Research Progress in The Application of CRISPR Gene Editing Technology in Virus Detection and Treatment

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Abstract. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) gene editing (GE) technique is the latest generation of GE technology, which can carry out targeted and precise modification of the genome. In this article, a comparison of the Zinc-Finger Nucleases (ZFNs) , CRISPR/Cas and Transcription Activator-Like Effector Nucleases (TALENs) is presented, which indicates that CRISPR/Cas has significant advantages. For example, synthesis and screening are simpler and faster, and multiple genes can be edited simultaneously. To keep abreast of the times, current progress in the application of CRISPR in virus detection as well as treatment will be introduced in this paper. Firstly, CRISPR and its characteristics will be briefly introduced. Then the current application of CRISPR about detecting and treating viruses will be reviewed. Finally, the paper summarizes the shortcomings of CRISPR and looks forward to its future development.This article attempts to give readers a brief introduction to the emerging technology based on CRISPR and highlights its potential applications in virus detection and treatment.

Keywords: CRISPR, Virus Detection, Gene editing, Therapy.

1. Introduction

Viruses is a kind of microorganism without cellular structures and can infect various organisms. Some viruses can infect humans and cause diseases, which seriously threaten human health and cause huge social and economic costs. For example, the outbreak of SARS in Wuhan in 2019 caused huge economic losses throughout the world, and tens of thousands of people were killed.In addition to human immunodeficiency Virus (HIV), Zika Virus (ZIKV) , Ebola Virus (EBV), Influenze Virus can also cause diseases. To control the virus and detect infected persons early, a sensitive and specific detection method is crucial. Early detection, early diagnosis and early treatment can effectively reduce the mortality rate and the incidence of sequelae. Existing detection methods such as antigen-antibody analysis, polymerase chain reaction (PCR)-based technology, and genome sequencing have some shortcomings which are high false positive rate, long time-consuming, high cost and dependence on expensive instruments and professional technicians. The optimal pathogen detection method should be rapid, sensitive, specific, affordable and instrument-free. Several assays have been developed based on CRISPR gene editing. It makes up for the shortcomings of some traditional detection methods. It is believed that with further optimization and improvement, the existing detection methods can be innovated. Clinically, treatment of viral infections still mainly relies on supportive treatment, antiviral drugs and antibodies produced by vaccination in advance. Taking the new coronavirus infection as an example, some studies show that even if multiple vaccinations are given, multiple infections may still be accompanied by some sequelae. Some diseases caused by viral infection are still incurable, such as AIDS and hepatitis B. It is gratifying that the sequencing technology and some progress in gene function research have made antiviral therapy based on gene editing possible.

2. CRISPR-Cas system

2.1. Development

CRISPR is a special structure first noticed by Yoshizumi et al. in 1987 when they studied the conversion of alkaline phosphatase isoenzymes regulated by the iap gene of Escherichia coli. Then a

special sequence was found to have spaced repeat sequence including 30 bases in archaea. It is named as CRISPR, and the protein encoded by CRISPR-related genes is called Cas protein. In 2005, it was found that the spaced repeat sequence are highly homologous to the phage genomes and plasmids invaded by foreign sources, so it is speculated that the CRISPR is related to immune function. Afterwards, the immunologic defence mechanism of it was clarified. CRISPR began to be used for gene editing after the discovery that the Cas9 protein that binds to CRISPR RNA (crRNA) can bind and cut specific sites in DNA sequences in vitro. At present, CRISPR has developed at a high speed to have various applications and constantly developed new uses.

2.2. Mechanism

The mechanism of CRISPR is as follows. Taking CRISPR-Cas9 as an example, the specific process is as follows: First, exogenous phages or plasmids carrying a protospacer adjacent motif (PAM) sequence invade bacteria, and the Cas1 and Cas2 proteins in the bacteria recognize the PAM sequence of the invading foreign gene, and the adjacent sequence of the sequence is used as a candidate protospacer sequence. Then it is cut by the Cas1 protein and Cas2 protein complex and inserted with the mature protospacer sequence into the CRISPR sequence at the aid of enzymes. Next when the same foreign DNA invades again, the corresponding protospacer sequence is transcribed to generate pre-crRNA (precursor RNA). The pre-crRNA binds to the multi-subunit crRNA-Cas protein and is cleaved into mature crRNA under the catalysis of endonuclease RNA III. Finally, the loop region on the Cas9 protein can recognize and bind to crRNA. Cas9 protein has nuclease function, HNH nuclease and RuvC-like nuclease domains, and it is able to separately cleave complementary and noncomplementary target DNA strands and lead to exogenous gene break.

2.3. Classification

More than 40 Cas proteins have been discovered and investigated for now. In accordance with the subunits' number, they can be classified into two categories. One requires multiple subunit effector proteins to function together, and the other requires only a single effector protein to function. In the second type of protein, it is further divided into 3 types according to different effector proteins. Cas9, Cas12a (cpf1) and Cas13a (C2c2) proteins are more widely used in gene editing. The difference between these three types of proteins on target DNA is that Cas9 protein cleaves dsDNA, Cas12a protein cleaves ssDNA, and Cas13a protein cleaves RNA. The difference in structure is that Cas9 protein cuts through HNH and RuvC domains, Cas1 protein contains a RuvC-like domain for cutting, and Cas13a contains two HEPN domains for cutting. The difference in the recognition site is that the Cas9 protein recognition site is NGG downstream of the spacer sequence, the Cas12 protein recognition site is TTN upstream of the spacer sequence, and the Cas13 protein recognition site is A, U, and C downstream of the spacer sequence.

3. CRISPR-Cas in virus detection

In recent years, many researchers have continuously innovated and improved the CRISPR and developed various detection methods. They are more efficient and accurate than traditional detection methods that are used in gene editing, disease treatment, model organism construction, plant breeding and other fields.

3.1. Detection method based on CRISPR-Cas9 system

Xiong exploited a method that based on CRISPR-Cas9 system [1]. It combines and assembles CRISPR with other technologies, including three-line side flow analysis (TL-LFA) and multiple reverse transcriptase recombinase polymerase amplification (RT-RPA). This method can be used to design multiple guide RNAs and detect a variety of virus genes simultaneously, and the sensitivity is not low, which can reach 4 copies/ μ L. A detection method is constructed with dCas9 technology by Moon [2]. This method designs specific guidance RNA to detect nucleic acid in virus lysates, and links

biotin, presentation oligonucleotide and PAM into a complex. Then streptavidin-horseradish peroxidase binding to the complex causes color changes through oxidation of 3,3′,5,5′-tetramethylbenzidine. Its result is able to be roughly judged by the naked eye without special instruments and equipment, so this method is very suitable for primary level hospitals.Marsic et al. optimized the Lateral flow assays (LFAs) [3]. This method designs special guide RNA to directly amplify viral nucleic acid from clinical samples.The selected amplification method is reverse transcription isothermal nuclear acid amplification.Its advantages are that it can be expanded at room temperature and take a short time, and it does not need expensive instruments and equipment. This combination makes the method applicable to a wide range, such as outdoor and grass-roots hospitals.Then uses VirD2-dCas9 complex to bind specific target sequences. Finally, nucleic acid detection was performed by lateral flow assay. This method has ultra-high sensitivity, and the detection sensitivity can reach 2.5 copies/μL. Although the Cas 9 protein is the first protein to be studied in the CRISPR system, it is not yet used for clinical diagnosis. It is believed that with the maturity and perfection of the technology, it will be used clinically in the near future.

3.2. Detection method based on CRISPR-Cas12a system

Cas12a protein is a type V protein of class II in the CRISPR. After it recognizes specific DNA sequence, it will be activated under the action of auxiliary enzymes, and then non-specifically cut single strand DNA (ssDNA). Broughton built a detection method,which uses a fluorescent molecule to link ssDNA with a quencher, and then amplifies the specific protein sequences of virus [4]. It uses the loop-mediated isothermal amplification reaction technology to amplify the virus sequence, because it is fast and sensitive, and the reaction can be carried out at room temperature. Then the specific Ribonucleoprotein complex links to and cleaves the specific sequence, and the fluorescent molecule is separated from the quencher. Qualitative detection can be carried out by naked eyes, and further quantitative detection can be carried out by fluorescence value. Lin H et al. developed a variety of devices to detect virus [5]. These devices improve safety and detection speed, and it only takes 25 minutes to perform diagnostics on extracted RNA samples in a closed space. Ding et al. built a new method to detect virus,which is cheap and time-consuming [6]. This method uses two pairs of crRNAs to target specific sequence of virus, which greatly improves the specificity. However, it has some disadvantages,compared with RT-PCR, it has lower sensitivity.

3.3. Detection method based on CRISPR-Cas13 system

Joung built a method to detect virus,which is named SHERLOCK (specific high -sensitive enzymatic reporter unlock) [7]. This method first performs isothermal nucleic acid amplification on obtained clinical throat swab or saliva samples to obtain a large number of products, and the Cas13a protein targeting specific protein sequences of virus specifically cuts the target sequence to generate ssRNA. Finally, the ssRNA fluorescent reporter probe in the system can detect the fluorescent signal. Many researchers have optimized and modified the system to significantly improve the sensitivity and specificity. Because it does not rely on RT-qPCR equipment and only takes about one hour to read the results, it has broad application prospects and is very suitable for primary medical institutions.Fozouni built a method to detect virus without amplification [8]. This method uses many crRNAs targeting diverse specific sequences of the virus to activate effect protein. With such a designing pattern the sensitivity can reach 100 copies/μL without RT-qPCR. Using it to observe RNA from nasal swabs requires only about 30 minutes of reaction time, and the results can be read directly through a smartphone.

3.4. Detection method based on CRISPR-Cas14 system

This protein is a type II protein in the CRISPR , and its amino acid composition is only about 400-700. It is the smallest known CRISPR effector protein that can cut DNA. Compared with the Cas12a protein, it has a stronger ability to specifically recognize ssDNA and is not limited by the PAM sequence, so it is often used in nucleic acid detection systems for single-base mutations.Harrington

et al. built a method to rapidly detect infectious pathogens and diagnosis of genetic mutations [9]. Jonathan and Cameron et al. develop a facile diagnostic method based on the CRISPR-Cas14 system [10]. Many new diagnostic methods currently diagnostic methods,which all use CRISPR technology, together with other technologies.

4. Application of CRISPR-Cas system in virus therapy

4.1. SARS-CoV-2 virus

SARS-CoV-2 was discovered in Wuhan, Hubei Province, China in 2019, which can cause a new type of coronavirus pneumonia. Since then, the epidemic has spread to the world and is the greatest public health threat since this century.Hoffmann et al. built a gene library with high coverage to confirm the host required for SARS-CoV-2 virus infection based on the CRISPR. Compared with traditional methods, the resolution of this method is higher [11]. Applying this method can better discover therapeutic targets of related viruses and explore the pathogenesis of various viruses. Wei et al. established a genome-wide screening system of SARS-CoV-2 in Vero-E6 cell line based on CRISPR-Cas9 to discover new therapeutic targets [12]. The application of this technology can quickly and efficiently find potential therapeutic targets in specific cell lines. Abbott et al. built a method of inhibiting virus , which called as prophylactic antiviral CRISPR in human cells (PAC-MAN) [13]. They identified crRNA targeting the conserved region of virus. The bioinformatics analysis showed six kinds of crRNA could inhibit more than 90% of coronavirus. With the development of drug delivery systems, PAC-MAN is likely to become an important method of pan-convirus suppression in the future. Nguyen built a new treatment method which used adenovirus vector and CRISPR-Cas13d system, which has entered the clinical trial stage [14]. This method is based on adenovirus vector and CRISPR-Cas13d system. The sgRNA targeting SARS-CoV-2 replicase transcriptase (ORF1ab) and spike protein is injected into patients. These sgRNAs will cut the virus and destroy its structure to inactivate it. Compared with the traditional method, the biggest feature of this method is that it directly kills the nucleic acid of the virus, and has a good application prospect.

4.2. HIV virus

Mutations of special receptors in some individuals will affect the entry of the virus into cells, thus inhibiting the spread and development of the virus. Many researchers have developed methods for treating and preventing HIV based on this. Induced pluripotent stem cells (iPSCs) can be chosen for research. In previous study, researchers knocked out the CCR5 gene in wild-type cells used CRISPR, and then differentiated the cells into monocytes or macrophages.The results showed they were resistant to HIV-1 force. A new therapeutic method has been proposed by designing specific guidance RNA to target specific regions of viral nucleic acid and transferring it into cells. In the experiment, it was observed that the expression of virus in T cells invaded by virus was weakened. It was further confirmed by sequence analysis that the system could efficiently cleave the LTR target sequence. Most surprisingly, the approach holds promise for eradicating dormant viral genomes from infected individuals. It can also be applied to clear virus' genome in cells. By this way, the virus gene integrated in cells can be effectively knocked out, and the fragment length can reach 9709bp.

4.3. Other viruses

Herpes virus treatment can be investigated using the CRISPR-Cas system. Specifically, the CRISPR was applied to guide specific sequences to slice herpes genome latent in cells. The virus proliferation was stagnated and the viral load was also reduced after being cut. CRISPR can also help to construct vaccines. While viral replication, the system can enter the host cell and target specific sequences in the DNA viral genome to break them. It uses non-homologous end joining and homology-directed repair to slice specific sequence and insert some genes. After that CRISPR can restrain the specifc virus growth. The recombinant virus can rapidly proliferate. Only one round of

selection can obtain the purified recombinant virus. Zhen built a method to knock out specific genes in human papillomavirus (HPV), the expression was significantly interrelated to the occurrence of cervical cancer [15]. They used CRISPR guiding HPV 16 E6/E7, and transduced it into cervical HPV-16 positive cell line SiHa. The in vitro proliferation ability of cervical cancer cells was reduced. They conducted further verification in tumor animal models and found that after the gene-edited cells were introduced into mice, tumorigenesis and growth in mice were significantly inhibited.

5. Conclusion

CRISPR has made gene editing easier, faster, and less costly. At the same time, it has been used in many fields.In the detection and treatment of viruses, CRISPR has great promise. Several new detection ways developed based on it have significant advantages over traditional detection methods in that they are less time-consuming and do not require expensive equipment. In terms of virus therapy, the emergence of CRISPR makes it possible to revolutionize the existing treatment methods and treat the virus from the root.

Nevertheless,it is still a long way from clinical application, and there are still many aspects that need to be optimized, such as accuracy and off-targets; finding more efficient methods for introducing into cells, etc. With continuous development, CRISPR/Cas GE techniques will definitely play an important role in the detection and therapeutics of viruses in the future.

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