Application of CRISPR Technology in Influenza Detection

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Abstract: This study explores the application of CRISPR technology in influenza detection, detailing its technical principles, advantages, practical applications, and challenges. Originating from the bacterial adaptive immune system, CRISPR has demonstrated significant potential in detecting influenza A and B viruses due to its high specificity, sensitivity, and rapid detection capabilities. The CRISPR-Cas system, particularly Cas12 and Cas13, enables precise recognition of viral RNA or DNA, making it a promising tool for early and accurate diagnosis. However, the lack of standardized protocols remains a key challenge, affecting the reproducibility and comparability of test results across different laboratories. Despite these limitations, ongoing advancements in CRISPR-based diagnostic platforms, such as SHERLOCK and DETECTR, are expected to enhance the efficiency and accessibility of influenza detection. With continuous optimization, CRISPR holds great promise for strengthening infectious disease surveillance and prevention, providing a crucial safeguard for global public health.

1 INTRODUCTION

Influenza has always been a serious challenge in global public health. According to the World Health Organization, seasonal influenza epidemics infect 5% to 15% of the world's population each year, and the number of deaths due to influenza-related respiratory and circulatory diseases reaches 29 to 650,000. Traditional influenza virus detection methods, such as virus culture and immunofluorescence detection, have disadvantages such as low sensitivity and long detection time, which are difficult to meet the current needs of rapid and accurate detection (Gootenberg et al., 2018).

The rapid development of gene editing technology has brought new opportunities for influenza detection, and the CRISPR system stands out for its high efficiency and accuracy. The application of CRISPR in influenza detection has achieved many results, and it has shown a wide range of applications in the detection of influenza A and B viruses and drug resistance monitoring based on the characteristics of identifying and cutting specific nucleic acid sequences. The purpose of this study was to deeply analyze the application potential and practical clinical value of CRISPR in influenza detection.

2 PRINCIPLES OF CRISPR

CRISPR is derived from the adaptive immune system of bacteria, and its core components are Cas protein and guide RNA (gRNA). The gRNA can specifically complement and bind to the target nucleic acid sequence, guiding the Cas protein to accurately recognize and cleave the target DNA or RNA.

In influenza detection, gRNAs are designed to match the unique genetic sequences of influenza viruses, especially conserved gene segments. Influenza virus genes are unique, taking influenza A virus as an example, although its HA (hemagglutinin) and NA (neuraminidase) genes are prone to mutation, there are relatively conserved regions within it. Through in-depth analysis and study of these conserved regions, researchers have designed specific gRNAs. When the gRNA forms a complex with the Cas protein, if the influenza virus nucleic acid is present in the sample, the gRNA will accurately recognize and bind the target sequence by relying on the principle of base complementarity pairing, and guide the Cas protein to cleavage it (Chen et al., 2019).

The cleavage reaction triggers a series of signaling responses, and commonly used reporter molecules, such as fluorescent molecules, are activated after the

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Cas protein cleaves the influenza virus nucleic acid, emitting a fluorescent signal. By detecting the presence and strength of the fluorescence signal, it is possible to determine the presence and content of influenza virus in a sample. This principle lays a solid foundation for the application of CRISPR in influenza detection, so that the detection can be realized at the technical level.

3 ADVANTAGES OF CRISPR IN INFLUENZA DETECTION

3.1 High Specificity

The CRISPR system can recognize the target nucleic acid sequence with an accuracy of up to the base level, which can effectively distinguish the subtle genetic differences between different subtypes of influenza virus, avoid cross-reactivity with other respiratory pathogens, greatly reduce the false positive rate, and ensure the reliability of the detection results. Research by Gootenberg et al. has shown that CRISPR has high precision specificity in identifying specific nucleic acid sequences.

In practical applications, there are many subtypes of influenza viruses, such as H1N1 and H3N2 subtypes of influenza A viruses, and their gene sequences are different. The gRNA in the CRISPR system can precisely match the specific gene sequence of the target subtype, and the Cas protein is guided by gRNA, and only the target sequence is cleaved, so that other respiratory pathogens will not be misjudged (Myhrvold, et al., 2018). For example, during the influenza season, there may be multiple respiratory pathogens in patient samples at the same time, and traditional detection methods are prone to cross-reactivity leading to false positive results, while CRISPR can accurately capture the genetic characteristics of influenza viruses and achieve specific detection due to its accurate identification ability. This high specificity is of great significance in epidemic prevention and control, which can avoid the waste of medical resources and the deviation of epidemic prevention and control direction caused by misdiagnosis.

3.2 High Sensitivity

By optimizing the reaction system and signal amplification strategy, CRISPR can detect very low copy number influenza virus nucleic acids, which can be accurately diagnosed when the viral load is low in the early stage of infection, and the infection can be detected earlier than traditional methods, which buys valuable time for epidemic prevention and control. Chen et al. used a CRISPR-based amplification detection method to achieve sensitive capture of trace influenza virus nucleic acids.

In the early detection of influenza patient samples, traditional detection methods such as virus culture require a certain amount of virus to be successfully cultured and detected, while in the early stage of infection, the viral load is extremely low, and it is often difficult for traditional methods to detect the presence of virus. The CRISPR-based amplification detection method can amplify a very small amount of viral nucleic acid with the help of an optimized reaction system, and enhance the weak detection signal through the signal amplification strategy, so as to achieve effective detection of trace viral nucleic acid. This characteristic is very important in epidemic prevention and control, which can detect potential infectious sources in a timely manner, and take prevention and control measures such as isolation and treatment to effectively curb the spread of the epidemic and ensure public health safety.

3.3 Quick and Easy

Some CRISPR-based detection methods can be completed on portable devices or even simple test strips without the need for complex instruments and equipment, which are simple to operate, and can quickly give results in the field and primary care units, breaking the dependence of traditional testing on professional laboratories. A CRISPR-based on-site rapid detection platform developed by Myhrvold et al. enables influenza virus detection in a short time (Corman, et al., 2020).

For example, in primary medical institutions or epidemic sites, CRISPR-based test strip detection methods only need to collect a patient sample (such as a throat swab), add dropwise to the test strip, and determine whether the influenza virus is infected by the color change on the test strip within 15 minutes. This detection method does not need to be operated by professional technicians, and it is simple and easy to understand, which greatly improves the convenience of detection. During the period of epidemic prevention and control, fast and convenient testing methods can quickly obtain test results, take timely prevention and control measures, and effectively control the spread of the epidemic, which

provides great convenience for epidemic prevention and control.

4 APPLICATIONS

4.1 Influenza A

4.1.1 CRISPR-Cas13-Based Nucleic Acid Detection of Influenza A Virus

The research team designed gRNA for the unique conserved gene fragment of influenza A virus and developed a detection protocol using the CRISPR-Cas13 system. When influenza A virus nucleic acid is present in the sample, Cas13 is activated, and the reporter RNA is cleaved to produce a fluorescent signal, which can be detected with high sensitivity in less than 30 minutes with the help of a portable fluorescence detector. In areas with localized outbreaks of influenza A, the test has been initially trialed, providing an effective aid for epidemic screening. The clinical trial report published by Li et al. details the practical application of CRISPR-Cas13-based detection of influenza A virus (Li, et al., 2021).

During the outbreak of influenza A in a certain region, a large number of samples of suspected cases were detected using a CRISPR-Cas13-based detection method. Compared with the traditional PCR detection method, this method not only greatly shortens the detection time, but also performs the same in terms of detection sensitivity. In some samples of patients with mild symptoms, the traditional detection method needs to repeat the test many times to determine the result, but the CRISPR-Cas13-based detection method can quickly and accurately detect the viral nucleic acid, providing timely and accurate data support for epidemic prevention and control. This rapid and sensitive detection method can quickly identify infected people in epidemic screening, and timely isolation and treatment measures can be taken to effectively control the further spread of the epidemic.

4.1.2 Application of CRISPR in the Study of Pathological Mechanisms of Influenza A

Through CRISPR gene editing technology, host cell genes related to influenza A virus infection and replication can be accurately knocked out or modified, and the interaction mechanism between viruses and host cells can be deeply explored. For example, the study of the replication efficiency of influenza A viruses in cells, the extent of infection, and the impact on the immune response of host cells after the deletion of specific genes provides a rationale for the development of more effective antiviral drugs and treatment strategies (Lee, et al., 2021).

Researchers used CRISPR to knock out a key gene in host cells, which is thought to be closely related to the entry of influenza A viruses into cells. The experimental results showed that after knocking out this gene, the replication efficiency of influenza A virus in cells was significantly reduced, and the infection range was also significantly reduced. Further studies have found that the immune response of host cells is altered after gene knockout, and the immune response originally suppressed by the virus is partially restored. The results of this study provide a theoretical basis for the development of antiviral drugs targeting this gene target, and are expected to develop new anti-influenza A virus drugs, block the process of virus infection in cells, and provide new ideas and methods for clinical treatment.

4.2 Influenza B

4.2.1 CRISPR Influenza B Detection Platform with Microfluidic Chip

The integration of CRISPR and microfluidic chip realizes the integration of sample processing, nucleic acid amplification and detection according to the characteristics of influenza B virus. The microchannels in the chip precisely manipulate the fluid, automatically complete the complex reaction process, and use the high specificity of CRISPR to identify influenza B virus subtypes. The whole testing process is completed within 1 hour, and the results can be read through the mobile APP, which is very suitable for small clinics, customs quarantine and other scenarios, which greatly improves the detection efficiency. Wang et al.'s study delves into this innovative application model and demonstrates the potential of multi-technology convergence to improve the detection performance of influenza B (Wang, et al., 2022).

In the customs quarantine scenario, when influenza detection for inbound and outbound personnel, the CRISPR detection platform combined with microfluidic chip has obvious advantages. Traditional testing methods require samples to be sent to a specialized laboratory for testing, which takes a long time and poses a risk of crowding and virus

transmission. The testing platform can quickly complete the test on site, and the test results can be read in real time through the mobile phone APP, which is convenient and fast. In a customs quarantine work, the platform was used to test hundreds of people entering and leaving the country, and successfully detected a number of cases of influenza B virus infection, effectively preventing the cross-border transmission of the virus. This integrated and convenient testing platform provides strong technical support for the prevention and control of influenza B, improves the detection efficiency, and reduces the risk of epidemic transmission.

4.2.2 CRISPR Facilitates the Study of the Mutation Mechanism of Influenza B Virus

Influenza B viruses mutate during transmission, affecting their pathogenicity and immune evasion ability. CRISPR can be used to construct models of influenza B viruses carrying different mutation sites, simulating the mutation process of the virus in the natural environment. By comparing the infection characteristics of different mutant virus strains in cells and animal models, researchers can reveal the pattern of influenza B virus mutation and its impact on the biological characteristics of the virus, providing key information for the development and updating of influenza vaccines.

Researchers have used CRISPR to construct a variety of influenza B virus models with different mutation sites, infecting cells and animal models, respectively. Studies have found that certain mutation sites can cause changes in the surface protein structure of the virus, thereby enhancing the immune escape ability of the virus, allowing it to evade the recognition and attack of the host immune system (Aubry, et al., 2019). At the same time, these mutations also affect the pathogenicity of the virus, and the symptoms are more severe in animal models after infection with strains of the virus carrying specific mutations. Based on these results, vaccine developers can optimize the design of influenza vaccines for these key mutation sites, improve the effectiveness of vaccines, and provide more effective prevention and control methods for dealing with influenza B virus mutations.

4.3 Standardization Issues

The CRISPR influenza detection methods developed by different laboratories and research teams have differences in operation procedures and reagent formulations, and lack of unified standards, which seriously affects the comparability and mutual recognition of test results and hinders the wide application of the technology. Liu et al. called for the establishment of industry standards for CRISPR detection technology to promote its clinical promotion and application translation （Liu, et al., 2022）.

In practice, due to the lack of uniform standards, different laboratories may obtain different results when testing the same sample using their own developed CRISPR assays. This not only brings trouble to clinical diagnosis, but also affects the communication and sharing of scientific research results. For example, in multicenter clinical trials, different CRISPR detection methods are used in each center, which makes it difficult to integrate and analyze data and accurately assess the effectiveness of detection technology. The establishment of a unified standard will help standardize the application of CRISPR in influenza detection, clarify the operation process and reagent quality standards, improve the accuracy and reliability of detection, promote the popularization and application of this technology in the world, and promote the effective development of clinical diagnosis and epidemic prevention and control.

5 CONCLUSIONS

CRISPR has shown significant advantages in the field of influenza detection, which is based on the principle of bacterial adaptive immune system, and realizes the detection of influenza viruses with high specificity, high sensitivity, and fast and convenient. CRISPR has played an important role in the detection of influenza A and B viruses and the study of related mechanisms, providing new methods and ideas for influenza prevention and control, whether it is used for virus nucleic acid detection or to help study pathological mechanisms and mutation mechanisms.

However, at present, this technology faces the challenge of lack of standardization, and the differences in testing methods in different laboratories affect the reliability and mutual recognition of test results. In the future, it is necessary for scientific researchers to work together to establish a unified industry standard and standardize the application of technology. At the same time, we continue to optimize the technical system, further improve the detection performance, and expand the

application scenarios. With the continuous improvement of technology, CRISPR is expected to play an irreplaceable role in influenza detection and even the entire field of infectious disease prevention and control, providing strong technical support for protecting public health.

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