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REVIEW

The Trend of CRISPR-Based Technologies in COVID-19 Disease: Beyond Genome Editing

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Abstract

Biotechnological approaches have always sought to utilize novel and efficient methods in the prevention, diagnosis, and treatment of diseases. This science has consistently tried to revolutionize medical science by employing state-of-the-art technologies in genomic and proteomic engineering. CRISPR–Cas system is one of the emerging techniques in the field of biotechnology. To date, the CRISPR–Cas system has been extensively applied in gene editing, targeting genomic sequences for diagnosis, treatment of diseases through genomic manipulation, and in creating animal models for preclinical researches. With the emergence of the COVID-19 pandemic in 2019, there is need for the development and modification of novel tools such as the CRISPR–Cas system for use in diagnostic emergencies. This system can compete with other existing biotechnological methods in accuracy, precision, and wide performance that could guarantee its future in these conditions. In this article, we review the various platforms of the CRISPR–Cas system meant for SARS-CoV-2 diagnosis, anti-viral therapeutic procedures, producing animal models for preclinical studies, and genome-wide screening studies toward drug and vaccine development.

Keywords CRISPR–Cas systems · SARS-CoV-2 · Diagnosis · Anti-viral approach · Genome-wide association study · Animal model

Introduction

The SARS-CoV-2 virus, a member of the *Coronaviridae* family, *Betacoronavirus* genus, and the causative agent of Coronavirus Disease 2019 (COVID-19), is responsible for the third large-scale human outbreak of its family in the last two decades. This zoonotic virus is a non-segmented enveloped positive-sense single-stranded RNA virus with a large genomic size that has high animal-to-human and human-to-human transmission compared to its family members [1]. The genomic sequence of this new emerging virus has a high rate of mutation and recombination, due to its unique self-replication phenomenon [2]. At the beginning of the pandemic, the complete sequence of the viral genome was shared through metagenomic approaches and the three-dimensional structure of its key proteins was determined [3]. The fourteen open reading frames (ORF) of the SARS-CoV-2 genome encode sixteen nonstructural proteins to form replicase complex (such as 3-chymotrypsin-like protease (3CLpro), papain-like protease (PLpro), helicase, and RNA-dependent RNA polymerase (RdRp), nine accessory proteins, and four structural proteins (spike (S), membrane (M), envelope (E), and nucleocapsid (N)). Spike protein has two functional segments (S1 and S2) that are activated by host cell proteases (cathepsin L and transmembrane protease serine 2 (TMPRSS2) [4]. In SARS-CoV-2 the receptor-binding domain (RBD) of spike protein binds to the human cell

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receptors, Angiotensin-Converting Enzyme 2 (ACE2), and defines the tropism and the pathogenicity of the virus, While the spike protein of other human coronaviruses can bind to the other cellular entry receptors such as aminopeptidase N (APN) and dipeptidyl peptidase 4 (DPP4) too. Mutation in RBD enhances the binding affinity of the virus to human ACE2 and consequently higher transmissibility compared to SARS-CoV and MERS-CoV [1, 5].

The creation of new and fast platforms at three compasses of prevention, diagnosis, and treatment of virus infections are remarkable biotechnological approaches in the COVID-19 pandemic. Therefore, knowing more about the biology and pathogenesis of the virus will help in diagnosis, treatment regimens, and vaccine design. Regions of RdRP gene sequences in the ORF1ab, envelope protein gene (E), and nucleocapsid protein gene (N) are hotspot regions used for molecular detection of the SARS-CoV-2 virus due to their conserved sequences [2]. The use of intervention strategies, such as targeting the virus cell entry pathway, including RBD, as well as the virus replication complex, including RdRp, are critical targets in the design of anti-viral molecular therapeutic platforms [6, 7].

CRISPR–Cas System

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and its associated proteins (Cas) are encoded by bacteria and archaea as defensive mechanisms against invasive genetic agents such as viruses and plasmids through a three-stage process of adaptation, maturation, and interference [8, 9]. This two-component system (CRISPR array and Cas protein) is classified into class I and class II, including different types and subtypes based on computational sequence and protein analysis of their effector subunits. The discovery of the CRISPR system has revolutionized biotechnology approaches because of its genomic sequence recognition and enzymatic cleavage ability to genetic engineering of the genome and beyond [10]. Types II, V, and VI from class II of the CRISPR/Cas system have enzymatic activity with a similar nuclease domain in their effector protein through the detection of target DNA or RNA sequences. In CRISPR/Cas complex, the CRISPR RNA (crRNA) is responsible for target sequence (RNA or DNA) identification and can depend on protospacer-associated motif (PAM) or protospacer flanking site (PFS) based on CRISPR types. In addition to crRNA and Cas complex, type II and type V-B have additional trans-activating RNA (tracrRNA) which mediates the binding between them. The single-guide RNA (sgRNA) sequence is chimerically designed with about 20 nucleotides complementary of the target sequence, instead of the crRNA-tracrRNA complex, in the laboratory to use as a biotechnological tool. In these complexes, the nuclease domains of the Cas protein have cleavage activity, resulting in blunt or overhang on-target sequences [11, 12]. Table 1 summarizes the three most applied types of class II CRISPR–Cas systems [13–16].

Applications of CRISPR–Cas Systems in COVID-19 Disease

Some aspects of the CRISPR system beyond genome editing have been considered by researchers during this course of the COVID-19 pandemic. In this review, we screened research articles from PubMed and LitCovid for CRISPR–Cas system application in SARS-CoV-2 using ''COVID-19 and CRISPR'' as keywords for the period 2020 and 2021. We returned 290 articles and after deleting articles that were unrelated, review, and preprint articles, we were left with 86 articles focusing on diagnosis, anti-viral therapeutics, preclinical models, and genome-wide screening. The percent distribution of the diagnosis researches and the other categorized research articles is presented via a pie diagram in Fig. 1.

Table 1 Applied types of class II CRISPR–Cas system

Type	Subtypes	Nuclease domain	PAM/PFS	Cleavage activity	Applications
II (Cas9)	II-A II-B II-C	HNH and RuvC domains	3′ G-rich motif	dsDNA blunt cleavage activity	Diagnostic platforms Genome-wide screening Animal model designing
V (Cas12)	V-A (Cas12a/Cpf1) V-B (Cas12b/C2c1)	RuvC and Nuc domains RuvC domain	5′ T-rich motif	dsDNA overhang cleavage activity and ssDNA collateral cleavage activity	Diagnostic platforms
VI (Cas13)	VI-A (Cas13a/C2c2) VI-D (Cas13d)	2 HEPN domains	3′ none G PFS None	ssRNA overhang cleavage activity and ssRNA collateral cleavage activity	Diagnostic platforms Anti-viral therapy

PAM protospacer adjacent motif, *PFS* protospacer flanking site

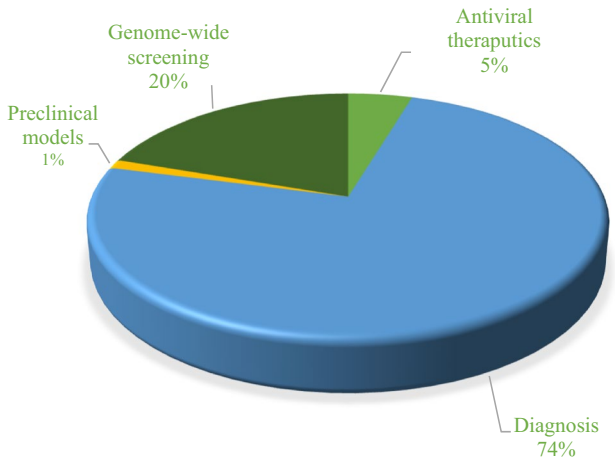


Fig. 1 Diagram of published research articles related to CRISPR-based studies for COVID-19

CRISPR-Based Diagnosis Assays

For most in vitro molecular diagnostic assays of infectious agents, such as viruses, the target nucleic acid must first be amplified. Thermocycling-based amplifications, namely, polymerase chain reaction (PCR) and real-time-PCR methods, are the gold standard techniques. Also, isothermal-based amplification methods, namely, loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), are other available rapid nucleic acid amplification tests. Parameters such as sensitivity, specificity, efficiency, accuracy, simplicity, cost, and especially in this case, the speed of reaction are significant criteria for creating a suitable molecular diagnostic platform for identification of agents [17, 18]. The goal of designing diagnostic platforms is receiving point-of-care (POC) methods, considering detection with the least tools and even outdoors away from the laboratory. Therefore, simultaneous detection of multiple agents in the shortest time is changing approach from routine methods to novel methods [19, 20].

One of the new applications of the CRISPR–Cas system, beyond genome editing, is the diagnosis of infectious and non-infectious diseases. Since 2017 CRISPR-based diagnosis (CRISPR-DX) platforms based on nucleic acid detection have been developed. The design of CRISPR-DX platforms has been able to consider most of the necessary parameters by incorporating other amplification and detection methods to develop optimal systems with high performance [21]. To date, there is an urgent need to develop new POC detection methods in the emerging condition of the COVID-19 pandemic. In 2020 the CRISPR-based detection systems for SARS-CoV-2 have been able to receive the US food and drug administration (FDA) emergency use authorization (EUA) approval [22].

Due to the structure of the CRISPR–Cas system, designing different and innovative CRISPR-based diagnostic tools often considers the diversity in classes and types of Cas nucleases. Cas subunit with their enzymatic RNase activity properties along with crRNA can identify the target DNA or RNA and make cleavage [23]. The most important classes of this enzyme, applicable in detection, are class II (types II/Cas9, V/Cas12a/b, and VI/Cas13), with attomolar sensitivity for detecting RNA or DNA viruses. Usually, using each Cas protein type for virus genome detection should combine with amplification methods, especially isothermal methods, to improve the target sample sequence. Then, through the crRNA and Cas complex, the target sequence can be identified more accurately with visual fluorescent-based readout or lateral flow assay (LFA) in final detection step [10, 24]. In cases with low viral RNA copy numbers, it is difficult to interpret by visual detection. On the other hand, there is a correlation between LFA signals and Ct values in Real-Time PCR results. So, the visual results can be semi-quantified through machine learning tools to enhance the analytical sensitivity. There have been efforts to use smartphone applications for detection and quantification of POC SARS-CoV-2 CRISPR-DX platforms by imaging [25]. In fluorescent-based readouts using diode laser [26], capturing of image by companion smart-phone application [27] and 3D printing instrument for helping smart-phones detection [28] are used to develop the POC purposes.

For detecting the SARS-CoV-2 virus, different CRISPR-based platforms are using the diverse Cas nuclease enzymes listed in Table 2. The Cas9 endonuclease protein in combination with gRNA targets the dsDNA sequence. One of the advantages of using the Cas9 type is the ability to identify single-nucleotide polymorphisms that are useful for virus genotyping as well as low-frequency gene mutations [29, 30]. This type of capability has enabled the Cas9 from *Campylobacter jejuni* NCTC11168 (CjeCas9) to be used to distinguish the SARS-CoV-2 and its D614G ($Asp^{614} \rightarrow Gly$) variant with single-base resolution in patient samples [31]. Amplification-free electrochemical detection methods are based on binding affinity between the dCas9 (dead activity-Cas9) and the viral genome on graphene-based FET chips, which also have the potential for SARS-CoV-2 detection [32].

The first diagnostic platform to be designed based on Cas12 was a DNA endonuclease-targeted CRISPR trans reporter (DETECTR) for the detection of HPV in human patient samples. In this platform, the crRNA-Cas12a complex was used to bind and cleave the amplified virus genome sequence. Collateral cleavage of a non-specific short ssDNA sequence, which is a fluorophore quencher (FQ) labeled as a reporter was used to visualize this binding by light emission [52]. The DETECTR platform has been redesigned for SARS-CoV-2 detection from the extracted RNA of a

Table 2 Notable CRISPR–Cas-based diagnostic platforms for SARS-CoV-2 designed in 2020 and 2021

CRISPR–Cas type		Technique name	Attendant methods		Target genes	Limit of detection (LOD)	Duration time	References
			Amplification method	Detection method				
Type II (Cas9)	Cas9/D10A with nickase activity	CRISPR array-mediated primer exchange reaction-based biochemical circuit cascades	PER	Electrochemical biosensing	Nucleocapsid (N)	5 nM for synthesized SARS-CoV-2 genome in the human cell lysate	Three steps (CRISPR reactant, PER reactant, and detection) within 110 min	[33]
	Francisella novicidae Cas9	CRISPR/Cas9-mediated triple-line lateral flow assay (TL-LFA)	RT-RPA	Lateral flow visual readout	Simultaneous dual genes; envelope (E) and Orf1ab	100 RNA copies per reaction (25 µl)	Two steps at 37 °C within 40 min	[34]
	Campylobacter jejuni NCTC11168 (CjeCas9)	Leveraging engineered tracrRNAs and on-target DNAs for parallel RNA detection (LEOPARD)	RT-PCR or free-amplification	Polyacrylamide gel electrophoresis or Bioanalyzer	Spike (S)	One copy, or 1.7 aM in the original dilution, of RNA compared with 3×10^8 copies, or 0.6 nM in the original dilution, without preamplification	unspecified	[31]
Type V (Cas12)	Cas12a (Cpf1)	DNA endonuclease-targeted CRISPR trans reporter (DETECTR)	RT–LAMP	Lateral flow visual readout	Nucleocapsid (N) and envelope (E)	10 copies per µl of nasopharyngeal swab samples	Three steps within 30–40 min	[35]
		CRISPR-based fluorescent diagnosis system for COVID-19 (COVID-19 CRISPR-FDS)	RT-RPA	Fluorescence-based detection in 96-well microtiter plate and adaptable to smartphone-read chip format	Nucleocapsid (N) and envelope (E)	2 copies per µl of nasal swab samples	Three steps within 50 min	[36, 37]
		In vitro Specific CRISPR-based Assay for Nucleic acid detection (iSCAN)	RT-LAMP	Lateral flow and fluorescence-based detection	Nucleocapsid (N) and envelope (E)	10 copies per µl of Nasopharyngeal swab samples	One and two steps at 62 °C within 60 min	[38]
		CRISPR–Cas12a naked eye readout (CRISPR–Cas12a-NER)	RT-RAA	Fluorescence-based detection	orf1a, orf1b nucleocapsid (N) and envelope (E)	10 copies per µl input	Three steps within 60 min	[39]

Table 2 (continued)

CRISPR–Cas type	Technique name	Attendant methods		Target genes	Limit of detection (LOD)	Duration time	References
		Amplification method	Detection method				
	Specific enhancer for PCR-amplified Nucleic Acid (SENA)	rRT-PCR	Fluorescence-based detection	Orf1ab (O) and nucleocapsid (N)	1.6 copies per µl of pharyngeal and nasopharyngeal swabs with 95% confidence	Three steps within rRT-PCR time and CRISPR detection time	[40]
	Manganese-enhanced Cas12a detection (MeCas12a)	RT-RAA	Fluorescence-based detection	Envelope (E)	5 copies with Mn2+ compared to 10 copies with Mg2+	Three steps within 45 min	[41]
	ENHANCE system	RT-LAMP	Fluorescence-based assay and lateral flow assay	Nucleocapsid (N)	3–300 copies per µl	Three steps within 40–60 min	[42]
	One-pot visual SARS-CoV-2 detection system named (opvCRISPR)	RT-LAMP	Fluorescence-based assay	Spike (S)	5 copies per µl	Three steps within 45 min	[43]
	All-In-One Dual CRISPR–Cas12a (AIOD-CRISPR)	RT-RPA	Fluorescence-based assay	Nucleocapsid (N)	5 copies per µl	One-step amplification and detection at room temperature (37 °C) within 20 min	[44]
	Microfluidic Isotachophoresis (ITP)-CRISPR-based	RT-LAMP	Fluorescence-based assay	Nucleocapsid (N) and envelope (E)	10 copies per µl of Nasopharyngeal swab samples	Within 30 min	[45]
	Digital warm-start CRISPR (DWS-CRISPR)	RT-DAMP	Fluorescence-based assay	Nucleocapsid (N)	5 copies/µl RNA in the chip tenfold higher sensitivity than tube-based bulk assay format	One step in QuantStudio 3D digital chip initiating at above 50 °C	[46]
Cas12b (C2c1)	CRISPR-assisted detection (CASdetec)	RT-RAA	Fluorescence-based assay	RdRp	10 copies per µl	one step at 42 °C within 60 min	[47]
	SHERLOCK Testing in One Pot (STOP)	RT-LAMP	Fluorescence-based assay and lateral flow assay	Nucleocapsid (N)	100 copies/reaction	One-step amplification and detection at 60 °C within 45 min	[48]

Table 2 (continued)

CRISPR–Cas type		Technique name	Attendant methods		Target genes	Limit of detection (LOD)	Duration time	References
			Amplification method	Detection method				
Type VI (Cas13)	Cas13a (C2c2)	Specific High-sensitivity Enzymatic Reporter unlocking (SHERLOCK)	RT–RPA and T7 transcription	Fluorescence-based assay and lateral flow assay	Spike (S), nucleoprotein (N) replicase polyprotein 1ab (Orf1ab)	42 RNA copies per reaction of Nasopharyngeal swab	Three steps within 60 min	[26, 49]
		Combinatorial arrayed reactions for multiplexed evaluation of nucleic acids (CARMEN-Cas13)	PCR or RT-RPA	Fluorescence-based assay using mixes of color-coded amplified sequences	spike (S), nucleoprotein (N) replicase polyprotein 1ab (Orf1ab)	10^4 copies per µl for synthetic targets	Three steps	[50, 51]
		Streamlined highlighting of infections to navigate epidemics (SHINE)	RT–RPA and T7 transcription	Fluorescence-based assay and lateral flow assay	ORF1a	10 copies per µl with 100% specificity	One step	[27]
		Cas13 assisted saliva-based & smartphone integrated testing (CASSPIT)	RT-RPA and T7 transcription	Lateral flow assay integrated with a smartphone application	S and Orf1ab	200 copies/reaction for the S gene with a corresponding Ct value of 35.4	Two steps dual-heat inactivation (37 °C for 10 min and 95 °C for 5 min)	[25]

PER primer exchange reaction, *LAMP* loop-mediated isothermal amplification, *rLAMP* RNA transcription following LAMP, *RAA* recombinase-aided amplification, *RPA* recombinase polymerase amplification, *DAMP* dual-priming isothermal amplifications

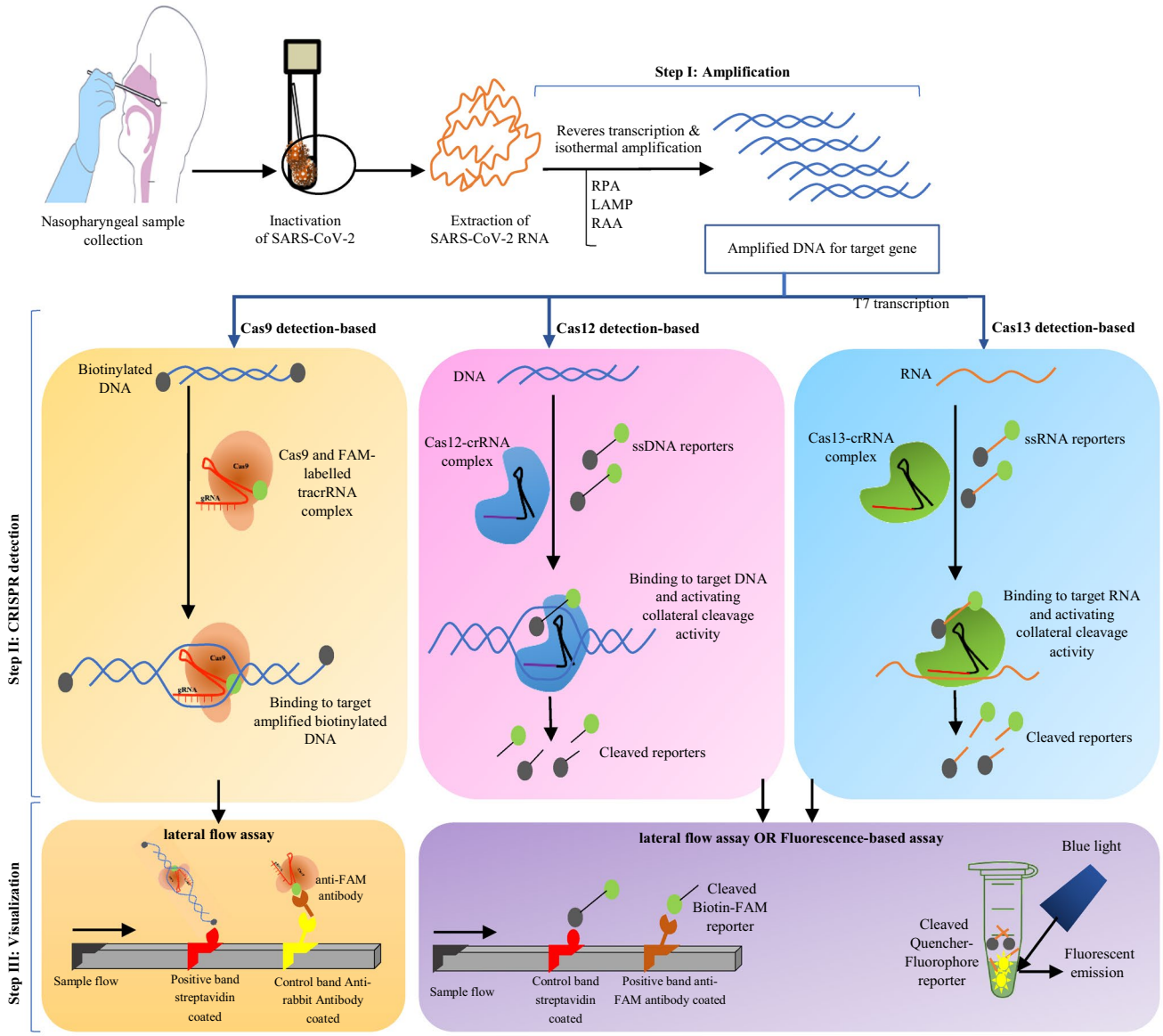
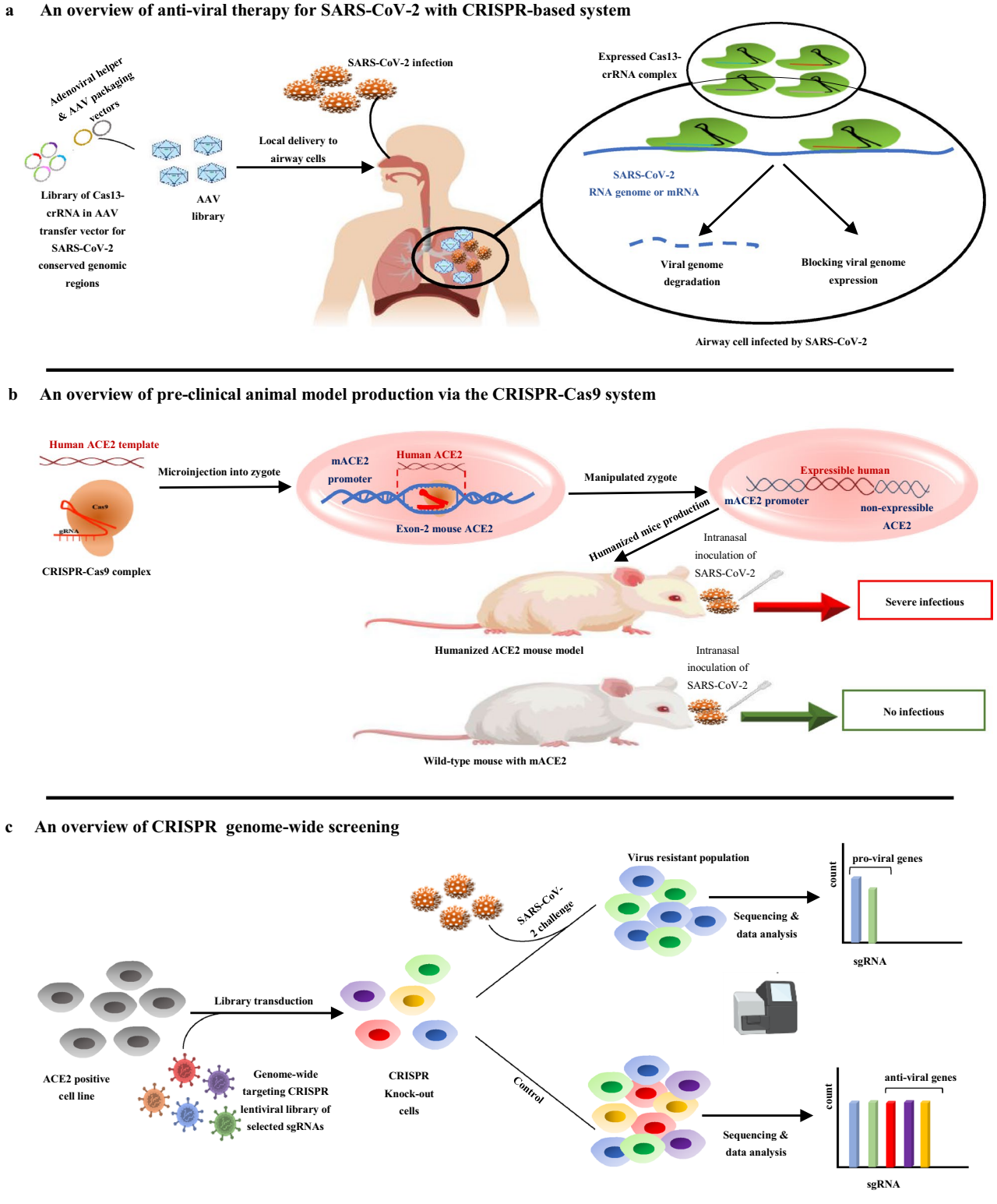


Fig. 2 An overview of CRISPR-based diagnostic assays with three types of Cas enzymes; Cas9, Cas12, and Cas13 detections with names of FELUDA, DETECTR, and SHERLOCK, respectively, after extraction of RNA of SARS-CoV-2, pass through three steps and can be visualized through the lateral flow assay or fluorescence-based assay

respiratory swab, by lateral flow assay. The collaboration of this method with reverse transcriptase-loop-mediated isothermal amplification (RT-LAMP) to convert and amplify RNA virus to DNA effectively increased the detection efficiency [35]. The advantages of this method are laboratory instrument independence and rapidness, along with acceptable accuracy and specificity [28, 53]. Other DETECTR-like platforms for COVID-19 are listed in Table 2.

The pioneer of the CRISPR-DX system is Specific High-sensitivity Enzymatic Reporter unlocking (SHERLOCK) that uses type VI/Cas13 for detection [54]. This platform was designed to detect pathogens, including viruses, in combination with reverse transcriptase-recombinase polymerase amplification (RT-RPA) and T7 transcription to increase detection accuracy using a quenched fluorescent ssRNA reporter suitable for visual or lateral flow readout [55]. This platform was successful in the diagnosis of the SARS-CoV-2 virus during its pandemic. It is the first CRISPR-based platform to be used on clinical samples with the US FDA-EUA approval and has acceptable results compared with other diagnostic methods, such as next-generation sequencing (NGS) and RT-PCR (q-PCR) [22, 49, 56]. Discrimination of the wild and mutated SARS-CoV-2, such as D614G mutation with Cas13a-gRNA and Cas12a-gRNA



CRISPR-based platforms, is valuable for monitoring of epidemiological analyses and can improve the ability of POC tests [57, 58]. Other notable CRISPR–Cas13-based platforms for SARS-CoV-2 virus detection are listed in Table 2. Figure 2 shows the steps of the available three types of CRISPR-DX platforms.

◂**Fig. 3** **a** Cas13-crRNA complex through variable delivery systems (such as AAV delivery method) can target virus genes (such as ORF1ab, RdRp, S, and N genes) to degrade viral genome and block genome expression of virus. **b** Production of humanized ACE2 mouse model through the microinjection of CRISPR–Cas9 complex and human ACE2 template sequence in mouse zygote to substitute mouse ACE2 gene and express in the lung, intestine, and brain of a mouse under its promoter. This humanized model is compared with a wild-type mouse in SARS-CoV-2 infectious conditions. **c** The pooled or arrayed CRISPR genome-wide screening is done for analysis of top-ranked gene clusters in the pathway of virus infection in host cells. In this direction, designing of sgRNA library for targeting candidate host cell gene to production of knocked out cells and challenging with SARS-CoV-2 virus are done for determining of anti-viral and pro-viral genes from sensitive and resistant cells

CRISPR-Based Therapeutics

Conventional therapies for treating viral diseases are usually based on preventing the virus infections by targeting biomolecules in the entrance pathway up to proliferation. In the emergence of viruses, the first step in anti-viral therapy is drug repurposing to use existing drugs for the benefit of treatment in the shortest possible time. The second approach could be target-based drug designing by focusing on the genomics and proteomics of the desired virus [59, 60]. Based on these two approaches, drug candidates for the treatment of SARS-CoV-2 virus infectious usually focus on disruption of the enzymatic function of the virus (especially RNA-dependent RNA polymerase), prevention of virus endocytosis, blocking viral proteins (envelope, membrane, nucleocapsid, and accessory proteins), helping innate immunity or suppressing the excessive inflammatory response, and prevention of SARS-CoV-2 replication [61]. On the other hand, viruses that have new mutations and cause pandemics usually cannot respond to old drugs and vaccines. These limitations in the treatment and prevention of the COVID-19 could be applied to other viruses of the same family, such as SARS and MERS. Therefore, under these conditions, there is an urgent need for new therapies based on targeting the genomic sequences of the virus. The disruption of gene function in anti-viral therapy incorporates biotechnological tools, such as siRNA or CRISPR–Cas system. One of the well-known applications of the CRISPR–Cas system is the possibility of identifying the DNA or RNA sequence of the virus to cut them and destroy their function [62].

Related studies have suggested CRISPR an acceptable candidate for targeting human pathogens such as HIV, hepatitis B virus (HBV), herpesviruses, human papillomavirus (HPV), and JC virus (JCV) [63]. The Cas9 and Cas13 as endonuclease enzyme subunits in the CRISPR system can identify the DNA and RNA of viruses in infected mammalian cells. Studies have shown that Cas9 has a lower cleavage efficacy for ssRNA and also the ability to create off-targets in the DNA sequence of virus-infected host cells [15, 64].The CRISPR–Cas13 system is not dependent on the detection of PAM and the crRNA targets ssRNA coding sequences of the virus without interfering with the human transcriptome, causing sequence degradation, interrupting gene expression, and eventually blocking viral function [65]. Therefore, using CRISPR–Cas13 is superior to CRISPR–Cas9 as an anti-viral programmable inhibition system.

Prophylactic anti-viral CRISPR in human cells (PAC-MAN), effectively can identify and degrade the virus sequence and its mutants in the human lung epithelial cells. The PAC-MAN system uses the class 2, type VI-D, CRISPR–Cas13d system derived from *Ruminococcus flavefaciens* XPD3002. PAC-MAN simultaneously identifies more than 90% of all coronaviruses using a combination of 6 crRNAs by identifying protected regions (such as ORF1ab, RdRp, and N genes) as well as detecting virus ssRNA in their replication and transcription phases [66]. The comprehensive set of bioinformatics methods for receiving in silico optimal crRNA candidates can improve the efficiency of laboratory tests for combating SARS-CoV-2 in the least time [67]. Besides in silico designing and human cell line tests, using animal models will improve preclinical studies with the simulation of respiratory infections in vivo and will help to screen the safety and effectiveness of CRISPR-based platforms for anti-viral approaches [68]. The advantages of the CRISPR-based anti-viral systems are high flexibility in identifying viral sequences, speed of detection, and directness against the virus [65, 66]. The Cas13d expression vector could be transmitted via the adeno-associated virus-packaging system (AAV delivery method) with high tropism for respiratory tissues and expressed under the induction of expression promoters in specific tissues, especially airway cells [65]. The basis of the anti-viral CRISPR-based system for SARS-CoV-2 is depicted in Fig. 3a.

CRISPR in preclinical Researches

Preclinical researches related to COVID-19 require suitable animal model that can be infected with the SARS-CoV-2 virus and subsequently access mild-to-severe disease. As in human cells, the target cells in the selected animal should possess ACE2 as the SARS-CoV-2-binding receptor to let virus cell entrance. Animals such as hamsters, ferrets, African green monkeys, cynomolgus, and rhesus macaques have similar human ACE2 receptors. For extensive researches the best candidate is mice because of reducing costs and increasing the number of animals but mouse ACE2 has much lower binding affinity for the viral spike protein, compared to its human counterpart. This low binding affinity will develop a mild disease in mice models that is not suitable for multi-aspect studies

Table 3 Genome-wide CRISPR screens of pathways and processes involved in SARS-CoV-2 infection

Screened pathways & process	Screened cell line	Top-ranked gene clusters in pathway		Highlights	Similar small molecule	References
		Resistant genes	Sensitize genes			
Cholesterol biosynthesis pathway (part of the endosomal entry pathway from the vacuolar ATPase proton pump, Retromer, and Commander complexes)	A549	ATP6AP1 ATP6V1A NPC1 RAB7A CCDC22 PIK3C3		Loss of resistant genes such as RAB7A reduces viral entry by sequestering the ACE2 receptor inside cell	RNA-sequencing of cells treated with amlodipine small molecule shows a similar differential gene expression profile as seen in CRISPR knock-out of genes in cholesterol biosynthesis pathway	[84]
Rab-GTPase requirements Glycosylphosphatidylinositol-anchored biosynthesis Cholesterol biosynthesis	HAP1 Huh-7.5-Cas9	SREBP/SCAP HS2ST1 EIF4E2 RAB2A RAB10 RAB14	MRPS2 MRPS5 MRPS25 MRPS27	SCAP regulates lipid and cholesterol homeostasis by sequestering SREBPs in the ER in the presence of sterols	Fatostatin molecule as SCAP inhibitors shows anti-viral properties 27-Hydroxycholesterol (27OHC) and 25OHC have SARS-CoV-2 anti-viral activity in VeroE6 cells	[83]
Replication cycle	A549	TMEM41B		SARS-CoV-2 require TMEM41B for replication cycles in cell	None	[85]
Chromatin remodeling Histone modification Cellular signaling RNA regulation	Huh7.5 VeroE6	ARID1A DYRK1A KDM6A CTSL ACE2 SMARCA4 DYRK1A KDM6A HMGB1 HIRA CABIN1 TRIP12 BPTF PIAS2	TARDBP	Viral RNA-RNA and RNA–protein interactions reveal specific SARS-CoV-2-mediated mitochondrial dysfunction during infection pro-viral genes and pathways, including HMGB1, and the SWI/SNF chromatin remodeling complex are SARS-CoV-2 lineage HMGB1 is critical for SARS lineage viral entry with critical role in ACE2 expression	PFI-3, which targets the bromodomains of the SWI/SNF proteins SMARCA4 and SMARCA2 SIS3, which targets the pro-viral gene SMAD3 identified in the screen	[80, 81]
Glycosaminoglycan biosynthesis SREBP signaling Glycosylphosphatidylinositol biosynthesis Cholesterol biosynthesis	Huh-7.5 hepatoma cells (Huh-7.5-Cas9)	SCAP TMEM106B TMEM41B VAC14 ACE2 HMGCS1 MVK PMVK RAB6A RAB10		Absolute requirement for the VTT-domain containing protein TMEM41B for infection by SARS-CoV-2	None	[86]

Table 3 (continued)

Screened pathways & process	Screened cell line	Top-ranked gene clusters in pathway		Highlights	Similar small molecule	References
		Resistant genes	Sensitize genes			
Phosphatidylinositol phosphate biosynthesis Cholesterol homeostasis Heparan sulfate biosynthetic genes	Huh7.5.1 hepatoma cells	TMEM106B B3GALT6 B3GAT3 B4GALT7 EXT1 EXT2 EXTL3 FAM20B NDST1 SLC35B2 UGDH XYLT2 SREBP/SCAP		Deletions in TMEM106B caused defects in lysosome trafficking, impaired acidification, and reduced levels of lysosomal enzymes but its precise molecular function remains enigmatic	None	[87, 88]
O-glycan biosynthesis N-glycan biosynthesis	HEK293T	C1GalT1 MGAT1		Knocking-out N-glycan biosynthesis on Spike-abrogated viral entry	Kifunensine small molecule inhibits N-linked glycosylation to reduce viral entry	[89]
Cholesterol biosynthesis pathway (part of the endosomal entry pathway from the vacuolar ATPase proton pump, Retromer, and Commander complexes)	A549	ATP6AP1 ATP6V1A NPC1 RAB7A CCDC22 PIK3C3		Loss of resistant genes such as RAB7A reduces viral entry by sequestering the ACE2 receptor inside cell	RNA-sequencing of cells treated with amlodipine small molecule shows a similar differential gene expression profile as seen in CRISPR knock-out of genes in cholesterol biosynthesis pathway	[84]
Rab-GTPase requirements Glycosylphosphatidylinositol-anchored biosynthesis Cholesterol biosynthesis	HAP1 Huh-7.5-Cas9	SREBP/SCAP HS2ST1 EIF4E2 RAB2A RAB10 RAB14	MRPS2 MRPS5 MRPS25 MRPS27	SCAP regulates lipid and cholesterol homeostasis by sequestering SREBPs in the ER in the presence of sterols	Fatostatin molecule as SCAP inhibitors shows anti-viral properties 27-hydroxycholesterol (27OHC) and 25OHC have SARS-CoV-2 anti-viral activity in VeroE6 cells	[83]
Replication cycle	A549	TMEM41B		SARS-CoV-2 require TMEM41B for replication cycles in cell	None	[85]

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Screened pathways & process	Screened cell line	Top-ranked gene clusters in pathway		Highlights	Similar small molecule	References
		Resistant genes	Sensitize genes			
Chromatin remodeling Histone modification Cellular signaling RNA regulation	Huh7.5 VeroE6	ARID1A DYRK1A KDM6A CTSL ACE2 SMARCA4 DYRK1A KDM6A HMGB1 HIRA CABIN1 TRIP12 BPTF PIAS2	TARDBP	Viral RNA-RNA and RNA–protein interactions reveal specific SARS-CoV-2-mediated mitochondrial dysfunction during infection pro-viral genes and pathways, including HMGB1, and the SWI/SNF chromatin remodeling complex are SARS-CoV-2 lineage HMGB1 is critical for SARS lineage viral entry with critical role in ACE2 expression	PFI-3, which targets the bromodomains of the SWI/SNF proteins SMARCA4 and SMARCA2 SIS3, which targets the pro-viral gene SMAD3 identified in the screen	[80, 81]
Glycosaminoglycan biosynthesis SREBP signaling Glycosylphosphatidylinositol biosynthesis Cholesterol biosynthesis	Huh-7.5 hepatoma cells (Huh-7.5-Cas9)	SCAP TMEM106B TMEM41B VAC14 ACE2 HMGCS1 MVK PMVK RAB6A RAB10		Absolute requirement for the VTT-domain containing protein TMEM41B for infection by SARS-CoV-2	None	[86]
Phosphatidylinositol phosphate biosynthesis Cholesterol homeostasis Heparan sulfate biosynthetic genes	Huh7.5.1 hepatoma cells	TMEM106B B3GALT6 B3GAT3 B4GALT7 EXT1 EXT2 EXTL3 FAM20B NDST1 SLC35B2 UGDH XYLT2 SREBP/SCAP		Deletions in TMEM106B caused defects in lysosome trafficking, impaired acidification, and reduced levels of lysosomal enzymes but its precise molecular function remains enigmatic	None	[87, 88]
O-glycan biosynthesis N-glycan biosynthesis	HEK293T	C1GalT1 MGAT1		Knocking-out N-glycan biosynthesis on Spike-abrogated viral entry	Kifunensine small molecule inhibits N-linked glycosylation to reduce viral entry	[89]

[69, 70]. The application of transgenic mice can solve this problem by inserting the human ACE2 receptor gene into the mouse embryo genome in an engineered manner that will pass on to the next generation. In this case, humanized ACE2-incorporated mice will be more sensitive to SARS-CoV-2 infection via intranasal inoculation. Notably, the pathological outcomes and subsequent lung damages will be similar to human infection [71]. In addition to the biotechnology-based techniques used to produce transgene mice, CRISPR technology could also insert the target gene into the mouse genome by the knock-in process. For COVID-19 preclinical studies, the CRISPR–Cas9 knock-in technology can help the expression of the human ACE2 receptor in various mouse tissues. Humanized mice sensitize to SARS-CoV-2 intranasal inoculation and intragastric inoculation [72], which is depicted in Fig. 3b. The production of humanized mice has facilitated anti-viral therapeutic research, the development of vaccines, studying virus transmission and pathogenesis, and screening severe symptoms in COVID-19.

Genome-Wide Screening by CRISPR

Functional studies of genes via conditional mutagenesis in mammalian cells have been considered for decades. Genome engineering tools such as Cre-recombinase, RNAi, and designer nucleases such as ZFN and TALENs have been used for forwarding genetic screening until 2012. The discovery of the CRISPR–Cas system as a novel biotechnological method of designer nucleases generation is an alternative technique to overcome the limitations. One of the advantages of genome-wide CRISPR screening is the designing of sgRNA libraries to target thousands of genes simultaneously for pooled or arrayed screening and directly identify the candidates of desired phenotype [73, 74]. In addition to genome-wide mutagenesis through CRISPR knock-out approach, this system is a suitable tool for insertion and deletion of the target genes. Also, site-directed base editing and up/down gene regulation via gene-specific transcription factors can be done with the new modifications of CRISPR system. CRISPRing has provided an opportunity to understand the biology of mammalian cells related to diseases, such as cancers and pathogen infectious, including viruses, and designing the pharmacological studies through the creation of targeted mutant cell libraries [75, 76].

Usually the host genes that coordinate the virus entrance and pathogenesis processes are unknown. The identification of principal host genes in the viral infection, regulation, or suppression pathway can provide a better understanding of the virus function in the host cell and can help to find novel potential therapeutic marks such as antagonist drugs and design an appropriate vaccine. All of these studies in the host cell lines can be done by CRISPR–Cas9 genome-wide mutagenesis screening. Recently screening of the Noroviruses, Zika virus, West Nile virus, and HIV in host cell lines has been performed by CRISPRing [77–79].

The necessity to identify the pathogenesis of the SARS-CoV-2 virus pandemic, new screening studies are extended for revealing of viral entry and pathogenesis of host genes through genome-wide pooled or array CRISPR libraries in the appropriate cell lines. In these studies, the candidate host genes are determined by systems biology approaches and host–viral protein interactome [61]. The targeted sgRNA libraries are designed for candidate host genes to produce knock-out cell lines. These mutant cells will be challenged by virus and finally, the sequencing of the target region of the sgRNAs on mutant cell genome will determine the causative genes of resistance and sensitivity [80–82]. The process of the CRISPR genome-wide screening of SARS-CoV-2 host cell is depicted in Fig. 3c. After knocking-out steps the genes that make the virus-resistant cells are pro-viral, and the genes that make the virus-sensitive cells are anti-viral. By propagating resistant knock-out cells and sequencing data analysis, anti-viral drugs would be designable based on these gene pathways [83]. In Table 3, the CRISPR-based genome-wide screening of important cellular pathways and processes to determine top-ranked resistant and sensitive genes involved in SARS-CoV-2 infection is reported. In some cases, the candidate small molecules that mimic anti-viral gene pathways or blocking pro-viral gene pathways have been specified. The studies show that due to the lack of adequate knowledge about specific genetic pathways involved in cytotoxicity and metabolism associated with drug repurposing in SARS-CoV-2, research in this field has begun by genome-wide CRISPR.

Conclusion

After less than two years of the COVID-19 pandemic, CRISPR technology has shown some potential in all applications, from diagnosis to treatment of this disease. All of these capabilities are as a result of CRISPR's high sensitivity, flexibility, adaptability, and developable platform. In addition, based on new SARS-CoV-2 mutations and the need for newly designed vaccines, we have to find other treatment strategies like CRISPR technology for COVID-19 and other future viral infections. In addition to all advantages of using the CRISPR system in the detection of the virus variants, producing a preclinical animal model for drug and vaccine researches, its application in anti-viral therapeutics based on eliminating the virus in the infectious cells, and studies on genes involved in the disease; this system certainly has shortcomings.

One of the disadvantages of this system in diagnosis is difficult to access results for clinicians in clinical studies, so diagnostic protocols based on CRISPR-DX platforms should be toward automation as well as better reading using the mobile application with provision for offline or online mode.

In studies related to anti-viral applications of the CRISPR system, the biggest limitation is the development of effective and safe in vivo delivery methods. Therefore, more studies on the transfer of structures are needed. Also, sufficient expression of CRISPR/Cas in the target cells has a direct effect on achieving efficient viral inhibition through viral genome cleavage.

Off-target effects and limited on-target activity as well as low knock-out efficiency for designed sgRNAs are other problems facing CRISPR genome-wide screening researches, which require optimizing CRISPR systems through structural engineering and more precise design of sgRNAs for the target genome.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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