Potential of CRISPR-Cas System Treatment to Eradicate the COVID-19 Pandemic Caused by SARS-COV-2

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Abstract

Over the past two years, scientists' efforts to develop vaccines and antiviral treatments against the COVID-19 pandemic have failed due to the lack of a proven therapy and the inefficacy of currently available vaccines against the virus' mutant forms. Therefore, the COVID-19 pandemic has forced scientists to develop highly reliable diagnostic and therapeutic procedures to quickly prevent the infection's spread. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated protein (Cas) system is an RNA-directed adaptive immune system used as a gene-editing technology. Recent CRISPR application studies have shown that CRISPR effectors may limit the replication of double-stranded DNA or single-stranded RNA (ssRNA) viruses in mammalians. Because SARS-COV-2 contains a single-stranded, positive-sense RNA genome and structure, CRISPR-Cas systems are suitable for therapeutic use. Similarly, many clinical studies have investigated the CRISPR-Cas systems' potential for diagnosing SARS-COV-2 and have proven their effectiveness. CRISPR-Cas systems' success in quickly diagnosing SARS-COV-2 serves as a model for applying CRISPR-Cas systems to treat the Covid-19 pandemic. This review aims to investigate the uses of CRISPR-Cas-based gene-editing systems (CRISPR-Cas9, CRISPR-Cas12, CRISPR-Cas13) when treating the COVID-19 pandemic, to analyze related articles, and to examine this novel technology's limits and possibilities.

Keywords

Antiviral strategy; Covid-19 pandemic; CRISPR-Cas systems; Gene editing; SARS-COV-2.

Introduction

Identifying SARS-COV-2 positive individuals and treating them is essential to control the COVID-19 outbreak. [1] While reverse transcription-polymerase chain reaction (RT-PCR) is currently the gold standard for molecular detection, its drawbacks include expensive equipment costs, staff training requirements, the risk for false-positive/negative results, and long processing times. CRISPR-Cas systems were determined to successfully and quickly diagnose SARS-COV-2 cases. Thus, they were used for diagnoses in the COVID-19 outbreak. [2] To

exemplify, Sherlock Biosciences' Sherlock™ CRISPR SARS-COV-2 kits were licensed by the Food and Drug Administration (FDA) for emergency usage to alleviate COVID-19 detection bottlenecks in the United States. [3] CRISPR-Cas systems' efficacy in quickly diagnosing SARS-COV-2 showcases its potential success in treating the COVID-19 pandemic. Similarly, CRISPR-Cas systems may be used therapeutically since CRISPR effectors limit DNA and RNA structures' replication, such as SARS-COV-2. For example, the CRISPR-Cas13-based approach for COVID-19 treatment successfully destroyed SARS-COV-2 sequences and a live IAV genome. The system is called Prophylactic Antiviral CRISPR in human cells (PAC-MAN) to suppress SARS-COV-2. [4] This review provides in-depth examinations of the examples above of CRISPR-Cas systems' usages to analyze how and to what extent they help limit and treat the COVID-19 pandemic. To strengthen one's understanding of these examinations, the review first discusses the COVID-19 pandemic and the virus and the general history, mechanism, and technicalities of CRISPR-Cas systems.

Discussion

SARS-COV-2 and COVID-19 Pandemic

While (severe acute respiratory syndrome coronavirus) SARS-COV-2 was first identified as an atypical respiratory tract infection agent in Wuhan, a city in the Hubei province of China, in December 2019, the World Health Organization declared a SARS-COV-2-related pandemic on March 11, 2020, just one year later. Unfortunately, the Covid-19 epidemic remains significant as a worldwide hazard. Many countries' healthcare systems have been strained to breaking point, with some collapsing under the weight of the pandemic, and meticulous precautionary efforts to slow virus transmissions, such as quarantine, travel restrictions, and social isolation, have had catastrophic consequences for the global economy and society. [5]

SARS-COV-2 is a spherical, enclosed coronavirus with a single-stranded, positive-sense RNA genome encased in an extracellular membrane comprising spike glycoproteins. [6] It is a positive-sense ssRNA that primarily infects the upper and lower respiratory tracts. Furthermore, it causes illness by direct cytotoxicity and activation of host cytokine-mediated inflammation. [7] The infection process starts with the virus entering the cell by endocytosis or direct fusion of the viral envelope with the host membrane. Then, the virus uncoats and releases RNA into the cytoplasm. [8]

While serological tests for COVID-19 as an immunological assay can mainly detect viral antigens in respiratory secretions or antibodies in the blood, molecular methods for COVID-19 are based on SARS-COV-2 RNA detection in nasopharyngeal samples since droplets primarily transmit the disease. [8,9] COVID-19 may be seen in asymptomatic people (30%). After an incubation period of 4–14 days, most people have mild to severe symptoms (55%) with anorexia,

myalgias, anosmia, dysgeusia (dysfunction of the sense of taste), a sore throat, a headache, or rhinorrhea (runny nose). Around day five, nearly 30% of individuals experience severe dyspnea (shortness of breath) and fever. Patients with severe symptoms often deteriorate in the second week and need hospitalization for hypoxemia and bilateral pneumonia (75%). Most hospitalized patients receive conventional treatment, but 20% may rapidly worsen with severe complications, including acute respiratory distress syndrome, acute cardiac injury, acute kidney injury, and septic shock. [10]

At present, there is not a single specific antiviral therapy for COVID-19, and symptomatic supportive care remains the mainstay of treatment. [10,11] Nonetheless, several drugs are being evaluated for their efficacy as antiviral agents and are suggested for use in the National Institutes of Health COVID-19 therapy recommendations. Convalescent plasma treatment is an exemplary example of a modern therapeutic technique.[12]

History of the CRISPR-Cas system

In 1987, during a study regarding Escherichia coli, Y. Ishino and colleagues discovered an unusual genetic structure composed of alternating repeat and non-repeat DNA sequences. [13] In 2002, Jansen and colleagues revealed that prokaryotes possess multiple distinguishing features in their chromosomes, with repetitive “spacer” sequences placed on each side of distinct ones. They developed the abbreviation CRISPR for “Clustered Regularly Interspaced Short Palindromic Repeats,” referring to the results of their study. The researchers then discovered that numerous clusters of hallmark CRISPR-associated Cas genes were highly preserved and often next to repetitive elements. [14] The finding of sequence similarities between the spacer sections of CRISPRs and those of bacteriophages, archaeal viruses, and plasmids in the early 2000s provided insight on CRISPR's immune system role. The CRISPR arrays’ specific sequences were discovered in 2005 by three different research teams (Mojica and colleagues, Bolotin and colleagues, and Pourcel and colleagues) through the systematic examination of the spacer sequences between individual direct repeats. [15, 16, 17]

Many researchers proposed that CRISPR spacers operate as RNA-like guides to degrade viral transcripts or spacer-matching CRISPR spacers drive Cas enzymes to cleave viral DNA at spacer-matching areas. [16,18] In a subsequent study published by Rodolphe Barrangou et al. of Danisco, a yogurt company, researchers employed Streptococcus thermophilus bacteria as models. They noticed that they were implanted by inserting new pieces into areas of bacteria that survive a viral assault. Additionally, they determined that the DNA sequence of these spacers is similar to that of segments of the viral genome. Moreover, the researchers altered it by eliminating spacers and inserting new viral DNA sequences. They could modify the bacterium's resistance to a particular viral attack. Consequently, the researchers established that CRISPR is crucial for bacterial immunity regulation. [19] Individual spacers to guide Cas nuclease activity.

[20] In 2013, Cas was confirmed as an RNA-guided endonuclease. [21] Makarova et al. discovered that one or more operons having a cluster of Cas genes producing the system's effector enzymes are located near the CRISPR array. [22] Then, CRISPR-Cas was applied in eukaryotic cells. In 2014, genome-wide functional screening using Cas studies was done. [23] In 2016, Cong and Hsu successfully made in vivo genome editing via homolog independent targeted integration. [21, 24]

The function of the CRISPR-Cas system

CRISPR-Cas is an RNA-directed adaptive immune system that occurs naturally in about 48% of bacteria and 95% of archaea. The system is triggered by the invasion of foreign genetic material. [9, 25] It generates an immune response in three stages: adaptation, pre-crRNA expression/processing, and interference. [26] Firstly, during the adaptation step, short and direct repeats separated by spacers constitute CRISPR sequences. Then, a foreign DNA termed protospacer is cleaved and incorporated into the CRISPR array. As a result, the integrated pieces function as new spacers. [26] The second step is the expression, during which the CRISPR array is transcribed to generate precursor CRISPR-derived RNA (pre-crRNA), which is subsequently matured to develop CRISPR-derived RNA (crRNA). [27] This is proceeded by the interference phase, which attaches the mature crRNA to the processing complex and serves as a guide RNA, recognizing identical sequences in the invading viral RNA. Viral RNA is subsequently cleaved and inactivated by one of the Cas proteins. [28]

CRISPR-Cas systems' capacity to produce a double-strand break (DSB) at a particular genomic locus ensures their use for genome editing. The occurrence of DSBs relies entirely on the host cell's DNA repair mechanism to fix the lesion created by these systems. The repair methods may be homology-directed repair (HDR) or non-homologous end joining (NHEJ). HDR repairs the DSB by using a homologous template DNA to the break site, such as an unbroken sister chromatid or homologous chromosome. Then, it delivers exogenous DNA Templates to the host genome to effect a user-defined modification. In contrast, NHEJ relies on the direct combining of the DSB's broken ends, rendering NHEJ the more error-prone of the two mechanisms. NHEJ may be used to damage genes, while HDR enables the introduction of new genetic data or the direct repair of a single locus's sequence. The NHEJ is a repair mechanism in which DSB junctions undergo insertions and deletions. Although the HDR process needs the presence of a homologous DNA template, it is very accurate in repairing DSBs, and exogenous homologous sequences may be used to direct genome editing. [29]

Classification of the CRISPR-Cas systems

Each with its unique composition and method of action, CRISPR-Cas systems have six CRISPR-Cas types in total, and at least 29 subtypes 6 -8, and both the type and subtype lists are

constantly expanding. [29] The CRISPR-Cas system is classified into two distinct groups, depending on the arrangement of its effectors. [9] The Class 1 system cleaves the target genome sequence using crRNA and a multi-effector complex. In the class I system, the ribonucleoprotein (RNP) complex has several protein subunits and crRNA. In contrast, the Class 2 system cleaves the target genome sequence using a single multidomain Cas protein and crRNA for interference. The class II system contains just one protein and crRNA to target invasive viral RNAs. [30] Class 2 CRISPR-Cas systems are found exclusively in bacteria and combine a crRNA with a Cas protein to form a ribonucleoprotein complex. The crRNA is programmed to recognize a PAM sequence adjacent to the target DNA. [31] These multidomain effector proteins interfere through complementarity between the crRNA and the target sequence. [30] Each of the two classes is further subdivided into three subtypes, Type I, III, and IV in Class I and Type II, V, and VI in Class II. [18]

CRISPR-associated (Cas) genes are a group of genes with varied orientations and sequences that code encoding Cas proteins. They are critical for acquiring and destroying foreign sequences. A total of 93 distinct Cas genes have been recognized to date. Based on sequence similarity, these genes were categorized into 35 families. This review focused on Cas proteins: Cas9, Cas12, Cas13, classified as type II, type V, and type VI enzymes. [31]

The most widely characterized CRISPR-Cas system is the type II subtype II-A found in Streptococcus pyogenes (Sp), which uses SpCas9. Cas9 was the first Cas-protein engineered for use in gene editing [32], and it is the most efficient genome-editing machinery for targeting double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA). Cas9 uses trans-activating CRISPR RNA (tracrRNA) and RNase III to process the pre-crRNA in the type II CRISPR-Cas system. [33] In comparison, the Cas12 and Cas13 proteins in the type V and VI systems process the pre-crRNA directly. While Cas12a and Cas12b proteins break dsDNA upon identification by matured crRNA, Cas13 protein cleaves ssRNA. Additionally, Cas13 is not dependent on the presence of a PAM in the target RNA, while Cas12 needs a PAM in the dsDNA target but not in the ssDNA target.

CRISPR-Cas systems are being used as an antiviral, targeting genes in human and viral genomes. Studies have effectively targeted viral and host genes involved in viral entry, replication, and persistence. [34] The invention, advancement, and ease of using Type II CRISPR-Cas9 systems have expedited their adoption and implementation across various applications.

CRISPR-Cas systems versus pathogenic viruses

Class 2 systems are used in various fields, including genome editing, which can be accomplished using a type II or type V effector enzyme to evaluate potential developing therapies and target ssDNA/RNA and for viral disease detection and treatment. [35]

Current Cas9 applications have shown that CRISPR effectors may limit replication of dsDNA or single-stranded RNA (ssRNA) viruses with DNA intermediates in mammalian cells. [7, 36, 37, 38, 23, 39] Liu et al. found from their research that HBV-specific gRNA/Cas9 systems inhibit HBV replication in different genotypes. HBV templates are cleaved and cleared by HBV-specific gRNA/Cas9 systems, and so they proved the inhibition of HBV in vivo by the gRNA/Cas9 system induces clear. [7] Then, the work by Ophinni et al. revealed that CRISPR-Cas9 might target the HIV-1 proviral genome and suppress replication in latency models. [36] Later, Ramanan et al. demonstrated that the CRISPR-Cas9 system could selectively target and cleave conserved areas of the HBV genome, resulting in potent inhibition of viral gene expression and replication. [37] Lastly, Roehm et al. revealed that Cas9 and gRNA delivery by Lentivirus reduces HSV-1 infection and protects cells against infection. [38]

Cas9 and other DNA-targeting effectors defend against invading DNA bacteriophages. However, around two-thirds of viruses capable of infecting humans have ssRNA genomes, and just 2.5 percent of those viruses include DNA intermediates that could be targeted with Cas9. [] Recently identified Cas9 orthologs that target RNA and DNA are less likely to satisfy this demand, since they have poor RNA cleavage performance and may produce off-target effects on cellular DNA. [41]

It was then discovered that Cas 12 and Cas13 effectors, which are associated with type III and VI CRISPR systems, may contribute to the defense of mammalian cells against DNA and RNA viruses. [42] CRISPR-Cas12 is often referred to as CRISPR-associated endonuclease. From Prevotella and Francisella 1 (cpf1), it is a type V-associated nuclease belonging to the Class 2 family. [43, 44] The CRISPR-Cas12 system is an efficient method to generate staggered cuts in both ssDNA and dsDNA, and it utilizes only the crRNA to make staggered cuts at the targeted region. In rodent models of influenza and SARS-COV-2 infections in 2021, Cas13a was shown to attenuate the severity of influenza and SARS-COV-2 in vivo. [45] Cas13a was supplied in a synthetic mRNA that allows for repeat dosing since the expression is temporary. It was the first time that Cas13a has been tested as a therapy for influenza infection after it had been administered in vivo. There were no in vivo tests for treating SARS-COV-2 conditions, since Cas13a was administered before infection.

Applications of the CRISPR-Cas systems in the diagnosis of SARS-COV-2

RNA-guided CRISPR-Cas nuclease-based nucleic acid detection has recently shown tremendous promise for developing next-generation molecular diagnostics technologies because of its high sensitivity, reliability, and specificity. Due to the challenges associated with heterologous expression of multiple cascade complexes, the class 2 CRISPR-Cas system is more

convenient and quick in responding to disasters such as COVID-19 than the class 1 system. For instance, since single-gene testing may produce false-negative results, Xiong et al. offered dual-gene testing, a widely adopted strategy by clinical-approved RT-qPCR diagnostic kits. They improved the CRISPR-Cas9-mediated triple-line LFA (TL-LFA) design combined with multiplex reverse transcription-recombinase polymerase amplification (RT-RPA). As a result, an examination of 64 clinical samples taken from the nose revealed that the CRISPR-Cas9-mediated T-LFA approach is as analytically specific and sensitive as the gold-standard RT-qPCR procedure. [46]

For rapid, specific, and sensitive detection of SARS-COV-2, Ali et al. developed Bio-SCAN (biotin-coupled specific CRISPR-based assay for nucleic acid detection) for pathogen detection. Ali et al. tested Bio-SCAN's ability to identify SARS-COV-2 variations by focusing on the spike polypeptide alterations that increase infectivity and disseminate COVID-19 worldwide. They purposed to demonstrate that one could use the Bio-SCAN platform to forecast the emerging trends and prevalence of specific variant(s) in a particular sample collection from a population group or region by designing five sgRNAs with a specific focus on the PAM or seed region of the target sequence of the prevalent variants. Bio-SCAN diagnosed SARS-COV-2 with acceptable accuracy, demonstrating its use for routine examination in non-laboratory POC situations. Bio-SCAN can accurately identify individual variations, enabling trend prediction or identification of the most common SARS-COV-2 variant(s) during screening tests. [47]

Cas13d is one of the best subtypes because of its high precision, strong knockdown efficiency, and short coding sequence, making it easy to spread. The unique aspect of the Cas13d enzyme is that its cleavage does not depend on PAM-like sequences. This makes it easier to target constantly changing viruses because the rapid development of crRNAs makes it easier to target them. To illustrate, Nguyen et al. used CRISPR-Cas13d technology to build a flexible and efficient technique for targeting RNA in the laboratory. To reduce the capacity of production SARS-COV-2, they preferred to use the CRISPR-Cas13d system because of its flexibility in making guide RNAs and the unnecessary use of PAM. Also, they offered that adeno-associated virus (AAV) could be used to deliver the Cas13d effector to people who have been infected with SARS-COV-2. Because of the small size of the Cas13d effector, it can be used for an "all-in-one" AAV delivery with a gRNA array. In addition, AAV has serotypes specific to the lung, which is the main organ infected by SARS-COV-2. This means that the CRISPR system can be delivered to the right place. A similar strategy can also be used to fight other RNA viruses. Nguyen et al. developed the system with engineered crRNAs and optimized conditions to detect various clinically relevant nucleic acid targets, including human immunodeficiency virus, hepatitis virus C, and SARS-COV-2, with high sensitivity. [48]

The review had previously given examples of the potential of CRISPR-Cas systems, including Cas9, Cas12, and Cas13 proteins, for diagnosing SARS-COV-2 infection in recently

completed studies. Following the Cas9 protein, the Cas12 and Cas13 proteins focus on a viral illness diagnosis. Cas12 cleaves both ssDNA and dsDNA, while Cas13 cleaves only ssRNA; hence, both proteins are critical in diagnosing SARS-COV-2. [48]

As an example of Cas12 utilization, Brandsma et al. developed a diagnostic platform based on the Cas12a collateral cleavage of a reporter nucleic acid composed of an ssDNA called DETECTR (DNA endonuclease-targeted CRISPR trans reporter) to recognize and detect SARS-COV-2 gRNA. Comparing DETECTR with qRT-PCR to diagnose SARS-COV-2, they found that both techniques are equally sensitive in detecting SARS-COV-2, and DETECTR was found 100% specific for SARS-COV-2 relative to other human coronaviruses. [49] HOLMES, a one-hour low-cost, highly efficient system, is another Cas12a-based detector for precise target DNA detection. [44] Another example would be the All-In-One Dual CRISPR-Cas12 (AIOD-CRISPR) assay, introduced by Ding et al. as an ultra-sensitive and faster assay to detect the target nucleic acid. These AIOD-CRISPR assay mixtures are incubated at 37 °C to prevent contamination, simplifying the detection procedure. Utilizing AIOD-CRISPR assay, they caught the RNA of HIV and SARS-COV-2 within 60 minutes. [50]

As opposed to Cas9, Cas13 causes loss-of-function phenotypes without causing chromosomal deletion of the targeted gene. So, it meets the demand for quick gRNA creation to target virus strains that change and elude traditional drugs. Using Cas9, which cleaves DNA, has several disadvantages over Cas13, which cleaves RNA. Off-target cleavage and mismatches may cause unintended mutations and malignancy. Moreover, Cas9 often cleaves DNA at the proper site, but the cellular repair process fails. Thus, CRISPR-Cas13 may be employed directly in tissues damaged by SARS-COV-2 as a possible treatment strategy. [51]

An example of Cas13 usage is the SHERLOCK technology, which utilized a quenched fluorescent ssRNA reporter. It is the first platform built on CRISPR-Cas13 systems, enabling bio-sensing with sensitivity for DNA and RNA virus detection with single-base distinction. This Cas13a based system also has been used for the robust detection of Zika and dengue viruses. [52] As a result, SHERLOCK for SARS-COV-2 detection is significantly faster than qRT-PCR and has a sensitivity of 93.1 percent. Gootenberg et al. developed SHERLOCK further to create STOP (SHERLOCK Testing in One Pot) for specific point-of-care (POC) diagnosis of COVID-19. They demonstrated significant sensitivity and specificity in detecting Zika and Dengue viruses using SHERLOCK. Additionally, SHERLOCK was used to detect the Ebola and Lassa viruses. When the COVID-19 epidemic began, Feng Zhang's team recreated SHERLOCK to make SARS-COV-2 detection possible. They modified different primers and guided RNA to deliberately target the open reading frame 1ab (ORF1ab) and spike (S) genes. Clinical verifications indicated that the SHERLOCK test result is equal to RT-qPCR. They demonstrated that SHERLOCK is a flexible, reliable method for detecting RNA and DNA that may be used for quick diagnoses, such as infectious disease applications and sensitivity genotyping. [51]

Sherlock Biosciences was recently licensed by the Food and Drug Administration (FDA) for emergency usage to alleviate COVID-19 detection bottlenecks in the United States. [3]

Because SARS-COV-2 viral load varies during the day and illness phases, a quantitative reverse transcriptase PCR (qRT–PCR) diagnostic approach may be negative when the viral load is low, necessitating a more reliable test. Rauch et al. devised a CREST method (Cas13-based, rugged, equitable, scalable testing) for detecting SARS-COV-2. They confirmed that CREST is equivalent sensitivity to the gold standard reverse transcription-quantitative PCR (RT-qPCR) method. [54] The accuracy in detecting target templates through the Cas-sg/crRNA complex makes CRISPR-Cas technologies an outstanding alternative to PCR-based approaches. [53]

Cas13d is significant among all subtypes because of its high efficiency and strong knockdown efficiency, and its simplicity of viral administration due to the effector domain's short coding sequence. Recent research focused on repurposing the RNA-guided RNA endonuclease activity of Cas13d in mammalian cells against SARS-COV-2 and live influenza A virus (IAV). PAC-MAN, which stands for "prophylactic antiviral CRISPR in huMAN cells," is the first Cas13 antiviral strategy to fight SARS-COV-2. Abbott et al. presented a CRISPR-Cas13-based approach for COVID-19 treatment called Prophylactic Antiviral CRISPR in huMAN cells (PAC-MAN) to suppress SARS-COV-2. [4] In this study, Cas13d is guided by pan-coronavirus crRNAs to destroy the viral genome and suppress gene expression. As there was no access to live SARS-COV-2 strains at the time of this investigation (April 2020), the researchers resorted to synthesized fragments of SARS-COV-2 (besides using live H1N1 IAV). Abbott and colleagues used bioinformatic screens to identify highly conserved regions across the viral genomes. They found a group of six crRNAs that could target 91% of all coronaviruses and a group of 22 crRNAs that could target all coronaviruses with no mismatches. The PAC-MAN approach successfully destroyed SARS-COV-2 sequences and a live IAV genome. Cas13d may specifically target conserved genomic areas of SARS-COV-2. This way, CRISPR-Cas technology might target viral RNA for destruction and limit virus reproduction in host cells, restricting transmission of the virus. [4]

The CRISPR-Cas system has been used to regulate epigenetic modifications in eukaryotic systems to correct genetic errors and improve hereditary traits. Cas12 and Cas13 are CRISPR proteins that are efficient agents for diagnosing and combating ssRNA viruses. The development of COVID, RNA viruses have been a primary worldwide concern, and Cas13 has garnered considerable attention as an antiviral due to its ability to cut ssRNA, such as that seen in SARS-COV-2. Since ssRNA viruses account for the bulk of viruses capable of infecting people, a Cas13 antiviral system was created and confirmed in mammalian cells before COVID. [42] Freije et al. tested Cas13's action against three different ssRNA viruses in the laboratory [lymphocytic choriomeningitis virus (LCMV), influenza A virus (IAV), and vesicular stomatitis virus (VSV)]. They created Cas13-assisted restriction of viral expression and readout (CARVER)

by combining this antiviral activity with Cas13-based diagnosis. The researchers identified Cas13 crRNA target sites in viral RNA. They then used cell culture models to create a series of antiviral crRNAs that could be multiplexed in a combinatorial fashion, demonstrating that the crRNA-directed Cas13 enzyme effectively inhibits viral RNA replication in mammalian cells. [42] The researchers demonstrated in their study that the CARVER system for quick diagnostics and antiviral drug development and CRISPR RNAs (crRNAs) targeting highly conserved regions of the viral genome were advantageous to avoid escape mutants. They noticed that this technique is remarkably adaptive to resist probable viral evolution since it employs multiplexing (targeting many loci) with pooled crRNAs. [42]

Conclusion

This study examined the potential efficacy and utility of CRISPR-Cas technology in limiting the COVID-19 pandemic. For this purpose, we evaluated the mechanism of CRISPR-CAS systems, functions of Cas9, Cas12, and Cas13, their applications in diagnosis and targeting SARS-COV-2, and the history of CRISPR technology and the pandemic. Cas13d was determined to be the most significant among all subtypes because of its high efficiency, strong knockdown efficiency, and ease of viral administration due to the effector domain's short coding sequence. Cas13 effectors have been successfully cut complementary target ssRNA, making it a more reliable and protected alternative to Cas9 since it produces loss-of-function phenotypes without triggering chromosomal loss of the targeted gene. Moreover, Cas13 does not need Cas9 to act on a PAM, making the CRISPR-Cas13d system adaptable in guiding RNA design. There have been reports of CRISPR-based antiviral agents utilizing Cas9 inducing mutations in target sites [23], but no crRNA target-site mutations were identified after Cas13 treatment. [42] These findings suggest that the Cas13 enzyme can be an efficient antiviral agent in treating SARS-COV-2 infection. As a result, CRISPR-Cas12 systems satisfy the need for fast gRNA generation to target various virus strains that mutate and evade conventional treatments. Although these results are promising, CRISPR-Cas systems are still not ready to be used for fighting acute viruses since various problems require solutions before antivirals are utilized in in-body work. There is a risk that genome editing therapies for viruses could change the human genome. The consensus opinion is that genome editing in somatic cells is acceptable if the goal is to treat horrible diseases, but gene editing is fairly disheartening (National Academy of Sciences, 2020). In this way, genome editing treatment methods for viral infectious diseases are likely to be less controversial because they only affect cells in the body. [55] Furthermore, the immunogenicity of Cas proteins has not yet been thoroughly explored, and further safety studies are required to determine how and when they are safely expressed in people. [56]

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