Manipulation of genes could inhibit SARS-CoV-2 infection that causes COVID-19 pandemics

Arnab Banerjee, Sandip Mukherjee and Bithin K Maji
Department of Physiology (UG & PG), Serampore College, Serampore, Hooghly 712201, India
Corresponding author: Bithin K Maji. Email: bm_scp@yahoo.in

Abstract
The year 2020 witnessed an unpredictable pandemic situation due to novel coronavirus (COVID-19) outbreaks. This condition can be more severe if the patient has comorbidities. Failure of viable treatment for such viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is due to lack of identification. Thus, modern and productive biotechnology-based tools are being used to manipulate target genes by introducing the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas (CRISPR-associated) system. Moreover, it has now been used as a tool to inhibit viral replication. Hence, it can be hypothesized that the CRISPR/Cas system can be a viable tool to target both the SARS-CoV-2 genome with specific target RNA sequence and host factors to destroy the SARS-CoV-2 community via inhibition of viral replication and infection. Moreover, comorbidities and COVID-19 escalate the rate of mortality globally, and as a result, we have faced this pandemic. CRISPR/Cas-mediated genetic manipulation to knockdown viral sequences may be a preventive strategy against such pandemic caused by SARS-CoV-2. Furthermore, prophylactic antiviral CRISPR in human cells (PAC-MAN) along with CRISPR/Cas13d efficiently degrades the specific RNA sequence to inhibit viral replication. Therefore, we suggest that CRISPR/Cas system with PAC-MAN could be a useful tool to fight against such a global pandemic caused by SARS-CoV-2. This is an alternative preventive approach of management against the pandemic to destroy the target sequence of RNA in SARS-CoV-2 by viral inhibition.

Keywords: COVID-19, SARS-CoV-2, viral, CRISPR/Cas system, PAC-MAN, antiviral strategy

Introduction
SARS-CoV-2 causes the COVID-19 pandemics; in China, people could have been infected due to the use of seafood from China’s local market and also due to the exposure to infected animals. On the contrary, in some cases, investigations showed that people with no previous record of visiting the seafood market or exposed to infected animals in the market were also infected. Further, the virus spread over the population via sneezing, coughing, and aerosols from one person to another, leading to a severe infection of the lungs through the nasal cavity or mouth; until now, seven types of coronaviruses caused infection in humans. The severe acute respiratory syndrome coronavirus and Middle East respiratory syndrome coronavirus were originated from a zoonotic population in humans in 2003 and 2012, respectively. Among other coronavirus strains, the seventh one is severely contagious SARS-CoV-2 which causes severe respiratory disease and the current pandemic situation worldwide. The positive-sense RNA virus SARS-CoV-2 mainly infects the upper and lower part of the tracks of the respiratory system of humans. However, coronavirus infection alters the redox-status by generating reactive oxygen species (ROS) followed by oxidative stress (OS) by manipulating host cell machinery, which causes different abnormal situations in the human body. Moreover, regular consumption of a high-lipid diet alone or monosodium glutamate also disturbs the redox-equilibrium, so it can hypothesize that such types of diets...
cause OS, which may indirectly stimulate the viral infection. Therefore, it can predict that OS plays a pivotal role in SARS-CoV-2 disorders that alter redox signaling to increase the severe acute respiratory syndrome risk. As there is no such effective treatment for the condition of contagious SARS-CoV-2, the best way to stop the pandemic is to find a possible effective antiviral strategy of management against COVID-19. The present review offers the development of the antiviral prophylactic strategy against SARS-CoV-2 via manipulation of genes by the CRISPR/Cas system.

**CRISPR/Cas system**

Nowadays, genetic engineering-based work makes it researchers and scientists easy to trace the master regulator of any disease and treat them accordingly. The CRISPR/Cas system has been found in most prokaryotes such as bacteria and archaea, conferring adaptive immunity against invading nucleic acids. CRISPR/Cas is a molecular tool in genetic engineering and helps to identify the target genes in various diseases. Besides editing deoxyribonucleic acid (DNA), they have also played a crucial role in editing and target specific ribonucleic acid (RNA) sequences. It also acts as a DNA/RNA-guided/targeting tool in prokaryotic organisms with inherited adaptive immunity to combat against xenobiotics. The systems have two classes; the class 1 system includes type I, III, and IV CRISPR/Cas system, which uses crRNA with multi-effector complex to find out the target sequence and then cleave them. On the other hand, class 2 includes type II/Cas9, type V/Cas12, and type VI/Cas13, which uses a single multi-domain Cas protein with CRISPR RNA (crRNA) for involvement. The second class of CRISPR/Cas system has been vital for genomic editing and diagnostic markers of different infections. On the contrary, recently, CRISPR/Cas12a, CRISPR/Cas13a, and CRISPR/Cas13b have been utilized to establish an accurate diagnostic tool to detect bacteria and virus-mediated infections in humans. Recently, molecular tool-based technology is being used to fight against various bacteria or virus-mediated infections by modifying gene sequences. Therefore, we hypothesize that it may be a viable tool to combat against SARS-CoV-2-mediated infection in the body.5

**CRISPR/Cas system versus human pathogenic viruses**

Apart from targeting human genomic DNA, the “molecular scissors” (i.e. CRISPR/Cas system) can also target any double-stranded viral invaders in humans’ case. There is a plethora of evidence where the CRISPR/Cas technology inhibits the number of viral infections in human in vitro and in vivo models. CRISPR/Cas technology has a potential antiviral strategy against human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), Herpes simplex virus (HSV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papillomavirus (HPV), human polyomaviruses or John Cunningham virus (JCV), human cytomegalovirus (HCMV), African swine fever virus (ASFV), and Pseudorabies virus (PV) most likely mediated via inhibition of the translation, replication of the viruses, or direct disruption of the viral genome. Moreover, in the case of HCV, this single-stranded RNA virus causes inflammation of liver and hepatocytes infection worldwide. CRISPR/Cas plays an anti-viral tool to disrupt the genome of the HCV to combat against the HCV-mediated infections. In this case, the earlier study suggested that the potential power of the Cas9 from *Francisella novicida* (FnCas9) targets mRNA of bacteria and induced repression of viral genes. Therefore, the CRISPR/FnCas9 system’s antiviral strategy via targeting the mRNA to fight against HCV infection in eukaryotic organisms was unique. Previously, researchers also pointed out the HCV translation inhibition by inhibition of D11A/H969A (the catalytically inactive version of FnCas9). Therefore, there is no need for direct degradation of RNA in the virus for the stoppage of translation of viral protein by the CRISPR/FnCas9 system. Alternatively, the connection of FnCas9 with the RNA of HCV genome appeared to be sufficient for inhibition of the translation and replication of the virus. Hence, it can infer that the specification of applying CRISPR/Cas in case of RNA viruses seems to be little bit different from that of DNA viruses.

**SARS-CoV-2 and pathogenesis**

The coronavirus consists of various proteins such as spike protein, membrane, envelope, and nucleocapsid. The heavily glycosylated spike protein (type I) present on the external surface is attached with the host angiotensin-converting enzyme two receptors (ACE2R). This glycosylated type I protein is a valuable determinative of the SARS-CoV-2 insertion into the host cells as the receptor-binding domain for the ACE2R, and direct membrane fusion plasma membrane with the SARS-CoV-2 that is present on the head of the virus. Besides that, SARS-CoV-2 also gets entry to the host cells via clathrin-dependent and clathrin-independent endocytosis. Subsequently, the RNA genome of SARS-CoV-2 released into the cytoplasm, translated into two polyproteins and structural proteins to begin the replication of the viral genome. Primarily, polyprotein 1a/1ab is translated from the genomic RNA, which ultimately constructs the replication-transcription complex in a double-membrane vesicle after the infection of SARS-CoV-2. Eventually, in a discontinuous manner, the copy numbers of RNAs are increased by the replication-transcription complex. Additionally, the viruses containing the vesicles are fused with the plasma membrane to liberate the virus from the infected cells. Viral replicase genes have also been present in novel coronavirus with a variable number open reading frame (ORF), which codes essential proteins for replicating the virus, the formation of nucleocapsid and spikes, etc. About 2/3rd of the RNA of SARS-CoV-2 principally placed in the 5’-terminal in the first ORF translates two polyproteins which code for 16 different non-structural proteins (NSP); on the other hand, remaining ORFs codifies eight accessory proteins interceding with the nonspecific defense mechanisms. Also, there are some intraviral protein-to-protein
communications with these proteins, ultimately leading to such complicated situations.

The pathogenesis of the SARS-CoV-2 depends upon various factors, including the interaction with the receptor, responsiveness of the immune system, and genetic factors. The pathogenicity of the SARS-CoV-2 has occurred in multiple stages; primarily when the SARS-CoV-2 enters the body via nasal cavity or mouth and ultimately goes to the lungs, the S1 subunit of S protein ties-up with the ACE2R on type II alveolar epithelial cells. It leads to the conformational alteration of the S2 subunit. It stimulates the association between heptad repeat (HR) 1 and HR2 domains to construct 6-HB, which assists the cell membrane and viral membrane in adjacent positions a fusion. This fusion induced damage of the lungs which is the primary infection site by SARS-CoV-2. The lungs’ infection site thereby induced activation of CD4+ and CD8+ T cells; alteration of pro-inflammatory and anti-inflammatory affects lungs and cytokines levels, increased chemokines, lymphocytopenia, increased production of IgM and IgG, cytotoxicity of the lymphocytes, severe organ dysfunction due to abnormal regulation of host response to SARS-CoV-2 infection. On the other hand, lung tissue inflammation, acute respiratory distress syndrome, inflammation-induced lung injury, failure of the respiratory system and other organs, and death. Moreover, the virus SARS-CoV-2 also has harmful impacts on other organs such as the brain to cause cerebral damage and neurologic disturbances, or cardiomyocytes of the heart with escalated angiotensin II. Additionally, it could generate oxygen-free radicals by hypoxia. Hypoxia-reperfusion can also lead to cardiac injury, injury to renal tubular cells of kidney and hepatocytes, or bile duct cells of the liver. All these events can eventually induce hepatocellular anomalies via binding on ACE2R, respectively. The spike proteins of the SARS-CoV-2 recognize the receptors on the cell surface to penetrate the host cells just like the HIV GP 120 protein does, but the only difference is the specificity of the receptor. In the case of SARS-CoV-2, it is ACE2R to penetrate mucosal epithelial cells and cluster of differentiation four receptors (CD4R) for HIV to penetrate CD4+: T cells; Figure 1 shows the overall mechanism pathogenicity of SARS-CoV-2 and how it invades into the epithelial cells of the lungs. The mechanism of cellular entry of the SARS-CoV-2 via ACE2R by fusion with the cell membrane and then it ultimately liberated its RNA. Further, in the endoplasmic reticulum or in the cytoplasm, it synthesizes viral proteins, and some of the viral protein (i.e. membrane glycoproteins, spike proteins, envelope proteins, and nucleocapsid protein) and RNA ultimately construct a replication complex to enhance the synthesis of RNA, or it increases the copy numbers of RNA. Next, it is packaged with the Golgi apparatus and then released into the circulatory system. Therefore, it is common speculation that via the circulatory system or blood flow for spreading the virus not only affects lungs but also poses a potential threat to the other important structure of the human body. Additionally, ACE2R plays a crucial role in the body, and it has a high affinity to Angiotensin II type 1 and 2 receptors with diverse physiological functions. ACE2R is expressed in various organs, tissues in the human body controlling renal, reproductive, and cardiovascular function. Therefore, some reports stated the knockdown of this receptor, application of ACE2R inhibitor, or silencing the angiotensin-converting enzyme 2 can reduce the infection of the SARS-CoV-2. Still, the after-effect in the body is very unfavorable as it regulates different physiological functions. Thus, in the present review, we propose using the CRISPR/Cas system as an alternative way of an antiviral management strategy against muscular SARS-CoV-2 without or fewer side effects.

**CRISPR/Cas to combat against SARS-CoV-2**

The world’s present pandemic situation is because of the SARS-CoV-2; patients with SARS-CoV-2 infection and comorbidities such as obesity, diabetes, non-alcoholic fatty liver disease, cardiovascular disorder, etc. strengthen the mortality rate. On the other hand, failure of effective treatments, or lack of effective drugs or drugs with lots of adverse effects, and absence of vaccines make it a serious health concern and ultimately leads to a pandemic situation. The CRISPR/Cas system has concentrated on trace and degrades the intracellular viral mRNAs and genome. In contrast, the other common vaccines or drugs depend on humans’ immune systems to trace the viral proteins and some components to reduce the virus’s entrance into the cells. In this context, the present review suggests an alternative antiviral approach of management to combat this pandemic situation by using the CRISPR/Cas system.
Accumulating evidence suggests that the CRISPR/Cas system has antiviral potential to treat virus-mediated diseases via genomic editing or degrading the DNA or RNA sequences to stop the viral replication and translation. Although, in the present scenario, COVID-19 is not a genetic abnormality; where SARS-CoV-2 is the master player of such infection which belongs to the family of RNA viruses, enters the epithelial cells of the human to infect them, liberates its RNA genome into the cytoplasm, and then synthesizes to increase the copy number of viral RNA to construct a new virus to infect adjacent cells. However, to combat the novel coronavirus, it is necessary to utilize RNA research, which may explain an important way of developing an antiviral strategy to fight against the virus and terminate the infections. On the CRISPR-based other hand, DNA is the genetic material transcribes into the RNA in the human body, but when the DNA is in the cell’s nucleus, the genetic information is carried by RNA to the cell. Moreover, COVID-19 caused by SARS-CoV-2 is also a sneaky RNA virus that causes the human body’s protein synthesis machinery to fault it for RNA constructed by our DNA. Once the SARS-CoV-2 infects the human body, it continuously liberates RNA; long viral proteins are produced from the human body’s hijacked cells, which are the great source of virus, and it fails to heal our immune system to protect us from SARS-CoV-2 infections. After that, the virus rapidly spreads all over the body via body fluids and infects other humans through the fluidic medium.

In this context, as an alternative antiviral strategy of management to fight against the SARS-CoV-2-mediated infection, the present review proposes the CRISPR/Cas system, the Cpf1 and C2c2/Cas13 for editing of genes in case of RNA virus or SARS-CoV-2. An earlier study demonstrated that the Cas9 targets endogenous mRNA and thereby controls the expression of target genes. Therefore, it can be speculated that to manipulate the SARS-CoV-2-like RNA virus or host factor’s mRNA, an RNA-induced RNA targeting system could be an alternative antiviral strategy of management. Moreover, till now, lack of effective antiviral strategy of management to combat against RNA virus such as Dengue Virus (DENV) and Zika Virus (ZIKV), West Nile Virus (WNV), Influenza Virus, Ebola Virus (EBOV), SARS-CoV, MERS-CoV are the hottest topic of current research field. To survive this, it is important to understand the host’s interaction with the virus. CRISPR/Cas system-based work has now gained importance to uncover the underlying mechanism and knockdown the target genes to inhibit viral replication and infection. RNA targeting the CRISPR system could be used to silence the transcriptional genetic factors. Recently, CRISPR (type VI)/Cas13 system has been suggested as an important tool for gene silencing because there are various important Cas13 target sites that exist in the genome of the single-stranded RNA (ssRNA) virus, and Cas13 is an efficient antiviral against different ssRNA viruses and lastly it acts as programmable scissors to cut the complementary RNA to its crRNA. Moreover, recent reports suggested that Cas13 has significant antiviral activity against some ssRNA viruses; it has also documented that the antiviral activity of the Cas13 assisted restriction viral expression and readout (CAVER) in which Cas13 identifies and destroys the viral RNA.

Additionally, the RNA-guided as well as targeting manipulation of the gene via the Cas13 enzymes permit the sorting out of the target genes; this characteristic feature of Cas13 may be effective therapeutic tools in genetic engineering-based COVID-19 treatment by eliminating the target sequences in the genome; moreover, a recent study suggested that Cas13 fight against the transcriptional as well as splicing-associated RNA binding protein and furthermore they also used the guide RNA as a tool for further analysis.

Besides the antiviral application of Cas13 in targeting and knocking down viruses can also be utilized to evaluate as tool for replication, localization, and evolution of virus. This approach is well supported by earlier studies which reveal that the catalytically dead mutants of Cas13 (Cas13d) can be mainly utilized to investigate the localization of viral RNA, and the RNA-editing ability of Cas13d fusion proteins could be an alternative marker to recognize the viral polymorphisms functionally. Therefore, the CRISPR/Cas13 system could be an antiviral tool for diagnosis and treatment of virus-mediated infections. By these tools, researchers can accurately reach the research’s peak and show the way to a quick cure against the virus.

Very recently, a group of researchers of Stanford University stated that CRISPR/Cas13 specifically targets and knockdown viral RNA sequences liberated from the SARS-CoV-2 to fix the COVID-19; they suggested that CRISPR-based technology inhibits viral infections. Prophylactic antiviral CRISPR in human cells (PAC-MAN) can elicit strong viral degradation by neutralizing the SARS-CoV-2 and inhibiting intracellular replication; the enzyme Cas13 is a viral degrading enzyme that degrades nucleic acid sequences in the genome of coronavirus. PAC-MAN has now been suggested to kill the virus-mediated infections via potentially degrading the RNA of the sequence from live influenza A virus and SARS-CoV-2 of humans. PAC-MAN uses CRISPR/Cas13 for the inhibition of the SARS-CoV-2. It can be hypothesized from the earlier report that when SARS-CoV-2 infects the cell of human body, it releases RNA and thereby more copies of viral mRNA and genomes are produced, and the Cas13d tool acts as an inhibitor to attenuate the replication of the virus and function too via immediately cut the viral positive-sense RNA of SARS-CoV-2. A recent report suggested that the CRISPR/Cas13 system reduced the viral load of epithelial cells of the lungs of humans infected with the H1N1 strain of the flu and SARS-CoV-2. Therefore, we can speculate that it might be feasible from the pharmacological standpoint to develop any such drug which is approved to use in the case of a human trial. A group of researchers and scientists already found that two highly conserved zones of the genome of SARS-CoV-2 can be suitable to be directed by PAC-MAN as an antiviral strategy to inhibit the replication and spreading of the virus by RNA-dependent RNA polymerase gene (RdRP gene) in the open reading frame (ORF1a/b) region; it regulates the multiplication of coronavirus and nucleocapsid...
cases, there are some issues. On the contrary, delivery directed adjacent to various genomic sites, still in some been sorted out by the application of multiple gRNAs higher rate of virus pathogenicity. Although this issue has reports have demonstrated that CRISPR/Cas-based tech- its therapeutic potential in humans. Moreover, earlier using the CRISPR/Cas system. Therefore, it fails to explain virucide potential in humans. Moreover, earlier reports have demonstrated that CRISPR/Cas-based tech- nology incited virus escape mutants and developed a higher rate of virus pathogenicity. Although this issue has been sorted out by the application of multiple gRNAs directed adjacent to various genomic sites, still in some cases, there are some issues. On the contrary, delivery of Cas9:guide RNA (gRNA) is an important therapeutic component to confirm the success and potentiality of consideration of viral vectors. Therefore, to develop a safe and efficient antiviral strategy against any viral disease via CRISPR/Cas system, it is very important for the success of the system in models, clinics, targeting of organs, tissues, and cells of humans yet to be a challenge. Furthermore, there are some known provocations that can stimulate the reactivation of the virus and subsequent spreading of the replication of the virus, which causes serious problems like pandemics all over the world. Additionally, the PAC-MAN strategy limitation is the delivery method in the body; it can be delivered through chemical polymers or nanoparticles of lipid. Moreover, liposome-mediated delivery is another option also such as lipitoids, HEDGES platform. On the other hand, it can deliver via ribonucleoprotein complex with Cas13d protein tagged renin-angiotensin with crRNA, engineered amphiphilic peptides. Additionally, the advance- ment of gene therapy-based approaches with safe and efficient non-viral delivery systems such as self-assembled peptide-poloxamine nanoparticles could also be an alternative one. Figure 2 shows a hypothetical target pathway of gene manipulation and an alternative strategy of management to combat deadly SARS-CoV-2 mediated infection to weaken the COVID-19 pandemics. Therefore, it can be assumed that all these antiviral techniques can be given to patients by using a nebulizer or a nasal sprayer. Further studies are required to identify the technology’s barriers or disadvantages before initiating it directly to fight against novel coronavirus. However, it is crucial to recognize and investigate the disadvantageous impacts of the use of single-guide RNAs (sgRNAs) or CRISPR/Cas system or PAC-MAN on the physiology of the host. However, if we can limit the adverse effect on the integrity of the genome and transcriptome profiles, then PAC-MAN and CRISPR/Cas13d system will be an attractive and useful therapeutic strategy against SARS-CoV-2-mediated infection via inhibition of viral replication and function by cleavage of the specific sequences of the target RNA of SARS-CoV-2.

Conclusions

As a tool of molecular biology and genetic engineering, CRISPR/Cas13d along with PAC-MAN acts as an antiviral tool with high flexibility and specificity for the target disease to inhibit RNA virus-mediated infection. Further studies are required to evaluate the success rate and safety of this antiviral tool-based technology to fix the SARS-CoV-2-mediated infection in animals before its potential therapeutic use to SARS-CoV-2-infected patients. If it proves to be
effective, it could be used as a weapon to prevent SARS-CoV-2-induced infections worldwide as a pattern of the advancement of medical science via knockdown or degradation of specific RNA sequence in SARS-CoV-2 and associated comorbidities.

AUTHORS’ CONTRIBUTIONS

All authors were equally involved in construction of the manuscript, reviewing, editing and approved the final version submitted for publication.

ACKNOWLEDGEMENTS

Authors are grateful to Prof. Dhrubajyoti Chattopadhyay (Vice Chancellor of Sister Nivedita University, Kolkata) and Dr. Sumit Sarkar (Research Biologist, Division of Neurotoxicology, National Center for Toxicological Research, US Food and Drug Administration, Jefferson, USA) for their continuous encouragement and valuable suggestions. Authors are indebted to Ms. Aparna Shil (Department of Life Sciences, Presidency University) for her valuable help.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Arnab Banerjee https://orcid.org/0000-0002-8727-0227
Bithin K Maji https://orcid.org/0000-0001-7017-1399

REFERENCES

1. Lotfi M, Rezaei N. CRISPR/Cas13: a potential therapeutic option of COVID-19. Biomed Pharmacother 2020;131:110738
2. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 2019;17:181–92
3. Liu J, Zheng X, Tong Q, Li W, Wang B, Sutter K, Trilling M, Wang B, Fang M, Dittmer U, Yang D. Overlapping and discrete aspects of the pathology and pathogenesis of the emerging human pathogenic coronaviruses SARS-CoV-2, MERS-CoV, and 2019-nCoV. J Med Virol 2020;92:491–4
4. Suhail S, Zajac J, Fossum C, Lawower H, McCracken B, Severson N, Laatsch B, Narkiewicz-Jodko A, Johnson B, Liebau J, Bhattacharyya S, Hati S. Role of oxidative stress on SARS-CoV (SARS) and SARS-CoV-2 (COVID-19) infection: a review. Protein J 2020;39:644–56
5. Checconi P, De Angelis M, Marconcini ME, Fraternali A, Magnani M, Palamara AT, Nencioni L. Redox-modulating agents in the treatment of viral infections. Int J Mol Sci 2020;21:4084
6. Banerjee A, Das D, Paul R, Roy S, Bhattacharjee A, Prasad SK, Banerjee O, Mukherjee S, Maji BK. Altered composition of high-lipid diet may generate reactive oxygen species by disturbing the balance of antioxidant and free radicals. J Basic Clin Physiol Pharmacol 2020;31:15–20
7. Banerjee A, Das D, Paul R, Roy S, Das U, Saha S, Dey S, Adhikary A, Mukherjee S, Maji BK. Mechanistic study of attenuation of monosodium glutamate mixed high lipid diet induced systemic damage in rats by coccinella andriscus. Sci Rep 2020;10:15443
8. Xiang Q, Qian K, Zhang Z, Lin F, Xie Y, Liu Y, Yang Z. CRISPR-cas systems based molecular diagnostic tool for infectious diseases and emerging 2019 novel coronavirus (COVID-19) pneumonia. J Drug Target 2020;28:727–31
9. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR-cas systems. Science 2013;339:819–23
10. Pardee K, Green AA, Takahashi MK, Braff D, Lambert G, Lee JW, Ferrante T, Ma D, Donghia N, Fan M, Daringer NM, Bosil I, Dudley DM, O’Connor DH, Gehrke L, Collins JJ. Rapid, low-cost detection of Zika virus using programmable biomolecular components. Cell 2016;165:1255–66
11. Guk K, Keem JO, Hwang SG, Kim H, Kang T, Lim EK, Jung J. A facile, rapid and sensitive detection of MRSA using a CRISPR-mediated DNA FISH method, antibody-like dCas9/sgRNA complex. Biosens Bioelectron 2017;95:67–71
12. Myhrvold C, Kellner MJ, Paul LM, Parham LA, Garcia KE, Barnes KG, Chak B, Mondini A, Nogueira ML, Isen S, Michael SF, Lorentzen I, Yozwiak NL, Macmynn BL, Bosil I, Gehrke L, Zhang F, Sabeti PC. Field-deployable viral diagnostics using CRISPR-Cas13. Science 2018;360:444–8
13. Goottenberg JS, Abudayyeh OO, Lee JW, Essefizchelbier P, Dy AJ, Joung J, Verdine V, Donghia N, Daringer NM, Freie CA, Myhrvold C, Bhattacharyya RP, Livny J, Regev A, Koonin EV, Hung DT, Sabeti PC, Collins JJ, Zhang F. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science 2017;356:438–42
14. Goottenberg JS, Abudayyeh OO, Kellner MJ, Joung J, Collins JJ, Zhang F. Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science 2018;360:439–44
15. Chekani-Azar S, Gharib ME, Birhan M, Yousefi M. CRISPR/Cas9 gene knockdown or degradation of specific RNA sequence in SARS-CoV-2 and associated comorbidities. Front Microbiol 2020;11:01019
16. Pardee K, Green AA, Takahashi MK, Braff D, Lambert G, Lee JW, Ferrante T, Ma D, Donghia N, Fan M, Daringer NM, Bosil I, Dudley DM, O’Connor DH, Gehrke L, Collins JJ. Rapid, low-cost detection of Zika virus using programmable biomolecular components. Cell 2016;165:1255–66
17. Guk K, Keem JO, Hwang SG, Kim H, Kang T, Lim EK, Jung J. A facile, rapid and sensitive detection of MRSA using a CRISPR-mediated DNA FISH method, antibody-like dCas9/sgRNA complex. Biosens Bioelectron 2017;95:67–71
18. Myhrvold C, Kellner MJ, Paul LM, Parham LA, Garcia KE, Barnes KG, Chak B, Mondini A, Nogueira ML, Isen S, Michael SF, Lorentzen I, Yozwiak NL, Macmynn BL, Bosil I, Gehrke L, Zhang F, Sabeti PC. Field-deployable viral diagnostics using CRISPR-Cas13. Science 2018;360:444–8
19. Chekani-Azar S, Gharib ME, Birhan M, Yousefi M. CRISPR/Cas9 gene knockdown or degradation of specific RNA sequence in SARS-CoV-2 and associated comorbidities. Front Microbiol 2020;11:01019
28. Wu J, Deng W, Li S, Yang X. Advances in research on ACE2 as a receptor for 2019-nCoV. *Cell Mol Life Sci* 2020;78:531–44.

29. Lu CY, Huang HY, Yang TH, Chang LY, Lee CY, Huang LM. siRNA silencing of angiotensin-converting enzyme 2 reduced severe acute respiratory syndrome-associated coronavirus replication in vero E6 cells. *Eur J Clin Microbiol Infect Dis* 2008;27:709–15.

30. Costa FF, Rosário WR, Ribeiro Farias AC, de Souza RG, Duarte Gondim RS, Barroso WA. Metabolic syndrome and COVID-19: an update on the associated comorbidities and proposed therapies. *Diabetes Metab Syndr* 2020;14:809–14.

31. Abbott TR, Dhamdhere G, Liu Y, Lin X, Goudy L, Zeng L, Chemparathy A, Chmura S, Heaton NS, Debs R, Pande T, Endy D, La Russa MF, Lewis DB, Qi LS. Development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza. *Cell* 2020;181:865–76.e12.

32. University of Rochester-COVID-19: what’s RNA research got to do with it?, https://www.rochester.edu/newscenter/COVID-19-rna-coronavirus-research-428952/ (accessed 28 April 2020).

33. Sampson TR, Saroj SD, Llewellyn AC, Tzeng YL, Weiss DS. A CRISPR/Cas system mediates bacterial innate immune evasion and virulence. *Nature* 2013;497:254–7.

34. Chen S, Yu X, Guo D. CRISPR-Cas targeting of host genes as an antiviral strategy. *Virus* 2018;10:40.

35. Science daily-New kind of CRISPR technology to target RNA, including new viral genes. https://www.sciencedaily.com/releases/2020/03/200316141514.htm (accessed 16 March 2020).

36. Wessels HH, Méndez-Mancilla A, Guo X, Legut M, Daniloski Z, Sanjana NE. Massively parallel Cas13 screens reveal principles for guide RNA design. *Nat Biotechnol* 2020;38:722–7.

37. Freije CA, Myhrvold C, Boehm CK, Lin AE, Welch NL, Carter A, Metsky HC, Luo CY, Abudayyeh OO, Gootenberg JS, Yozwiak NL, Zhang F, Sabeti PC. Programmable inhibition and detection of RNA viruses using Cas13. *Mol Cell* 2019;76:826–37.

38. Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, Verdin E, Cox DBT, Kellner MJ, Regev A, Lander ES, Voytas DF, Ting AY, Zhang F. RNA targeting with CRISPR-Cas13. *Nature* 2017;540:280–4.

39. Abudayyeh OO, Gootenberg JS, Franklin B, Koob J, Kellner MJ, Ladha A, Joung J, Kirchhutter P, Cox DBT, Zhang F. A cytosine deaminase for programmable single-base RNA editing. *Science* 2019;365:382–6.

40. Cox DBT, Gootenberg JS, Abudayyeh OO, Franklin B, Kellner MJ, Joung J, Zhang F. RNA editing with CRISPR-Cas13. *Science* 2017;358:1019–27.

41. Nguyen TM, Zhang Y, Pandolfi PP. Virus against virus: a potential treatment for 2019-nCoV (SARS-CoV-2) and other RNA viruses. *Cell Res* 2020;30:189–90.

42. Gergen J, Coulon F, Creneuy A, Elain-Duret N, Gutierrez A, Pinkenburg O, Verhoeven E, Anegon I, Nguyen TH, Halary FA, Hasopot F. Multiplex CRISPR/Cas9 system impairs HCMV replication by excising an essential viral gene. *PloS One* 2018;13:e0192602.

43. Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *J Control Release* 2017;266:17–26.

44. Hendel A, Bak RO, Clark JT, Kennedy AB, Ryan DE, Roy S, Steinfeld L, Lunstad BD, Kaiser RJ, Wilkens AB, Bachetta R, Tsalenko A, Dellingler D, Bruhn L, Porteous MH. Chemically modified guide RNAs enhance CRISPR-Cas genome editing in human primary cells. *Nat Biotechnol* 2015;33:985–9,

45. McKinlay CJ, Vargas JR, Blake TR, Hardy JW, Kanada M, Contag CH, Wender PA, Wyomouth RM. Charge-altering releasable transporters (CARTs) for the delivery and release of mRNA in living animals. *Proc Natl Acad Sci U S A* 2017;114:E448–56.

46. Sago CD, Lokugamage MP, Paunovska K, Vanover DA, Monaco CM, Shah NN, Gamboa Castro M, Anderson SE, Rudolf TG, Lando GN, Munmlal Tiwari P, Kirschman JL, Willett N, Jang YC, Santangelo PJ, Bryksin AV, Dahlman JE. High-throughput *in vivo* screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing. *Proc Natl Acad Sci U S A* 2018;115:E9944–52.

47. Xu CF, Chem GJ, Luo YL, Zhang Y, Zhao G, Lu ZD, Czarna A, Gu Z, Wang J. Rational designs of *in vivo* CRISPR-Cas delivery systems. *Adv Drug Deliv Rev* 2021;168:3–29.

48. Handumrongkul C, Ye AL, Chmura SA, Soroceanu L, Mack M, Ice RJ, Thistle R, Myers M, Urus SJ, Liu X, Kashiuri-Sabet M, Heath TD, Liggitt D, Lewis DB, Debs R. Durable multimtransgene expression in vivo using systemic, nonviral DNA delivery. *Sci Adv* 2019;5:eaax017.

49. Huang CY, Uno T, Murphy JE, Lee S, Hamer JD, Escobedo JA, Cohen FE, Radhakrishnan R, Dvarki V, Zuckermann RN. Lipotidoids–novel cationic lipids for cellular delivery of plasmid DNA *in vitro*. *Chem Biol* 1998;5:345–54.

50. Amirkhanov RN, Stepanov GA. Systems of delivery of CRISPR/Cas9 ribonucleoprotein complexes for genome editing. *Russ J Bioorg Chem* 2019;45:431–7.

51. Krishnamurthy S, Wohlford-Lenane C, Kandimalla S, Sartre G, Meyerhofs DK, Théberge V, Hallée S, Duperre AM, Del’Guidice T, Lepełlet-Stoffaes JP, Barbeau X, Guay D, McCray PB Jr. Engineered amphiphilic peptides enable delivery of proteins and CRISPR-associated nucleases to airway epithelia. *Nat Commun* 2019;10:4096.

52. Guan S, Munder A, Heddité F, Braubach P, Glage S, Zhang L, Lienkensl SA, Schultz A, Hasenpach G, Garrels W, Stanke F, Miskely C, Johler SM, Kumar Y, Tümmler B, Rudolph C, Ivics Z, Rosencker J. Self-assembled peptide-poloxamine nanoparticles enable *in vitro* and *in vivo* genome restoration for cystic fibrosis. *Nat Nanotechnol* 2019;14:287–97.