CRISPR-CAS Replacing Antiviral Drugs against HIV: An Update

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ABSTRACT: Various antiretroviral drugs do not kill or cure the human immunodeficiency virus (HIV) but do prevent the replication of the virus. The combination of antiretroviral drugs is known as highly active antiretroviral therapy (HAART). Current drug therapies effectively suppress HIV-1 replication but do not inactivate the provirus that persists in latent reservoirs. Guide RNA (gRNA)-directed CRISPR/Cas9 system can be used for sequence-specific attacks on this proviral DNA. The biggest achievement might be the complete elimination of HIV from infected cells. A study revealed that the tail injection, in transgenic mice and rats having HIV-1 genome, of an adenoassociated virus (AAV) vector expressing a short version of the Cas9 endonuclease (saCas9) and the gRNAs resulted in the cleavage of integrated HIV-1 DNA and excision of a DNA fragment spanning between the LTR and Gag gene in the spleen, liver, heart, kidney, and circulating lymphocytes. HIV-1 has capacity to escape the attack on its genome from most of inhibitors. Thus, to achieve successful antiretroviral treatment, combinations of several antiviral therapies have been applied that are based on two important facts. The first is that multiple drugs lead to synergistic or additive inhibition, and the second is that the combinational therapy increases chances of drug resistance. The success that has been achieved with the help of the genetically engineered tool CRISPR is that dCas9 protein alone can efficiently silence viral gene expression in bacteria with sgRNA. All the reported investigations have indicated that CRISPR/Cas9 can be used as immune machinery into human cells in the form of novel antivirus tools.

KEY WORDS: antiretroviral drugs, CRISPR/Cas9, gRNA, HIV

I. INTRODUCTION

Human immunodeficiency virus (HIV) can cause acquired immunodeficiency syndrome (AIDS) if not treated. The human body cannot dispose of HIV completely even with the treatment, unlike other viral infections. Therefore, if someone is infected with HIV once, he or she will remain infected for their entire lifetime. The body's immune system helps to fight against the viral infection, and HIV specifically targets the CD4 cells (T cells) in the immune system. When HIV attacks, the number of CD4 cells in the immune system decreases and the person becomes more vulnerable to viral infections. With time, HIV can lead to the destruction of various CD4 cells, making the person unable to combat viral infections and diseases. Hence, the weak immune system of a person leads to the last stage of HIV infection, which is AIDS.[1]

Antiretroviral therapy is used to treat HIV. The drugs in this therapy do not kill or cure HIV, but they do prevent the growth or replication of the virus. Antiretroviral drugs are referred to as ARV, and when medicines are taken in combination it is called highly active antiretroviral therapy (HAART).[2] ARVs act by blocking the various stages of the life cycle of the virus; as a result, the virus cannot replicate and cause infection. If treatment progresses without interruption, the virus becomes latent and unable to cause infection. If the treatment is interrupted, however, the virus can reappear as the viral genome is still present in the host.[3]

The advances in the knowledge of HIV and improvement in antiretroviral therapy (ART) in the

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past 30 years are unique in the history of medicine. Although HIV has been controlled and the death rate reduced to a significant number, it is still regarded as an incurable disease. This essentially occurs because there is no adequate treatment to get rid of HIV completely from its host cell and the virus becomes persistent because it acts as a latent provirus. Due to the persistence of HIV, lifetime antiretroviral drugs are required to restrain the HIV viral particles into the bloodstream of the patient. Moreover, treatment failure and drug resistance against HIV has developed as a result of interrupted drug intake. Due to the high mutation rate of the virus, HIV develops drug resistance against antiretroviral therapy. In addition, toxicity can also develop as a result of long-term drug use. Although antiretroviral therapy is life-saving, it does not provide a cure against HIV due to viral persistence. These issues motivate scientists to find more advanced methodologies to cure HIV disease.[4]

Bacteria and Archaea have developed a particular defense system made up of CRISPR (clustered regularly interspaced short palindromic repeat) along with the Cas9 (CRISPR associated system) protein against the foreign plasmids and viruses. Recently, Cas9 proteins with single guided RNA (gRNA) have been used for the targeted genome editing in various organisms, including human cells.[5] These molecular scissors can also be used to remove the HIV-1 provirus from the host genome. The CRISPR/Cas9 system is adapted to human cells to directly target and destroy the viral RNA genome during their life cycle within the host and provides the defense against foreign DNA and viruses. The CRISPR/Cas9 system open a new way for research and can be used as a new therapeutic strategy against viral infections. The CRISPR/Cas9 system is cost-effective, simple to perform, and highly specific in targeted genome editing.[6]

II. ROLE OF CRISPR/CAS9-BASED GENOMIC ENGINEERING IN HIV TREATMENT

Previously, scientists had been investigating a method that would permanently eliminate HIV infection; unfortunately, the strong resistance due to virus plasticity that leads to presence in a latent state within the host, even in the presence of antiretroviral drugs or other similar preventive measures, has placed a permanent block in such investigative techniques.

One recent invention that proved to be helpful in developing a cure for HIV is known as the CRISPR/Cas9 system, specifically "clustered regularly interspaced short palindromic repeats." CRISPR are a specific type of short portions of DNA repetitions with additional short segments of selective DNA snippets exposed from viral phage. The Cas genes (CRISPER-associated genes) have the capability to remove specific genetic portions and insert new ones.[7]

RNA interference (RNAi) was previously used for silencing the expression of genes; this was composed of a combination of small RNAs and mRNAs for the degradation of target transcripts.[8] Other methods that previously helped in altering gene expression by fusing to repressors or transcriptional activators are known as zinc fingers and transcription activator-like effectors (TALEs).[9] The limited effectiveness, such as high off-target effects of RNAi and complications in construction and delivery of zinc fingers and TALEs into the cell, make them less functional compared to advanced CRISPR technology, which offers more efficient, robust, multiplexable, and designable approach for genome-wide activation or repression.[10–14]

CRISPR technology works by fusing transcriptional repressors (CRISPRi for repression) and transcriptional activators (CRISPRa for activation) with a catalytically inactive form of Cas9 protein, termed as dCas9. Targeting of dCas9 to the genome is dictated by a single guide RNA (sgRNA) containing a designed 20-nucleotide sequence complementary to the DNA target, which is adjacent to a short DNA motif, termed the proto-spacer-adjacent motif (PAM).[15] The dCas9 fusion protein complexed with a sequence-specific sgRNA binds to the target DNA and is engineered such that it localizes a repressive or activating effector domain to turn down or turn on transcription of the target genes. CRISPR interference and CRISPR activation has now been helping investigators to systematically interrogate gene expression.

The biggest achievement of this tool has been seen in the complete elimination of HIV virus from

infected cells. The tail injection of an AAV vector expressing a short version of the Cas9 endonuclease (saCas9) and the gRNAs resulted in the cleavage of integrated HIV-1 DNA and excision of a DNA fragment spanning between the LTR and Gag gene in the spleen, liver, heart, lung, and kidney as well as in the circulating lymphocytes. Furthermore, retroorbital inoculation of rAAV9:saCas9/gRNA in transgenic rats eliminated a targeted segment of viral DNA and decreased the level of viral gene expression in circulating blood lymphocytes. Such results were the first evidence revealing removal of HIV-1 DNA from infected cells with the help of the genetically engineered tool CRISPR. After testing on transgenic rats, scientists further tested this method on a humanized mouse model having infected human immune cells with HIV virus; the tests revealed 96% removal of HIV-1 virus in the mice, confirming that this method could be used in prophylactic treatment.[16]

HIV-1 has the capacity to escape the attack on its genome from most of inhibitors such as anti-viral drugs, RNAi zinc finger nucleases, or transcription activator-like effector nucleases.[17,18] However, to achieve successful antiretroviral therapy, combinations of several antiviral treatments have been applied that are based on the two important facts. The first one is that multiple drugs lead to synergistic or additive inhibition, and the second is that the combinational therapy increases genetic threshold for the acquisition of drug resistance. Similarly, combinational RNAi was proved to be effective in stopping viral replication and viral escape (Fig. 1).[19]

The success that has been achieved with the help of CRISPR is that dCas9 protein alone can efficiently silence viral gene expression in bacteria with a well-designed sgRNA but moderately in mammalian cells; however, when the similar protein was fused with repressive KRAB (Kruppel-associated box) domain of Koxl strong gene silencing was observed when cell stably expressed, leading to multiple gene silencing with coexpression of multiple sgRNAs.[10,12]

Furthermore, multiple genes can be repressed or activated simultaneously by codelivering multiple cognate sgRNAs to analyze multiple genes' interaction.[10,12,20–22] For activating and repressing multiple

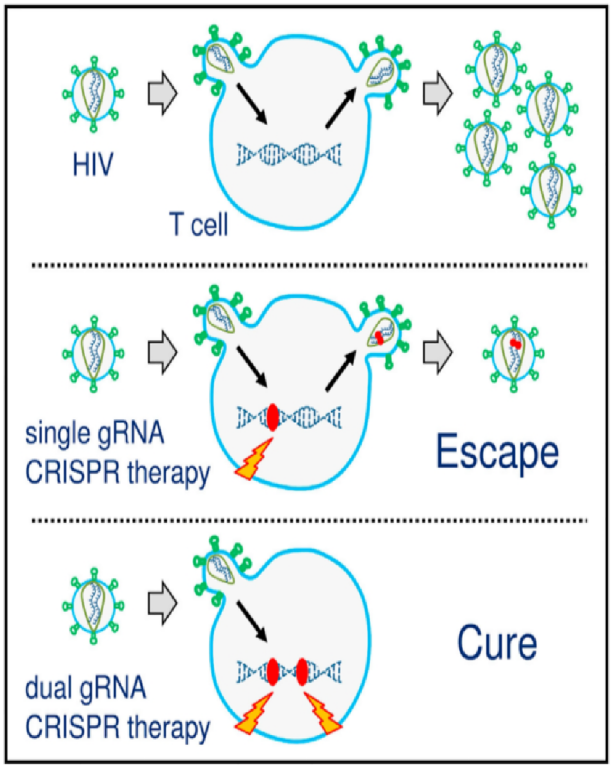

FIG. 1: Targeting HIV-1 DNA with single and dual gRNAs (Reprinted from Wang et al.,[29] with permission from Elsevier, Copyright 2016)

genes in the same cell scaffold RNAs (scRNAs) were engineered with the fusion of sgRNAs to orthogonal protein-binding bacteriophage RNA such as MS2, PP7, and Com.[14] It has been shown that codelivery of MCP–VP64 and COM–KRAB with a dCas9 protein allows simultaneous activation of CXCR4 (a chemokine receptor) and repression of B4GAL4NT1 (encoding β-1, 4-N-acetyl-galactosaminyl transferase) in the same cell.[14] Thus, engineered scRNAs provide a versatile platform for multigene modulation for recruiting diverse effectors to different genomic loci.

Cas9 proteins have been abundant across the bacterial kingdom, varying in sequence and size. The Cas9 enzymes comprise an HNH domain for cleaving DNA strand complementary to the guide RNA sequence (target strand), and a RuvC nuclease domain required for cleaving the noncomplementary strand (non-target strand), yielding double-strand DNA breaks (DSBs). At a desired location these

DSB can open up the DNA. The ability of genetic engineering of Cas9 proteins such as cleavage of DNA at specific sites is due to guide RNAs. When directed to target loci in eukaryotes by either dual crRNA: tracrRNA guides or chimeric single-guide RNAs, Cas9 generates site-specific DSBs that are repaired either by nonhomologous end joining or by homologous recombination, which can be exploited to modify genomic sequences in the vicinity of the Cas9-generated DSBs. The opened DNA can then be targeted by crRNA: tracrRNA segments that remove and replace the targeted DNA or by a chimeric single-guide RNA, which accomplishes this all in one step (Fig. 2). This is the second step in CRISPR gene targeting and reengineering.[7]

Furthermore, catalytically inactive Cas9 alone or fused to transcriptional activation or repression domains can be used to control transcription at sites defined by guide RNAs.[23] Both type II-A and type II-C Cas9 proteins have been used in eukaryotic genome editing. Smaller Cas9 proteins, encoded by more compact genes, are potentially advantageous for cellular delivery using vectors that have limited size such as AAV and lentivirus.[7]

All these present investigations have indicated that CRISPR/Cas9 can be used as immune machinery into human cells in the form of novel antivirus tools. It has been observed that Cas9 proteins can either target the viral coding or noncoding regions during their preintegration or provirus stages; meanwhile, targeting coding regions can directly disable viral genes through mutations, insertions, or deletions.

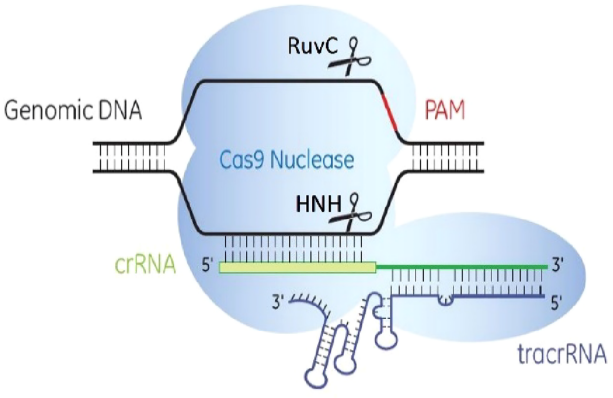

FIG. 2: CRISPR: cut and paste at the genome level

The next step would be testing the permanent cure of HIV using CRISPR/Cas9 with a primates model, which will allow further investigations in latent T cells reservoirs and privileged sites for HIV-1 such as brain cells. Nevertheless, the most crucial step would be cranial trials with human patients in which securing ethical concerns and human genome integrity would be the priority. CRISPR/Cas9 would become the main human genetic tool for correcting many genetic disorders. Many technical advances are allowing researchers to address some of the off-target and reversible CRISPR/Cas9 problems.[16]

III. FUTURE PERSPECTIVES

Taking all the recent advances into consideration, it is safe to say that CRISPR-Cas9 is the next step in human genetic engineering. It will be the answer to many genetic disorders and might even be required for mechanisms to correct unwanted epigenetic modifications. Technical advances have already addressed some of the off-target problems, and reversible CRISPR-Cas9 is being tested. However, a recent ethical concern regarding the modification of the human genome and the right to preserve genetic diversity has raised concerns at different levels. Some future applications of CRISPR/Cas9 genome engineering include human gene therapy, screening for drug target ID, agriculture, crops and animals, viral gene disruption, pathogen gene disruption, programmable RNA targeting, synthetic biology pathway engineering, and ecological vector control.[24]

IV. FUTURE CLINICAL TRIALS

CRISPR/Cas9 is replacing the antiretroviral therapies against HIV, and further innovations are also evolving in CRISPR/Cas9 with time. CRISPR/Cas9 has also been employed to remove HIV-1 DNA by making gRNAs that target the long terminal repeat (LTR) promoter DNA of viruses or essential viral genes. It was first reported that Cas9 and LTR targeting gRNA inhibited the HIV LTR-driven reporter gene expression. It was also evident from the sequencing analysis that CRISPR/Cas9 treatment caused insertions and deletions in the LTR and removed the viral DNA between the 5' and 3' LTRs. To demonstrate the efficient

editing of DNA in HIV transgenic mouse models, Kaminski and Yin used the recombinant AAV vector to express and deliver gRNAs and saCas9 into mice. Providentially, by the help of two properly designed gRNAs, escape of HIV-1 from CRISPR/Cas9 attack can be overcome.[23–25]

According to recent studies, HIV-1 gRNAs do not suppress various viral quasispecies within patients, which continue to multiply; due to this observation, the genomes of 23 HIV-infected patients were sequenced in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES). Then some gRNA strategies were designed. One was personalized based on HIV-1 sequences of the patient; the other was the broad spectrum based on the consensus sequences of the large number of patients. This bioinformatics algorithm strategy increases the excision capability of CRISPR/Cas9, which controls the molecular heterogeneity of HIV-1 that persists after long antiretroviral therapy.[26]

The sequential application of long acting slow effective release (LASER ART) rilpivirine, myristolyated dolutegravir, lamivudine, and abacavir and deletion of viral DNA by CRISPR/Cas9 in the HIV-1–infected mouse will led to the removal of viral DNA with both therapies. In contrast, HIV-1 was readily detected in all infected animals treated with LASER ART or CRISPR-Cas9 alone. Thus, viral sterilization is possible by the administration of LASER ART and CRISPR/Cas9 in combination within the HIV-1–infected humanized mice.[27]

China is progressing to perfect gene therapies in the global race. Some patients have seen improvement in these experiments, but scientific papers have not been published yet regarding these experiments. There have been a total 15 deaths, but scientists reported that they were unrelated to CRISPR/Cas9 treatment; those deaths were due to previous medical conditions. Out of the 15 deaths, seven of those were in one trial.

CRISPR/Cas9 trials on humans have been slow because the institutions or the scientists first have to seek advice from the National Institutes of Health to assess the risks and benefits of CRISPR/Cas9 therapies and then get approval from the US Food and Drug Administration; it takes years to obtain the ethical approval for the experiments. Researchers in Pennsylvania received the approval for CRISPR/Cas9 based therapy on 18 cancer patients in two years. Moreover, the genetic disorder beta-thalassemia can be treated with CRISPR/Cas9, and it is hoped that a Phase I clinical trial regarding beta-thalassemia will begin soon. CRISPR trials are also slow because there is no strong scientific evidence on the safety of CRISPR in humans; moreover, scientists are afraid of unintended mutations caused by CRISPR. If any of the experiments become successful, it will open doors for many innovations in the field of medical sciences.[28–30]

V. CONCLUSION

HIV can cause AIDS and specifically targets the CD4 cells (T cells) in the immune system, making the person more vulnerable to viral infections. Antiretroviral therapy does not kill or cure the HIV but prevents the growth or replication of the virus. Due to the high mutation rate of the virus, HIV develops drug resistance against antiretroviral therapy and persists. The CRISPR/Cas9 system proved to be helpful in developing a cure for HIV. The biggest achievement of this tool has been seen in the complete elimination of HIV virus from infected cells. The CRISPR/Cas9 system is adapted to human cells to directly target and destroy the viral RNA genome during its life cycle within the host and provides the defense against foreign DNA and viruses. The CRISPR/Cas9 system open a new way for research and can be used as a new therapeutic strategy against viral infections. The CRISPR/Cas9 system is cost-effective, simple to perform, and highly specific in targeted genome editing.

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