Progress in the application of CRISPR/Cas system in the precise detection and treatment of diseases associated with viral infections

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Abstract. Various pathogens can cause infectious diseases, which can spread from one person or animal to another or even between humans and other animals. Microorganisms, viruses, and parasites make up the majority of pathogens. The disease can typically be spread through direct contact with infected people, contact with their bodily fluids and feces, their contaminated objects, vertical transmission (mom-to-child transmission), soil transmission, water transmission, food transmission, contact transmission, and oral transmission of feces and bodily fluids. The importance of treating infectious diseases is heightened by the fact that they are difficult to totally eradicate and are disseminated so widely. Now, CRISPR/Cas9 has been applied in the field of detection and treatment of diseases related to viral infection. It has the characteristics of high specificity and low off-target rate, and is an emerging approach for rapid and accurate detection of viral infection and safe and effective treatment of diseases related to viral infection. In recent years, the system has been successfully applied in the detection and treatment of many viruses such as HIV, HBV, HPV and multiple herpes virus, and the development prospect is broad. This review introduced the RNA-targeting CRISPR/Cas system's composition, structure, molecular mechanism, and potential application, focused on the research progress of the CRISPR/Cas system in the detection and treatment of viral infection-related diseases, and summarized and forecasted the development of relevant systems and related research.

Keywords: CRISPR/Cas system; Gene editing; Virus infection.

1. Introduction

Infectious diseases caused by viruses in the current relevant research is very concerned about the direction, especially for HIV, HBV, HPV and a variety of herpes viruses and other related viruses, these viruses will pose a great threat to the life and health of infected people, and there is no good cure, because of this, CRISPR technology in this direction of the application came into being.

Genome editing technology is a technology that uses specific nucleases to edit and modify specific genomic sites, and CRISPR-Cas9 is currently the most advanced and widely applied with high efficiency and easy design. Compared with the previous two gene-editing technologies, ZFNs and TALENs, CRISPR/Cas9 has great advantages. CRISPR/Cas9 is based on the molecular mechanism by which bacteria defend themselves against phage infection. It is a gene editing technology that is recognized by sgRNA, uses the Cas9 protein to perform targeted cutting of the genome, and then realizes gene insertion, such as or knockout, through the body's repair mechanism. This technology has broad application prospects in basic research, virus infection detection, gene therapy and other fields [1].

At present, CRISPR/Cas technology is widely used in the field of virus detection, with high specificity and sensitivity, and has been put into clinical use. At the same time, CRISPER technology is also widely used in the field of gene targeted knockout, and the treatment of cancer has been put into clinical practice and has achieved good results, but CRISPR/Cas technology is still in the exploration stage in the treatment of viral infection.

2. CRISPR-Cas system

Clustered Regularly Interspaced Short Palindromic Repeats is known as CRISPR. CRISPR sequence and CRISPR associated (Cas) protein form the CRISPR/Cas system [2].

Prokaryotes use the CRISPR/Cas system as a self-defense mechanism to protect themselves against the invasion of foreign genetic material [3]. Three phases make up its immunological response: adaptation, expression, and interference. Three stages of immune activity, including interference, expression, and adaptability. In the adaptation step, bacteria employ the Cas1 and Cas2 proteins to capture invasive foreign nucleic acids and integrate them into their own genome's CRISPR sequence to create a memory function, enabling bacteria to mount an immune response when the next invasion occurs. Precursor crRNA (pre-crRNA) and trans-activating crRNA (tracrRNA) were produced by the CRISPR sequence during the expression stage of transcription. Pre-crRNA underwent processing to form crRNA to match foreign gene sequences, and single-guide RNA (sgRNA) was created by base-pairing of crRNA and tracrRNA for Cas protein recruitment. Sgrnas direct the Cas protein to relocate to its homologous foreign nucleic acid target during the interference phase, where it activates its endonuclease activity to cleave the target nucleic acid and performs the immunological function [2].

In 2015, Makarova et al. analyzed the protein families of all known CRISPR/Cas systems and established a library consisting of 394 position-specific score matrices (PSSMs), among which 229 PSSMS were newly added. Using these matrices, they searched 2,751 fully annotated bacterial and fungal genomes and predicted the loci of 1,694 complete Cas genes. Finally, 1 574 complete loci were grouped into previously separated types or newly classified Type IV and Type V systems. After analysis and summary, Makarova et al. believe that the CRISPR/Cas system should be classified from a broader and more basic perspective, that is, the crRNA effect complex with multiple subunits should be classified into one category, while the few protein subunits that perform functions are classified into another category, and the downward classification can be divided into 5 types and 16 subtypes [4]

2.1. CRISPR-Cas9 system

Bacteria have evolved the CRISPR-Cas9 system in order to eliminate the foreign invasion genes of viruses from their own genomes. Using the CRISPR-Cas9 system, bacteria can cut the virus genes from their own genomes [5]. CRISPR-Cas9 system can be divided into typeI, typeII and typeIII. Type of II system composition is simple, only one Cas protein, namely Cas9 protein, its nuclease cutting function domain is composed of Ruv C and HNH structure domain, respectively cutting target double-stranded DNA. CRISPR - Cas9 technology is transformed from the system.

The CRISPR-Cas9 gene editing system has multiple advantages in the treatment of DNA viruses. First, the technology is simple and economical. The carrier to build is simple. Second, the CRISPR - Cas9 biological safety system, through backcross separation can be obtained without exogenous genes similar to the natural mutation of the mutant strain. In addition, the CRISPR - Cas9 technology can edit multiple target genes at the same time, applied to the rapid treatment of virus infection caused by the disease, can be in to integrate their own genes and host cells in the treatment of viral infection provides a new train of thought [3].

2.2. CRISPR-Cas13 system

CRISPR Cas13 is a newly discovered CRISPR - Cas9 series nuclease editing system, specific targeted RNA, achieve the fixed point of intracellular RNA editing, splicing regulation, low and elimination. It belongs to the first type VI CRISPR/Cas system, is a kind of RNA mediated by RNA enzymes, CRISPR/Cas13 system can be divided into VI-A, VI-B, VI-C and VI - D. Unlike most CRISPR/Cas proteins, Cas13 / crRNA effect complex DNA enzyme activity, but with RNA enzymes, through targeted degradation of RNA at the transcriptome level to achieve the purpose of gene editing. Cas13X have the highest specificity and efficiency. Cas13 is also the smallest type VI CRISPR effector protein found so far, with only 775 amino acids. In 2022, the group to further develop high

fidelity Cas13X variants hfCas13X, will be in the field of gene therapy based on RNA editing shows great potential applications. The related protein, Cas13d, is smaller at about 930 amino acids and was discovered in 2018 [6].

The CRISPR/Cas13 gene-editing system offers multiple advantages for the treatment of RNA viruses. First of all, when CasRx targeting RNA, RNA targets both ends don't need PFSFS (protospacer flanking sequence, PFS; Equivalent to Cas9 PAM on DNA sequences), so the design of sgRNAs can be more flexible and can target RNA at almost any position. Secondly, compared with gene silencing techniques such as CRISPRi and shRNA, CRISPR-CasRx system has higher efficiency and specificity for targeted knockdown and lower off-target effects, which is a more advantageous gene knockdown tool in the field of gene editing. CasRx and NLS expression within the nucleus fusion, can timelier and more efficient on low expression of mRNA and other RNA in the cell nucleus [7].

3. Nucleic acid detection based on CRISPR/Cas technology

Structural analysis revealed that Cas13a protein has a "two-leaf" structure, including a "crRNA recognition" lobe and a "nuclease" lobe. When the crRNA-target RNA complex passes through the nuclease channel of the Cas13a protein, it triggers the movement of HEPN1 toward the HEPN2 domain, which causes a conformational change. After activation, the HEPN catalytic site on the outer surface of Cas13a protein began to exert dual nuclease activity, one is to process pre-crRNA into crRNA, and the other is to cleave the target RNA specifically [8]. Similar to Cas12a, the specific recognition of RNA by Cas13a is usually limited by the PFS at the 3 'end of the target RNA. Upon cleavage of the target RNA, the rnase activity of the Cas13a protein complex is activated, which can cleave nearby single-stranded RNA without discrimination. These structural aspects of the basic research for subsequent Cas13a in the diagnosis of gene editing and molecular laid a foundation [9].

Based on Cas13a Trans cutting activity, the researchers developed a variety of tools for RNA detection. In 2017, GOOTENBERG based on Cas13a SHERLOCK detection system was developed, such as the general principles of diagnosis technology are as follows: The specific nucleic acid sequence fragments were first amplified by *in vitro* simultaneous nucleic acid amplification (RPA) technology, and then converted into RNA by T7 RNA polymerase. When Cas/13acrRNA complex specifically recognized and bound to the target sequence, the ribonuclease activity of Cas13a was activated, and the target RNA was cleaved with a fluorescent group at one end of the system. The single stranded RNA probe with quenching group at the other end was used for diagnosis by detecting the corresponding fluorescence signa [10]. In 2018, the team developed SHERLOCK II, which was further optimized and improved in terms of sensitivity and on-site detection. In 2017, Zhang Feng's team discovered the VI-B CRISPR/Cas system in the gene library by bioinformatics methods. Cas13b is an RNA-guided nuclease that can specifically recognize and cleave single-stranded RNA, with two accessory proteins, Csx27 and Csx28. Based on this, Cas13b has been divided into two different isoforms VI-B1 and VI-B2. Csx27 can inhibit the RNA interference activity of Cas13b by inhibiting the nuclease activity of HENP domain, while Csx28 can enhance the RNA interference activity of Cas13b. In addition, Cas13b was superior to Cas13a in terms of recognition efficiency and specificity. At present, Cas13b is mainly used for gene editing, but it is worth noting that Cas13b has multiple spacer sequences on the crRNA, which can target multiple RNA transcripts at the same time, which will provide new possibilities for multiple detection of CRISPR/Cas system.

With the further research on CRISPR/Cas system, KONERMANN et al. discovered the VI-D CRISPR/Cas system (Cas/13d) in prokaryotes in 2018. In 2019, ZHANG et al. resolved the crystal structure of the binary complex of UrCas13d-crRNA. Except for the HEPN domain, Cas13d has no obvious sequence similarity with other type VI Cas proteins. The accessory proteins containing the WYL domain can promote Cas13d to cleave RNA, which opens up a new idea for the regulation of Cas13d activity. The full length of Cas13d protein is about 930 amino acids, which is about 20% smaller than the other three Cas13 proteins, and its recognition sequence is independent of PFS.

Therefore, Cas13d has the advantages of wide targeting range and high efficiency. In 2020, ZHOU et al. used Cas13d to knock down PTBP1 (polypyrimidine tract binding protein 1) gene in mouse retinal Muller glial cells, successfully converted mouse glial cells into optic nerve cells, and improved the vision of mice with permanent vision loss. Cas13d reveals the great potential of application in disease treatment. In 2020, BROGAN et al. used Cas13d for molecular diagnosis for the first time, with a minimum detection rate of 100 copies of target RNA per reaction, indicating that Cas13d is expected to become a new generation of powerful molecular diagnostic tools. The Cas13 system only targets RNA, and the nucleic acid probe added to the detection system also needs to be RNA. Because RNA is easily affected by ribonuclease, the detection technology based on the Cas13 system has higher requirements for the preservation, transportation and field environment of reagents than Cas12a and Cas9, and the cost is relatively higher [8].

4. Treatment of viral infections based on CRISPR/Cas technology

At present, there are two main methods of virus prevention and treatment based on CRISPER technology: one is to edit the virus genome, which does not need to be carried out in the human body, and can be treated before, during and after infection; The second is to cut off the link between the viral genome and the host cell through gene editing. According to the current research situation, the biggest advantage of this method is that it will not affect the biological function of the host cell, and when treating embryos and immature cells, it has lower cytotoxicity compared with other related technologies [11].

4.1. HIV

AIDS is mostly brought on by the pathogen HIV-1, which invades CD4+T cells in the human immune system. dsDNA of the virus integrates into the cell genome to form a previrus and replicates in the cell, damaging immune cells directly or indirectly. The immune function of the body is unbalanced or impaired, which leads to opportunistic infections or tumors in AIDS patients [12]. Many drugs are currently available to treat AIDS, which work mainly by blocking the replication cycle of the virus, but this therapy fails to clear the latent reservoir of HIV-1 virus in cells and stopping antiretroviral therapy can easily lead to virus reactivation and disease progression to acquired immune deficiency. At the same time, such treatment will lead to a gradual increase in drug resistance, and the infection cannot be eradicated, and people infected with HIV face lifelong antiretroviral treatment [13]. The CRISPR/Cas9 gene editing technology has great potential application value in the fight against HIV infection because it can remove the provirus lurking in the body cells.

Currently CRISPR/Cas9 gene editing technology has two targets in the treatment of AIDS: the HIV-1 genome or host cells. With Long terminal repeat (LTR) at both ends of HIV-1 genome, including U3 sequence, R sequence, and U5 sequence, which contains promoter, enhancer, and other transcriptional regulatory binding sequences, CRISPR/Cas9 technology has the potential to edit and organize the genome expression of HIV-1. The CRISPR/ Cas9 system can effectively cut and mutate LTR targets, inhibit the expression of activated previruses and the reactivation of latent viral reservoirs, and also remove internal viral genes from host cell chromosomes [14].

Another target of CRISPR/Cas9 is a co-receptor in the host cell. The receptor of HIV is the first-level receptor CD4 molecule, and the co-receptor is the chemokine receptor CXCR4 or CCR5. As CD4 is indispensable for the function of normal CD4+ T cells, the second-level receptors CXCR4 and CCR5 of HIV are easily deleted and destroyed by CRISPR/Cas9 technology. Therefore, knocking down the expression of CXCR4 or CCR5 gene in cells can effectively inhibit HIV-1 infection or delay the course of the disease [15]. The precise and efficient genome editing targeting CXCR4 gene obtained by HouP et al. through a series of experimental studies provides a new strategy for the treatment of HIV-1 infection [16]. Wang W et al. demonstrated the high specificity of this method by using Cas9 system for specific editing of CRR5 gene [17]. The technique needs to be improved, according to Xu L et al., who used CRISPR/Cas9 to remove CCR5 receptors from hematopoietic

stem cells and progenitor cells before transplanting them into HIV patients. [18]. By modeling HIV infection in non-human primates, cutting and removing integrated proviral DNA fragments via the CRISPR/Cas system is a key reference for eradicating the HIV genome in humans [19].

4.2. HBV

HBV is a common blood-borne pathogen that infects humans via reverse transcriptional replication and causes acute and chronic hepatitis, as well as cirrhosis and liver cancer. The reverse transcription of covalently closed circular DNA (cccDNA) and the establishment of immunological tolerance are the primary causes of hepatitis B virus infection [20]. Standard HBV medications include nucleotide reverse transcriptase inhibitors (NRTIs) and interferon. Approved antiviral medicines can suppress viral replication and, to some extent, delay the onset of liver cancer, but they cannot stop viral reverse transcription to generate cccDNA and cannot entirely cure HBV infection. [21].

Blocking cccDNA is the key to treat hepatitis B virus infection and apply gene editing technology. Using CRISPR/Cas9 in HepG2 cell lines expressing HBV receptors, a tissue culture system open to HBV was established to directly target cccDNA, confirming that cccDNA can be used as a target for Cas9-induced double-stranded DNA breaks [22]. With HCAdVs, using CRISPR/Cas9 to target three HBV target genes HBsAg-open reading frame, proteins X-ORF and C-ORF, a complete TALENs was constructed, and the plasmid expressing nuclease was transfected into human liver cancer cell line Huh7, which showed a significant decrease in HBsAg concentration. It has been confirmed that this method can inhibit HBV replication [23].In summary, CRISPR/Cas mediated cccDNA targeting and specific cutting is a potential strategy for the treatment of chronic HBV infection. However, this treatment approach has a drawback: Treating HBV with the CRISPR/Cas system may cause the host genome to rearrange damage. To address this shortcoming, Yang YC et al developed SpCas9-BE based on the original CRISPR technology, which can inactivate HBV gene expression without cutting DNA [24]. Experimental evidence has shown that SpCas9-BE can attach to specific gRNAs to successfully edit polymerase and surface genes, as well as insert nonsense mutations into particular locations in the HBV genome. Little base insertion or deletion can result from decreased HBV gene expression in cells with HBV integrated genomes. Additionally, nonsense mutations in the polymerase genes and viral surface genes that integrate the HBV genome and cccDNA resulted in a marked decrease in HBsAg production and viral replication, two essential steps in the treatment of HBV.

4.3. HPV

Human papillomavirus (HPV) is a highly species-specific epitheliophilic virus. More than 200 types have been found, and different types can cause different diseases. According to the pathogenicity or carcinogenic risk of HPV subtypes, HPV is divided into high-risk types and low-risk types. At present, there is no radical treatment plan for HPV infection, and there are currently preventive and therapeutic HPV vaccine, drug therapy, physical therapy and surgical treatment for this disease. Existing methods can only prevent and reduce the possibility of HPV infection, but cannot completely eliminate the impact of HPV on organs, nor can completely eliminate the virus in the body [12].

The widespread adoption of CRISPR technology in the management of high-risk HPV is a result of its many benefits. The CRISPR/Cas9 method was demonstrated by Fujii W et al. to be effective in genome editing and to selectively abolish E6 and E7 functionalities, demonstrating tremendous promise for the treatment of HPV-positive cervical cancer [25]. Zhen et al. created a CRISPR/Cas9 system that targets E6 and E7, and they employed SiHa cells to assess the therapeutic effects of knocking down E6 or E7 genes. Studies have demonstrated that the CRISPR/Cas9 system can efficiently, specifically, and persistently decrease HPV-16 E6 and E7 expression both in vivo and in vitro. Additionally, CRISPR/Cas9 has been found to be able to slow the growth of cervical cancers in mice [26]. At the same time, Yoshiba et al. injected AAV sgE6 directly into subcutaneous tumors and transfected the Cas9 gene into HeLa, HCS 2, and SKG I3 HPV-positive cervical cancer high-risk

cell lines. They also conducted in vitro detection of gene mutation, frequency, protein expression level, apoptosis and proliferation of these cell lines. AAV-sgE6 was found to play a part in a mouse model of cervical cancer that was conducted in vivo. The outcomes demonstrated a concentration-dependent inhibition of cell proliferation by AAV-sgE6 transducers. As a result, targeting E6 expression in high-risk HPV with CRISPR/Cas9 can be a successful and incredibly targeted tactic [27].

For the CRISPR treatment of low-risk HPV, Liu YC et al. tested the possibility of applying the reprogrammed CRISPR/Cas9 system against the E7 gene of low-risk HPV (HPV6/11) by creating a reprogrammed CRISPR/Cas9 system targeting the E7 gene homologous sequence of HPV6/11 [28]. For this study, the CRISPR/Cas9 system could, in theory, destroy two gene loci simultaneously by co-expressing a Cas9 protein with two sgRNAs. Experimental data showed that sgRNAs directed to highly conserved regions could inactivate HPV-11 E7 and HPV-16E7 [28], suggesting that reprogrammed CRISPR/Cas9 has great potential for dealing with cross-species related viruses.

4.4. Other infectious diseases

A study published in PLOS Pathogens on June 30, 2016 showed that CRISPR-Cas9 technology attacked herpes virus DNA, could inhibit viral replication, and in some cases it could eliminate the virus. The study, led by Robert Jan Lebbink and his colleagues at the University Medical Center Utrecht in the Netherlands, concluded that CRISPR/Cas9 could target and mutate potential herpesvirus DNA in infected cells, potentially preventing herpesvirus-related diseases [7].

Herpes simplex virus type 1 (HSV-1), which causes cold sores and herpetic keratitis, was the main focus of the study in order to obtain reliable data. the most frequent viral cause of birth abnormalities (when the virus is passed from the mother to the fetus) is human cytomegalovirus (HCMV); Additionally, EBV, which causes infectious mononucleosis and several types of cancer, was found to be extremely effective and highly specific in the removal of tumor cells harboring latent infections, while HSV-1 and HCMV replication in human cells was only marginally reduced, indicating that although CRISPR-Cas9 cannot guide the genome engineering of dormant HSV-1, the use of anti-HSV-1 gRNAs can effectively inhibit viral replication after dormant HSV-1 reactivation[30]. These results demonstrated the enormous promise of CRISPR technology for precise and efficient treatment of diseases caused by viral infections.

5. Conclusions

In the field of nucleic acid detection, compared with the traditional pathogen detection methods, CRISPR/Cas technology has the characteristics of long detection cycle, complex process, and high dependence on technicians and complex equipment. The pathogen detection technology established by CRISPR/Cas combined with isothermal amplification technology has similar or even more prominent sensitivity and specificity than qPCR. At the same time, it has great advantages such as strong scalability, low cost, portability and speed. It has successfully broken through the bottleneck of conventional pathogen detection technology, and has shown strong competitiveness and application potential in pathogen detection and prevention.

In this review, several pathogen detection technologies based on Cas9 and Cas13 protein paired with nucleic acid amplification technology were also introduced. The biological importance, categorization, and principle of the CRISPR/Cas system were also briefly summarized. According to its features, this evaluation also covered the CRISPR technology's current state of research in the field of treating diseases.

Although CRISPR-Cas technology has obvious advantages in the treatment of viral infections, it also has some shortcomings, mainly in that related research has not yet achieved practical conclusions. This hypothesis is still in the verification stage, and there is no conclusion on whether CRISPER technology can be applied in the treatment of viral infection-related diseases.

CRISPR technology as a simple, high efficient, low cost and high security technology, has greatly improved the efficiency of gene editing and fixed-point treatment and promoted the study on prevention and treatment of various diseases. CRISPR technology has a good application prospect in virus defense. Using Cas9 and Cas13 protein of virus genes or receptor gene mutation to prevent bacteria invasion, is an effective method of antiviral research, in the prevention and control of HIV virus will own gene into host cells such as areas is a very good research direction.

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