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ORIGINAL ARTICLE

The CRISPR-Cas system as a tool for diagnosing and treating infectious diseases

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Abstract
Emerging and relapsing infectious diseases pose a huge health threat to human health and a new challenge to global public health. Rapid, sensitive and simple diagnostic tools are keys to successful management of infectious patients and containment of disease transmission. In recent years, international research on Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-related proteins (Cas) has revolutionized our understanding of biology. The CRISPR-Cas system has the advantages of high specificity, high sensitivity, simple, rapid, low cost, and has begun to be used for molecular diagnosis and treatment of infectious diseases. In this paper, we described the biological principles, application fields and prospects of CRISPR-Cas system in the molecular diagnosis and treatment of infectious diseases, and compared it with existing molecular diagnosis methods, the advantages and disadvantages were summarized.

Keywords CRISPR-Cas · Infectious diseases · Application · COVID-19

Introduction

Emerging and relapsing infectious diseases pose a huge health threat to human health and a new challenge to global public health. Since the 21st century, many emerging infectious diseases have emerged and spread in the world. Zika, swine flu, avian flu, Ebola, SARS and MERS, and the COVID-19 pandemic in late 2019 have done great harm to human health, present some recent examples of a global epidemic of emerging infectious diseases [1–6]. The early detection and timely diagnosis and treatment of emerging infectious diseases are of great significance to the prevention and control of emerging infectious diseases. Traditional methods of diagnosing emerging infectious diseases include traditional culture tests, multiple real-time PCR tests, metagenomic-based tests, and imaging tests [7–9]. However, the existing diagnostic methods have disadvantages such as low detection accuracy, slow detection speed and high detection cost [10–12]. Improvements in existing diagnostic methods for emerging infectious diseases are increasingly being explored by researchers. In recent years, diagnostic technologies based on Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-related proteins (Cas) have gradually matured, It has brought about significant changes in our diagnostic techniques [13]. Its application will provide a new idea for the rapid diagnosis of infectious diseases in hospitals, communities and families, as well as a new method for the timely treatment and intervention of infectious diseases and epidemiological monitoring. In this paper, we described the biological principles, application fields and prospects of CRISPR-Cas system in the molecular diagnosis and treatment of infectious diseases, and compared it with existing molecular diagnosis methods, the advantages and disadvantages were summarized.

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CRISPR-Cas biology and principle

The CRISPR and CRISPR-associated endonuclease (Cas) is an important part of the adaptive immune system of bacteria [14]. It can be used to protect bacteria by recognizing specific exogenous nucleic acid sequences and cutting them specifically [15] [16]. The CRISPR adaptive immune system can protect prokaryotic cells from foreign nucleic acid invasion by using nucleases guided by CRISPR RNA (crRNA). It can target RNA cleavage, catalyze RNA degradation, and different Cas proteins have different catalytic activity, can recognize orthogonal crRNA sets, and have different ssRNA cutting specificity [17]. Some studies have found that CRISPR system, as a gene programming tool, can improve the gene diagnosis ability, and at the same time, target to kill RNA viruses [18, 19]. The application of CRISPR has important implications for the treatment of human genetic diseases, the discovery of new drugs and rapid diagnosis of diseases [20–22]. In the context of emerging infectious diseases, the CRISPR-Cas system, as a fast, accurate and convenient genome editing tool, provides a fast, inexpensive and highly sensitive diagnosis when used for nucleic acid detection [23, 24].

Classification of CRISPR-Cas system

Existing CRISPR systems are classified into 2 classes, 6 types and 33 subtype. The first class of CRISPR system has effectors composed of multiple cas proteins that act on the binding and processing of target proteins, while the second class of CRISPR system consists of a single protein with multiple domains [25]. The class 1 system accounts for about 90%, which can target DNA and RNA to play their biological role[26]. It includes types I, III, and IV, of which types I and III are more common, often occurring in archaea, and can drive targeting and DNA cleavage [27]. However, type IV system is rare and lacks highly conserved adaptive modules and nucleic effector enzymes, which are the signature components of CRISPR-Cas system, and can play an important regulatory role by targeting foreign plasmids [28]. The class 2 system includes types II, IV, and VI. It contains three effector proteins, Cas9, C2c1 and Cpf1 [29]. The protein effect modules in class 2 CRISPR-Cas system are often derived from mobile genetic elements, which have been successfully used in genome engineering. In recent years, the diversity of the CRISPR-Cas system has increased dramatically as technology advances and developments allow researchers to combine computation and experiments, and the classification of the CRISPR system has entered a new era with the increase of new variants [30].

Current molecular diagnostic tools for infectious diseases

In the diagnosis of emerging pathogens, mNGS (Metagenomic Next-Generation Sequencing) and RT-PCR (Real-time reverse transcription polymerase chain reaction) are the most commonly used molecular diagnostic methods [31, 32]. MNGS performed a comprehensive analysis of all nucleic acids in the sample to directly identify infectious microorganisms[33]. RT-PCR is currently recognized as the gold standard nucleic acid amplification experiment [34, 35]. They play an important role in determining the pathogen of emerging infectious diseases, but they also have the disadvantages of long detection time and high experimental conditions, which can be addressed by the CRISPR system. Nucleases such as Cas12a, Cas12b and Cas13a, which bind to crRNA targets, have unique enzymological properties and can detect nucleic acids by cleavage of surrounding single-stranded DNA [36, 37]. Using CRISPR-Cas system in clinical samples to detect pathogens of emerging infectious diseases is superior to other testing methods in terms of time and cost [38]. Table 1 compared the three molecular detection methods. Rapid nucleic acid testing is considered to be an important component of many techniques related to human health and biology. It can help identify and diagnose many infectious diseases. In recent years, the CRISPR-Cas system has begun to be used for molecular diagnosis of diseases. And many tools based on CRISPR-Cas system methods have been used to diagnose and treat emerging infectious diseases [39]. The engineering of the CRISPR tool involves structural modification of the Cas protein to enhance specificity to target nucleic acids [40]. Using the CRISPR-Cas system is critical for rapid diagnosis and treatment of infectious diseases during the global epidemic.

How CRISPR-Cas system is used to diagnose and treat infectious diseases

In 2013, CRISPR-Cas system's multi-genome editing capabilities made possible powerful applications in basic science, biotechnology and medicine [41]. This also brought new ideas and methods for the diagnosis and treatment of emerging infectious diseases. The CRISPR-Cas system is a prokaryote driven gene editing tool [42]. It can recognize specific DNA and RNA sequences in various living cells [43]. The CRISPR-Cas system detects required DNA or RNA sequences on a disease diagnostic platform through the CRISPR-Cas enzymology and amplification process [44]. Cas9, Cas12, and Cas13 protein are common nucleic acids used to diagnose infectious diseases[45–47]. Figure 1 introduced the mechanism detection platform based on CRISPR/

Table 1 Comparison of three molecular diagnostic methods

Method	Time required	Response procedures	Advantages	Disadvantages
mNGS	20 h	Library preparation, mNGS sequencing, bioinformatics analysis	Comprehensive analysis of all nucleic acids, rapid and preliminary identification of new pathogens	Expensive equipment, complexity of operation, and not all genomes were available
RT-PCR	1.5 h	Reverse transcription, RNA-cDNA hybridization denaturation, PCR amplification	Gold standard, currently the most common detection method	Complex laboratory infrastructure required, specialized technical personnel
CRISPR-Cas	0.6 h	DNA amplification, Cas reaction	Low cost, high sensitivity, no need for complex instruments and equipment, fast and convenient for field testing	Not widely used in clinical trials, pending clinical validation

Cas9, CRISPR/Cas12 and CRISPR/Cas13. CRISPR-associated protein 9 (Cas9), derived from the CRISPR Type II bacterial immune system, was an RNA-guided DNA endonuclease that could program for new sites by changing the sequence of its guided RNA [48]. It was developed as a tool to make sequence specific gene editing easier. Since the development of RNA editing technology in the Type II CRISPR-Cas9 system in 2012, a series of CRISPR technologies have been developed for the diagnosis and treatment of emerging infectious diseases [49]. Pardee et al. used nucleic acid sequence amplification (NASBA) in combination with CRISPR-Cas9 to accurately distinguish Zika virus strains in the plasma of a macaque. The researchers attached the synthesized trigger sequence to the viral RNA amplified by NASBA and used the sgRNA-Cas9 complex to cut off dsDNA. The diagnosis was performed by activating sensor switches that, when incorporated into the diagnostic workflow, provided precise genetic information for use in the field within hours [50]. Quan et al. showed that CRISPR-Cas system can be used to detect and diagnose antibiotic resistant sequences, thus treating resistant microorganism [51]. Dronina et al. discussed the role of genome editing tool CRISPR-Cas9, and the results showed that DNA modification enzymes including CRISPR-Cas9 system can be used as DNA and RNA sensors, and then used for rapid detection of infectious pathogens and detection of the occurrence of infectious diseases [52]. The CRISPR-Cas12a (Cpf1) protein was an RNA-guided enzyme that bound and cuted DNA as part of the bacterial adaptive immune system. This was based on the production of targeted double-stranded DNA breaks. Chen et al. demonstrated that RNA-guided DNA binding could release the cleavage activity of arbitrary single-stranded DNA (ssDNA) by completely degrading the Cas12a of the ssDNA molecule, and that the targeted activation of non-specific single-stranded deoxy RNA nuclease (ssDNase) cleavage was also characteristic of other V-type CRISPR-Cas12 enzymes. The researchers also created a method called DNA endonuclease-targeted CRISPR trans reporter (DETECTR), which combined Cas12a ssDNase activation with isothermal amplification and had extremely low sensitivity to DNA detection. It is a molecular diagnostic technology that can detect human papillomavirus (HPV) quickly and specifically [53]. The Cas12a-CrRNA complex binds and sects the target HPV dsDNA, thereby activating trans-secting of ssDNA. Fluorescent reporter genes coupled with ssDNA produce fluorescent signals when they are cleaved. It also provided a platform for efficient and convenient detection of HPV subtypes associated with cervical cancer [54]. Studies have also shown that Cas12a is more accurate and effective for diagnosis [55]. Ding et al. developed a rapid and accurate method for real-time detection of Hepatitis B virus (HBV) based on LAMP-Cas12a. It solved the problems of extracting nucleic acid from samples and detection in 10 min on site. The analysis results are displayed with fluorescent readings and transverse flow test bars. Transverse flow test strip technology makes results be visible to naked eyes, while fluorescence readings enable real-time, highly sensitive detection. The LAMP-Cas12-based HBV test relies on minimal equipment, provides fast, accurate results and low cost, and provides important practical value for real-time detection of HBV [56]. Luo et al. first found CRISPR-Cas12a had trans-cutting activity, and combined its trans-cutting capability with Au-nanobeacon to establish a new medical early diagnosis tool with rapid and high sensitivity [57]. Cas13 is a programmable single-effect RNA-guided ribonuclease, which belongs to type VI CRISPR-Cas system. It can be used to target a wide range of ssRNA viruses, and the developed Cas13-assisted virus expression and readout restriction system (CARVER) has a wide range of potential utility for rapid diagnosis of emerging infectious diseases and antiviral drug development. In 2018, Gootenberg et al. developed a platform called SHERLOCK (Specific Highly Sensitive Enzyme Report Unlocking) that detected individual molecules of RNA or DNA through isothermal pre-amplification combined with Cas13. The platform highlighted its potential as a multi-channel, simple, rapid and accurate nucleic acid detection platform through the characterization and application development of

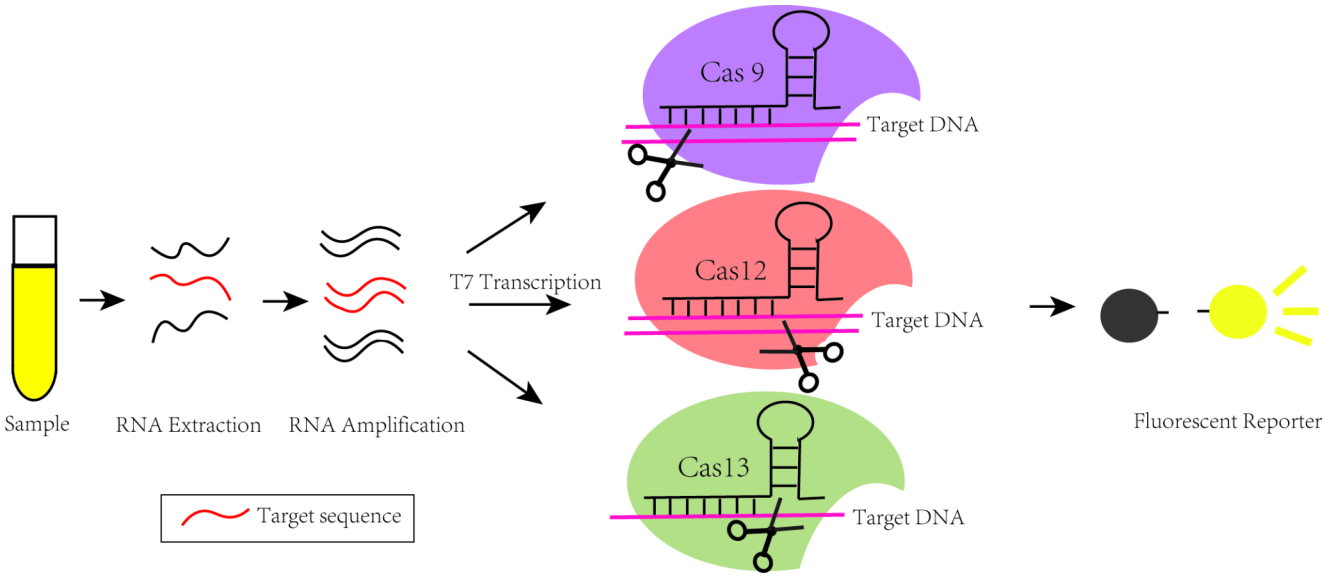

Fig. 1 Based on the mechanism of CRISPR/Cas9, CRISPR/Cas12, CRISPR/Cas13 detection platform

CRISPR enzymological properties for side-flow detection of Dengue or Zika virus [58]. To sum up, CRISPR-Cas system has been used to detect RNA viruses, diagnose diseases, and identify various bacterial pathogens [59]. The CRISPR-based platform is considered a good diagnostic platform for infectious diseases. The emerging CRISPR diagnostic tool will have great potential for detecting viral and tumor-derived nucleotides, DNA methylation, and single nucleotide polymorphisms [60].

During chronic and latent viral infections, the virus can remain in the host cell indefinitely, and treatment becomes challenging when the virus takes over the host cell [61]. Based on the fact that viral genome replication completes its life cycle within the cell, CRISPR-Cas system mediated degradation of highly conserved and essential genetic elements to suppress the virus is considered to be a cheaper and effective antiviral strategy [62]. CRISPR-based systems can control viral genomes within host cells, making them unable to transcribe and replicate [63]. Therefore, CRISPR gene editing could treat chronic infections [64]. It is particularly suited to overcoming outbreaks of viral infections because their effective control relies on effective treatment, but also on rapid diagnosis to prevent disease transmission. The CRISPR toolkit provides DNA-and RNA-targeting nucleases, which will have important implications for fighting viral infections. They can be used to manipulate viral and host genomes for therapeutic purposes, as well as to prepare sensor devices for detecting viral nucleic acids. Research by Liao et al. has shown that the CRISPR-Cas9 system can destroy the latent viral genome and play a long-term defense against viral infection in human cells. They found that human induced pluripotent stem cells that express HIV-targeted CRISPR-Cas9 can effectively differentiate into human immunodeficiency virus (HIV) reserve cell types and maintain inhibition to HIV-1 [65]. The study of Wang et al. also showed that the gene editing technology based on CRISPR-Cas9 combined with drug therapy had a significant inhibitory effect on the growth of cancer cells [66]. These results revealed the great value of the CRISPR-Cas9 system in the treatment of viral infections [65]. It has been used as an antiviral treatment because it provided protective immunity against invading viruses [67]. Abbott et al. discovered a CRISPR-based therapy called the prophylactic antiviral CRISPR (PAC-MAN-T6) approach in human cells, using Cas13, with strong target lysis activity [68]. Aggregation of regularly spaced short palindromic repeats (CRISPR)/CRISPR-related proteins (Cas) and EDITING systems derived from CRISPR-Cas have changed the landscape of gene therapy. Their ability to edit genome sequences and enable gene destruction and modification has expanded the range of gene therapy targets and accelerated the development of many rare diseases that can be treated by transplantation or modification of hematopoietic stem cells. In short, the CRISPR-Cas system is an effective tool for treating biological diseases or chemotherapy as a therapeutic strategy to correct genetic defects [69]. It has become a promising therapeutic and molecular diagnostic technique.

COVID-19 is a novel pneumonia caused by the novel coronavirus. It is characterized by rapid transmission and high pathogenicity, which has caused great harm to human health [70]. Recent studies have found that the successful combination of CRISPR technology and nucleic acid amplification technology can achieve high sensitivity and rapid detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), thus having important implications for the diagnosis and treatment of COVID-19 [71].

Broughton reported a rapid (< 40 min), simple, and accurate CRISPR-Cas12-based side-flow assay for SARS-CoV-2 in RNA extracts from respiratory swabs. CRISPR-based DETECTR provides a visual, fast alternative to RT-PCR detection for SARS-COV-2, with a 95% positive and 100% negative predictive consistency [72]. Moon et al. reported a colorimetric virus detection method based on the clustered regularly spaced short palindromic repeats (CRISPR)/Cas9 intraconuclide death (dCas9) system. In this approach, RNA is directly identified by the CRISPR/dCas9 oligonucleotide system (PAMmer), which has a biotin-primordium protospacer adjacent motif (PAM). Streptavidin-horseradish peroxidase then binds to biotin and induces color changes by oxidizing 3, 3', 5, 5' -tetramylbenzidine. This method can identify severe acute respiratory SARS-CoV-2, pH1N1 and other viruses by color change [73]. Lin et al. found that the use of glycerin additive can further improve the detection efficiency of recombinant enzyme polymerase amplification (RPA)-CRISPR/Cas12a method. The improved CRISPR-Cas12a method is simple, sensitive and versatile, and can be used as a real-time nucleic acid monitoring and detection platform [74]. Meanwhile, the CRISPR-Cas system is also playing an important role in the development of improved antiviral. Wang et al. identified the specific viral fragment of SARS-COV-2 through bioinformatics methods, designed the crRNA library based on this, and screened out the most effective crRNA sequence, which has a strong cleavage effect on S protein and a strong side branch cleavage effect. Thus, a new antiviral tool against SARS-COV-2 based on CRISPR-Cas13 was provided [75]. Zeng et al. demonstrated that CRISPR-Cas13d, as a new broad-spectrum antiviral drug, can inhibit multiple SARS-CoV-2 variants and multiple human coronavirus strains, providing an important complement to existing antiviral strategies [76]. Liu et al. demonstrated that Cas13b has specificity, high efficiency and rapid deployment, which may provide a theoretical basis for the development of antiviral drugs that can inhibit and prevent a wide range of SARS-CoV-2 mutants [77]. Therefore, the application of CRISPR/Cas system in the diagnosis and treatment of emerging infectious diseases will be very promising and meaningful.

Application of CRISPR-Cas system in diagnosis and treatment of infectious diseases

Since the discovery of the CRISPR system, many CRISPR-related diagnostic and therapeutic techniques have been developed. The CRISPR-Cas System plays an important role in the diagnosis and treatment of various infectious diseases due to its advantages of high specificity, sensitivity, simplicity, speed and low cost [78](Fig. 2). The development of the CRISPR-Cas system has made the immediate detection of pathogens possible. Based on LAMP-Cas12a, Cas12a can detect HBV in 13-20 min [56]. The regulation of CRISPR-Cas9 genome editing on HIV-1 genome has become an effective tool for CRISPR-Cas9 system to treat HIV-1 [79]. Using a combination of isothermal amplification and CRISPR-Cas12a-mediated detection, HIV-1 genotypes were detected in 30 min under non-laboratory conditions [80]. Tuberculosis (TB) is difficult to diagnose quickly. The CRISPR-MTB method could complete DNA extraction, amplification and Cas12a detection in 1.5 h. This method can be used to diagnose mycobacterium tuberculosis rapidly on the same day [81]. Table 2 summarized the detection of some infectious diseases based on CRISPR-Cas system.

COVID-19, which is caused by SARS-CoV-2, has caused a global pandemic. So far, there have been multiple mutant strains, which are more contagious and have a higher fatality rate, posing a great challenge to the prevention and control of the epidemic. The management of emerging infectious diseases, such as COVID-19, requires a variety of measures, including social distancing and medical isolation of confirmed patients. Therefore, a highly sensitive diagnostic kit with rapid early detection capability is needed. RT-PCR is the most accurate method to detect COVID-19, but it requires advanced equipment and specialized personnel, and is not suitable as a screening method for COVID-19 [82]. Many people infected with COVID-19 have no clinical symptoms but remain highly contagious [83, 84]. Rapid detection of asymptomatic infected persons is key to controlling the epidemic, and the nucleic acid detection technology of the CRISPR-Cas system opens up a new path in mass screening. Such as SHERLOCK (Cas13a), DETECTR (Cas12a), CDetection (Cas12b) and CAS14-DETECTR in a variety of pathogen detection applications [64]. Since the outbreak of COVID-19, research on CRISPR-Cas detection of SARS-COV-2 has developed rapidly. An AUNP-based visual analysis method combined with Cas12a-assisted RT-LAMP (CLAP) could detect 4 copies /μL of SARS-CoV-2 RNA within 40 min under optimal conditions [85]. A study found that wild type and mutated SARS-CoV-2 could be detected by loop-mediated isothermal amplification (LAMP) at 60–65°C using the engineered Cas12a enzyme. The use of hybrid DNA-RNA guides increased response speed, allowing the test to be completed within 30 min [86]. Cas13a crRNAs can specifically target SARS-CoV-2 or detect related coronavirus strains broadly, and the indirect effects of Cas13a are combined with a generic autonomous enzyme-free hybridization chain reaction (HCR) to trigger the downstream HCR circuit by designing a cleavage hairpin reporter release promoter sequence [87]. The detection

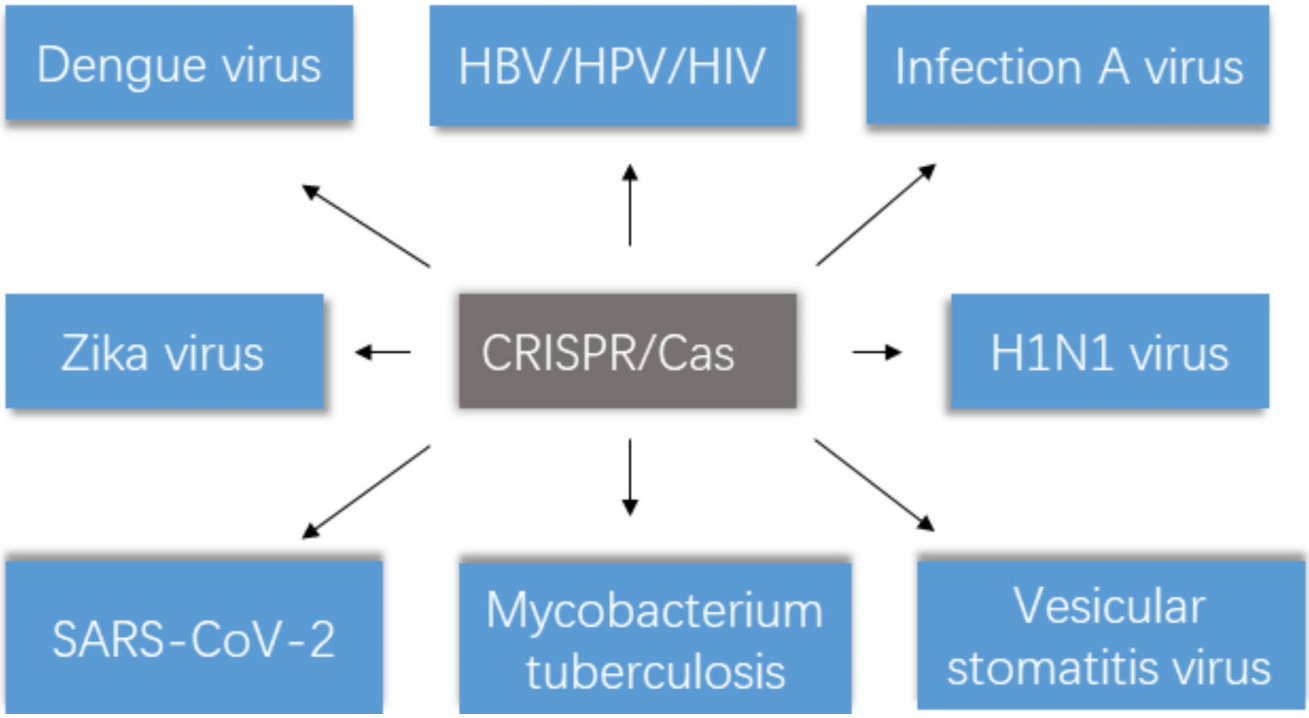

Fig. 2 Application of CRISPR/Cas system in diagnosis and treatment of infectious diseases

method based on the CRISPR-Cas13 amplification strategy is expected to be used to monitor the transmission of SARS-CoV-2 associated with objects [88].

In recent years, various detection methods combined with isothermal amplification and CRISPR-Cas system have become diagnostic tools for rapid detection of SARS-CoV-2 virus RNA. Huang et al. developed a CRISPR-based analysis method. Target amplifiers produced by standard RT-PCR or isothermal recombinase polymerase amplification (RPA) were detected using a customized CRISPR Cas12a/gRNA complex and fluorescent probes to enable sensitive detection at sites that do not have the RT-PCR system required for qPCR diagnosis. The method was highly sensitive to the detection of SARS-COV-2 virus, with a reaction time of about 50 min and a detection limit of 2 samples per sample. The CRISPR analysis diagnostic results obtained from nasal

Table 2 The characteristics of CRISPR-based diagnostic methods

CRISPR-based method	Pathogen	Enzyme/Effector	Type of Nucleic acid	Characteristics	Time required	References
Cas9	Zika virus	Cas9	dsDNA	Isothermal amplification nucleic acid sequence-based amplification (NASBA) was combined with Cas9 cleavage activity	180 min	50
Cas12	HPV	LbCas12a	ssDNA	Recombinase polymerase was coupled with LbCas12a for isothermal amplification	< 60 min	53
	HBV	Cas12a	ssDNA	The test results were visualized by fluorescence readout and lateral flow strips	13-20 min	56
	TB	Cas12a	dsDNA	The test was combined with the Recombinant Polymerase Amplification (RPA) step and the subsequent Cas12a detection step	90 min	81
	SARS-CoV-2	LbCas12a	ssRNA	Isothermal amplification was combined with CRISPR-Cas12 DETECTR technology	< 40 min	72
	HIV-1	Cas12a	ssDNA	The test was combined with the isothermal amplification and CRISPR-Cas12a-mediated detection	< 30 min	80
Cas13	SARS-CoV-2	Cas13a	ssRNA	A biosensing technology was based on HCR coupled CRISPR-Cas13a	< 60 min	87
	Dengue or Zika virus	LwaCas13a、PsmCas13b	ssRNA	A combination of isothermal preamplification and Cas13 was used to detect RNA or DNA single molecules	< 90 min	58

swab samples from suspected COVID-19 patients were comparable to quantitative RT-PCR tests and superior to the same clinical laboratory tests [89].

Currently, there is no specific treatment for some emerging infectious diseases, such as COVID-19, so it is critical to find some effective ways to diagnose and treat emerging infectious diseases. A recent study has shown that CRISPR-Cas13 has become an effective system for protecting host bacterial cells from phage infection via specific cRNA [90], so this strategy could also be used to design therapeutic drugs targeting the ssRNA genomes of emerging infectious diseases. Abbott et al. developed and demonstrated a CRISPR-Cas13-based strategy, PAC-MAN (Prophylactic Antiviral CRISPR in Human Cells), that degraded SARS-CoV-2 sequences and Influenza A virus (IAV) RNA in cells, identification of functional crRNA targeting SARS-COV-2. The method reduces viral load in cells. A group of just six crRNAs can target more than 90% of coronaviruses, making PAC-Man potentially an important pan-coronavirus suppression strategy [91]. The development of CRISPR-based tools to treat emerging infectious diseases has good research prospects.

Limitations of the CRISPR-Cas system in diagnostic therapy

The biggest limitation to the widespread use of the CRISPR-Cas system is its inability to recognize specific nucleic acids for diagnostic and therapeutic use [92, 93]. The efficient delivery of the CRISPR-Cas system can address this off-target effect by increasing the modification of the CRISPR protein to enhance the specificity of the target nucleic acid [94]. The off-target effect can usually be detected by bioinformatics methods. The improved Cas9 can reduce the off-target effect of the system [95]. In the future research, we should strengthen the study of off-target effect and improve the detection method.

Applying CRISPR-Cas system prevention and control of infectious diseases, including through the determination of target cells in the body delivery tools, often use as a carrier to carry virus. However, CRISPR's carrier function was limited to the size of the virus genes, and most of the Cas protein is big molecular weight protein [96]. In order to overcome this problem, researchers need to look for Cas proteins with low molecular weight [97]. In addition, the CRISPR-Cas system is used to treat infectious diseases by providing Cas proteins, which are often derived from prokaryotes. These proteins were toxic, and when they enter the human body, they can cause an immune response that produces specific antibodies. And specific antibodies interfere with CRISPR's immune response in the body. In the future development of CRIPSR tool, the efficiency of CRISPR-Cas system should be enhanced in order to reduce the immune response.

Cas9 and Cas12 proteins can recognize and cut dsDNA in a PAM-dependent manner, so as to specifically target specific gene sequences in target genes [98]. However, some highly specific sequences are not available, so it is necessary to strengthen the CRISPR tool for target selection. In addition, the widespread existence of RNA enzymes leads to RNA instability, thus affecting the diagnostic efficiency of CRISPR.

Conclusions

In modern infectious disease prevention and control, although we have made great progress, in the face of some newly emerging infectious diseases, such as COVID-19, we still need more accurate, portable, easy handled diagnose technology of low cost. As the virus continues to mutate and vaccinate, the number of asymptomatic infected people around the world is increasing. Therefore, early diagnosis of coronavirus recognition will be vital for prevention and control of COVID-19. Exporing more sensitive practical methods for early detection is critical for control of the COVID-19 pandemic.

The CRISPR-Cas system is a new generation of gene editing technology that is commonly used for rapid molecular diagnosis and treatment. CRISPR-based diagnostics are easy to use, easy to carry, and take less time than the real-time PCR-based assay currently in use. CRISPR-Cas system has been shown to be useful in diagnosing and treating dengue, Zika, HIV, tuberculosis, hepatitis B and more. DETECTR and SHERLOCK technologies based on CRISPR diagnosis are fast, effective and low-cost, while RNA viruses are unstable and easy to mutate. Therefore, it will be a challenge to develop a therapy to treat RNA viruses. In therapeutics targeting CRISPR-Cas technology, besides viral genome, host virus replication is also involved. CRISPR-Cas system can resist the entry of novel coronavirus into host cells by delivering CRISPR-13d. In the future, further efforts should be made to develop more sensitive and effective CRISPR technologies, as well as to evaluate the safety and potential toxicity of future CRISPR applications.

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Declarations

Competing interests The authors have declared that no competing interests exist.

Ethics approval Not applicable.

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