

Efficient CRISPR Cas13 in The Treatment of The COVID-19

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Abstract. Currently, CRISPR Cas13 is one of the rapid advancement and growing potential in the fields of diagnostics and therapeutics. It has proven highly effective in detecting RNA viruses like SARS-CoV-2, making it an essential tool for identifying COVID-19 variants. However, since CRISPR Cas13 is a newly emerged technology in the CRISPR system, there are still some problems that need to be tackled. This article aims to discuss the application of CRISPR Cas13 in treating COVID 19. Two CRISPR Cas13 based diagnostic tools, PAC-MAN and CARMEN, hold potential for detecting variants, with research indicating their ability to degrade viral RNA and suppress viral gene expression. Despite this potential, challenges remain, particularly in achieving efficient and safe delivery systems for Cas13. While it is not yet fully implemented in clinical settings, the progress made so far highlights CRISPR Cas13's significant role in addressing viral outbreaks, specifically COVID 19 and its potential for future applications in infectious diseases and other RNA-based conditions.

Keywords: CRISPR Cas13; COVID-19; RNA-targeting.

1. Introduction

COVID-19, a global viral pandemic caused by SARS-CoV-2 that burst out in 2019, has offered scientists and researchers great challenges due to its rapid mutation and the consistent emergence of new variants. As a result, it is urgent to detect and differentiate between these variants quickly and accurately to keep public health safe and under control. CRISPR Cas13 is a powerful and versatile gene-editing tool derived from the CRISPR system, which is an adaptive immune system used by bacteria against viruses. While almost every CRISPR system targets DNA, which is not suitable for the application of COVID-19 as it is an RNA virus, CRISPR Cas13 is noted for its precision in targeting and cleaving RNA. This unique distinction makes Cas13 to be very well-suited for the detection of RNA viruses, such as SARS-Cov-2, an RNA virus responsible for COVID-19. CRISPR Cas13 plays a key role in COVID-19 treatment through targeting the SARS-Cov-2 genome.

PAC-MAN, in full term, prophylactic antiviral CRISPR in human cells, is a CRISPR Cas13-based strategy used for detecting and degrading SARS-Cov-2 was developed after the first COVID-19 case was announced. The Cas13 enzymes with gRNA help recognize the SARS-Cov-2, which can block the protein transcription and therefore, repress the viral replication effectively. Besides targeting the viral genome, CRISPR Cas13 can also interfere with SARS-Cov-2 amplification by targeting the host cells. For the purpose of detecting SARS-Cov-2 variants, the development of CARMEN and mCARMEN enables a sensitive and straightforward RNA detection utilizing CRISPR-based diagnostics and microfluidics, allowing researchers to test different variants of COVID, including Delta and Omicron. CRISPR-Cas13 has emerged as a key technology in addressing these challenges. Its RNA-targeting capabilities have been harnessed to develop advanced diagnostic tools and explore novel therapeutic approaches. By leveraging Cas13's precision in targeting viral RNA, researchers aim to improve the detection of SARS-CoV-2 and its variants, ultimately contributing to more effective control measures and treatment options. Studying CRISPR Cas13 can not only treat current diseases but may also offer potential treatments for new diseases in the future.

This paper will dive into the application of CRISPR Cas13 and COVID-19 by giving a thorough overview of how Cas13 is used in COVID-19 variants detection, antiviral drug discovery, as well as potential vaccines designed. Off-target effect is a key problem that every gene-editing tool has to



3.1. The structure of COVID 19

The structure of the SARS-CoV-2 virus could be important for patients who suffer from COVID-19 to receive the CRISPR Cas13 treatment. People accidentally infected with SARS-CoV-2 through mouth, nose, or eyes may experience mild to moderate respiratory illness such as persistent cough, hyperventilation, and erratic breathing [4]. Having the Cas13 enzyme as an RNA-targeted nuclease is a valuable property of CRISPR Cas13. This unique characteristic offers scientists and researchers great opportunities to address the challenges of COVID-19. SARS-CoV-2 viruses are composed of four main structural proteins: N (nucleocapsid), S (spike), M (membrane), and E (envelope) [5]. N stands for nucleocapsid. It coats and binds with the genome/RNA protects and gathers the genome inside the viral particle to facilitate viral transcription [6]. Understanding the structure of coronavirus is crucial for scientists to do further research that applies CRISPR Cas13 to the virus.

3.2. Detection of the Mutant Strains

Mutation is a natural process for survival and reproduction. Viruses mutate and become more effective in binding with the host human cells. Identifying newly emerged variants promptly is essential for scientists to come up with practical solutions to deal with the viral situation. There are two main reasons why CRISPR Cas13-based diagnostic tools struggle to detect SARS-CoV-2 variants: the virus mutates rapidly and unexpectedly, and the high specificity of Cas13 means that even a single mismatch can prevent detection [6]. To tackle this issue, several innovative strategies have been developed. Until now, many technologies have been designed to help detect virus variants. For example, PAC-MAN is a newly developed method aimed at detecting variants of SARS-CoV-2, the virus that leads to COVID-19. PAC-MAN, short for prophylactic antiviral CRISPR in human cells, is a CRISPR Cas13-based strategy that uses Cas13d (could also be other Cas13-based enzymes) along with specially designed guide RNAs (gRNAs) to detect and degrade SARS-Cov-2 as well as other virus like influenza A [7]. Study has shown that PAC-MAN is able to suppress expression of coronavirus fragment in human lung epithelial cells. The crRNAs should be adequately designed as it may affect the effectiveness of inhibiting SARS-CoV-2 [8]. However, several challenges remain to be addressed with this technology before the PAC-MAN strategy can be implemented. For instance, achieving sufficient expression of crRNA and Cas13, along with developing safe and effective delivery methods, is crucial. Additionally, managing the immunogenic response against foreign Cas's proteins is an important consideration for the success of this approach [8]. Besides PAC-MAN, CARMEN is another cost-effective solution for SARS-CoV-2 variant detection. Researchers process samples and Cas13-crRNA complexes individually before combining them for detection using fluorescence microscopy [6]. This process enables the identification of 169 human-associated viruses from 8 samples simultaneously. mCARMEN is an upgraded version of CARMEN. mCARMEN helps with clinical testing of SARS-CoV-2 variants like Delta and Omicron and provides direct RNA detection [6]. To sum up, both PAC-MAN and CARMEN are CRISPR-Cas13-based diagnostic tool that are designed to detect SARS-CoV-2 variants due to the virus's rapid mutation and the high specificity of Cas13. They are key technologies that are developed to address this issue.

4. COVID Vaccine Research and Development

CRISPR Cas13 can play a significant role in developing and enhancing COVID-19 vaccines. A valid target is indispensable for the development of a vaccine. CRISPR Cas13 can be helpful in identifying and validating potential vaccine targets within the SARS-CoV-2 genome. By investigating how viruses and the host cells interact with each other, it provides a strategic approach in vaccine development. This process is crucial as it informs the researchers which parts of the virus elicit a robust immune response. Since CRISPR Cas13 is able to detect new variants along the rapid mutation, it gives researchers signs about the new variants and ensures that vaccines remain effective against emerging strains. Vaccine efficacy could also be monitored using CRISPR Cas13 diagnostic tools to detect the presence of the virus or its variants in vaccinated individuals, ensuring a smooth tracking of immune responses. Lastly, CRISPR Cas13 may also be explored for therapeutic uses, potentially

complementing vaccines by providing an additional layer of protection against the virus through RNA targeting. Overall, the Cas13 technique offers a versatile toolkit that enhances both the development and application of COVID-19 vaccines.

5. Development of Antiviral Drugs

As for now, there are no specific antiviral drugs for COVID-19 that have been fully developed and approved using CRISPR Cas13. However, research is ongoing in this area, and CRISPR Cas13 is proven to be a potential gene editing tool for targeting and degrading SARS-CoV-2, which could potentially lead to the development of new antiviral therapies.[9]. As mentioned in the previous article, scientists are applying PAC-MAN in the field of COVID-19. PAC-MAN is capable of effectively degrading SARS-CoV-2 in human epithelial cells. While CRISPR Cas13 has not yet resulted in an approved antiviral drug for COVID-19, it holds significant potential for developing future treatments.

6. Problems and Optimization

Although the overall picture is very promising, there are still limitations and problems within CRISPR Cas13. One of the biggest challenges in applying CRISPR Cas13 and the rest of the CRISPR system is to design sgRNAs with high on-target efficiency [10]. Scientists and researchers are trying to avoid off-target effects. Off-target effects are unexpected consequences that occur during CRISPR gene editing. For example, the Cas protein cleaves the DNA or RNA sequence at unwanted sites, potentially leading to adverse, irreversible effects. However, compared with other CRISPR systems, CRISPR Cas13 is noted for its precision in genome editing as it targets RNA sequences with high accuracy. Besides off-target effects, delivery methods could also be one of the problems. For example, a primary challenge exists in using PAC-MAN clinically to create effective and safe methods for in vivo delivery [8]. It is not promised that these therapies will get into the body in a way that directly reaches its target cells and work as intended without causing any harm.

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