

CRISPR Technology in Infectious Disease Management: Principles, Applications, and Future Challenges with Emphasis on SARS-CoV-2, HIV, and HBV

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Abstract. Retroviruses and double-stranded DNA (dsDNA) viruses enter cells by integrating their genetic materials into host DNA. During this period, the virus stays dormant and can escape host immune surveillance, which is difficult to detect and kill clinically. Thus, viral diseases, especially those caused by RNA viruses, pose a huge threat to public health. Currently, more research shows that clearing the genome of the virus has become a direct and effective way to treat viral diseases. However, viral diseases such as SARS-CoV-2 exhibit significant genetic diversity, with over 300000 mutants discovered, covering the entire genome of the virus, which greatly limits the traditional gene editing techniques for diagnosing and clearing the virus sequence. CRISPR-Cas can target multiple regions, exhibiting stronger catalytic activity compared to other gene editing methods. At present, it has been widely used in the diagnosis and treatment of viral diseases. Therefore, understanding the principles of CRISPR-Cas technologies and identifying their potential applications in viral diseases is of great significance. This paper reviews the principles of this technology and its current applications in three aspects, COVID-19, HIV, and HBV. Additionally, this paper summarizes the difficulty in applying CRISPR-Cas technology and future expectations.

Keywords: CRISPR-Cas; COVID-19; HIV/AIDS; HBV.

1. Introduction

Gene editing is a technique that precisely alters genome sequences to induce genome insertion, deletion, or base substitution. Many diseases occur with the changes in gene expressions, especially genetic diseases caused by individual gene mutations, thus these diseases at the gene level are expected to be controlled by gene editing technology. The newest gene editing technology with the widest application nowadays is clustered regularly spaced short palindromic repeats (CRISPR)/CRISPR related protein (Cas) 9. This technology directs Cas proteins to specific regions of the genome by modifying the guide RNA's base sequence in a small segment, thereby making gene editing technology more efficient and applicable.

CRISPR-Cas9 is the most widely used technology of all. It consists of two important parts, one is the Cas9 protein that performs DNA double-stranded cleavage function, and the other is the guide RNA (gRNA). The recognition process of gRNA is accompanied by motifs (PAMs). The preferred PAM for Cas9 is NGG [1], which is common in the genomes of most organisms, thus promoting the use of this technology in biomedical fields. With the ability to change the nucleotide sequence of a small portion of the gRNA, CRISPR-Cas9 can precisely target almost any desired genomic site, thereby rectifying or silencing mutated genes.

CRISPR-Cas13 is also an effective way of disease diagnosis. The system was first reported in 2015 [2]. Unlike traditional CRISPR-Cas9, CRISPR-Cas13 directly targets RNA, does not cause irreversible DNA damage, and has high specificity and targeting efficiency. More interestingly, CRISPR-Cas13 exhibits a significant collateral effect in the in vitro response system [3]. In a

complete reaction system in vitro, gRNA recognizes the target sequence and activates it by binding to the Cas13 protein. On the one hand, it can degrade the target RNA sequence, and on the other hand, it can also cleave non-specific nucleic acid sequences that are close to their physical positions. Based on this characteristic, CRISPR-Cas13 technology is widely used for the detection and treatment of infectious diseases. At present, multiple COVID-19 diagnostic technologies based on this system have been validated at the clinical sample detection level (such as mCARMEN, SHINE, CASCADE, Cas13C, etc.), demonstrating the application prospects of this technology in SARS-CoV-2 detection. In terms of treatment, some studies have shown the potential of applying it to the treatment of COVID-19, HIV, and so on.

However, there is a lack of effective means to deliver the CRISPR system to diseased cells in the body, and the use of this technology to modify cells in vitro and infuse them back into the body can treat very limited diseases. Therefore, to make CRISPR technology a common treatment mode, it is necessary to achieve a steady, efficient, and secure in vivo delivery.

This article discussed the principles of CRISPR technology, including CRISPR-Cas9 and CRISPR-Cas13. Then we focus on its application in infectious diseases. The following examples are SARS-CoV-2, HIV, and HBV. Finally, the review summarizes other today's applications and future prospects.

2. Principle and mechanism

Barrangou et al. [4] validated the hypothesis that CRISPR sequences may be involved in the adaptive immunity of bacteria or archaea. They mixed *Streptococcus thermophilus* with bacteriophages and observed that a new spacer sequence was inserted into the CRISPR sequence of the screened strain with anti-phage resistance. When the spacer sequence was removed, the bacterial anti-phage resistance disappeared. This finding proved that the CRISPR sequence and Cas gene are responsible for bacteria's ability to obtain anti-phage resistance. The specificity of resistance depends on the characteristics of the phage septal sequence, demonstrating that CRISPR-Cas is an "acquired immune system" of bacteria.

The CRISPR-Cas system is made up of a discontinuous repeat (R), spacer sequences (S), a leading sequence leader (L), and other components. These Cas genes have the ability to cut foreign DNA sequences that are adjacent to the CRISPR sequence. Three categories of the system have been created by researchers, namely Cas3, Cas9, and Cas10, represented by Type I, Type II, and Type III, based on the different Cas gene expression proteins.

The CRISPR-Cas9 system, which cleaves invasive DNA linked with RNA spacers, is now the most popular system that has to be utilized. It works via Cas9 helicase attaching to RNA produced from host DNA palindromic repeats. Cas9 and gRNA cooperate. The target sequence from the DNA is located at the 5' end of the CRISPR RNA (crRNA), whose 3' end is coupled with tracrRNA. To make the crRNA target sequence move to complementary areas (protoseptal regions) that are on the DNA molecule, Cas9 captures tracrRNAs and forms binary complexes. Cas9-gRNA-protospacer which is a ternary complex is created when the crRNA and protospacer pair to split the corresponding second DNA strand. In addition to base pairing, loops are used. The prototype spacer sequence's two DNA strands are cut by Cas9 using the crRNA target sequence as a guide, depending on the existence of the prototype spacer and the prototype spacer proximity PAM [5]. CrRNA and tracrRNA are designed as single chimeras known as sgRNAs (single guide RNAs), in which two molecules are not only base pairing but also linked through loops[6]. This allows for easier genome editing.

Contrary to Cas9, after recognizing the target, Cas12a and Cas13a show incidental nonspecific action against random ssDNA(single-stranded DNA) or ssRNA, respectively. The T-rich PAMs are recognized by the programmable RNA-guided DNA nuclease Cas12a, which does not require transactivation of the crRNA (tracrRNA) for crRNA maturation. CRISPR-Cas13, on the contrary, is a CRISPR-Cas9 variant in which Cas13 cuts RNA rather than DNA [7]. This technique uses crRNA, which consists of an adjacent stem-loop structure known as a direct repeat (DR) and a target sequence

(spacer) [7]. Cas13 seizes DR and directs the crRNA so that the nucleotides are coupled and the spacer matches the corresponding sequence on the specimen's RNA. The distal end of the RNA targets combined with the spacer is quickly cut by Cas13 [7]. Latitudinally cutting depends on targeted cutting, and Cas13 has extra trans (paraphyletic) cleavage activity functioning around non-targeted ssRNA [7]. Depending on Cas13 homology, flank cleavage prefers particular dinucleotides in different ways [3]. The diagnosis of Cas13 is based on its lateral cleavage activity.

3. Application

Viral diseases, such as COVID-19, HIV, and HBV leave a great impression on public health, with millions of people dying from HIV and HBV every year. Additionally, the death toll of COVID-19 in the past three years has exceeded six million. All three viruses enter cells by integrating their own genetic material into host DNA. CRISPR-Cas technology can target the genome sequence of viruses and directly clear the virus genome, it can also specifically knock out host genes that assist in virus infection and replication, inhibiting virus invasion and amplification, which is widely applied to the diagnoses and treatments of viral diseases. In this part, we discuss the current research progress of the system for the diagnosis and treatment of COVID-19, HIV, and HBV.

3.1. COVID-19

3.1.1 Treatment

Cas13d can target multiple regions and has stronger catalytic activity, without requiring any strict PAM/pre-spacer sequence flanking sequences to limit the cleavage of targets, making CRISPR-Cas13d a hot topic in the development of antiviral therapy.

Abbott et al. [8] invented a new technology based on CRISPR-Cas13d, the prophylactic antiviral CRISPR (PAC-MAN) system in human cells. By comparing the genome sequences of 47 patients with COVID-19 and Middle East Respiratory Syndrome, a crRNA library targeting highly conserved RNA-dependent RNA polymerase and N gene was established which has less miss effect than the human genome. In human lung epithelial cell lines, PAC-MAN inhibits SARS CoV-2 by 85% and 70% respectively. Studies have shown that the PAC-MAN system can target and cleave more than 90% of coronavirus genes with only 6 crRNAs, including SARS, Middle Eastern respiratory syndrome and coronavirus related to COVID-19.

However, how to safely and effectively deliver CRISPR components to human cells and continue to function is the main challenge for the clinical application of new antiviral therapies based on the system [9]. Adeno-associated virus (AAV) and lentivirus vectors are representative of viral vector delivery systems. In order to effectively deliver Cas to infected cells, Zhang Feng's team discovered the smallest Cas13 effector protein, Cas13bt (only half the size of other Cas13 proteins), which can be directly loaded into a single AAV vector for delivery [10], Nguyen et al. [11] used lung-specific AAV serotype vectors to transmit Cas13d effector protein and specific crRNA targeting SARS-CoV-2 ORF1ab and S genes to lung epithelial cells of SARS-CoV-2 infected individuals, degrading the genome of this virus and inhibiting virus replication.

In addition, antibody and Cas fusion (ABACAS) technology can achieve the connection of CRISPR components by fusing Cas13 nuclease with the S protein. ABACAS will recognize the S protein on SARS-CoV-2 and promote Cas13 to be selectively delivered to infected cells together with the virus. Once ABACAS is transmitted to infected cells, it will recognize and cleave viral RNA, Relying on the selective transmission of the virus itself can also reduce extracellular effects. In addition to selectively delivering the CRISPR-Cas system, the neutralizing activity of ABACAS antibody fragments may also interfere with the interaction between the S protein and angiotensin-converting enzyme 2 receptor, and minimize virus entry into host cells [12].

Nonviral vector delivery includes lipid nanoparticles, electroporation, nuclear transfection, and microinjection methods. Among them, lipid nanoparticles stand out due to their non-toxic, low immunogenicity, and ability to increase the expression time of Cas enzymes. Blanchard et al. [13]

transported the synthesized Cas13a mRNA and lipid nanoparticles targeting SARS-CoV-2 ORF1ab and N genes to the respiratory tract of infected hamsters through an atomization device. The results showed that CRISPR-Cas13a can reduce SARS-CoV-2 replication and alleviate symptoms in hamsters. The synthesized mRNA transiently expresses the Cas13a effector in hamsters, greatly reducing the immune response and the possibility of mRNA integration with the subject's own genetic material. Fareh et al. [14] reported a programmable CRISPR-*pspCas13b* targeting the mRNA after transcription of the S and N genes of SARS-CoV-2, which inhibits SARS-CoV-2 with infectious replication ability, including non-mutated strains in monkey and human epithelial cells, D614G variants, and the recently highly anticipated B.1.1.7 variant. Their comprehensive mutation analysis further demonstrates the tolerance of a single crRNA to single nucleotide mismatch and the maintaining catalytic activity against mutated RNAs appearing in new variants, providing a conceptual validation for the development of therapeutic strategies for a wide range of the mutants. However, these studies require more extensive preclinical studies and further understanding of their immunogenicity, delivery of CRISPR components, timing of Cas protein expression, and off-target effects.

3.1.2 Diagnosis

SARS-CoV-2 spreads globally due to inadequate testing programs, exacerbating the COVID-19 pandemic. Countries with the highest infection rates faced the largest testing issues [15, 16]. Medical diagnostics encompass a broad range, and presently, no assay can simultaneously detect pathogenic messenger RNA (mRNA) and single-nucleotide mutations from the entire genome while satisfying the requirements and limitations of any clinical setting. Much progress has been made in the search for holistic solutions by creating a diagnostic assay using the CRISPR-Cas13 with clustered regularly interspaced short palindromic repeat. Diagnostic tests for CRISPR-Cas13 show an excellent degree of sensitivity, specificity, cost, speed, and portability.

CRISPR-Cas type 2 systems include Cas13a and Cas12a. After recognizing and cutting the target sequence, they experience incidental nucleic acid cleavage activity (also called trans-cleavage) [17]. This incidental activity is responsible for the sensitive and specific *in vitro* detection of virus-derived nucleic acids [18]. The researchers in this case created detection of nucleic acids techniques inspired by CRISPR-Cas13a [3] and CRISPR-Cas12a [19].

SHERLOCK was the first diagnostic assay based on CRISPR-Cas13a [3] (Fig. 1). If there is sample RNA containing the target sequence, Cas 13 will cleave the sample RNA and in parallel cleave the RNA sensor to release the fluorophore. The detection system has been applied to the nucleic acid detection of Zika virus and the point-of-care detection of COVID-19, with a maximum sampling rate of 100 samples per hour [20].

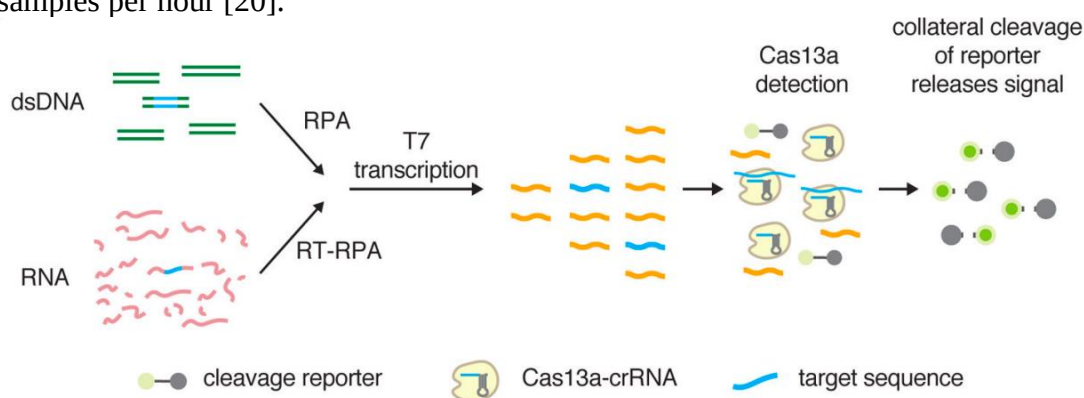


Fig. 1 Schematic of SHERLOCK [22].

Joung et al. [21] also made changes to the STOPCovid approach, which used the heat-stable Cas enzyme AapCas 12b for LAMP/Cas 12 detection of SARS-CoV-2. With the use of CRISPR-Cas 12a and RT-LAMP coupling methods for RNA for SARS-CoV-2 sensitivity detection extracted from respiratory swabs, Broughton et al. reported the efficient (less than 40 min) and sensitive (10 copies per response) identification of SARS-CoV-2 [22]. In order to immediately and quantitatively identify

RNA extracted from clinical samples and directly detect RNA from nasal swabs without amplification, an amplification-free CRISPR-Cas13a assay was established by Fozouni et al. This assay can accurately detect SARS-CoV-2 RNA in nasal swabs within 5 minutes [23].

3.2. HIV/AIDS

In 2022, there were about 1.3 million new cases of HIV infections and approximately 630,000 AIDS-related deaths [24]. Undoubtedly, HIV is a continuing and life-threatening infectious disease. Recently, some studies have found that CRISPR-Cas technology is able to provide new methods for the treatment of HIV.

In the previous research, the CCR5 or CXCR4 gene disruption has been demonstrated to provide resistance to HIV-1 infection that is R5-tropic or X4-tropic, respectively. Yu et al. simultaneously ablated CXCR4 and CCR5 (co-receptors for HIV-1) genes in CD4-positive T cells by CRISPR-Cas9, which can produce resistance to X4- and R5-tropic infection [25]. Yin and other researchers assessed the effectiveness of this technique for preventing HIV-1 infection by concentrating on the gene coding regions and the viral long terminal repeat. According to their findings, Cas9/gRNA is able to target and modify HIV-1 DNA in the cytoplasm and nucleus. The indels in the viral DNA as well as the decrease in viral DNA levels are both considered to be responsible for Cas9's inhibitory effect on HIV-1 [26]. Darcis et al. discovered that dual-guide RNA combinations can permanently inhibit HIV-1 replication and reduce the probability of viral escape have been discovered. Meanwhile, the tentative treatment of different HIV-1 isolates with double gRNA combinations was evaluated. They also found the replication of almost all virus isolates can be blocked by one gRNA combination at least. It was confirmed that the Cas9/NHEJ-mediated gene mutation and the excision of the integrated viral DNA inactivated the provirus and had inhibitory effects on different HIV-1 isolates [27]. Hartweiger et al. have been able to edit human B cells in vitro using this system to express broad-spectrum neutralizing antibodies against HIV-1 and retain their ability to participate in humoral immunity for use in HIV treatment [28].

Recently, some research shows that employing CRISPR-Cas to target the aspartic acid residue in position 366 and changing it to asparagine (D366N), abrogating miR-146a expression by employing lentiviral CRISPR-Cas9-mediated gene editing and using specific target sgRNAs and a small-sized SaCas9 are also potential for the treatment of HIV [29-31].

Although the attempts to use CRISPR-Cas-based technology in HIV are promising and potential, viral escape, off-target effects, immunogenicity, and effective delivery in patients continue to be significant obstacles to creating an effective and curative therapy. About the evaluation of off-target effects, compared with the detection method of direct amplification after software prediction of off-target sites, and then Sanger sequencing or deep sequencing, the off-target effect detected by whole genome sequencing can more comprehensively evaluate the off-target effect, because it can detect both coding and non-coding regions [32].

3.3. HBV

The disease called Chronic hepatitis B virus (HBV) infection is a worldwide concern of both infected and healthy people nowadays, with approximately one million people dying every year due to its prevalence and fatality [33]. Recently, scientists found it potential that the CRISPR-Cas9 system can cut the conserved regions in the HBV genome and make the cccDNA genome (a template for virus replication and amplification) unable to work. This is a promising progress as it reduces the cccDNA and other related viral genes's expression and translation and can furthermore play a therapeutic role.

Lin's team came first when they tried to apply the CRISPR-Cas9 method in the laboratory in order to attack this specific virus. The CRISPR-Cas9 mechanism directed by the use of a synthetic gRNA base paired to the target DNA sequence is a novel technique for cleaving targets' DNA at specified sites. If the HBV-expression vector (a genetic material isolated from HBV) was transferred into the Huh-7 cells, this system would drastically decrease the synthesis of HBV core and surface proteins.

Two effectively screened gRNAs proved useful among the trial targets. This team subsequently demonstrated that this approach potentially breaks the plasmid carrying the intrahepatic HBV genome and enhances its removal in vivo, utilizing an animal model for hydrodynamics-HBV persistence and causing serum surface antigen levels to drop. These results suggested that through the inactivation of the HBV-expressing templates both in or outside the body, it will be possible for this CRISPR system to completely eradicate persistent HBV infection [34].

In hydrodynamics-HBV in vivo models, several researches suggest that infusing CRISPR-Cas9 vectors along with HBV-encoding plasmid DNA into a test animal's tail vein could boost the synthesis of antiviral gRNAs, and this proves that it would be efficient in terms of destroying the virus production.

Dong and his team members created a cccDNA expression model in vivo by using a CRISPR-Cas9 target. In order to test, four sgRNAs were created and were directed at the conserved HBV areas. The gene expression of these sgRNAs with the new method resulted in a reduction in viral production not only in Huh7 cells but also the HBV-replication cell HepG2.2.15 respectively. Additionally, they discovered that the HBV cccDNA in transfected cells experienced direct breakage and breakage-mediated MUT. The low level of HBV prion protein and cccDNA in the novel mouse model with HBV cccDNA was caused by infusion of the same sgRNA-Cas9 plasmids as Lin's team through the fast tail vein. In conclusion, the created CRISPR-Cas9 technology can accurately and effectively hit HBV cccDNA and stop the virus from multiplication. This strategy could potentially used as an effective remedy for persistent HBV infection [35].

A recent study found that the introduction of SaCas9 and S gene targeting gRNA into HepG2.2.15 cells substantially decreased HBV replication indicators. The researchers looked into the usage of ssAAVs to deliver shorter DNA sequence-encoded infections of *Staphylococcus aureus* (Sa) CRISPR-Cas9. In AAVs that also produced Cas9, these RNAs were created using equivalents in the S of HBV Open Reading Frame(ORF). An intended targeted alteration of the HBV DNA became apparent after the all-in-one plasmids were used to transduce the cells. The effectiveness of cccDNA inactivation over HBV-infected hNTCP-HepG2 cells served as proof of its efficiency. No unexpected sequence alterations were found after analysis of potential off-target mutagenesis. It is a new way to use ssAAVs to supply every element needed for SaCas9 to disable cccDNA, and the technology has the potential to treat HBV infection. SpCas916 is around 1 kb smaller than the recently discovered *Staphylococcus aureus*-derived orthologous Cas9. To alter naturally occurring genes in the living cells of the intestines sequences, encoding SaCas9 has been paired with regulating elements, the sgRNA cassette, and a ssAAV. They added cassettes encoding SaCas9 and sgRNAs to ssAAVs to expand the use of technologies that are based on CRISPR-Cas9 in order to treat the HBV infection. Within cells that were cultured, the HBV's ORF was specifically targeted, thereby inactivating HBV replication and causing cccDNA mutation [36].

4. Conclusion

Compared with traditional methods, CRISPR-Cas technology has many advantages: high sensitivity, high specificity, portability, low cost, low level of by-products, simple operation, and easy reading of results. The development of this technology has promoted the rapid expansion of gene editing applications. Currently, besides COVID-19, HIV, and HBV, CRISPR-Cas technology has also been widely studied and discussed in the treatment of other diseases. Additionally, this technology can also be used to discover new disease treatment targets and detect biomarkers.

Considering the fact that various types of clinical drug resistance are becoming more and more serious currently, CRISPR-Cas technology can be applied to the detection of clinical bacterial drug resistance as another perspective of using this technology to treat infectious diseases, which is crucial to alleviate the current situation of drug resistance and common clinical drug-resistant bacterial infections. This technology can also be used in vaccine development by combining with other technologies for preventing infectious diseases.

The system is indeed a potential and promising technology used in the field of gene therapy, however, there are still some problems in applying it. In the future, developing safe and effective vehicles for the CRISPR-Cas, designing specific sgRNAs, exploring new strategies to decrease off-target effects, and understanding the exact mechanism of viral escape could help to use CRISPR-Cas technology to treat infectious diseases.

In conclusion, although CRISPR-Cas-based gene editing technology has some problems now, its superior performance shows broad application prospects and will bring revolutionary changes to the detection and treatment of infectious diseases. Deep analysis of the new types of CRISPR-Cas systems will make it possible to create more rapid, simple, and accurate methods.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

References

- [1] Li J, Xu R, Qin R, et al. Genome editing mediated by SpCas9 variants with broad non-canonical PAM compatibility in plants. *Molecular Plant*, 2021, 14(2): 352–360.
- [2] Shmakov S, Abudayyeh Omar O, Makarova Kira S, et al. Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems. *Molecular Cell*, 2015, 60(3): 385–397.
- [3] Gootenberg J S, Abudayyeh O O, Lee J W, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, 2017, 356(6336): 438–442.
- [4] Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science (New York, N.Y.)*, United States: 2007, 315(5819): 1709–12.
- [5] Garneau J E, Dupuis M-È, Villion M, et al. The CRISPR-Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature*, 2010, 468(7320): 67–71.
- [6] Jinek M, Chylinski K, Fonfara I, et al. A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science*, 2012, 337(6096): 816–821.
- [7] Abudayyeh O O, Gootenberg J S, Konermann S, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*, 2016, 353(6299): aaf5573.
- [8] Abbott T R, Dhamdhare G, Liu Y, et al. Development of CRISPR as an Antiviral Strategy to Combat SARS-CoV-2 and Influenza. *Cell*, 2020, 181(4): 865-876.e12.
- [9] Safari F, Afarid M, Rastegari B, et al. CRISPR systems: Novel approaches for detection and combating COVID-19. *Virus Research*, 2021, 294: 198282.
- [10] Kannan S, Altae-Tran H, Jin X, et al. Compact RNA editors with small Cas13 proteins. *Nature Biotechnology*, 2021, 40(2): 194–197.
- [11] Nguyen T M, Zhang Y, Pandolfi P P. Virus against virus: a potential treatment for 2019-nCov (SARS-CoV-2) and other RNA viruses. *Cell Research*, 2020, 30(3): 189–190.
- [12] Lino C A, Harper J C, Carney J P, et al. Delivering CRISPR: a review of the challenges and approaches. *Drug Delivery*, 2018, 25(1): 1234–1257.
- [13] Blanchard E L, Vanover D, Bawage S S, et al. Treatment of influenza and SARS-CoV-2 infections via mRNA-encoded Cas13a in rodents. *Nature Biotechnology*, 2021: 1–10.
- [14] Fareh M, Zhao W, Hu W, et al. Reprogrammed CRISPR-Cas13b suppresses SARS-CoV-2 replication and circumvents its mutational escape through mismatch tolerance. *Nature Communications*, 2021, 12(1).
- [15] Pascarella G, Strumia A, Piliago C, et al. COVID-19 diagnosis and management: a comprehensive review. *Journal of Internal Medicine*, 2020, 288(2).
- [16] Ravi N, Cortade D L, Ng E, et al. Diagnostics for SARS-CoV-2 detection: A comprehensive review of the FDA-EUA COVID-19 testing landscape. *Biosensors & Bioelectronics*, 2020, 165: 112454.
- [17] Liu L, Li X, Wang J, et al. Two Distant Catalytic Sites Are Responsible for C2c2 RNase Activities. *Cell*, 2017, 168(1-2): 121-134.e12.

- [18] Palaz F, Kalkan A K, Can Ö, et al. CRISPR-Cas13 System as a Promising and Versatile Tool for Cancer Diagnosis, Therapy, and Research. *ACS Synthetic Biology*, 2021, 10(6): 1245–1267.
- [19] Chen J S, Ma E, Harrington L B, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, 2018, 360(6387): 436–439.
- [20] Joung J, Ladha A, Saito M, et al. Point-of-care testing for COVID-19 using SHERLOCK diagnostics. *medRxiv*, 2020.
- [21] Joung J, Ladha A, Saito M, et al. Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing. *New England Journal of Medicine*, 2020, 383(15): 1492–1494.
- [22] Broughton J P, Deng X, Yu G, et al. CRISPR–Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*, 2020, 38.
- [23] Fozouni P, Son S, Díaz de León Derby M, et al. Amplification-free detection of SARS-CoV-2 with CRISPR-Cas13a and mobile phone microscopy. *Cell*, 2020, 184(2).
- [24] The path that ends AIDS: UNAIDS Global AIDS Update 2023. [www.unaids.org. https://www.unaids.org/en/resources/documents/2023/global-aids-update-2023](https://www.unaids.org/en/resources/documents/2023/global-aids-update-2023).
- [25] Yu S, Yao Y, Xiao H, et al. Simultaneous Knockout of CXCR4 and CCR5 Genes in CD4+ T Cells via CRISPR-Cas9 Confers Resistance to Both X4- and R5-Tropic Human Immunodeficiency Virus Type 1 Infection. *Human Gene Therapy*, 2018, 29(1): 51–67.
- [26] Yin L, Hu S, Mei S, et al. CRISPR-Cas9 Inhibits Multiple Steps of HIV-1 Infection. *Human Gene Therapy*, 2018, 29(11): 1264–1276.
- [27] Darcis G, Binda C, Klaver B, et al. The Impact of HIV-1 Genetic Diversity on CRISPR-Cas9 Antiviral Activity and Viral Escape. *Viruses*, 2019, 11(3): 255.
- [28] Hartweger H, McGuire A T, Horning M, et al. HIV-specific humoral immune responses by CRISPR-Cas9-edited B cells. *Journal of Experimental Medicine*, 2019, 216(6): 1301–1310.
- [29] Lampi Y, Van Looveren D, Vranckx L S, et al. Targeted editing of the PSIP1 gene encoding LEDGF/p75 protects cells against HIV infection. *Scientific Reports*, 2019, 9(1).
- [30] Teng Y, Luo M, Yu T, et al. CRISPR-Cas9-mediated deletion of miR-146a enhances antiviral response in HIV-1 infected cells. *Genes & Immunity*, 2018.
- [31] Xiao Q, Chen S, Wang Q, et al. CCR5 editing by Staphylococcus aureus Cas9 in human primary CD4+ T cells and hematopoietic stem/progenitor cells promotes HIV-1 resistance and CD4+ T cell enrichment in humanized mice. *Retrovirology*, 2019, 16(1).
- [32] Xu Y. Characterization of HIV-1 drug resistance mutations and a novel method for HIV-1 treatment using CRISPR-Cas9 technology. Zhejiang University: 2021.
- [33] Wang S, Li H, Kou Z, et al. Highly sensitive and specific detection of hepatitis B virus DNA and drug resistance mutations utilizing the PCR-based CRISPR-Cas13a system. *Clinical Microbiology and Infection*, 2021, 27(3): 443–450.
- [34] Lin S-R, Yang H-C, Kuo Y-T, et al. The CRISPR-Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates In Vivo. *Molecular Therapy - Nucleic Acids*, 2014, 3: e186.
- [35] Dong C, Qu L, Wang H, et al. Targeting hepatitis B virus cccDNA by CRISPR-Cas9 nuclease efficiently inhibits viral replication. *Antiviral Research*, 2015, 118: 110–117.
- [36] Scott T, Moyo B, Nicholson S, et al. ssAAVs containing cassettes encoding SaCas9 and guides targeting hepatitis B virus inactivate replication of the virus in cultured cells. *Scientific Reports*, 2017, 7(1).