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Novel CRISPR-Cas9 approach to combat SARS-CoV-2

Jahanzaib Ali [1*]

[1] Department of Biotechnology, Faculty of Life Sciences, University of Okara, Renala Khurd, Road Grand Trunk Rd, N-5 Okara, Pakistan, 56300
[*] Correspondence: writter1008@gmail.com

Abstract

Background: The recent coronavirus outbreak has affected people worldwide, so WHO declared it a pandemic. This pandemic has become a major problem for people, health professionals, and research institutes on how to combat SARS-CoV-2. WHO is highly concerned with developing more effective treatment for SARS-CoV-2. This review article aims to provide a brief study on how CRISPR/Cas9 can be used to combat SARS-CoV-2. **Methods:** The comparison of three genome editing techniques (ZNF, TALEN, and CRISPR/Cas9) was done. The mechanism of action of CRISPR/Cas9 is discussed. **Results:** The studies in this review article suggest that it is difficult to recognize this virus as they have their metabolic pieces of machinery, replicate themselves in host cells, and consume host cellular products to perform their functions. The COVID-19 proteins may contain specific areas that are suitable targets for therapy, such as tiny inhibitor molecules for viral polymerase or impede the attachment of viruses to the receptor sites. For example, viral coat proteins can use for vaccination purposes. **Conclusions:** CRISPR-Cas9 can control the SARS-CoV-2 genome from replication and spread to other body parts as it can edit the genome quite efficiently. This proposed model will help target the SARS-CoV-2 genome more precisely.

Keywords: Combat with Corona Virus; CRISPR Cas9; Applications of CRISPR Cas9.

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Introduction

SARS-CoV-2 is the virus that causes the global pandemic illness COVID-19 (Harrison, Lin, & Wang, 2020). The SARS-CoV-2 virion is 60–140 nm in diameter and possesses a positive-sense, single-stranded RNA genome of 29 891 base pairs (Hossain, Javed, Akter, & Saha, 2021; Zhou et al., 2020). The alignment of SARS-CoV-2 and its previous endemic SARS-CoV reveals that they have a 79.5% resemblance in their genome (Hu, Guo, Zhou, & Shi, 2021; Satija & Lal, 2007; Shi et al., 2020). The SARS-CoV-2 pandemic first appeared in Wuhan city of China (Rabaan et al., 2020; Yüce, Filiztekin, & Özkaya, 2021). Once it damaged people in Wuhan city, this pandemic affected worldwide. Over 100 million cases and approximately 1 million deaths are observed, estimated at the end of 31st August 2022 (Yamayoshi et al., 2020). SARS-CoV-2 infection, which results in severe acute respiratory syndrome sickness in humans, gave rise to COVID-19 in December 2019. When patients were spotted at the seafood market in Wuhan City, Hubei Province, it was for the first time isolated and recognized in them (Shi et al., 2020). The incubation period of SARS-CoV-2 is about 14 to 15 days (Lauer et al., 2020).

The World Health Organization's (WHO) international network coordinator received information regarding the deadly agent's high case fatality rate, global spread, and prompt response, which led to the rapid identification of the causal agent (Amin, 2021; Giovanetti et al., 2021). This pandemic can be attributed to the newly transmissible agent mot discovered earlier. The SARS-associated coronavirus-2 is the cause of this illness, which is now referred to as SARS-CoV-2 or COVID-19 disease (severe acute respiratory syndrome-2). The new

coronavirus was given the name SARS-CoV-2 by the Worldwide Virus Taxonomy Committee on February 11 (Kang & Xu, 2020; Majumder & Minko, 2021).

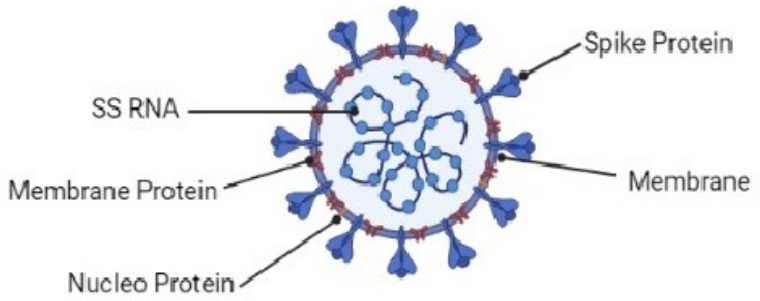

Figure 1. SARS-CoV is a single-stranded, positive sense RNA virus that causes severe acute respiratory syndrome in humans. Its size is 29.9 bp. SARS-CoV-2 symptoms include fever, coughing, pneumonia, and even death.

Method

Genome of SARS-CoV-2

When we observed this virus under the electron microscope, it showed crown-like structures. Due to its appearance this virus termed as coronavirus (Hsieh et al., 2005; Stadler et al., 2003). Among RNA viruses, the coronavirus genome ranges from 27 to 32 kilobytes (Rabaan et al., 2020). But the COVID-19 or SARS-CoV-2 genome size varies from 29 kb to 32.2 kb (Luk, Li, Fung, Lau, & Woo, 2019; Salian et al., 2021). The main signs and symptoms of COVID-19 include fever, chest pain, illness, and a little cough (Yi, Lagniton, Ye, Li, & Xu, 2020; Böger et al., 2021). Patients with COVID-19 have varying degrees of health; some patients exhibit severe symptoms while others do not, but it solely relies on the patient's body defense mechanism (Habibzadeh & Stoneman, 2020). The severity of COVID-19 disease and susceptibility to infection is influenced by several factors (Pairo-Castineira et al., 2021). While some COVID-19 patients have acute diarrhea, heart damage, kidney failure, and other organ damage, they may not exhibit any respiratory symptoms (Anka et al., 2021; Wiersinga, Rhodes, Cheng, Peacock, & Prescott, 2020).

The genetic material of SARS-CoV-2 is an encapsulated, positive sense, single-stranded RNA of about 29.9 kb (Petrosillo, Viceconte, Ergonul, Ippolito, & Petersen, 2020; Yüce et al., 2021). The glycoprotein that makes up the additional layer of the coronavirus envelope is generated from a part of the host cell membrane (Drosten, Preiser, Günther, Schmitz, & Doerr, 2003). The spike protein produced by the glycoprotein plays a role in the coronavirus's attachment to the host cell's ACE2 receptor (Sanyal, 2020; Xu et al., 2020). The capsid, a nucleocapsid consisting of a protein-rich subunit termed capsomeres, shields the viral DNA from damage. Two-thirds of the SARS-CoV-2 genome are at the 5' end and contain ORF1a and ORF1b, which encode the replication polyprotein. The remaining one-third of the genome is located at the 3' end and codes for structural proteins such as the spike, envelope, membrane, nucleocapsid, and non-structural polyprotein (Jin et al., 2020). Short untranslated regions are present in the SARS-CoV-2 genome at the 5' and 3' ends. RNA polymerase, papain-like protease, helicase, and chymotrypsin-like protease are just a few of the 16 naps that the protease created from the translational products of ORF1ab, which are encoded by SARS-COV-2 (Khan, Ahmed, Sarwar, Jamil, & Anwer, 2020; Mohamadian et al., 2021).

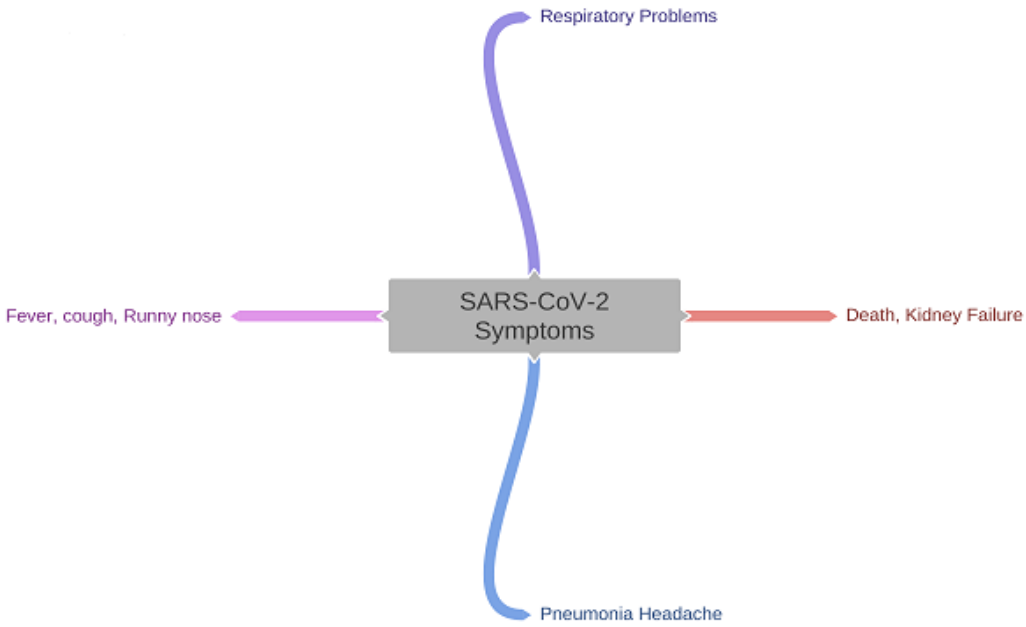

Figure 2. SARS-CoV-2 are the main signs and symptoms of COVID-19 include fever, chest pain, illness, and a little cough Patients with COVID-19 have varying degrees of health; some patients exhibit severe symptoms while others do not, but it solely relies on the patient's body defense mechanism While some COVID-19 patients have acute diarrhea, heart damage, kidney failure

Viral Characterization

SARS-CoV-2 are members of the Nidovirales order, family Coronaviridae, and genus Coronavirus and are responsible for the SARS-coronavirus sickness (Phan et al., 2018). These coronaviruses use nested RNA sets for replication and utilize RNA as their genetic material. There are four other genera in the coronavirus subfamily, including alpha-, beta-, gamma-, and delta-coronaviruses (Cui, Li, & Shi, 2019; Shi et al., 2020). The two coronavirus genera known as alpha and beta-coronavirus serve as the primary infectious agents in humans. The two beta-coronavirus strains, HCoV-HKU1 and HCoV-OC43, and two alpha-coronavirus strains, HCoV-229E and HCoV-NL63, primarily infect humans (Ludwig & Zarbock, 2020).

Techniques Used in Genome Editing

Recently, recombinant DNA technologies have been important in clinical research and agriculture, industry, food industry, and drug development (Khan et al., 2016). Recombinant DNA technology is being used to manipulate living organisms' genetic material to produce desired products, such as crops resistant to disease, treatments for cancer, genetic diseases, and viral diseases (Memi, Ntokou, & Papangeli, 2018). We proposed a model to edit the genomes of viruses and bacteria. In this model, we employ the specific method known as CRISPR/Cas9, which can identify certain nucleotide sequences inside viral genomes that would be a useful addition to the breakdown of viral genomes. There are additional genome editing methods that can use for this. These genome editing methods are divided into three types (Janik et al., 2020).

- ZNF
- TALEN
- CRISPR-Cas9

Zinc Finger Nucleases System

The Folkl restriction endonuclease's zinc finger was combined with specially designed Cys2-His2-Zinc finger proteins to make zinc-finger nucleases, a type of synthetic restriction enzyme that cuts both strands of DNA when bound to a certain complementary sequence inside the host cell (Kim, Li, & Chandrasegaran, 1994; Gaj, Guo, Kato, Sirk, & Barbas, 2012).

The host DNA's repair system fills in this double-strand break. This method makes it possible to alter an organism's genome precisely. Significant DNA fragments will be deleted from the target genome if two double-strand breaks are produced at a certain location within the target genome. Additionally, we can produce high fragment deletion at the chosen target site (Richardson, Ray, DeWitt, Curie, & Corn, 2016).

Transcription Activators like Effector Nuclease System

The second class, TALEN, is a type of effector nuclease similar to a transcription activator. A specific DNA sequence can be cut by pairing a TAL-effector DNA joining domain with an endonuclease restriction enzyme from this class (Wright, Li, Yang, & Spalding, 2014).

CRISPR/Cas9 System

CRISPR-Cas9 is the third and most potent class. The clustered regulatory interspaced short palindromic repeat (CRISPR)-associated nine genes is another name for it (Cas9). The CRISPR-Cas9 method offers exceptional control over modifying a specific genome (Gaj, Gersbach, & Barbas, 2013; Hryhorowicz, Lipiński, Zeyland, & Słomski, 2017). Here, we will address the CRISPR-Cas9 system's genome editing capabilities, which offer incredibly fine-grained control (Mali, Esvelt, & Church, 2013; Doudna & Charpentier, 2014; Hsu, Lander, & Zhang, 2014).

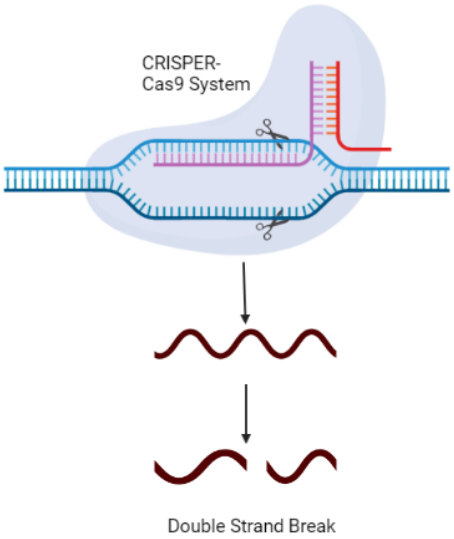

Figure 3. Genome Editing Technology and their Working Mechanism: Above diagram show the working principle of TALEN, ZNF, and CRISPR/Cas9. These are all techniques that produce double-strand breaks at specific sites and use to degrade the viral attack.

The CRISPR-Cas9 method is straightforward, simple to use, precise, potent, and keeps improving (Doudna & Charpentier, 2014). The CRISPR-Cas9 system is a natural defense mechanism found in some species of bacteria and Achaea (Bhaya, Davison, & Barrangou, 2011). The bacteria and archaea employ it to defend themselves against viral attacks. CAS protein and CRISPR loci were discovered after sequencing the bacterial and archaeal genomes. About 50% of bacterial genomes have the CAS restriction protein and CRISPR loci, but 90% of archaeal genomes or their resident plasmids do. Today, this defense mechanism controls the DNA's ability to degrade a particular target base sequence. The CRISPR-Cas9 contains two distinct components:

- A guided RNA (gRNA)
- An endonuclease (Cas9)

A mark's DNA or RNA sequence can be disturbed or altered when certain components, such as gRNA and Cas9, are co-expressed in a cell. The 20-base pair complementary guided sequence that makes up guided RNA is created in labs. This guided sequence uses the Watson-Crick base pairing method to attract guided RNA and a Cas9 endonuclease combination to its mark site (Cong et al., 2013).

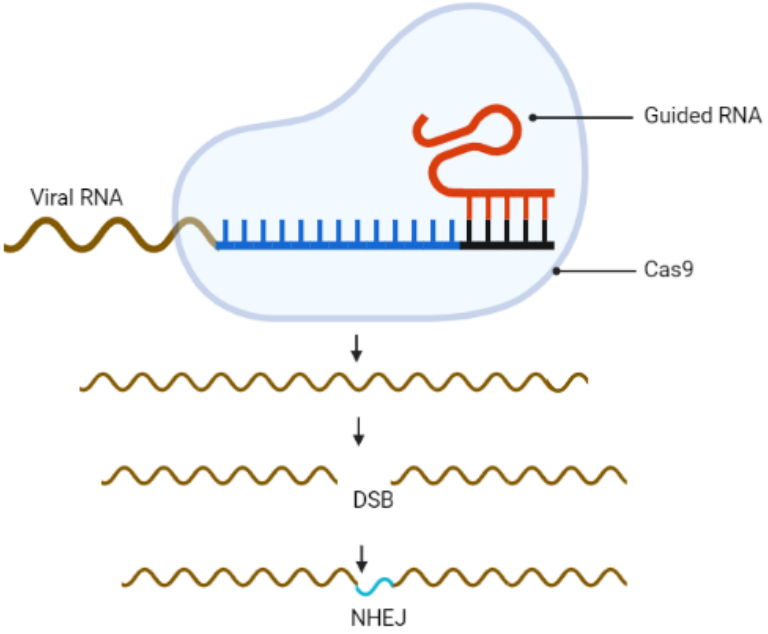

Figure 4. The working mechanism of CRISPR/Cas9: CRISPR/Cas9 contains a gRNA and a restriction endonuclease. The guided RNA helps the Cas9 reach its mark site, then Cas9 cleaves the mark sequence and produces a double-strand break that the cell repairing repairs.

A precise protospacer adjacent motif, or PAM sequence, which is a trinucleotide sequence, is needed for the guided RNA and Cas9 complex to correctly connect to the complementary mark sequence and disrupt the target region. Following the mark sequence is the PAM sequence. The endonuclease (Cas9) cleaves two RNA or DNA strands to cause a DNA or RNA double-strand break, which is known as a double or single-strand break (DSB or SSB). The protospacer adjacent motif sequence is four bases upstream of where the DSB occurs (PAM) (Doudna & Charpentier, 2014).

The non-homologous end-joining (NHEJ) route of DNA repair is used by the double-strand break (DSB) repairing mechanism. A piece of the DNA or RNA is deleted due to a double-strand break, which might result in frameshift mutations or stop codons. The mark gene's ORF1ab can successfully be disordered in this manner. Since the Cas9 endonuclease protein performs restriction activities, short-guided RNA, which is produced chemically, is required to transport the Cas9 to its mark site. The CRSIPR-Cas9 has been approved for use in thousands of solicitations over the preceding five years due to its ease of usage (Hsu et al., 2014; Kennedy & Cullen, 2015; Khalili, Kaminski, Gordon, Cosentino, & Hu, 2015).

Given the enormous size of the human genome, the restriction endonuclease Cas9 system is exceptional in that it achieves a significant step of specificity and provides nearly high-class on-target breakage. The SURVEYOR assay can assess off-target breakage and detect mismatched base pairs resulting from non-homologous end joining (NHEJ) or whole genome sequencing. However, the CRISPR-Cas9 technique continues to be improved and developed in recognition of its competence in genome excision and ability to lower the likelihood of off-target consequences (Hu et al., 2014; Veres et al., 2014; Yang et al., 2014). By using the newly developed paired Cas9 nickase method, the off-target action in the cell lines can be reduced 50–1500 times (Mali, Aach et al., 2013; Shen et al., 2014) and improve the site-specific induction of double-strand breaks (Cho et al., 2014; Ran et al., 2013).

The CRISPR-Cas9 method should, in theory, be suitable for any RNA or DNA viral eradication from cells. Human disease-causing viruses have recently been treated with the CRISPR-Cas9 system, which is used as a defense against harmful viruses (White, Hu, & Khalili, 2015).

Result and Discussion

Applications Of CRISPR-Cas9

Role of CRISPR-Cas-9 in COVID-19 cure

Scientists have used CRISPR-Cas technology to control the epigenetic mechanism in eukaryotic cells. It also controls RNA and DNA to correct genetic flaws and enhance hereditary features. It is frustrating for scientists worldwide to employ these tools to eradicate viruses from human cells. The potential use of the Cas13 enzyme to stop the replication cycle of ssRNA viruses has just been confirmed at Harvard University (Freije et al., 2019).

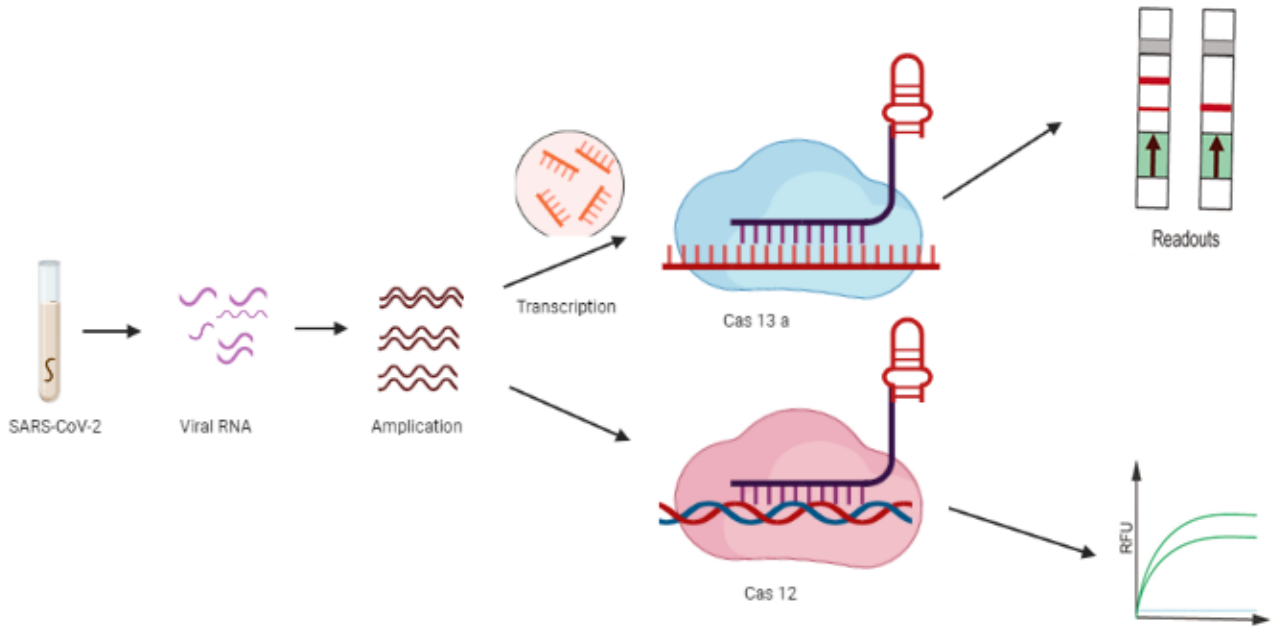

Figure 5. Experimental protocol for SARS-CoV-2 detection on CRISPR/Cas detection platform. (1) The extracted SARS-CoV-2 viral RNA is first pre-amplified (RT-RPA and RT-LAMP) into double-stranded DNA (dsDNA). (2) For Cas13a-SHERLOCK assay, dsDNA is first T7 trans

Elimination of COVID-19 by CRISPR-Cas-19

COVID-19 is a worldwide pandemic, so this disease diagnosis needs fast, precise, and easy-to-use approaches. CRISPR-based technology might solve this issue, identifying essential objects within 30 to 60 minutes. However, they are currently awaiting FDA approval. The collateral cleavage activity principle is used in the recently established CRISPR base diagnostic procedures using Cas13 or Cas12a nuclease. When crRNA targets the cleavage, the CRISPR technique's nuclease Cas12a/Cas13 enzymes are triggered. When crRNA is activated, the CAS proteins cause a cleave in the viral RNA or DNA strand (Chen et al., 2018; Esbin et al., 2020).

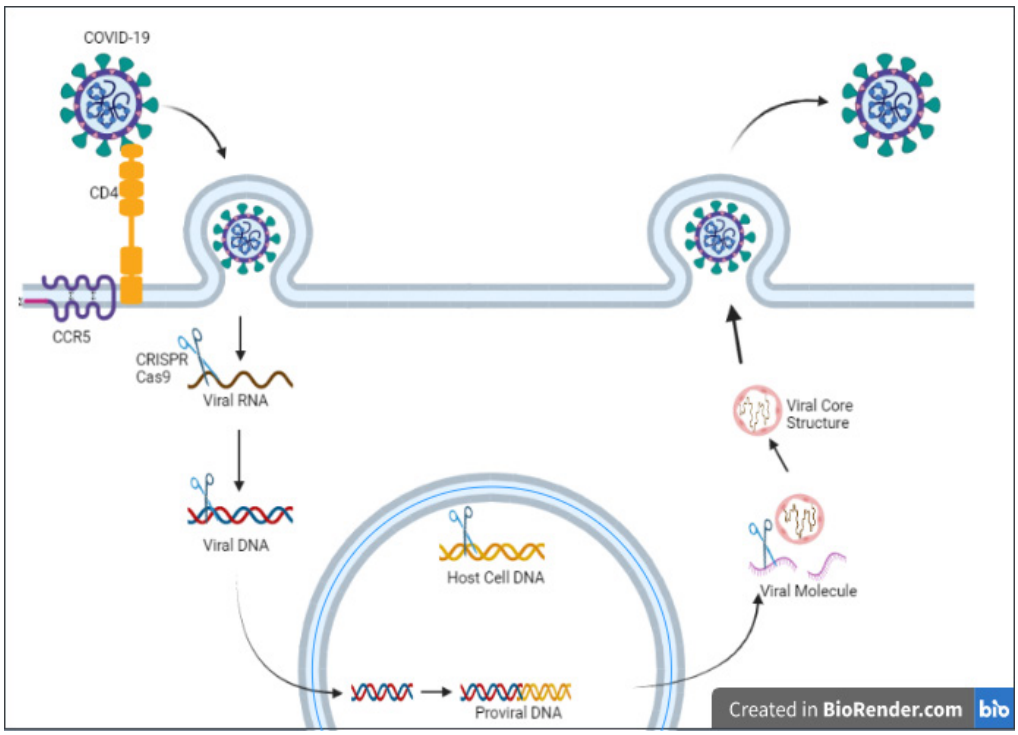

Figure 6. Mechanisms of CRISPR/Cas systematic elimination of SARS-CoV-2 by inhibiting its activity

Conclusions

The most practical method that is simple to utilize and has little off-target activity is CRISPR-Cas9. This device allows host cells to have their viral RNA/DNA degraded. For this reason, we create a guided RNA with the Cas9 protein attached to it. When the target complementary sequence is combined with gRNA and Cas9, breakage is caused. This results in the degradation of the viral DNA inside human cells. This technology is likewise of the next generation and can diagnose diseases quickly, precisely, and without medical experience. It also lowers the cost of disease diagnosis. We can treat the condition using a cheaper and faster technology to diagnose the disease than RT-PCR.

Declaration statement

The authors reported no potential conflict of interest.

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