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CRISPR-Cas9 System In Vivo Delivery to Combat HBV

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Abstract

Background: Hepatitis B Virus (HBV) infection remains a major global health issue despite the availability of HBV vaccine. The novel CRISPR-Cas9 gene editing technology efficiently helps to cure HBV by disruption or cleavage of HBV DNA. Aims: Several in vitro and in vivo studies have demonstrated the effectiveness of HBV-specific clustered regularly interspaced short palindromic repeat (CRISPR)/associated protein 9 (CRISPR/Cas9) systems in cleaving HBV DNA. **Methods:** In vivo, delivery of the CRISPR/Cas9 system at target sites remains a major challenge that needs to be resolved before its clinical application in gene therapy for HBV. **Results:** In this review article, we comprehensively evaluate the progress, challenges, and therapeutic potential of CRISPR-Cas9 gene therapy for HBV using adeno-associated virus (AAV) vectors as delivery vehicles. **Conclusion:** The CRISPR-CAS9, HBV, AAV, delivery methods of CRISPR-CAS9 component in vivo, challenges, and future perspectives in harnessing this innovative technology to combat HBV infection.

Keywords: hepatitis B virus; CRISPR/Cas9; delivery; AAV vectors; in vivo; Gene editing.

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Introduction

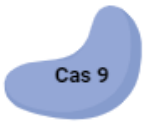
CRISPR, or Clustered Regularly Interspaced Short Palindromic Repeats, stands out as a precise tool for editing genomes by disrupting or cleaving viral or infected DNA. The CRISPR-Cas system, originally discovered in Escherichia coli bacterial adaptive immunity, is a natural defense mechanism against viral infections, particularly bacteriophages. This ground-breaking system was first elucidated by Ishino et al. in 1987.

Following the cleavage or disruption of viral DNA, it converts into a double-strand break (DSB). CRISPR effectively halts the functionality of viral DNA, preventing the formation of harmful proteins responsible for viral activity. The CRISPR-Cas system operates through two essential components for disrupting or cleaving viral DNA: guided RNA (gRNA) or CRISPR RNA (crRNA) and the CAS protein, an endonuclease enzyme responsible for cutting or cleaving viral DNA. The gRNA, complementary to the targeted DNA requiring cleavage, guides the CAS protein to the precise location. The specific DNA sequence where gRNA binds is termed the proto-spacer adjacent motif (PAM). After binding to the targeted DNA or PAM, the gRNA guides the endonuclease CAS protein to the exact cleavage location, forming an effector complex. Together, they cleave the viral DNA, transforming it into a double-strand break (DSB) and effectively ending its functionality after the conversion of DSB viral functionality is stopped or removed (Kayesh et al., 2020).

There are three main categories of CRISPR genome editing tools: For the activity of endonucleases, the CAS system types I, II, and III each have the cas3 protein, cas9 protein, and cas10 protein, respectively. Bacteria and archaea can protect themselves from intrusive genetic elements and viral infections using type I CRISPR-Cas systems. Their efficacy in

identifying and eliminating Cas3 foreign genetic material is attributed to their distinct modes of action, which involve the Cascade complex and the protein. Bacteria and archaea have microbial immune systems known as type II CRISPR-Cas systems. One of the most well-known and widely used genome editing tools, CRISPR-Cas9, belongs to the Type II CRISPR-Cas system (Redman et al., 2016). The single effector protein Cas9, which is in charge of target recognition and DNA cleavage, distinguishes type II systems. The cas10 protein in type III systems predominantly targets and degrades RNA molecules. Due to its unique RNA interference mechanism and possible uses in gene regulation, RNA targeting, and diagnostics, type III CRISPR-Cas systems have attracted attention. III CRISPR-Cas systems have attracted attention because of their unique RNA interference mechanism and possible uses in RNA targeting, gene regulation, and diagnostics. See Table 1.

Table 1. Mechanism and possible uses in RNA targeting, gene regulation, and diagnostics (Lin et al., 2014)

Types	Protein	Function	Mechanism
Cas I		Cas I systems are involved in interference and adaptation	Cas3 protein is responsible for the cleavage of target DNA.
Cas II		Cas II systems are primarily involved in interference (target DNA degradation)	The Cas9 protein uses its guide RNA to recognize and bind to target DNA. It then introduces DSB.
Cas III		Cas III systems may play a role in targeting both DNA and RNA	Cas10/Csm/Cmr complexes target and cleave both DNA and RNA

CRISPR-Cas9 is a member of CRISPR type II. The bacterium Streptococcus pyogenes is where the CRISPR-Cas9 system was first identified and described. In this system, the bacteria are protected from pathogenic viruses like bacteriophage. It is an antibacterial weapon (Lin et al., 2014). The RNA-guided DNA cleavage mechanism of the CRISPR-Cas9 system was discovered, which paved the way for the creation of the ground-breaking genome editing instrument that we use today. It consists of two parts. The Cas9 nuclease protein functions as a molecular scissor and cleaves viral double-stranded DNA to cause double-strand breaks (DSBs), disrupting the virus's destructive functioning. The second element is guided RNA (gRNA), a synthetic RNA molecule created to complement a particular target DNA sequence we wish to degrade into DSB. The nuclease cas9 protein is guided to the precise site of cleavage by this gRNA. This process refers to CRISPR/CAS9 as gRNA and the Cas9 nuclease protein work together (Ramanan et al., 2015).

gRNA is made up of two main parts. The first element is CRISPR RNA (crRNA). This brief RNA sequence comes from the CRISPR array, a section of the genome's DNA of certain bacteria) of the bacterial or archaeal host. The guide RNA (gRNA) plays a crucial role in the CRISPR-Cas9 system. It contains a spacer sequence that matches the target DNA sequence, guiding the Cas9 protein to the precise location for genome editing. The host organism acquires the spacer sequence through previous encounters with foreign genetic material, like viruses or plasmids.

The gRNA consists of two components: the crRNA, which carries the spacer sequence and guides Cas9, and the Trans-activating CRISPR RNA (tracrRNA), a separate RNA molecule providing structural support and stability to the gRNA. Together, they form a complex that

directs the gRNA to the target DNA. While tracrRNA is naturally present in some CRISPR systems, it has been engineered into the gRNA for enhanced efficiency and precision in the CRISPR-Cas9 system.

A single guide RNA (sgRNA) is generated to target a gene sequence by combining crRNA and tracrRNA. The Cas9 nuclease protein requires a proto-spacer adjacent motif (PAM), a specific DNA sequence where gRNA and Cas9 bind, as illustrated in Figure 1. Cas9 cleaves complementary and non-complementary DNA strands, creating a double-strand break (DSB).

After DSB formation, the cell's natural repairing pathways come into play. One primary pathway is Non-homologous End Joining (NHEJ), which attempts to reconnect the broken DNA ends, restoring DNA integrity. When DSB occurs, cellular proteins swiftly recognize the damage and initiate the repair process. Damaged or mismatched bases are removed, ensuring clean ends suitable for repair. This repair process may introduce insertions, deletions, or mutations at the repair site. In the context of CRISPR-Cas9 genome editing, NHEJ disrupts specific genes, leading to gene knock-out or loss of gene function (Lv et al., 2021).

Homologous Directed Repairing (HDR) is the second repair method. A homologous DNA template is required for HDR to fix the break precisely. High-fidelity homology repair (HDR) preserves the original DNA sequence with remarkable accuracy using an unaltered homologous template. It is necessary to preserve the integrity and stability of the genome. By offering an original repair template, HDR can be used with CRISPR-Cas9 genome editing to insert targeted modifications into the genome. Because of this, HDR is an effective approach for precise gene editing, enabling scientists to insert or change particular DNA sequences accurately (Ramanan et al., 2015).

The Cas9 protein features two crucial domains, namely HNH and RuvC, responsible for cleaving the two strands of targeted DNA during the genome editing process. The HNH domain, named after its characteristic conserved sequence motif (HNH), is a nuclease domain within Cas9. Its role is to cleave the complementary DNA strand in the target DNA. When guided to the target DNA sequence by the guide RNA (gRNA), the Cas9 protein's HNH domain specifically cleaves the DNA strand complementary to the gRNA.

On the other hand, the RuvC domain is another nuclease domain within Cas9, named after the structure-specific endonuclease found in E. coli (RuvC endonuclease). The RuvC domain cleaves the non-complementary DNA strand in the target DNA. In summary, these two domains play distinct roles in the precision of DNA cleavage during the CRISPR-Cas9 genome editing process. The RuvC domain cleaves the non-complimentary strand after the Cas9 protein has been led to the target DNA, and the HNH domain breaks the complementary strand. This creates the double-strand break (DSB) needed for genome editing.

Hepatitis B (HBV) infection is a major worldwide issue (Lin S. et al., 2014). Hepatitis B Virus (HBV), belonging to the Hepadnaviridae family, is the causative agent of hepatitis B, a potentially severe and chronic liver infection in humans. Extensive research on HBV indicates that approximately 296 million individuals worldwide were infected with HBV in 2020. It is estimated that hepatitis B causes 820,000 fatalities annually, with 25% of chronic infections progressing to liver cancer. Each year, around 1.5 million people globally acquire new HBV infections. In Pakistan, specifically, there are approximately 4.55 million people infected with HBV.

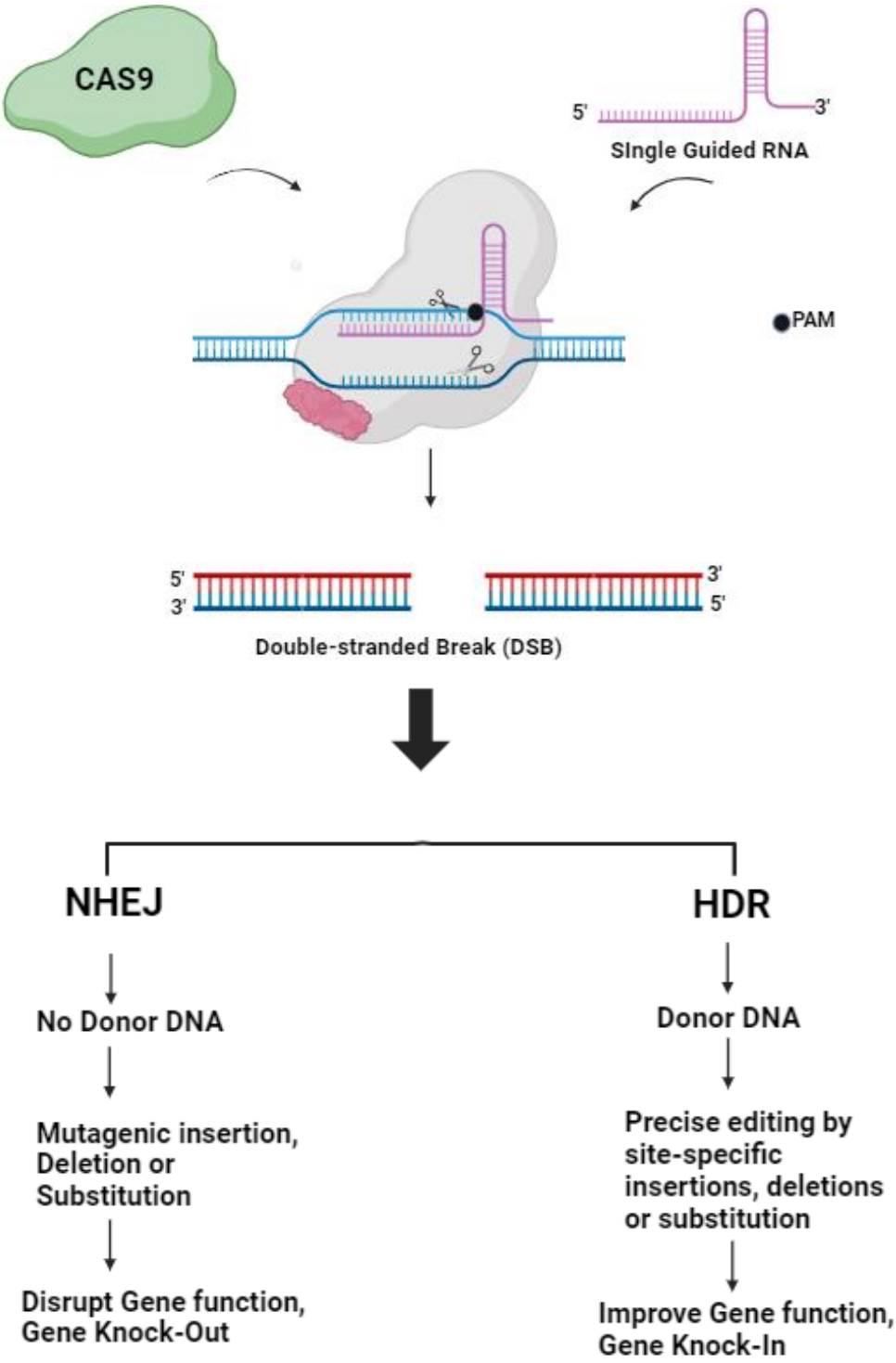


Figure 1. Describe the mechanism of CRISPR-CAS9 first. Cas9 nuclease protein combines with synthetic gRNA to form an effector complex that will cut targeted double-stranded DNA and convert it into DSB. After that, the repairing mechanism will activate the NHEJ and HDR mechanism to disrupt gene functionality or gene knock-out and improve the gene functionality or Knock-In, respectively (Redman M. et al., 2016).

HBV features a circular, partially double-stranded DNA genome, approximately 3.2 kb. This genome comprises four open reading frames (ORFs): PreS/S, Core, Polymerase, and X. These ORFs encode various viral proteins crucial for the virus's lifecycle. The viral core is enveloped by an icosahedral protein capsid composed of core protein (HBcAg), which encapsulates the viral DNA and plays a vital role in viral assembly. Additionally, HBV

possesses a lipid envelope derived from the host cell membrane. This envelope includes three viral surface antigens: Large (LHBs), Medium (MHBs), and Small (SHBs). These envelope proteins are essential for viral entry and immune recognition. Figure 2 provides a schematic diagram illustrating HBV's key components and structure.

The Hepatitis B Virus (HBV) initiates infection by binding to hepatocytes, which are liver cells, through the interaction of viral envelope proteins with host cell receptor sites. The virus can enter the human body if the host cell receptors are compatible with the HBV envelope proteins; otherwise, entry is prevented. The HBV genome is transported into the host nucleus upon entering the body. Within the nucleus, the relaxed circular DNA (rcDNA) is transformed into covalently closed circular DNA (cccDNA) using the host nucleus machinery. This cccDNA serves as a template for RNA transcription, generating viral RNAs, including genomic RNA (pgRNA), messenger RNAs (mRNAs), and noncoding RNAs. Host transcription and translation machinery are employed, translating these RNAs into viral proteins such as core proteins, envelope proteins, and polymerase.

The P ORF-encoded viral polymerase uses the pgRNA as a template to aid in reverse transcription, which creates new viral DNA. The pgRNA-DNA hybrid is encapsulated in the core protein, forming new virions. Virions assemble in the endoplasmic reticulum, where viral envelope proteins are integrated into the lipid envelope. Fully assembled virions are then released from the cell, often through a non-lytic mechanism Top of Form (Boni et al., 2007).

Despite the availability of an effective preventive HBV vaccine, chronic HBV infection remains a major global health problem. Therefore, novel alternative therapeutic strategies need to be developed to eradicate cccDNA with minimal side effects, and the CRISPR/Cas system appears to be a promising tool for achieving this goal.

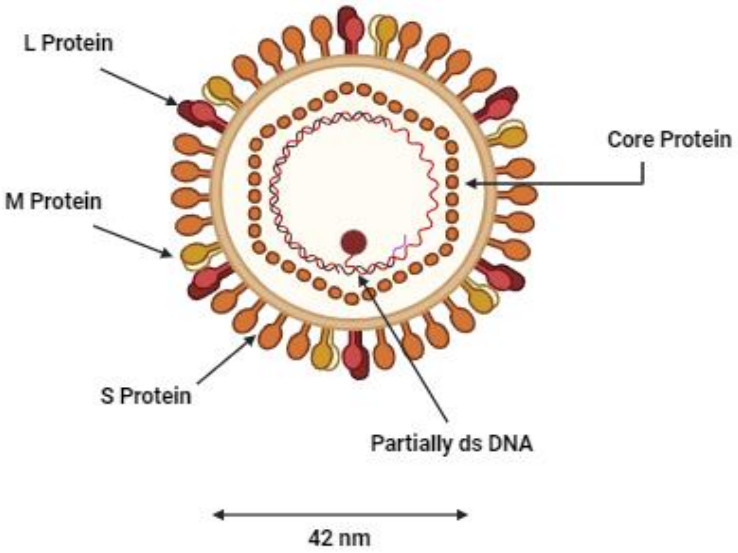


Figure2. Schematic diagram of hepatitis B virus (HBV) particles (Boni et al., 2007).

According to recent research, the CRISPR-Cas9 system can effectively target different diseases by cleaving double-stranded DNA into single-strand breaks. Moreover, it successfully blocks the Hepatitis B virus's ability to replicate and create cccDNA. The most common method for studying CRISPR-mediated gene therapy for HBV is Streptococcus pyogenes Cas9 (SpCas9). Guide RNA design approaches are used to specifically and successfully disrupt HBV sequences, including cccDNA and viral RNA transcripts (Zhu et al., 2016).

The CRISPR-Cas9 system and its constituent parts (gRNA and Cas9 nuclease protein) must be introduced into the host cell genome to be therapeutically effective. CRISPR-Cas9 components can be transferred to host cells using various methods, including in vitro and in vivo. In vivo—within living things—and in vitro—away from living things (Kayesh, M. E. H. et

al., 2020). The most popular technology for genome editing in in vivo therapeutic applications is the CRISPR-Cas9 system. For an appropriate delivery, in vivo models on animals were needed. A suitable and safe delivery mechanism is required to deliver CRISPR-Cas9 components to the host or targeted host cell. Several delivery techniques transfer CRISP-Cas9 components to the host cell for gene editing. The most popular delivery methods, such as non-viral and viral ones, enable the safe transportation of CRISPR-Cas9 components into host cells. Delivery is essential to the CRISPR-Cas9 system's ability to fight HBV (Kennedy, E. M. et al., 2015). We will talk about the CRISPR-Cas9 mediated suppression of HBV using the knowledge that is now available, with a particular emphasis on the delivery mechanism—more specifically, the adeno-associated virus (AAV) vector—that allows the CRISPR-Cas9 components to be delivered to the in vivo. In this review, we will also touch on non-viral vector-based delivery briefly.

Viral Vectors Based Deliver CRISPR-Cas9:

Delivery methods for delivering the CRISPR-Cas9 genome editing tools into target cells are frequently viral vectors. This method uses viruses' innate capacity to penetrate host cells and transfer genetic material. Modified viruses with their disease-causing genes deleted or rendered inactive are known as viral vectors. Although designed for safe and regulated delivery, they can infect cells and transfer genetic material (Schiwon et al., 2018). Adenoviral vectors, produced from adenoviruses and have high transduction effectiveness, are frequently utilized as viral vectors among the various virus types. This makes them ideal for delivering CRISPR-Cas9 components to various cell types. AAV vectors are flexible enough to infect cells that are not dividing as well as those that are. They are ideal for in vivo applications because of their low immunogenic response. Since lentiviral vectors can integrate into the host genome and are derived from retroviruses, they are an excellent choice for the steady, long-term expression of CRISPR-Cas9 components.

AAV Vector-Based Delivery CRISPR-Cas9 to Combat HBV:

The most popular viral vector for delivering genetic material in vivo for gene therapy is the adeno-associated virus (AAV) vector. The non-pathogenic adeno-associated virus is the source of AAV vectors. AAV is a member of the Dependovirus genus and family Parvoviridae (Lin G. et al., 2015). AAV vectors, characterized by their single-stranded DNA with a genome length of approximately 4.7 kb (refer to Figure 3), are widely recognized for their reliability in delivering genetic material in vivo. This popularity stems from their advantageous features, including low immunogenicity, non-pathogenic nature, stable and prolonged-expression in target cells, the availability of a diverse range of serotypes for targeted cell selection, and their existence as episomes (extra-chromosomal DNA). However, a notable limitation is their restricted capacity to carry genetic material compared to other vectors.

To overcome this limitation, researchers have devised a strategy of splitting the genetic material into two or three parts, creating dual or triple AAV vectors. This innovative approach allows the transformation of larger genetic materials in vivo. The size can be increased from the original 4.7 kb to 9 or 14 kb by employing dual or triple AAV vectors.

The AAV structure comprises various proteins, including four non-structural Rep proteins and three capsid proteins (VP1-VP3). In the human case, AAV exhibits 12 serotypes, ranging from AAV1 to AAV12. Each serotype possesses unique functionalities, and researchers can strategically select a specific serotype from these 12 based on the target cells or tissue, ensuring efficient delivery of genetic material.

To combat the Hepatitis B Virus (HBV) in vivo using CRISPR-Cas9, transferring CRISPR-Cas9 components, namely the Cas9 nuclease protein and guide RNA (gRNA), to an AAV vector is essential. The AAV vector facilitates the transfer of these components, combined or individually, based on their size limitations. As previously discussed, the AAV vector, with a size limit of 4.7 kb, can carry larger components by strategically splitting and forming dual vectors to transfer more extensive genetic material.

In the CRISPR-Cas9 system, the Cas9 protein can be sourced from two bacteria. The first is Streptococcus pyogenes Cas9 (SpCas9), which is larger and contains 1,368 amino acids. Dual AAV vectors are formed to deliver this protein to the host targeted cell, splitting the SpCas9 protein into two fragments. Recombination then occurs to create a fully functional protein from these two Cas9 fragments.

On the other hand, the second bacteria for the Cas9 protein is Staphylococcus aureus Cas9 (SaCas9), which is smaller in comparison, with 1082 amino acid residues. When delivering this protein to the host targeted cell, there is no need to form dual vectors; a single AAV vector can efficiently transfer to the host cell.

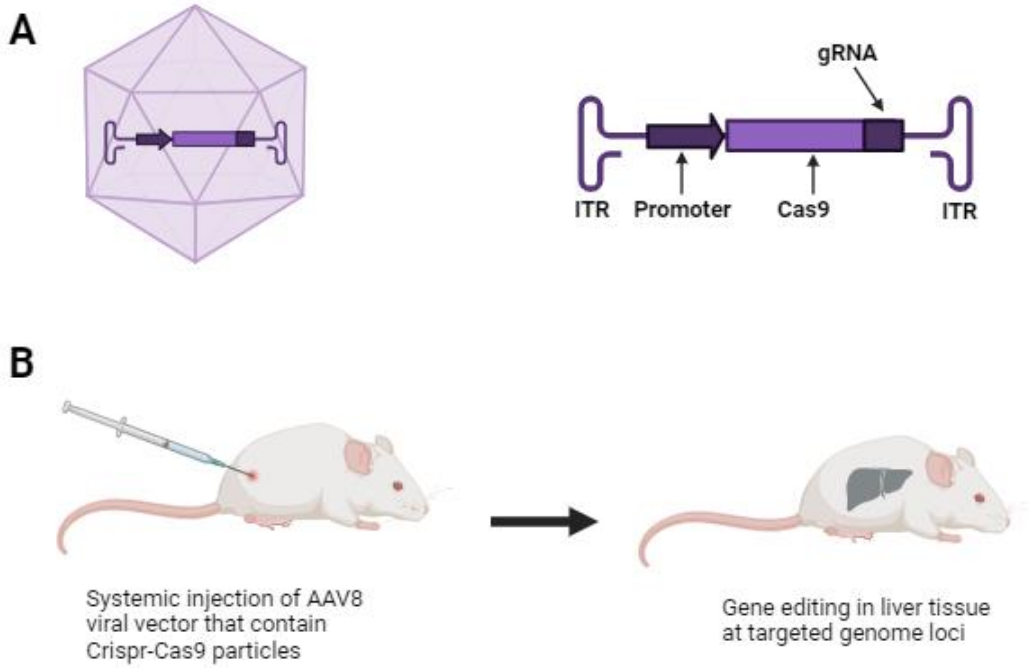


Figure 3. This diagram shows the AAV vector and its mechanism. (A) You can see the diagram of the AAV vector; in this phase, we prepare our AAV vector by loading CRISPR-Cas9 particles into it. In (B), after preparing the AAV vector, inject our AAV vector into mice. Injected AAV vector to mice will specifically transport to liver tissue and do genome editing at specific genome loci with the help of complimentary gRNA and Cas9 protein that cleave the DNA and inhibit the HBV (Yan, K. et al., 2021).

As explored earlier, animal models, particularly mice, are commonly employed in CRISPR-Cas9 gene therapy research. Among these models, humanized hepatocyte chimeric mice stand out as an ideal representation for studying HBV infection, closely mirroring the characteristics of human HBV infection. AAV8, a well-investigated serotype, is particularly notable for its liver-targeting capabilities. It naturally exhibits tropism for hepatocytes, making it highly effective in transducing the liver when administered systemically.

In a recent study, the inhibition of HBV was effectively displayed using CRISPR/SaCas9, delivered through the AAV8 serotype in C57BL/6 mice. The initial crucial step in this process involves designing and creating an AAV8 vector tailored for the intended application.

Through engineering, therapeutic genetic elements that target HBV would be carried via the AAV8 vector. Targeting crucial HBV genes like polymerase and HBsAg (surface antigen), AAV8 vectors may carry genes encoding antiviral proteins, such as small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs). We can refer to silencing gene expression as RNA interference (RNAi).

To damage viral DNA and prevent replication, AAV8 vectors can deliver the CRISPR-Cas9 system, which combines a guide RNA with the Cas9 nuclease protein to target specific

regions of the HBV genome (Yan et al., (2021). The engineered AAV8 vectors are administered to mice through intravenous injection or other suitable routes. Leveraging AAV8's liver tropism, these vectors efficiently transduce hepatocytes, the primary target cells for HBV infection. Once the engineered AAV8 vector is delivered to hepatocytes, its genetic elements are expressed using a specific approach.

If antiviral elements are expressed, such as the RNAi system, gene expression is suppressed or silenced, inhibiting protein production. On the other hand, if CRISPR-Cas9 elements are expressed, the HBV viral DNA is disrupted or cleaved through the CRISPR-Cas9 system, inhibiting DNA replication.

Results from these preclinical studies utilizing AAV8 vectors in mice yield valuable insights into the therapy's potential for combating HBV infection. Researchers thoroughly analyze the treatment's effectiveness, safety, and the associated immunological responses to assess its overall impact.

Another serotype that is employed to fight HBV is the AAV9 vector. Additionally, it works wonders in battling HBV. It may also transfer genetic material to hepatocytes, cells that make up the liver. Once within the hepatocyte, it will use CRISPR-Cas9 expression to stop HBV DNA replication and antiviral protein expression to quiet gene expression. Further research is necessary to translate promising preclinical study results into human therapeutics. This research must address possible immunological reactions, optimize vector dosage, ensure long-term safety and efficacy, and conduct thorough clinical trials. Research on using AAV9 vectors to fight HBV infection in preclinical animals is ongoing to create novel gene-based therapies for liver illnesses linked to HBV (Yan et al., (2021).

A recent study discovered AAV3B, a mutant AAV vector candidate with higher hepatocyte tropism. Compared to AAV8, AAV9, and AAV5, this serotype, AAV3B, is far more effective. Because of its effectiveness in transducing hepatocytes, AAV3B has become a useful tool for liver-targeted gene therapy. Treating a variety of liver-related illnesses, such as those brought on by genetic abnormalities or viral infections, such as the hepatitis B virus (HBV), requires liver targeting (Zhu, W. et al., 2016). Recent studies have delved into the potential of AAV3B for gene therapy applications, suggesting its utility in delivering therapeutic genes to the liver. This could address genetic disorders or enhance the expression of beneficial proteins within hepatocytes. Exploring AAV3B-based therapies extends to liver diseases, such as HBV, where they could deliver antiviral agents, disrupt viral replication, or boost the host immune response.

Distinct from AAV8 and AAV9, AAV3B is an emerging liver-targeted serotype under investigation. AAV8 is recognized for its liver-targeting capabilities, while AAV9 exhibits broader tissue tropism, making it suitable for various organs. The choice of serotype in gene therapy applications depends on specific therapeutic goals and the targeted tissues.

However, in the ongoing investigation of AAV3B and other serotypes for gene therapy applications, challenges such as immunogenicity, side effects, potential hepatotoxicity at high doses, and genome integration must be considered.

Adenoviral Vectors Based Delivery CRISPR-Cas9:

Another kind of viral vector that may be used to deliver CRISPR-Cas9 in vivo is the adenoviral vector. The genomic length of adenoviruses is between 26 and 46 kb. They include linear double-stranded DNA. These vectors are made from adenoviruses; adenoviral vectors are utilized in vivo gene therapy because of their huge DNA capacity, allowing them to convey significant genetic material to the targeted cells, effective gene transfer, and quick expression. These vectors have gained popularity in gene therapy and genetic research due to their ability to deliver genetic material into various dividing and non-dividing cells efficiently. In the context of CRISPR-Cas9, adenoviral vectors serve as vehicles for transporting the necessary components, including the Cas9 nuclease protein and guide RNA (gRNA), into target cells.

The following are important benefits and aspects of delivering CRISPR-Cas9 via adenoviral vectors:

Adenoviral vectors have a high transduction efficiency, meaning they can effectively enter target cells and deliver their genetic cargo. Adenoviral vectors typically result in transient expression of the delivered genes. This is advantageous when long-term expression is not required and helps minimize potential off-target effects.

Adenoviral vectors can infect various cell types, making them versatile tools for various tissue applications. Adenoviral vectors have a relatively large cargo capacity, allowing them to carry sizeable CRISPR-Cas9 components.

The process of adenoviral vector-based CRISPR-Cas9 delivery involves the following steps:

The adenoviral vector is engineered to carry the Cas9 gene and the gRNA sequences targeting specific DNA sequences. The engineered adenoviral vector is introduced into target cells, efficiently transducing the cells and delivering the CRISPR-Cas9 components.

Inside the target cells, the Cas9 protein, guided by the gRNA, recognizes and binds to the target DNA sequences. This binding triggers the cleavage of the DNA at the specified location. The cell's natural DNA repair mechanisms, such as Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR), are activated to repair the double-strand breaks induced by Cas9. This repair process can result in targeted gene disruption or modification. Effects The targeted DNA modifications lead to the desired cellular effects, such as gene knock-out, gene correction, or modulation of gene expression.

Lentivirus Vector-Based Delivery For HBV:

Utilizing lentivirus vector-based delivery for the CRISPR-Cas9 system to inhibit Hepatitis B Virus (HBV) represents an innovative approach in gene therapy research. Lentiviruses, a subtype of retroviruses, can integrate their genetic material into the host cell genome, ensuring stable and long-term gene expression. This integration is especially advantageous for sustained CRISPR-Cas9 activity.

The lentivirus vector carries the essential components of the CRISPR-Cas9 system, including gRNA that is complementary to the HBV viral DNA, along with the Cas9 nuclease protein responsible for cleaving or disrupting the HBV DNA. Moreover, the lentivirus vector can incorporate regulatory elements to control and optimize the expression of Cas9 and gRNA. Once inside the host cell, the lentivirus vector integrates the CRISPR-Cas9 system into the host cell genome, forming double-strand breaks (DSB).

The host cell's inherent DNA repair pathways, such as Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR), are then activated to mend the DSBs introduced by Cas9. In the case of NHEJ, errors in the repair process may disrupt the integrity of the HBV genome, rendering it non-functional and providing a potential therapeutic avenue (Kostyushev, D. et al., 2023). Researchers can use the HDR technique to make targeted alterations to the HBV genome, including gene disruptions or therapeutic sequence insertions.

Non-viral Vectors Delivery of CRISPR-Cas9 Delivery for HBV:

Employing lipid-based nanoparticles to deliver CRISPR-Cas9 to combat Hepatitis B Virus (HBV) represents an innovative strategy in gene therapy research. These nanoparticles, also termed lipoplexes, consist of lipid molecules that encapsulate and shield genetic material, including CRISPR-Cas9 components, during transport to target cells. As a subtype of non-viral vectors, lipid-based nanoparticles are among the most prevalent for transferring genetic material to specific cells.

The lipids in these nanoparticles form a protective shell around the genetic payload, enhancing stability and facilitating cellular uptake. Following administration to the target cells, the lipid-based nanoparticles are internalized through endocytosis. The nanoparticle lipids support fusion with the cell membrane, releasing CRISPR-Cas9 components into the cytoplasm.

Once in the nucleus, the Cas9 protein, guided by the gRNA, identifies HBV target sequences and initiates double-strand breaks. Cellular DNA repair mechanisms, such as Non-

Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR), are subsequently activated to disrupt the viral genome, impeding its replication.

An inventive method in gene therapy research targets the hepatitis B virus (HBV) by delivering CRISPR-Cas9 using polymer-based nanoparticles. This kind of vector isn't viral. Synthetic polymers are complexed with CRISPR-Cas9 components to create polymer-based nanoparticles or polyplexes (Liu et al., 2018). These nanoparticles promote cellular absorption while safeguarding the genetic material during delivery. Figure 4 shows the viral vectors and those that are not.

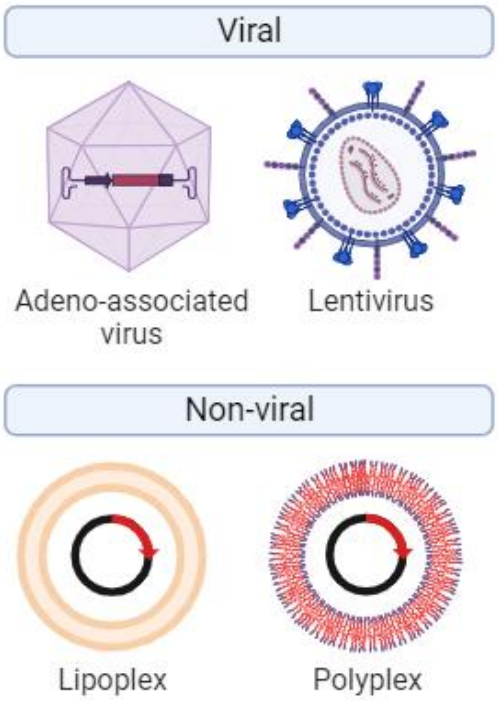


Figure 4. These are the schematic diagrams of viral and non-viral vectors. You can see all of these are different from each other; each of them has its specific shape and functions. These vectors are used to deliver the CRISPR-Cas9 system in vivo for gene therapy (Kennedy, E. M. et al., 2015).

Challenges & Future Perspectives:

For hepatitis B virus (HBV) CRISPR-Cas9 gene therapy to be successfully implemented in clinical practice, several issues and concerns frequently arising with this gene therapy must be resolved. CRISPR-Cas9 components must be delivered to target hepatocytes (liver cells) efficiently, so maximizing off-target effects on other cell types while maintaining high specificity for hepatocytes remains difficult.

Delivery vectors and the CRISPR-Cas9 system can trigger immune responses. Immune responses may result in safety issues and a decrease in therapeutic efficacy. Designing immunologically inert delivery devices and strategies to reduce immune responses is necessary.

It is important to ensure high specificity of the gRNA sequence to the HBV genome to prevent unintentional off-target effects on the host genome. Developments in genome-wide screening techniques, off-target prediction algorithms, and gRNA design partly address this problem.

Choosing safe and effective delivery vectors—viral or non-viral—that effectively transfer CRISPR-Cas9 components to hepatocytes is difficult. Vector optimization is crucial to alter genes while reducing side effects effectively.

The nucleus of infected hepatocytes becomes a stable reservoir of cccDNA due to HBV infection. Its structure and epigenetic changes make targeting and editing cccDNA difficult.

Creating methods to target and interfere with cccDNA is an important area of research. Achieving a long-term reduction in the viral load and sustaining the therapeutic effects are crucial. Long-term investigations are needed to ensure the altered cells remain resistant to HBV infection and replication.

Conclusions

A suitable delivery strategy is crucial to achieving the required CRISPR/Cas9 effects against HBV infection. The absence of an appropriate delivery method makes the therapeutic translation of the CRISPR/Cas system difficult. Scientists are putting a lot of effort into developing efficient CRISPR/Cas delivery strategies, which could alleviate the issue quickly. Despite being the most thoroughly studied delivery vehicle for CRISPR/Cas9 targeting of the HBV genome, AAVs have not been employed in any clinical trials. Future research can examine delivery methods based on non-viral nanoparticles, which could eventually replace viral vectors and offer fresh possibilities for effectively administering CRISPR/Cas9 against HBV infection.

Declaration statement

The authors reported no potential conflict of interest.

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