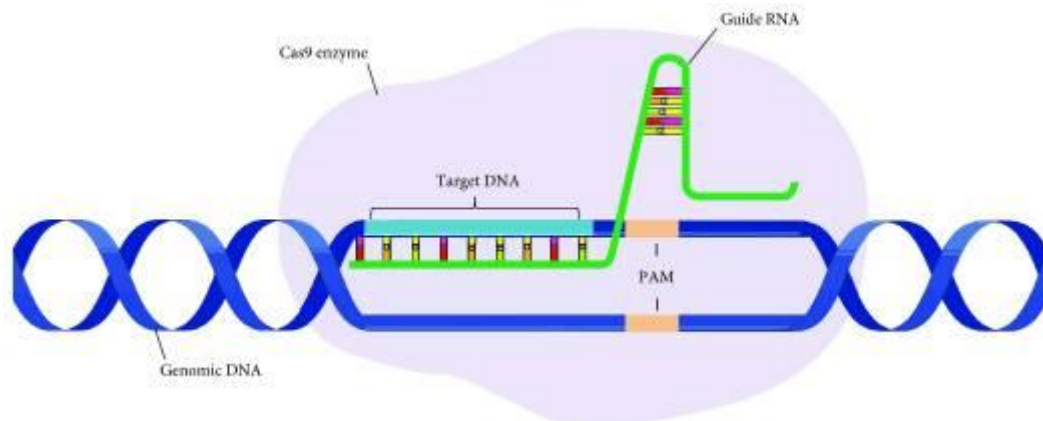
A glycoprotein that is essential for the viral envelope is encoded by env gene and is required for the guideline of the tropism of host cells. gp120 was classified into 5hypervariable districts with conserved districts. The positive and negative forces on the gene env contribute to the advancement of these districts. For the invasion of host cells, CD4 is required along with the coreceptors CXCR4 and CCR5. Thus, the tropism of HIV is mainly because of the articulation example of these coreceptors.

There are two main sorts of human immunodeficiency virus (HIV) that can infect the T cells of the body. These virus are called the white blood cell jungles and the macrophagic. They are most commonly transmitted by humans'-tropic virus can evolve into more robust strains because of their changes in the in the envelope protein. Evolution of these virus is also influenced by the coreceptor CXCR4. Some individuals with resistant status to infection of virus are not entirely resistant to virus instead a deletion of the 32-base pair of the CCR5 gene occurs in them. This reveals the frame shift and creation of a protein that does not requires to go into the cell surface. Even if heterozygous people have this mutation, they can still have a survival advantage over nonmutated individuals because of their decreased articulation of CCR5 which postpones replication of the virus. This survival advantage can be maintained even if they have a solitary duplicate of the gene.

CRIPSR-Cas9 Technology

Mojica *et. al.* reported in 1995 the revelation of extended lengths of couple rehashes (TBRs) in the archaea chromosome. These districts, which were first characterized by the first studies, were engages in the guideline of the biological function. The short palindromic rehashes were renamed to CRISPR after the sequence was discovered to be regularly interspaced. The Cas genes were also identified as adjacent to the CRISPR locus. These findings recommend that the cas gene may assume significant parts resistance to bacterial infection.

The relationship between bacterial immunity and CRISPR was established ten years after the first discovery. It was proposed that the special grouping of the CRISPR gene confers immunity to bacterial cells. The impact of complementarity on the translation of the CRISPR locus into specific RNAs was also observed. It was also observed that the transcription of the CRISPR locus leads to the advancement of CRISPR-derived RNAs that can target foreign DNA. Another example is trans-activating crRNA which acts as a little RNA that is capable of protecting the host from DNA. In 2012, the revelation of single-guide RNA (sgRNA) enabled the utilization of gene therapy to break DNA. This procedure



works by forming a solitary RNA strand that can guide Cas9 to break the DNA.

Figure 1

CRISPR-Cas9 Technology

Efficiency

In view of the distinctions in the parts and pathways of action of the CRISPR framework, it very well may be separated into two major classes: class 1 and class 2. In order to perform well in genome altering, a class 2 framework generally requires only a solitary RNA-guided endonuclease. Therefore class 2 are more alluring for genome editing. The capacity of bacterial CRISPR-Cas9 systems to adjust the outflow of cas gene in mammalian cells has been contemplated extensively. In this context, the generation of a chimeric helper RNA (gRNA) through the combination of the CRISPR RNA and the trans-activating ribonuclease (tracrRNA) has been suggested. Cas9 endonuclease and gRNA are the most broadly utilized components of the CRISPR-Cas9 system. The gRNA 5' end is utilized to complement the target DNA. Cas9 can focus any DNA focus of interest with its own locale of the gRNA. By tweaking a 20 district, Cas9 can focus any area of the DNA with a PAM-containing genomic locus. The cleavage effectiveness relies upon the DNA restricting and complementarity of the gRNA to the objective DNA. It is also influenced by the genomic setting and the neighbourhood GC content. The tools intended to foresee the effectiveness of gRNA targeting can likewise be utilized to alter the module furthermore, improve the efficiency. For example, by modifying the gRNA structure or the transcription rate, the efficiency can be improved. The cleavage productivity relies upon the DNA restricting and complementarity of the gRNA to the objective DNA. It is also influenced by the genomic setting and the nearby GC content. The tools intended to anticipate the productivity of gRNA targeting can likewise be utilized to alter the module and improve the efficiency. For example, by modifying the gRNA structure or the transcription rate, the efficiency can be improved. The CRISPR-Cas9 system can induce the non-homologous end joining and the

homology-directed repair pathways to repair double-stranded breaks (DSBs). These pathways are known to repair DSB lesions by inserting or deleting nucleotides.

Specificity and off-target effects

CRISPR-Cas9 can modify the duplex of RNA and DNA to allow for cleavage without causing unintended off-target sites. However, it can likewise endure defects in the duplex and cleavage can occur at unintended locations. Due to the intricacy and size of the human genome, off-target DNA cleavage represents an eligible hazard of creating into a serious disease. The safety of germline altering is not thought of for the moment. Several approaches have been created to minimize off-target effects, including the utilization of online tools for designing gRNAs and predicting off-target sites. The on/off-target ratio has improved significantly with the utilization of gRNA optimization and Cas9-based nuclease engineering. Cas9 nucleases can be developed with various modifications, for example, the utilization of a couple of nickases, the combination of the Cas9 to a restriction enzyme, or then again the advancement of a high-fidelity variant. Because of the nature of the bacterial inception of cas9 and other endonucleases, they are likely to produce a host immune response. These systems are not suitable for long-term studies. Cas12a, a Cas9 nuclease, has properties similar to those of Cpf1, but likewise can possibly create host immune response. This issue requires careful evaluation of these systems in their beginning stages of development.

Delivery

For transient CRISPR-Cas9 delivery, the cargo includes DNA, Cas9 mRNA and/or gRNA, just as ribonucleoprotein complexes. These can be transfected into cells through various methods. Although transient methods are safe, they cannot support long-term CRISPR-Cas9 activity. The improvement of more precise cell-targeting techniques could add to improving the safety and decreasing the toxicity. Since viral vectors are widely utilized to deliver CRISPR-Cas9 cassettes to various cell types, they have been known to be inefficient in delivering large transgene cassettes. Another common issue with viral vectors is their restricted bundling limit. Because of the diminished transduction titre and the AAV bundling capacity limitation, the utilization of CRISPR-SpCas9 components in applications other than CRISPR-directed gene altering is not feasible. The quest for elective CRISPR-based systems that are smaller in size is still imperative to address these problems. As of the late depicted framework that utilizes little *S. aureus* genome could moderate the AAV bundling issue, but it has restricted productivity and is not suitable for utilization in humans.

CRISPR-Cas for CCR5 Interruption

CCR5 is an objective of therapy for people with homozygous deletions of the 32-bp duplicate of the CCR5 gene. Those with this genetic defect are naturally resistant to R5 infection, though they may experience a viral switch if they encounter a bacterial infection. In the case of Timothy Ray Brown, who was diagnosed with HIV-1, allogeneic donation of his blood cells, which were referred to as HSPCs, led to the first “sterile cure” for HIV. Despite this encouraging outcome, it is still not widely believed that the virus can be eradicated from the body through the utilization of transplantation. Finding suitable bone marrow-matched individuals with a high sensitivity to HLA-matched individuals is not feasible in large-scale studies. Autologous transplantations are less toxic and are not required for successful engraftment. Recent research has found on the improvement of novel autologous constructs that can modify CD34+ HSPCs what’smore CD4+ T cells.

CRISPR/Cas9 technologies are an option in contrast to ZFN and TALEN-based methodologies for inducing interruption of CCR5. They are easier to utilization and have better specificity and on-target location. Along with a CRISPR/Cas9-based transposon benefiter succession Ye *et al* successfully reproduced the naturally-occurring deletion of CCR532 in incited pluripotent stem cells. These cells were then separated into monocytes and precursors resistant to infection by HIV-1. Mandalet.al used the CRISPR/Cas9 framework to target CD4+ T cells and CD34+ HSPCs. The impact of the system on CD4+ T cells was minimal, while the capacity to createcancellation was improved by utilizing a doubles gRNA approach.

Role of CRISPR-Cas for Interrupting CXCR4

G protein-coupled chemokine CXCR4 is a critical component of the chemokine CXCL12 that is responsible to control the migration of immaturehematopoietic microorganisms to the chemokine CXCL12, and the virus avail it to enter cells with tropism of X4. The coreceptor CXCR4 is significant for the bone marrow cells survival. In the setting of HIV-1, it is utilized by the virus to infect the cell with X4tropism and in latter stagesdouble-tropism strains occurs in R5 tropism. To prevent infection with these coreceptors, the creators utilized a strategy that involves the utilization of lenti viruses and their gRNAs (Hou *et al*)

Similar to the method used by Schumann *et al.*, Cas9 ribonucleases were utilized to emend human CD4+ T cells. HDR template was introduced to induce insertion or deletion in the CXCR4 gene and the results showed that 60% of the cells diminished the statement of the protein.

Since changes in the outflow of CXCR4 could affect the hematopoietic cells' physiology, it is critical to carefully consider the impact of these changes on CD4+ T cells and the coreceptor pertinence of the cells. In this study, the authors demonstrated that reducing the quantity of CD4+ T cells that expressed CXCR4 suppressed the contamination of HIV-1.

A more unpredictable technique is also needed at the same upset both CCR5 and CXCR4.The aftereffect of this examination showed that in blend with T cells, the indel mutations rate increased significantly for both coreceptors.

Reticence of infecting virus by CRISPR-Cas

The cycle of infection by HIV-1 is multistep, and it involves the formation of virus-cell hybrids, the turn around the record of various RNA atoms, and the integration of these into the host cell's genetic material. In this study, the outflow of Cas9 and gRNA, which are both constitutively expressed, induced a persistent reduction of viral contamination in T cells. The CRISPR-Cas9 framework was utilized to create anti-HIV pluripotent undifferentiated organisms that are able to do of replicating and developing into monocyte- and macrophage. After a few days, these cells became resistant to the infection with HIV-1. Kaminski *et al.* initiated a strategy to control the outflow of Cas9 by utilizing a promoter that is actuated by viral Tat. They tested this strategy in TZM. bI cells and discovered that the arrival of viral Tat promotes the creation of constitutive nucleases. Using the CRISPR-Cas9 system, Hu and colleagues previously utilized TZM. bI cells to prevent infection with human immunodeficiency virus-1 (HIV-1). The cells were contaminated with various strains of the virus and, in turn, maintained their stable articulation of Cas9 and GRNAs. These results support the possibility that the CRISPR-Cas9 system could be utilized to develop a vaccine against the virus.

Inhibition of replication of virus by CRISPR-Cas

The primary procedure to forestall the replication of HIV-1 was to utilize RNAs to specifically target the LTR area and other sites in the virus' genome. In HEK293T cells, the blend of these two strategies resulted in high efficiency, with the utilization of both gRNAs and the gag and LTR genes controlling the declaration of luciferase. Similar to the approach utilized by Yin *et al.*, the reduction in different qualities was seen when guide RNAs were focused to specific genes. The impact of these RNAs was greater in accordance to the alternative genes. The concealment of viral replication was reported in CD4+ T cells derived from robust individuals. Moreover, the reduction of duplicates of HIV-1 was also seen in the treated cells comparative examination utilizing CD4+ T cells and fringe blood mononuclear revealed a diminish in the quantity of viral copies in the plasma cells of seropositive individuals. The reduction in viral particles and articulation of p24 in the CD4+ T cells and PBMC was also significant. Inhibition of the replication of HIV-1 virus by S-T1 cells was likewise embraced by (Wang *et al.*, 2018). This effect could add to the viral escape and limit the control of infection. In addition to studying the impacts of Sup-T1 cells on the improvement of virus, they also focused on the utilization of certain gRNAs to read frames between genes. The results also showed that even though the quantity of tainted cells and the quantity of indels decreased during infection, the advancement of infectious particles remained constant. This suggests that the impact of indels on the virus' ability to generate and distribute viral particles is limited. Many researchers tried to fragmentise the HIV-1 genome before the incorporation into the host cell. In vitro contemplations were conducted to test the impact of the Cas9-mediated destruction of the aftereffect's DNA. The results of these experiments showed that the reduction of positive cells was possible even when the virus was released into the cytoplasm. It was also discovered that the CRISPR-Cas9 framework could inactivate both early and late DNA of the virus.

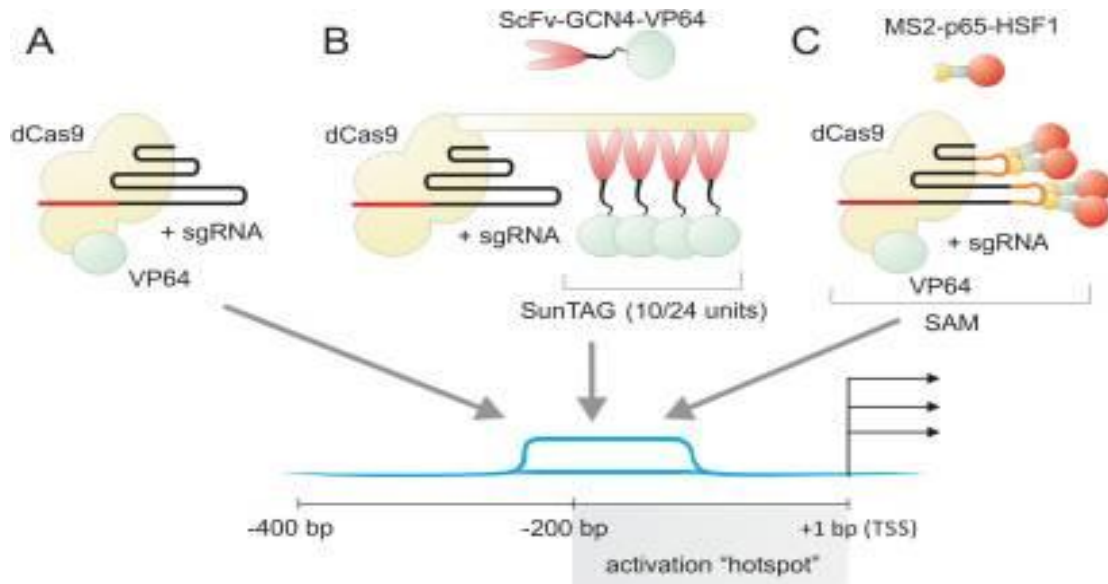
Possible role of CRISPR/Cas9-intervened dormancy in reactivating the virus

Essential technique for eliminating idle HIV repositories is to purge them by reactivating the latent virus. This strategy works by killing the latent cells through the active viral replication or by inducing the host immune framework to attack the contaminated cells this method is known as "shock and kill" method. Following the successful utilization of the histone deacetylase inhibitor beta deoxyisome hydroxamic acid (HDAC) to induce viral reactivation, it was estimated that the effect would only be limited to the actuation of memory CD4+ T cells. However, in a new study, the Siciliano lab has shown that the reservoir of viral protein is larger than previously thought and that the enactment of memory T cells is to great extent by the recurrence of events in the viral reservoir. The capacity to switch the latency of reservoir 29 through cell-reactivation or HDAC inhibitors is not feasible. In addition, the safety concerns associated with this approach are not ideal

Various forms of the CRISPR/Cas9 framework have been utilized to produce robust expression of targeted genes. These include the capacity to adjust the outflow of genes by coupling solitary sgRNAs with dCas9-VP64 fusion proteins. The systems can likewise be enhanced by selecting numerous dCas9-VP64 domains to a single dCas9 molecule. Different approaches can enhance the initiation of genes by selecting numerous dCas9-VP64 domains simultaneously. In this framework, the promoter's copy of dCas9 is connected to multiple VP64 districts through a scaffold called Sun Tag.

Through structural analysis, it has been revealed that dCas9 crystallized with sgRNA can cooperate with one another to advance the enrolment of effector area to the dCas9. In this work, the capacity to select numerous actuation areas was created through a combined activation mediator and short hairpin aptamers. (Koner mann *et al.*)

Through the utilization of CRISPR/Cas9, the capacity to target and induce the transcription of idle HIV-1 can be achieved. The most important factor influencing the initiation of the virus is the position of the objective site comparative to the start site (TSS). sgRNAs are known to reside in the vicinity of the TSS 32, and thus, could significantly job in the actuation of the virus. Future work will zero on creating sgRNAs which target the upstream promoter sequences of LTRs and achieve high-level activation. The ultimate goal is to build up a blend of these molecules that can activate both the viral promoter and the downstream



promoter sequences.

Figure 2

Methodologies to actuate HIV provirus using CRISPR/Cas9

Limitations of the CRISPR/Cas9 Application

Many diseases, such as Parkinson's disease and Duchenne muscular dystrophy, have been effectively treated using CRISPR/Cas9. The technology is safe and simple to utilize, and it has the characteristics of good proficiency and low cost. However, some limitations should be thought of before planning clinical trials.

Off-target effects are the fundamental concern in the improvement of new drugs. There are different kinds of off-target effects that can affect the target cell's ability to produce protein. Numerous endeavours have been made to limit askew impacts of Cas9 nucleases and other derivatives. These include the utilization of dimerization-dependent RNA-guided N-terminal FokI-dCas 9 nucleases and the utilization of truncated guide RNAs. (Wang *et al.*, 2015). The effect of this strategy on the CRISPR/Cas9 framework was evidenced by the reduction of the declaration of Cas9 in HIV-1 infectious cells (Kaminski *et al.*, 2016). Ifcoordinated to objective cells, then the Cas9 RNPs will degrade after editing the target DNA. Itappeared that a RNP complex can promote high-efficiency gene altering in human HSPCs with a reduced off-target editing ability. However, it can likewise trigger an innate immune response that can lead to cell cessation (Kimet *al.*, 2018). The immune response to Cas9 was not unique to humans and can be improved by various strategies. The improvement of these strategies and the evaluation of Cas9 immunogenicity ought to be contemplated before conducting clinical trials. (Crudele and Chamberlain, 2018),

Various lipid-based and nanoparticle-based approaches have been utilized to deliver CRISPR/Cas9. These include cationic polymer polyethyleneimine, lipid-based reagents, and nanoparticles. The blend of these strategies improved the productivity of the CRISPR/Cas9 framework by facilitating the repair of a human tyrosinemia gene. For the annihilation of latent HIV-1 reservoirs in the brain, the challenge is overcoming the blood brain barrier. The existence of the BBB is basic for the proper transport of large molecules and limited lipophilic molecules. Various nanoparticles have been utilized to focus the brain, such as gold nanoparticles, polymer nanoparticles, and magnetic nanoparticles. They have been investigated as the carriers for completing CRISPR/Cas9 system efficiently. The potential of using nanoparticles to convey CRISPR/Cas9 system to target HIV-1 reservoir in the brain is still not fully explored. Also, the conveyance, the delivery of VLPs and RNPs in human hematopoietic cells and bone marrow cells has been demonstrated to efficiently target EMX1, FTO, and other genes. It tends to be utilized to induce HDR expression in HEK293T cells and to generate transgenic mice with Tyr mutation. It can likewise be utilized to generate dCas9 expression in the liver. The potential of Nano blades for the advancement of therapeutic applications in iPSCs and HSCs is evidenced by their significant interest in iPSCs and HIV-1/AIDS treatment. However, prior to applying the technology in clinical therapy, safety what's more, off-target impact of the nano blades-based editing of host genes and their effect on primary cells and animal models should be tested.

Although it is considered as a promising tool to combat HIV-1, it has evolved escape mechanisms. For instance, by inducing the repair of NHEJ, the virus can get away inhibition by replicating without affecting the cleavage sites. The escape mechanism from CRISPR/Cas9 is also known as the NHEJ movement escape mechanism. This prompted the workers to formulate new solutions, likewise altering sgRNA or developing Cas9.

New Cas12a system

Several features of the Cas12a system were discovered, including increased specificity furthermore, a more modest cassette size. These advantages could help minimize off-target reactions and improve efficiency. The Cas12a system has lower gene editing proficiency relative to Cas9 and may have some flaws. We then focused on improving the Cas12a system by optimizing the statement of crRNA. Our results exhibit that small RNAs with a precise 5' end can be proficiently produced with the help of specific nucleotides. The results provided insights into how to improve the proficiency of CRISPR-Cas12a-based gene editing. They also permitted us to extend the duration of HIV inhibition experiments.

There is a difference in the genuine DNA cleavage occasion between the CRISPR and non-CRISPR systems. Cas9-induced cleavage is more basic for target DNA cleavage and less basic for binding and cleavage of gRNAs. Cas12a's ability to repair and cleave DNA is beneficial for inactivating genes because it allows the retargeted inclusions and erasures to be repaired. A more vigorous indel type mutation is more likely to be generated if the virus is replicating-incompetent

Animal Models for disrupting Introduced Virus

Although the productivity of CRISPR-Cas9 editing has been demonstrated to be beneficial in vitro, it is still not feasible to utilize it in vivo. For instance, Kaminski and his team demonstrated that Tg26 transgenic mice could successfully mimic the human HIV-1 virus infection. DNA from various organs was utilized to produce the sequences.

The consequences of the examination support the *in vivo* annihilation of human immunodeficiency virus-1 by confirming the presence of the similar parts as those found in the *in vitro* test. They also show that the evacuation of the hereditary material from target districts in circling lymphocytes is related with proviral eradication.

The adenovirus utilized AAV-DJ/8 and gRNA to focus the districts of HIV-1 provirus. They had the option to successfully destroy the virus in non-human primates without affecting the outflow of genes for the destruction of Cas9 and gRNA.

In live mice, the statement of viral genes was altogether diminished after 19 days. The authors noted that the conveyance of gRNAs and Cas9 suppressed the viral genome *in vivo*.

In a more relevant living model, the proficiency of anti-viral therapy was demonstrated in all more clinically significant animal. The model is known as BLT mice, which are formed from bone marrow, liver, and blood.

Using PBMC cells from three HIV-1-positive individuals, Bella *et al.* induced the cells to deliver LTR A and LTR B, which were coordinated to the LTR district. The outcomes showed that these two components reduced viral DNA significantly.

The utilization of CRISPR-Cas in the treatment of human immunodeficiency virus (HIV) led to the evacuation of the viral hereditary material from PBMC cells of the tainted individuals. The consequences of this examination support the thought of conducting human clinical preliminaries utilizing CRISPR-Cas.

CONCLUSION

The capability of CRISPR/Cas9 to block infection and promote the growth of other diseases is also attractive for the advancement of new therapies. CRISPR/Cas9-based activators are promising for the treatment of inactively tainted viral reservoirs, as well as for the removal of replicating virus. They can also generate deletion variants of CCR5, which provide important proof for the possibility of a functional cure. The studies done so far by the eminent workers proved to show the possibilities for managing particularly to reduce off-target impacts and the potential to fix and prophylaxis of HIV-1. The future studies should work in this field for exploring more about this study.

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