Applications of CRISPR in the Detection of Ebola Virus

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Abstract. Through the rapid development of the Internet today, people learned about viruses such as Ebola virus. Ebola virus has brought serious harm to human society. Different tests have been developed to detect the virus and provide assurance for its diagnosis and treatment, such as CRISPR technology. To this end, this research will analyze how this technology enables virus detection, and further understand the advantage and drawback of using CRISPR to detect Ebola virus. Studies have found that the use of CRISPR-Cas13a technology has the characteristics that other detection do not have, such as techniques of fast, no amplification, and sensitivity, but at the same time, it may also have the disadvantages of false positives in the detection. CRISPR tests for Ebola virus do better than harm. But because Ebola virus is an infectious virus with a very high fatality rate, detection techniques still need to be optimized, as soon as possible to find a better method or technology to detect Ebola virus.

Keywords: CRISPR, Ebola virus, Detection.

1. Introduction

Ebola virus disease is called Ebola Hemorrhagic Fever. Ebola broke out in 1976 in the Democratic Republic of Congo and Sudan, and there have been several cases in central Africa. Ebola is an acute infectious disease caused by Ebola virus infection. The main route of transmission is contact transmission. It is spread though close contact with the blood, secretions, organs, or other body fluids of infected animals. The main clinical manifestations are fever, bleeding and multiple organ damage. Ebola virus is a category A disease with the highest biosafety level, where research on live viruses needs to be carried out in a biosafety level 4 laboratory. The risks of Ebola include, but are not limited to severe clinical symptoms, highly infectiousness, multiple sequelae and highly mortality.

Some other methods like PCR-based diagnostics are not really practical because of high cost, advanced laboratory facilities and other problems. However, with current technology, Ebola virus can be detected by clustered regularly interspaced short palindromic repeats (CRISPR) cas13 protein as a type of RNA virus. The non-specific cleavage products of Cas13a can immediately measure by a custom-made integrated fluorometer, which is small enough to facilitate field diagnosis. It means with the help of enough Ebola-carrying bacteria, a rapid test can be carried out. The method of detecting the gene sequences through cas13 is rapid, accurate and sensitive. Reasonably use this technology helps scientists find the appearance of Ebola virus as it arises and prevent further infection.

CRISPR, is a kind of technology to edit gene which can modify the genome [1]. It is first found in bacteria, in which it can kill the DNA of the virus to protect themselves. There are some peculiarities of CRISPR, for example, it is easier to design and use than other gene editing technologies such as TALENs and ZFNs. Also, it is efficient and accurate. It can exactly find the place that it needs to be and edit it. CRISPR is universal as it can be used in most of creatures. Meanwhile, CRISPR can flexibly edit gene including knocking out genes, adding genes or exchanging them. Benefit from those peculiarities, CRISPR can be widely used in several aspects. For example, it can be used in modifying the genome, detecting viruses, treating hereditary diseases, improving crop varieties or researching biology.

As CRISPR is sensitive and highly specifically, it can be used to detect the EBOV. Researchers can design specific CRISPR-Cas system, which can find and combine with some special gene sequences. If they are matching with each other, CRISPR-Cas system will be activated and produce a signal that can be detected. With the signal, people can check the EBOV easily and accurately. Therefore, this research will analyze the Ebola virus detection method based on CRISPR technology, and discuss the advantages and disadvantages of this detection method in the application process.

2. Detection of Ebola virus based on CRISPR

2.1. Detection performance analysis

The specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) technology based on CRISPR/Cas13 system can be developed, which is aimed at cutting the RNA array. They added recombinase polymerase amplification (RPA) to extend target RNA and Cas13a is then activated under the guidance of crRNA, and the activated Cas13a cuts the RNA fluorescence reporter molecule so that the fluorescence signal can be detected [1]. It successfully made it possible to detect RNA virus quickly through CRISPR technology. In practice, Gootenberg et al. combined CRISPR-Cas13a's allele-specific sensing capabilities with a recombinant enzyme polymerase amplification method to detect specific RNA and DNA sequences [2]. Based on SHERLOCK diagnostic method, both fluorescence and transverse flow readings were used to demonstrate the sensitivity and specificity of the detection method against Ebola virus and Lassa fever virus in laboratory and clinical samples [2]. Also, the detection method is feasible in endemic areas. The method successfully detected the presence of Zika virus at atmospheric pressure levels, which means it may be practical in other viruses. And the high specificity of this technology can ensure low error rate. But the there are some challenges remain, such as high cost and high complexity.

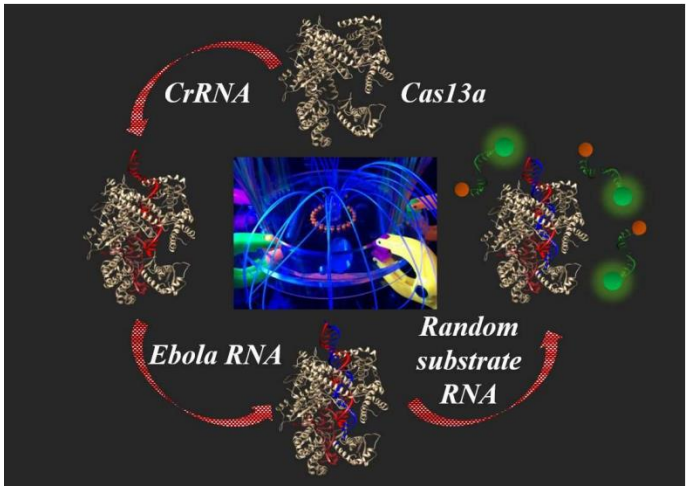

Figure 1. Ebola Virus detection based on CRISPR-Cas13a [3]

At the same time, as shown in Fig. 1, Qin et al. combined an automated and multiplexed CRISPR microfluidic chip with a custom-designed benchtop fluorometer for rapid and low-volume (~10 μL) Ebola virus detection. Using this integrated system, clinical diagnosis of any viral RNA can be achieved by programming crRNA spacer sequences that complement different RNA targets [3]. Previous studies have reported the advantages of CRISPR/CAS-based biosensors over traditional biosensors in terms of cost-effectiveness, simplicity, reliability, user-friendliness, repeatability, versatility, non-toxicity, high specificity, and biodegradability [4]. If the technology can be achieved all over the world, Ebola virus will not be such harmful.

The CRISPR technology can be used to detect MPXV. Based on both CRISPR-Cas13a and Cas12a, it can sensitively and quickly detect MPXV's coinfection with HIV and SARS-CoV-2. As a kind of zoonosis, MPXV is identified as a new global disaster by WHO and CDC. MPXV includes two kinds, MPXV-CA and MPXV-WA which has different pathogenicity. There is research that 51% of MPXV

patients are infected HIV [5]. During Covid-19 pandemic, the MPXV and SARS-CoV-2 coinfection cases are on the rise. These cases make disease management and control harder. This makes new detection more and more important.

A single target detection system about MPXV based on Cas13a and Cas12a has been developed. With a targeted design, they can distinguish MPXV-CA and MPXV-WA. By recombinase-assisted amplification, the detection sensitivity is further increased. RAA-Cas13a-Fluo and RAA-Cas12a-Fluo system's lowest detection even achieves 4 copies. These systems have a high sensitivity and specificity which transcend qRT-PCR, especially at reducing FNRs and increasing MPXV-WA's detection sensitivity. For patient infected different viruses, researchers combine Cas12a and Cas 13a together. In this way, health care workers can detect MPXV's coinfection with SARS-CoV-2 or HIV at the same time and support a quick and effective way to diagnose coinfection.

CRISPR can be a new way in fighting against COVID-19. CRISPR-Cas12a and CRISPR-Cas 13a can target single strand RNA virus such as SARS-CoV-2 and detect and degrade them. By recognizing RNA sequences paired by CRISPR RNA, it can specially target RNA viruses and provides possibility of quick detection and treatment for COVID-19. In terms of diagnose, there are some new diagnostic platforms based on CRISPR-Cas system, such as SHERLOCK and DETECTR. Combined with same-temperature amplification technology, they can examine SARS-CoV-2 RNA more quickly and sensitively than traditional RT-PCR. Meanwhile, these diagnostic tools based on CRISPR-Cas are cheap and easy to use, so it is possible to use in poor places.

In terms of treatment, CRISPR-Cas system is also potential, especially PAC-MAN tactic. This tactic can efficiently cut and degrade the RNA of viruses and inhibit viruses copy themselves by designing CrRNA aiming at the specific places in SARS-CoV-2 genomes [6]. And there is a new way, ABACAS, which reaches accurate targeting and effective inhibition of viruses by mixing Cas-13a protein with the antibody of SARS-CoV-2's prickly protein [6]. There are still some difficulties in using CRISPR-Cas system to detect and treat the disease, including protein and RNA delivery efficiency may be not so high, or this system may have safety problems. But it is still a great tool to help people responding to the epidemic.

DETECTR, a technology based on CRISPR-Cas12, can be used in quick diagnosis of SARS-CoV-2. Since December 2019, COVID-19 epidemic around the world. Traditional SARS-CoV-2 diagnosis relies on RT-PCR, but it needs one day to get the results, which imposes restrictions on controlling the spread of the disease. As a result, developing a quick, effective and sensitive way to diagnose is extremely urgent. DETECTR uses CRISPR-Cas12 combining with side-flow detection technology and it can diagnose SARS CoV-2 from RNA extraction in respiratory swab. It makes the diagnosis's time reduced to 40 minutes. With samples from America, DETECTR has a high accuracy of 95% in positive samples and 100% of negative samples [7]. DETECTR is quick and accurate. It can use respiratory swab samples to extract and detect, without expensive laboratory equipment. Unlike traditional qRT-PCR, DETECTR uses isothermal signal amplification, which don't need thermal cycling. DETECTR don't have a long term to develop, its development and validation only takes 2 weeks [7], and it is easy to change for new kinds of viruses. So, it gives possibility of rapid diagnosis for future epidemic situation. It develops an important tool of global public health safety.

2.2. Perspectives

No prior nucleic acid amplification is required, and direct detection is possible [8]. The minimum detection limit for most CRISPR-CAS based assays to detect unamplified nucleic acid substrates is picomole quantities. Although there are certain requirements for the concentration of the sample, however, the detection sensitivity can be improved through the optimization of the method to meet the needs of clinical sample detection. The first is to improve the activity of Cas endonuclease in the reaction system. The second is that the reaction signal can be amplified by CTOSPR Class III effector molecule Csm6.

CRISPR and LAMP techniques in combination, CRISPR and RPA techniques in combination. Through the collaboration of CRISPR-Cas system and other different detection technologies, he

detection platform based on CRISPR-Cas system has gradually improved in terms of detection sensitivity and specificity. Reducing the concentration of CRISPR-Cas and guide gRNA does not cause off-target effects. The method was optimized and improved through nucleic acid extraction process, nucleic acid amplification process and result reading process, Optimize the design of storage and transport equipment to maintain the stability of reagents.

Off-target effects can lead to false positives and false negatives in detection, therefore, attention needs to be paid [9, 10]. The off-target effect can be partially solved by constructing high-fidelity Cas9 molecules, optimizing the structure of guide RNA, and high GC content. Some nucleic acid amplification methods are inevitably contaminated with amplification products, which increases the complexity of detection. The method can be optimized and improved from three aspects: accounting extraction process, nucleic acid amplification process and result reading process. Most endonuclease effects require PAM sequences, which limits the application of this method. The solution is that when there is no suitable sequence at the target sequence site, an additional sequence can be inserted. During in vitro nucleic acid amplification, PAM sequences are designed at special locations of primers, which can be subsequently recognized by endonuclease. Therefore, the CRISPR-Cas system can be used to detect any gene location. Realize quantitative detection, multiple detection and standardized detection. SHERLOCKv2 enables quantitative detection over a relatively large sample concentration range by analyzing the biochemical properties of different members of the CRISPR-Cas13a family, improve the sensitivity of detection.

3. Conclusion

CRISPR-Cas13a is fast, sensitive and need not amplification. Though there may be some false-positive results, CRISPR can detect Ebola virus RNA effectively. As a result, CRISPR has so much benefits such as improving the sensitivity and accuracy of the detection, making the inspection process easier. By optimizing the technology, it will reduce false-positive results and make detection faster. The research gives a new way to detect the Ebola virus, which need not preamplification, be quickly and accurately. It gives a new idea for detecting RNA viruses such as Ebola virus. This takes an important place in improving the efficiency and accuracy during the epidemic, and it provides some ideas for the other researchers. Though CRISPR-Cas system is outstanding in detecting Ebola virus, it has some shortcomings as there may be false positive results. The future researches should be aiming to optimize CRISPR-Cas system further and improve its specificity and reduce false positive results. Also, there needs more technologies of Ebola virus as it has high fatality rate and contagiousness. In this way, it can give powerful technologic support for Ebola virus's prevention and control with a bigger range and a higher efficiency of the detection.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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