SciForschen
Open HUB for Scientific Research
Journal of Emerging Diseases and Virology
ISSN 2473-1846 | Open Access

REVIEW ARTICLE
Volume 5 - Issue 2

Use of CRISPR to Enhance and Combat Human Viral Infections as HIV and SARS-Cov-2

Asaad Mohammed Ali Ataa[1,*], and Amru Mansour Ali Ataa[2]

[1]Department of Biochemical Technology, Faculty of Applied Science, Thamar University, Dhamar-Yemen
[2]Department of Laboratory Medicine, Hamad Medical Corporation, Medical Laboratory Technologist, Doha, Qatar

[*]**Corresponding author:** Asaad Mohammed Ali Ataa, Department of Biochemical Technology, Faculty Applied Science, Thamar University, Dhamar, Yemen, Tel: 00962785504595; E-mail: asaad.ataa@tu.edu.ye

Received: 17 Apr, 2020 | **Accepted:** 11 May, 2020 | **Published:** 19 May, 2020

Citation: Ataa AMA, Ataa AMA (2020) Use of CRISPR to Enhance and Combat Human Viral Infections as HIV and SARS-Cov-2. J Emerg Dis Virol 5(2): dx.doi.org/10.16966/2473-1846.151

Abstract

Viruses depend on organisms to complete their life period, there are many vaccines and antiviral for viruses, but some viruses such as HIV did not show any sensitivity or effect for vaccines or anti-viruses. Recently, researchers are focusing on CRISPR/Cas9, which is a technique that allows for genome editing in organism cells. This technique has changed the concept of genetic engineering and it can be applied to repair genetic disorders in future therapies. Besides targeting the human genome, it can be applied to target specific sites of genetic material for viruses, hence can cure viral infections, and therefore, use to treat SARS-CoV-2 as an emergency solution to treat infections coronavirus, there are increasing numbers of infections coronavirus in the world. Here, we discussed recent studies on the use of CRISPR to enhance and combat human viral infections, with a focus on CRISPR and HIV, CRISPR/Cas9 studies, challenges, and explore mechanisms to apply it in the future. Finally, CRISPR technology is a revolutionary development in engineering resistance against viral infections. But this technique needs more and more studies before applying it to humans.

Keywords: CRISPR/Cas9; Coronavirus; COVID-19; SARS-CoV2; Viral infections; HIV

Introduction

Viruses depend on intracellular pathogens on host cellular components for replication. They connect to the receptor's cell surface to enter the cell, and therefore, enforce cellular functions and organelles to replicate [1].

Host cells can counteract infections by sensing pathogen-associated molecular patterns (PAMPs) including viral nucleic acids, carbohydrates, or proteins to sense viral pathogens in infected host cells wherein triggers the expression of antiviral genes [2].

The availability of antiviral strategies is yet limited to specific virus types, such as coronavirus, (HIV) Human Immunodeficiency Virus. These viruses can mutate easily, and it is not easy to discover the early stage of infection. Therefore, the availability of antiviral approaches so far is ineffective [3].

Recent research has depended on using microorganisms antiviral defense mechanisms to improve immunity against eukaryotic viruses, for example, most Archaea and Bacteria have defense antiviral mechanisms that target bacteriophages at different stages of their life-period which is known as the innate immunity. Moreover, bacterial have several independent mechanisms to defend themselves against viral infection or invasive DNA, such as associated plasmids. One of these mechanisms is the blocking adsorption; therefore bacteria prevent the binding of phages with cell receptors by mutation or masking for receptors, which about infection. On the other side, DNA injection for Phage is failed to enter the cell. Other systems operate directly on invasive DNA, such as restriction/modification and (associated with CRISPR). Failed infection systems are a form of selflessness defense, which causes cells to die upon injury. Thus, these defensive systems can operate independently of each other. As a result, it prevents phage adsorption and genome insertion, receptors mutation; also cancel infection by host cell death [4].

In 1987, Yoshizumi Ishino and his colleagues accidentally discovered a new accurate technique for gene modification but did not know an explanation of what they saw then. With the development of molecular genetics and the scientists reading the DNA of many organisms, scientists observed in 2002 that these strange parts that Japanese scientists talked about existed in many types of bacteria, and one of the Netherlands scientists called them (CRISPR). CRISPR/Cas9 is a simple and efficient tool for genome editing and has experienced rapid progress in its technology applications in the pest time [5].

Overly, CRISPR/Cas9 technique can be used to inactivate viral infection such as human immunodeficiency virus type one (HIV-1) in human cells. Whereas the expression and replication of the virus are stopped by change non-coding or coding sequences during the provirus stages, suggesting that the CRISPR/Cas9 technique perhaps a novel therapeutic strategy against infectious viruses especially HIV and COVID-19 [5,6].

In late December, a new strain of coronavirus appeared, known as SARS-CoV-2. And by May 14, 2020, the virus had caused 4258666 infections and 294,190 deaths worldwide [7]. A great effort has been made to find effective drugs against the virus in China, France, Germany, USA, and many countries, but they are still experimenting and have not produced any treatment that limits the spread of infections with SARS-CoV-2 [7-10].

Therefore, this revision will discuss recent advances in using CRISPR to enhance and combat human viral infections and providing examples to recruit the CRISPR technique against human virus infections. Also, it will give insights into the applications of using genome editing such as CRISPR/Cas9 and CRISPR/Cas13d techniques to improve active antiviral defenses. Furthermore, we will show the challenges of using the CRISPR/Cas technique to enhance and combat human viral infections and suggest challenging strategies to overcome such hurdles.

Viruses definition and infection

A virus is the smallest infectious agent that depends on a host to complete its replication, but it is not living outside the organism or the host. Viruses can cause damage to any type of life forms such as animals, plants, and microorganisms such as bacteria [11].

Viruses classification depends on genetic material, consist of: (i) DNA with capsids (virus cover) such as bacteriophage. (ii) RNA material genetic and envelope protein coat, with enzyme and some viruses have lipids in the out surface such as HIV. (iii) There is a classification depends on a simple structure or more complex structures for viruses [12].

Some studies mentioned that the origins of viruses in the evolutionary history of life are unclear: so, little has evolved from plasmids pieces of DNA that transfer to cells while others side may have evolved from bacteria such as bacteriophages. They depend on the host's cellular to replicate and spread. The viral infection is one of the most important sources that threaten global public health. Host cells have developed enormous antiviral defenses, including innate and adaptive immunity mechanisms such as the ability to identify viral (PAMPs). But many viruses can avoid host defenses or attenuate it [3,12].

Various strategies have been improved to combat viruses, also strategies that directly target specific sites in viruses that inhibit any virus replication and target host factors required by viruses to replicate and persist [3].

As an opportunistic pathogen, viruses have devolved many unclear strategies to manipulate host cells for infectious and replication [13].

CRISPR Technology

Clustered Regularly Interspaced Short Palindromic Repeats is part of DNA sequences discovered within the genomes of bacteria like *E.coli* [1].

The CRISPR sequences are found from DNA fragments of bacteriophages and used as a defense against any infectious previously infected. For example, one types of CRISPR-Cas such as CRISPR-Cas 2 systems, require a tracrRNA which plays a role in the maturation of crRNA. Therefore, this hybrid acts as a guide for the endonuclease Cas9, which cleaves the invading nucleic acid. Also, the spacers can ability the adaptable and gene-specific inactivating mechanism of the CRISPR system. Spacers are short segments (26 to 72 bp) of sequences that are homologous to phage or plasmid DNA. Then to identify and damaged DNA from similar phages during later infections. Therefore, the CRISPR/cas9 system plays an important defense system of antiviral of prokaryotes [1,14-19].

Cas9 (CRISPR-associated protein 9) is an enzyme that depends on CRISPR sequences as a directory to identify and cleave specific strands of DNA that are complementary to the CRISPR sequence. CRISPR sequences with Cas9 enzymes work together as part to edit genes within organisms. This editing process has a wide variety of applications including basic biological research, improvement of biotechnology products such as food, drugs, and industrial biology. It is noted that CRISPR as biotechnological was available in 2012 mainly to the works of Doudna-Charpentier and Church-Zhang. It can also be used for the treatment of diseases [5,20].

Featuring technique CRISPR is an editing tool for gene or genome. It is faster, cheaper, and more accurate than previous techniques of DNA editing and has a wide range of applications in genetic engineering [5,21].

Since the discovery of this technique in the eighties until now, more than 17744 in PubMed and 19819 in Science Direct published articles had mentioned the CRISPR [20,22]. Many preliminary studies had used CRISPR to functionally inactivate genes in human cell lines, modify yeasts used to make biofuels for enhancing their products, also genetically modify crop strains to enhancing insect resistance, infection and increase production. Also, CRISPR has been used to change the structure of Anopheles Mosquito which resulted in preventing the transfer of diseases like Malaria. Where a study showed when targeting the gene (AgdsxF) dsx-female, by CRISPR-Cas9 across targeted of the intron 4-exon 5 boundary and that blocked the formation of functional AgdsxF, therefore cause to homozygous for the disrupted allele complete sterility for females to aimed less risk Malaria [22,23]. In 2019, scientists in America used CRISPR to experimentally treat a patient with a genetic disorder. The patient was suffering from sickle cell disease; she was a woman a 34-year-old. Researchers from China have also used the technique to make genetic modifications to human cells. According to Chinese medical documents posted online the last year, a team at Southern University of Science and Technology in Shenzhen, recruited couples in a try to create the first gene-edited babies in the world. They planned to eliminate a gene called CCR5 in hopes of rendering the offspring resistant to HIV, smallpox, and cholera. Although they still did not present complete evidence of this achievement [1,24,25].

CRISPR-Cas9 biology

A lot of bacteria have improved advanced RNA-guided adaptive immune systems directly by CRISPR loci and the accompanying CRISPR-associated (Cas) genes so that to provide acquired immunity against any enemy [26].

Emerging CRISPR/Cas tools technology that can be applied for research studies and experiments on viruses, extending beyond host patterns.

The methods of this technique used to generate both *in vitro* and *in vivo* as model organisms or non-model organisms to study

viral contagions, to edit viral genomes, and to improve genes drive systems that have the potential to combat viral disease vectors and the improvement of antiviral medications (Figure 1).

Creating laboratory systems using cell lines was a valuable tool for studying virus infection. But these systems have limitations to giving comprehensive insights into host physiology, pathology, immunity, and transmission during infection [1].

From the first revelation of its chance for genome editing, the CRISPR technique was rapidly performing as an exquisitely powerful tool for genome manipulation in a wide spectrum of organisms owing to its ease of styling, simplicity in use, and high efficiency [27]. Unlike the traditional mediated DNA editing techniques, the CRISPR/ Cas9 system has the potential to impose, and radically change the technique of gene editing and thus change our understanding of genetic engineering [5,28,29].

CRISPR avoids the need for more complex genome editing strategies that relay on protein domains such as protein engineering of DNA-identify domains for each DNA target site to be modified [30]. Therefore, profoundly supporting its applicability for large-scale genomic screening or manipulation and different adoption within the scientific community [27,30,31].

In figure 1, cas genome editing enables the generation of *in vitro* and *in vivo* models to study viral pathogenesis. This technique is not limited to editing genes of model organisms, such as mice, roundworms, and fruit flies, but it can also be applied to non-model organisms such as monkeys, bats, chickens, and other animals. Also, CRISPR is useful in engineering the genome of large DNA viruses such as smallpox. Also, CRISPR has been used to change the structure of Mosquito which resulted in preventing the transfer of diseases like Malaria [1].

CRISPR as adaptive immunity

In prokaryotes, CRISPR/Cas9 systems give adaptive immunity against bacteriophages and other infectious genetic elements [3].

CRISPR technique interposes immunity so that in the end, it leads to sequence-specific interference with foreign nucleic acids. Finally, the Cas9 enzyme to identify and destroy foreign genetic elements harboring a sequence complementary to the sequence of the viruses [3].

CRISPR as combating human pathogenic viruses

Human immunodeficiency virus (HIV): Many studies have been published regarding the use of antivirus CRISPR/Cas9 to HIV. Although antiretroviral therapy (ART) stops virus replication, it is ineffective in interfering with latent infections in CD4 with immune memory as T-cells in addition to the lack of any functional vaccine until now [32,33].

Recent studies suggest that the antiviral CRISPR/Cas9 system can inactivate the genome from latently infected cells *in vitro* for HIV. So that resulting in a (functional) cure from infection [32,34,35].

CRISPR/Cas9 targeting of the proviral long terminal repeat (LTR) region proved successful in impairing HIV gene expression and clearing provirus from infected cells. Another study focused on CRISPR/Cas9 targeting of essential genes of viral as HIV [32,33].

Yin L, et al. [36] explored a powerful inhibition of HIV-1 infection by Cas9/gRNA, which resulted from the salient decrease in the levels of RNA or DNA viral. Additionally, Limsirichai P, et al. [37] explained that CRISPR activation systems have the potential to enhance HIV-1 expression in cell-based models of latency.

CRISPR technique, in combination with cART, may in the future lead to new treatments for HIV-1 infection. The first reported *in vivo*

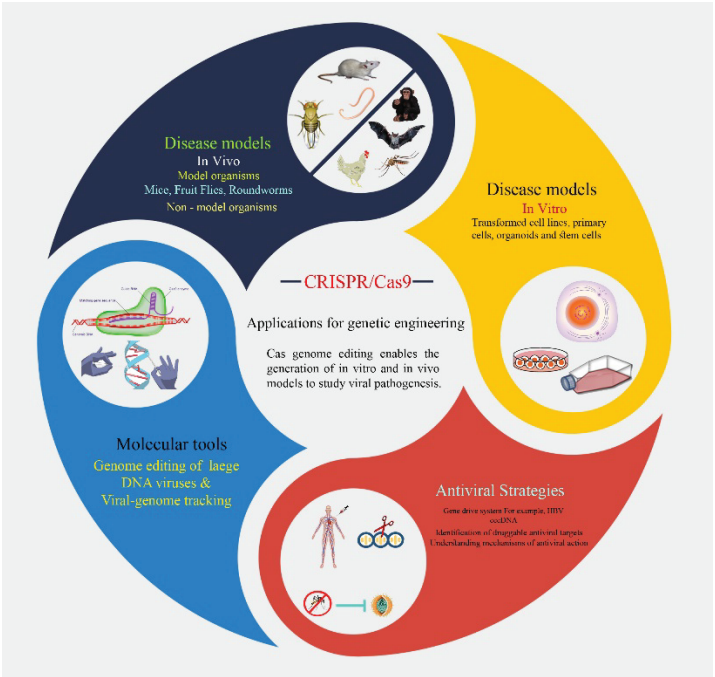

Figure 1: CRISPR/Cas9 applications for genetic engineering.

experiments of anti-HIV CRISPR therapy were by Kaminski R, et al. [38] they used associated viral (AAV) vector co-expressing two anti-HIV Cas9 therapy in clinical settings, enhancing delivery and efficacy *in vivo* models [32,34] (Figure 2).

In figure 2, we notice four general strategies for combating human viruses using CRISPR applications. Here we review the two most important strategies on direct targeting of viral genomes in infected cells by eradicating or disabling the viral DNA incorporated into the host genome and thus disrupting the work of viruses [32,38-40].

Finally, CRISPR technology has become the candidate method of choice for its efficacy in modifying the targeted HIV-1 genome as a part of HIV therapy.

Novel Coronavirus (SARS-CoV-2): The world is currently experiencing a novel coronavirus pandemic (COVID-19) disease, which is caused by severe acute Coronary Respiratory Syndrome 2 (SARS-CoV-2) [41].

Many research centers confirmed that scientists and researchers need to develop a safe and effective vaccine to prevent COVID-19, approximately within 12 to 20 months, to obtain a vaccine for reducing the spread of the infection. Thus, millions of people will be infected. Because this virus is characterized by being fast-spreading and highly contagious, according to the statistics shown daily by the World Health Organization and is accompanied by an increase in the number of deaths in the world. So, there is an urgent need to develop innovative methods to combat a widespread virus that may arise in the future [42,43].

So, we here used Cas13d which is characterized by a small size, the median size of Cas13d proteins is 190 to 300 a smaller than that of Cas13a-Cas13c. Moreover, the small size, minimal targeting constraints, and modular regulation of Cas13d effectors further expand the CRISPR toolkit for RNA manipulation. Therefore, it depends on this enzyme which can inhibit viral function and replication by directly targeting and cleaving all viral positive-sense RNA [42,44-46].

Some studies have found a relation between SARS-CoV-S Spike and SARS-CoV-2 Spike, through those SARS-CoV-2 mutations which can bind to receptors surface of sensitive cells after coming in contact with the surface of the airway [47-55].

The ACE2 plays a pivotal role as a mediator of infection that may mediate the virus's entry into target cells and viral reproduction. The binding of SARS-CoV-2 to ACE2 is not as strong as the SARS-CoV coronavirus (ACS2) binding to ACE2, but it is still far above the required threshold for infection with the virus [54,56-59].

Another study found that SARS-CoV-2 must bind to ACE2 to enter HeLa cells 9. Other studies showed a positive correlation between ACE2 expression and the infection of SARS-CoV *in vitro* [60-62].

Previous studies of coronaviruses that cause severe acute respiratory syndrome (SARS) revealed that they are bound to ACE2 in the lung

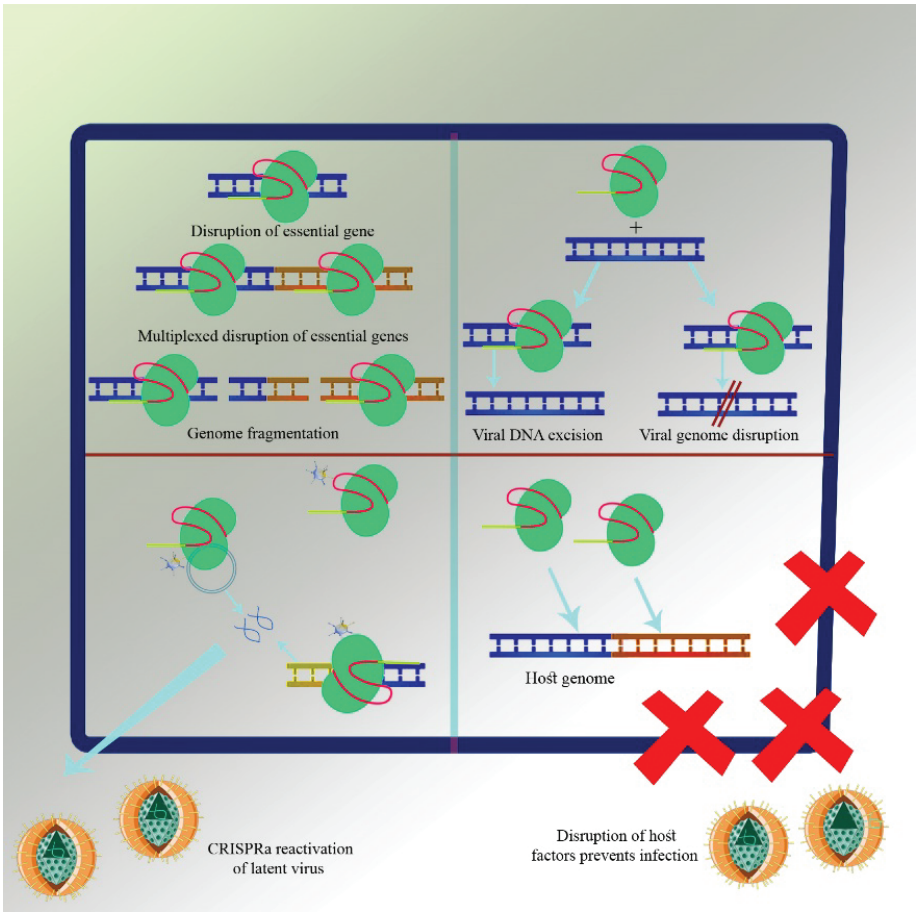

Figure 2: The strategies to combat human viruses using CRISPR.

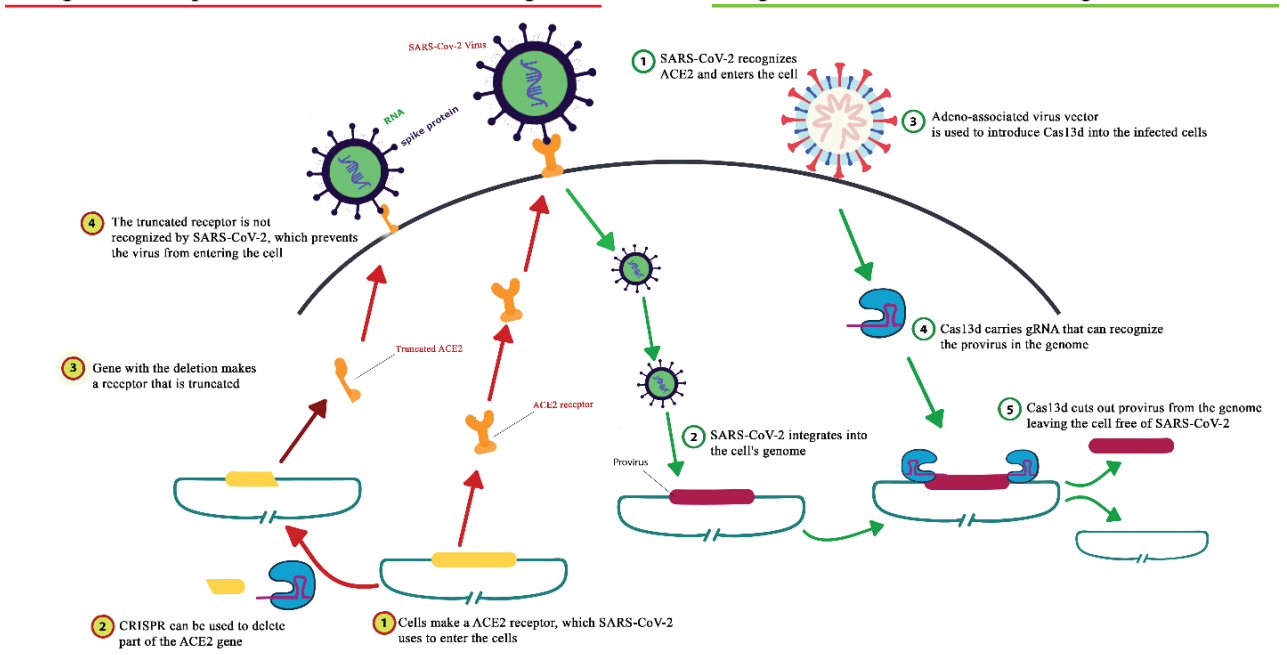

Figure 3: Using CRISPR-Cas13d against SARS-CoV-2 (COVID-19).

vesicles through surface elevation proteins and then cause lung damage and even lung function failure. ACE2 is likely to be the cellular receptor for SARS-CoV-2, but whether it is the only cellular receptor remains to be researched further [63-66].

Moreover, the high catalytic activity of Cas13d in human cells provides a potential mechanism for targeting SARS-CoV-2 for specific viral RNA genome degradation and viral gene expression inhibition. This is because it has several advantages as small size, strong catalytic activity, and high specificity. So Cas13d was the best choice to target and destroy RNA viruses [41,44-46].

Conclusion the CRISPR-Cas13d technique can inhibit SARS-CoV-2 to bond with ACE2 because of the CRISPR technique can direct it towards inhibiting angiotensin-converting enzyme (ACE), by truncated ACE2 receptor that will not be recognized by SARS-CoV-2, which prevents the virus from entering the cell. Thereby decreasing levels of the bond between SARS-CoV-2 with ACE2 receptors, and Cas13d can carry gRNA that can recognize the previous in the genome, which causes cuts out provirus from the genome leaving the cell-free of SARS-CoV-2. Therefore, Cas13d can remove the integrated infection without effect on cells (Figure 3). Thus, this may reduce the chances of infection with the Coronavirus.

In figure 3, the left above showed way using CRISPR to prevent SARS-CoV-2 from entering a cell. When SARS-CoV-2 encounters an immune cell, it has to bind a receptor on its surface. The receptor, called ACE2, acts like a lock into which SARS-CoV-2 inserts it's key and opens the door into the cell. A slight change in the ACE2 can prevent the key from fitting in. In the end, the truncated receptor is not recognized by SARS-CoV-2, which prevents the virus from entering the cell. While in the right above Cas13d carries gRNA that can recognize the previous in the genome, which causes cuts out provirus from the genome leaving the cell-free of SARS-CoV-2 therefore Cas13d can remove the integrated infection without effect on cells [54,67].

Limiting restrictions in anti-viral CRISPR therapies in humans

New techniques naturally emerge questions regarding restrictions and safety before using it. To arrangement facilitate CRISPR-based techniques in a clinical setting. Thereby reduce detection by the host immune system. Mostly, unknown triggers can result in virus reactivation and subsequent virus production and spread, so that causes more problems. Also, CRISPR techniques are not effective during the latent stage of infection. Many challenges remain for the use of CRISPR against HIV and SARS-CoV-2. However, more studies and experiments must be conducted to identify the disadvantages of this technology before starting to use it directly to combat human viruses [32,68,69].

Future Outlook

Viruses depend on the organisms to replicate and spread. However, infected cells counteract viral replication by sensing PAMPs and subsequently trigger the expression of antiviral genes. Therefore, CRISPR/Cas systems have caused a great effect on studies of virus biology on eukaryotic species [3,70].

Many studies raise warnings in harnessing DNA-targeting by CRISPR/Cas technique and need to be taken into consideration when using it, to combat viruses [3,71].

Escape mutations can produce Cas9-resistant variants and might lead to the emergence of altered viruses with greater pathogenicity. This may cause viruses of high resistance to appear [3,72].

Also, some studies showed that targeting certain genome-specific sites, for example, the non-coding intergenic sequences, within some plant virus genomes lead to the appearance of mutations that were deleterious to virus replication. This, in turn, affirms that carefully determining the target viral genome sequences can help reduce the formation of fugitive mutants and thus eliminate them [3,71].

Conclusion

Even though the full potential of the CRISPR/Cas technique for engineering defenses against eukaryotic viruses has not yet been fully exploited, this technology is rampage advancement in engineering resistance against human and plant viruses.

There are many preliminary studies focused on improving antiviral strategies based on some types of CRISPR/Cas9 system. But, there is still a great need to enhance and combat human viral infections. Furthermore, it will reveal new knowledge about the potential for site-specific integration of DNA into eukaryotic genomes, to opening additional genome engineering by CRISPR techniques.

In the left above showed way using CRISPR to prevent SARS-CoV-2 from entering a cell. When SARS-CoV-2 encounters an immune cell, it has to bind a receptor on its surface. The receptor, called ACE2, acts like a lock into which SARS-CoV-2 inserts its key and opens the door into the cell. A slight change in the ACE2 can prevent the key from fitting in. In the end, the truncated receptor is not recognized by SARS-CoV-2, which prevents the virus from entering the cell. While in the right above Cas13d carries gRNA that can recognize the previous in the genome, which causes cuts out provirus from the genome leaving the cell-free of SARS-CoV-2 therefore Cas13dcan remove the integrated infection without effect on cells [54,67].

Acknowledgments

We would like to thank Dr. Laith Al-Eitan, Shima Ataa, Hanan Aljammal, and Aiman Yaseen for helping us. Also, we introduce this article to our parents. Also, Asaad would like to thank DAAD (Deutscher Akademischer Austauschdienst) for helping him.

Conflict of Interest

The author declares that there are no conflicts of interest.

References

1. Puschnik AS, Majzoub K, Ooi YS, Carette JE (2017) A CRISPR toolbox to study virus-host interactions. Nat Rev Microbiol 15: 351-364.
2. Chan YK, Gack MU (2016) Viral evasion of intracellular DNA and RNA sensing. Nat Rev Microbiol 14: 360-373.
3. Mahas A, Mahfouz M (2018) Engineering virus resistance *via* CRISPR-Cas systems. Curr Opin Virol 32: 1-8.
4. Labrie SJ, Samson JE, Moineau S (2010) Bacteriophage resistance mechanisms. Nat Rev Microbiol 8: 317-327.
5. Mei Y, Wang Y, Chen H, Sun ZS, Ju XD (2016) Recent progress in CRISPR/Cas9 technology. J Genet Genomics 43: 63-75.
6. Liao HK, Gu Y, Diaz A, Marlett J, Takahashi Y, et al. (2015) Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells. Nat Commun 6: 1-10.
7. World Health Organization (2020) Clinical management of severe acute respiratory infection (SARI) when COVID-19 disease is suspected: interim guidance. Geneva, Switzerland.
8. Gao J, Tian Z, Yang X (2020) Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends 14: 72-73.
9. Lu H (2020) Drug treatment options for the 2019-new coronavirus (2019-nCoV). Biosci Trends 14: 69-71.
10. Zhang L, Liu Y (2020) Potential interventions for novel coronavirus in China: A systematic review. J Med Virol 92: 479-490.
11. Koonin EV, Senkevich TG, Dolja VV (2006) The ancient Virus World and evolution of cells. Biol Direct 1: 29.
12. Koonin EV, Starokadomskyy P (2016) Are viruses alive? The replicator paradigm sheds decisive light on an old but misguided question. Stud Hist Philos Biol Biomed Sci 59: 125-134.
13. Amara A, Mercer J (2015) Viral apoptotic mimicry. Nat Rev Microbiol 13: 461-469.
14. Barrangou R (2015) The roles of CRISPR-Cas systems in adaptive immunity and beyond. Curr Opin Immunol 32: 36-41.
15. Charpentier E, Richter H, Van der Oost J, White MF (2015) Biogenesis pathways of RNA guides in archaeal and bacterial CRISPR-Cas adaptive immunity. FEMS Microbiol Rev 39: 428-441.
16. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, et al. (2011) CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 471: 602-607.
17. Dong C, Qu L, Wang H, Wei L, Dong Y (2015) Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. Antiviral Res 118: 110-117.
18. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816-821.
19. Nasko DJ, Ferrell BD, Moore RM, Bhavsar JD, Polson SW, et al. (2019) CRISPR spacers indicate preferential matching of specific virioplankton genes. mBio 10: 1-16.
20. Lino CA, Harper JC, Carney JP, Timlin JA (2018) Delivering CRISPR: a review of the challenges and approaches. Drug deliv 25: 1234-1257.
21. Carbonell A, Lison P, Daros JA (2019) Multi-targeting of viral RNAs with synthetic trans-acting small interfering RNAs enhances plant antiviral resistance. Plant J 100: 720-737.
22. Alphey L (2016) Can CRISPR-Cas9 gene drives curb malaria? Nat Biotechno l34: 149.
23. Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, et al. (2018) A CRISPR-Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. Nat Biotechnol 36: 1062-1066.
24. Bernabe-Orts JM, Casas-Rodrigo I, Minguet EG, Landolfi V, Garcia-Carpintero V, et al. (2019) Assessment of Cas12a-mediated gene editing efficiency in plants. Plant Biotechnol J 17: 1971-1984.
25. Raposo VL (2019) The First Chinese Edited Babies: A Leap of Faith in Science. JBRA Assist Reprod 23: 197-199.
26. Mojica FJ, Rodriguez-Valera F (2016) The discovery of CRISPR in archaea and bacteria. FEBS J 283: 3162-3169.
27. Jiang F, Doudna JA (2017) CRISPR-Cas9 structures and mechanisms. Annu Rev Biophys 46: 505-529.
28. Chandrasegaran S, Carroll D (2016) Origins of programmable nucleases for genome engineering. J Mol Biol 428: 963-989.
29. Liu X, Wang M, Qin Y, Shi X, Cong P, et al. (2018) Targeted integration in human cells through single crossover mediated by ZFN or CRISPR/Cas9. BMC Biotechnol 18: 66.

30. Hu JH, Davis KM, Liu DR (2016) Chemical biology approaches to genome editing: understanding, controlling, and delivering programmable nucleases. Cell Chem Biol 23: 57-73.

31. Ihry RJ, Worringer KA, Salick MR, Frias E, Ho D, et al. (2018) p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat Med 24: 939-946.

32. de Buhr H, Lebbink RJ (2018) Harnessing CRISPR to combat human viral infections. Curr Opin Immunol 54: 123-129.

33. Pham HT, Mesplede T (2018) The latest evidence for possible HIV-1 curative strategies. Drugs Context 7: 212522.

34. Panchasara H, Patel S, Singh V (2020) Exploring the potential of CRISPR-Cas9 for the removal of human viruses. In: Singh V, Dhar PK (eds) Genome Engineering *via* CRISPR-Cas9 System. Academic Press 27-38.

35. Wang G, Zhao N, Berkhout B, Das AT (2018) CRISPR-Cas based antiviral strategies against HIV-1. Virus Res 244: 321-332.

36. Yin L, Hu S, Mei S, Sun H, Xu F, et al. (2018) CRISPR/Cas9 inhibits multiple steps of HIV-1 infection. Hum Gene Ther 29: 1264-1276.

37. Limsirichai P, Gaj T, Schaffer DV (2016) CRISPR-mediated activation of latent HIV-1 expression. Molecular Therapy 24: 499-507.

38. Kaminski R, Bella R, Yin C, Otte J, Ferrante P, et al. (2016) Excision of HIV-1 DNA by gene editing: a proof-of-concept *in vivo* study. Gene therapy 23: 690-695.

39. Chen S, Yu X, Guo D (2018) CRISPR-Cas targeting of host genes as an antiviral strategy. Viruses 10: 40.

40. Darcis G, Das AT, Berkhout B (2018) Tackling HIV persistence: Pharmacological *versus* CRISPR-based shock strategies. Viruses 10: 157.

41. Abbott TR, Dhamdhere G, Liu Y, Lin X, Goudy L, et al. (2020) Development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza. Cell 181: 1-12.

42. Li W, Shi Z, Yu M, Ren W, Smith C, et al. (2005) Bats are natural reservoirs of SARS-like coronaviruses. Science 310: 676-679.

43. Shi Z, Hu Z (2008) A review of studies on animal reservoirs of the SARS coronavirus. Virus Res 133: 74-87.

44. Yan WX, Chong S, Zhang H, Makarova KS, Koonin EV, et al. (2018) Cas13d is a compact RNA-targeting type VI CRISPR effector positively modulated by a WYL-domain-containing accessory protein. Mol cell 70: 327-339.

45. Gootenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, et al. (2018) Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 360: 439-444.

46. Smargon AA, Cox DBT, Pyzocha NK, Zheng K, Slaymaker IM, et al. (2017) Cas13b is a type VI-B CRISPR-associated RNA-guided RNase differentially regulated by accessory proteins Csx27 and Csx28. Mol Cell 65: 618-630.

47. Bosch BJ, van der Zee R, de Haan CA, Rottier PJ (2003) The coronavirus spike protein is a class I virus fusion protein: structural and functional characterization of the fusion core complex. J Virol 77: 8801-8811.

48. Belouzard S, Chu VC, Whittaker GR (2009) Activation of the SARS coronavirus spike protein *via* sequential proteolytic cleavage at two distinct sites. Proc Natl Acad Sci U S A 106: 5871-5876.

49. Burkard C, Verheije MH, Wicht O, van Kasteren SI, van Kuppeveld FJ, et al. (2014) Coronavirus cell entry occurs through the endo-/lysosomal pathway in a proteolysis-dependent manner. PLoS Pathog 10: e1004502.

50. Kirchdoerfer RN, Cottrell CA, Wang N, Pallesen J, Yassine HM, et al. (2016) Pre-fusion structure of a human coronavirus spike protein. Nature 531: 118-121.

51. Millet JK, Whittaker GR (2014) Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein. Proc Natl Acad Sci U S A 111: 15214-15219.

52. Millet JK, Whittaker GR (2015) Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. Virus Res 202: 120-134.

53. Park JE, Li K, Barlan A, Fehr AR, Perlman S, et al. (2016) Proteolytic processing of Middle East respiratory syndrome coronavirus spikes expands virus tropism. Proc Natl Acad Sci U S A 113: 12262-12267.

54. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, et al. (2020) Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181: 281-292.

55. Walls AC, Tortorici MA, Bosch BJ, Frenz B, Rottier PJM, et al. (2016) Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer. Nature 531: 114-117.

56. Klenk HD, Garten W (1994) Host cell proteases controlling virus pathogenicity. Trends Microbiol 2: 39-43.

57. Hulswit RJG, Lang Y, Bakkers MJG, Li W, Li Z, et al. (2019) Human coronaviruses OC43 and HKU1 bind to 9-O-acetylated sialic acids *via* a conserved receptor-binding site in spike protein domain A. Proc Natl Acad Sci U S A 116: 2681-2690.

58. Steinhauer DA (1999) Role of hemagglutinin cleavage for the pathogenicity of influenza virus. Virology 258: 1-20.

59. Tortorici MA, Walls AC, Lang Y, Wang C, Li Z, et al. (2019) Structural basis for human coronavirus attachment to sialic acid receptors. Nat Struct Mol Biol 26: 481-489.

60. Hofmann H, Geier M, Marzi A, Krumbiegel M, Peipp M, et al. (2004) Susceptibility to SARS coronavirus S protein-driven infection correlates with expression of angiotensin converting enzyme 2 and infection can be blocked by soluble receptor. Biochem Biophys Res Commun 319: 1216-1221.

61. Li W, Sui J, Huang IC, Kuhn JH, Radoshitzky SR, et al. (2007) The S proteins of human coronavirus NL63 and severe acute respiratory syndrome coronavirus bind overlapping regions of ACE2. Virology 367: 367-374.

62. Cao Y, Li L, Feng Z, Wan S, Huang P, et al. (2020) Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. Cell Discov 6: 11.

63. Gralinski LE, Menachery VD (2020) Return of the Coronavirus: 2019-nCoV. Viruses 12: 135.

64. Chan JF, Kok KH, Zhu Z, Chu H, To KK, et al. (2020) Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect 9: 221-236.

65. Tian X, Li C, Huang A, Xia S, Lu S, et al. (2020) Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg Microbes Infect 9: 382-385.

66. Li G, Hu R, Zhang X (2020) Antihypertensive treatment with ACEI/ARB of patients with COVID-19 complicated by hypertension. Hypertens Res.

67. Peng G, Xu L, Lin YL, Chen L, Pasquarella JR (2012) Crystal structure of bovine coronavirus spike protein lectin domain. J Biol Chem 287: 41931-41938.

68. Soppe JA, Lebbink RJ (2017) Antiviral goes viral: harnessing CRISPR/Cas9 to combat viruses in humans. Trends Microbiol 25: 833-850.

69. Van Diemen FR, Lebbink RJ (2017) CRISPR/Cas9, a powerful tool to target human herpesviruses. Cellular microbiology 19: e12694.

70. Wang A (2015) Dissecting the molecular network of virus-plant interactions: the complex roles of host factors. Annu Rev Phytopathol 53: 45-66.

71. Ali Z, Ali S, Tashkandi M, Zaidi SS, Mahfouz MM (2016) CRISPR/Cas9-mediated immunity to geminiviruses: differential interference and evasion. Sci Rep 6: 26912.

72. Yoder KE, Bundschuh R (2016) Host double strand break repair generates HIV-1 strains resistant to CRISPR/Cas9. Sci Rep 6: 29530.