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REVIEW ARTICLE

Advances of CRISPR-Cas13 system in COVID-19 diagnosis and treatment

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Abstract The ongoing global pandemic of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in over 570 million infections and 6 million deaths worldwide. Early detection and quarantine are essential to arrest the spread of the highly contagious COVID-19. High-risk groups, such as older adults and individuals with comorbidities, can present severe symptoms, including pyrexia, pertussis, and acute respiratory distress syndrome, on SARS-CoV-2 infection that can prove fatal, demonstrating a clear need for high-throughput and sensitive platforms to detect and eliminate SARS-CoV-2. CRISPR-Cas13, an emerging CRISPR system targeting RNA with high specificity and efficiency, has recently drawn much attention for COVID-19 diagnosis and treatment. Here, we summarized the current research progress on CRISPR-Cas13 in COVID-19 diagnosis and treatment and highlight the challenges and future research directions of CRISPR-Cas13 for effectively counteracting COVID-19.

Introduction

SARS-CoV-2 belongs to the β-coronavirus genus of the family *Coronaviridae*, which comprises four genera, α-coronavirus, β-coronavirus, γ-coronavirus, and δ-coronavirus (ICTV Virus Taxonomy:2019 Release). It is an enveloped virus with a single-stranded, positive-sense RNA genome of ~30,000 nucleotides in size.[1–3] The N-terminal 3/4 genome is translated into two polyproteins (pp1a and pp1ab), which are then processed into 16 nonstructural

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proteins that constitute the replication/translation complex.[3–5] The rest of the genome close to the C-terminus synthesizes spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins as well as accessory proteins including ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10 (Fig. 1A).[3,6,7] The SARS-CoV-2 genome has significant genetic diversity (https://nextstrain.org/sars-cov-2) with ~300 thousand *de novo* mutations found to date.[8] These mutations cover almost the entire genome (Fig. 1B), which makes sequence-specific targeted diagnosis and degradation difficult. Additionally, these mutations may also re-profile the viral entry and replication pathways, resulting in evolutionary-escape resistance to current prevention and therapeutics.[9–13] An effective reagent that can eliminate SARS-CoV-2 and its variants or stop virus entry/replication is of tremendous assistance in COVID-19 treatment. Although it has been almost 3 years since the first case of COVID-19 was reported in early December 2019 in Wuhan and over 12 billion doses of vaccine have been administered, people are still suffering from the pandemic worldwide with ~400 thousand new diagnosed cases every day (https://covid19.who.int/). Thus, rapid, low-cost, highly sensitive, and point-of-care detection tools are urgently needed for early diagnosis and quarantine to prevent the spread of the virus. Notably, recent accumulating evidence indicates the broad application prospects and values of the CRISPR-Cas13 system, a specific RNA-targeting platform, in viral (*i.e.*, SARS-CoV-2) infection diagnosis and treatment.

Characteristics and applications of CRISPR-Cas13

Differences between CRISPR-Cas13 and CRISPR-Cas9 systems

The CRISPR-Cas system comprises repeat sequences of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas).[14–16] It can be classified into two major classes based on the number of effectors, which are further divided into six types (type I–VI) (Fig. 2A).[17,18] CRISPR-Cas13, which belongs to type VI, was first reported by Shmakov et al in 2015 and subsequently implemented for nucleic acid detection, termed specific high-sensitivity enzymatic reporter unlocking (SHERLOCK)[19,20] and RNA elimination.[21,22] Six CRISPR-Cas13 system subtypes have been reported to date (Cas13a, Cas13b, Cas13c, Cas13d, Cas13X, and Cas13Y).[19,22–26] In contrast to other types of CRISPR-Cas systems, such as CRISPR-Cas9 that targets DNA templates,[27–30] type VI (CRISPR-Cas13) systems exhibit potent RNA knockdown capability by recognizing and targeting RNA templates specifically and efficiently (Fig. 2B).[22,31,32] CRISPR-Cas13 does not introduce unwanted and irreversible DNA changes, which is a major obstacle limiting CRISPR-Cas9's therapeutic application.[33] Additionally, Cas13 proteins are smaller than other Cas9 proteins (the smallest Cas13 protein is only 775 aa compared to Cas9 with 1400 aa[34]), which

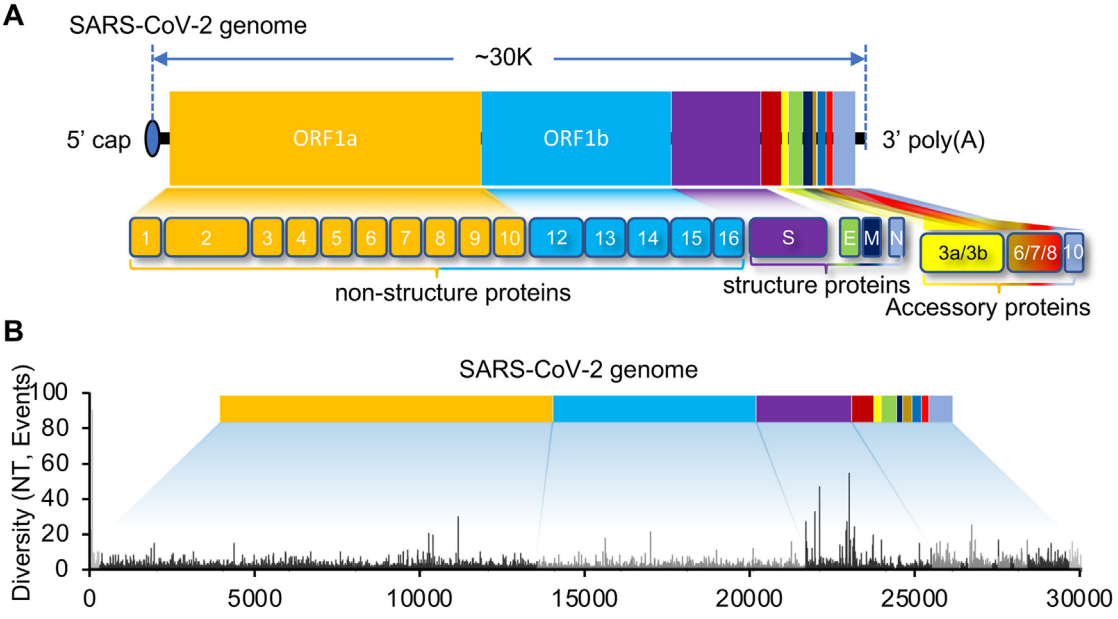

Figure 1 Genome coding potential and diversity of SARS-CoV-2. **(A)** The viral genome is a single-stranded, positive sense RNA with a 5′ cap and 3′ poly-A tail. ORF1a and ORF1b at the N-terminal encode 16 nonstructural proteins, whereas the 1/4 genome close to the C-terminal encodes structural proteins (S, spike; E, envelope; M, membrane; N, nucleocapsid; HE, hemagglutinin esterase) and accessory proteins. **(B)** The diversity events of SARS-CoV-2 nucleotide (NT) (data collection time range: 12/14/2010–07/16/2022; data taken from https://nextstrain.org/ncov/gisaid/global/6m).

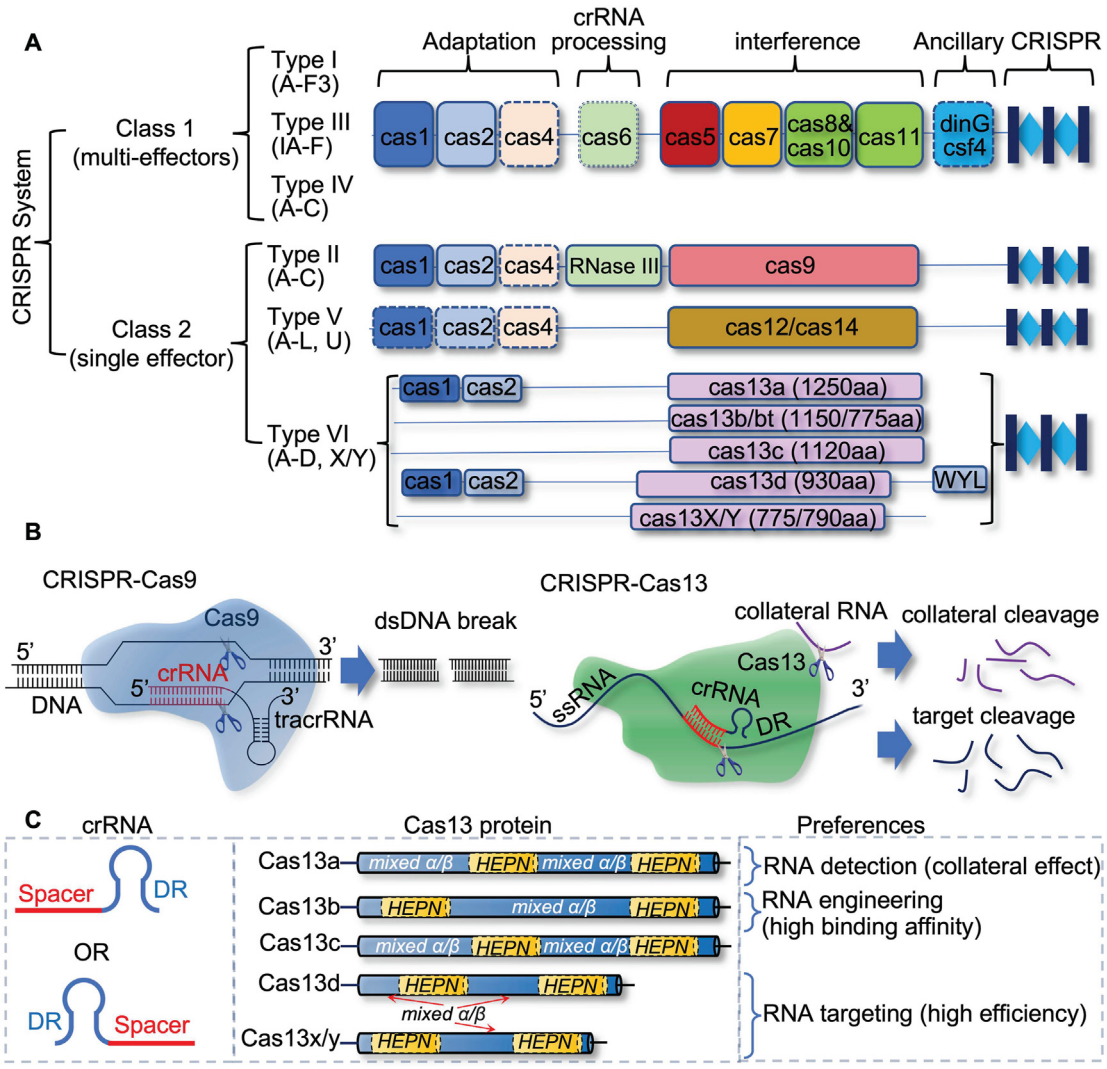

Figure 2 Classification and components of CRISPR-Cas system. **(A)** Class 1, including types I, III, and IV, contains multiple effectors, whereas class 2, including types II (CRISPR-Cas9), V (CRISPR-Cas12/Cas14), and VI (CRISPR-Cas13), has only one effector. The module missing in some subtypes is represented by dashed outlines. Five subtypes of the CRISPR-Cas13 system have been identified so far, with variant effector sizes ranging from 775 to 1250 aa. **(B)** Schematic illustration of RNA-guided cleavage of Cas9 and Cas13. Cas9 uses both a tracrRNA and crRNA to assist the recognition and cleavage of target DNA (left). The 5′ or 3′-terminal direct repeat (DR) interacts with Cas13 protein, and the crRNA specifically recognizes the target RNA sequence (red). The cleavage is performed by two HEPN domains. The binding of Cas13-crRNA to the target RNA also activates a collateral cleavage activity of Cas13 (right). The cleavage sites are depicted by scissors. **(C)** The components of different subtypes of CRISPR-Cas13 that are required for RNA detection, engineering, or treatments.

makes CRISPR-Cas13 suitable for AAV packaged delivery and molecular genetic manipulation *in vivo* (Fig. 2A).

Characteristics of different CRISPR-Cas13 systems

Since the first CRISPR-Cas13 (CRISPR-Cas13a, also termed C2c2) was reported by Shmakov et al in 2015,[19] there are six subtypes of CRISPR-Cas13 systems have been identified currently, including Cas13a-d, Cas13X, and Cas13Y.[19,22–26] As shown in Figure 2C, all of the Cas13 proteins share the same two conserved R(N)xxxH motifs that are characteristic of higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domains,[35,36] which possess RNase activity that is responsible for both "cis" and "trans"-RNA cleavage. The Cas13 proteins also contain a crRNA "recognition" lobe that helps in binding and recognizing the crRNA direct repeat (DR) sequence and act as a "bilobed" effector.[19,37] The most intuitive difference between these subtypes of CRISPR-Cas13 is the size of Cas13 proteins ranging from 1250aa to 775aa. The difference in size between Cas13 proteins impacts the *in vivo* delivery efficiency with AAV or nanoparticles, which also alters the RNA binding affinity and RNA targeting efficiency and specificity. Yang et al compared the *in vivo* RNA labeling affinity of eight Cas13 proteins and showed that Cas13b has the highest binding affinity to *in vivo* RNAs. Coincidentally, another two groups demonstrated that CRISPR-dCas13b alone or fused with

functional domains can be successfully used for RNA engineering such as alternative polyadenylation (APA) regulation and RNA methylation modification,[38–40] indicating that CRISPR-Cas13b may be suitable for RNA engineering. Moreover, studies comparing the RNA knockdown efficiency of Cas13X, Cas13Y, Cas13a, Cas13b, and Cas13d, demonstrated that Cas13X/Y and Cas13d have higher RNA knockdown efficiency on most tested genes, inferring that Cas13X/Y and Cas13d are promising for RNA elimination such as SARS-CoV-2.[22,26,41] Lastly, though all of the CRISPR-Cas13 systems were proven to collaterally cleave "non-target RNA", Cas13d and Cas13X exhibit lower collateral activity, and it is worth noting that CRISPR-Cas13a is the most frequently used platform for RNA detections, though whether other subtypes of CRISPR-Cas13 are more suitable for RNA detection needs further exploration. These studies together indicated different subtypes of CRISPR-Cas13 systems may have their advantages for various applications,[42] and future biochemical and structural studies are also required to help us to garner a better understanding of the differences in crRNA-loading, RNA binding, and cleavage specificity between Cas13 subtypes.

CRISPR-Cas13 for RNA interference

As an RNA-targeted CRISPR system, CRISPR-Cas13-mediated RNA interference achieves higher knockdown efficiency with minimal off-target effects compared with other RNA interference technologies, such as siRNA/shRNA,[22,31,43] which makes it an ideal tool for targeting RNA, including mRNA,[44,45] circRNA,[43,46] and RNA viruses.[47,48] Konermann et al compared the RNA knockdown efficiency of CRISPR-Cas13d with shRNA; transcriptome profiling showed that CRISPR-Cas13d dramatically down-regulated target gene expression without significant off-target changes, whereas shRNA induced hundreds of significant off-target changes with moderate target gene down-regulation.[22] Transcriptome analyses performed by several other research groups have also shown highly efficient and specific targeted RNA knockdown by CRISPR-Cas13, indicating its great potential for RNA targeting.[31,41] Accumulating evidence has shown that CRISPR-Cas13 targeting of aberrantly expressing disease-related genes has achieved success both *in vitro* and *in vivo*, especially in nanotherapies.[49–51] Furthermore, the high specificity of CRISPR-Cas13 also makes it a promising RNA engineering platform; for example, catalytically dead Cas13 can work alone or guide functional protein/domains to the desired RNA sites to exert diverse RNA-regulatory functions, including single-base RNA editing, alternative splicing, alternative polyadenylation, N^6-methyladenosine modification, and RNA localization tracking (Table 1).

CRISPR-Cas13 for RNA detection

In addition to their applications in RNA degradation and editing, CRISPR-Cas13 systems hold great promise as RNA sensors owing to their unique enzymatic activity that cleaves the target nucleotide sequence and subsequently neighboring non-target sequences, termed collateral cleavage activity.[20,52,53] This collateral cleavage activity on the one hand may act as the potential toxicity for RNA-targeted therapies,[54,55] which on the other hand was employed as a "switch" to turn on various types of reporter molecules.[21,56,57] Utilizing the collateral effect, Abudayyeh and East-Seletsky et al successfully engineered CRISPR-Cas13 (previously known as C2c2) for RNA detection.[19,58] Gootenberg et al later optimized the platform and established an LwCas13a-based detection platform, SHERLOCK, by combining the collateral effect of Cas13a with isothermal amplification, enabling RNA (*i.e.*, Zika virus) detection with attomolar sensitivity and single-base mismatch specificity *in vitro*.[20,59] Such a platform can also be used for non-coding RNA detection.[60–62] Bruch et al reported a CRISPR-Cas13a-powered electrochemical microfluidic biosensor system to detect miR-19b in serum samples from patients with brain cancer within a few minutes using <1 μL of a sample.[63] Taken together, these studies demonstrated the feasibility of the CRISPR-Cas13 system for RNA interference and detection.

CRISPR-Cas13 for COVID-19 detection

Development of COVID-19 detection platforms

Early diagnosis of viral infections allows for rapid intervention and minimizes the risk of disease transmission. PCR is currently the gold standard for diagnosing SARS-CoV-2 infections; however, limited qPCR equipment and materials

Table 1 Application of CRISPR-Cas13 system.

Characteristics	Effects	Applications
Targeted RNA cleavage	Targeted RNA interference	1. Knockdown of oncogenic genes or other disease-related genes for disease treatments[22,31,43–45] 2. Functional screening of mRNA/circular RNA[43,46,126] 3. RNA virus degradation[47,48]
Collateral effect	Collateral RNA cleavage	1. RNA virus infection detection[79,127–129] 2. Non-coding RNA detection[60–63]
Steric effect	Block the access of factors to the desired site	1. Alternative splicing manipulation[22,130] 2. Alternative polyadenylation regulation[38]
Guide modular	Guide protein/domain to the desired site	1. Single-base RNA editing[24,131] 2. N^6-methyladenosine modification[39,132,133] 3. RNA localization tracking[32,134,135]

impede diagnosis, especially in developing countries and areas.[64,65] Lateral flow assay systems to detect IgM, IgG, and IgA, as reviewed by Ince and Sezgintürk,[66] are simple-to-use, disposable, inexpensive diagnostic devices for testing biomarkers in blood and urine samples, and are also served as useful tools for SARS-CoV-2 infection screening.[67] However, they are not adequately specific and sensitive, especially for the highly contagious global pandemic. CRISPR-based diagnostics offer an alternative approach that promises to resolve these drawbacks by providing low-cost, specific, sensitive, quick, and straightforward diagnoses, which have recently shown great potential for detecting different RNA viruses.[59,68,69] Importantly, several CRISPR-Cas12-and CRISPR-Cas13-based SARS-CoV-2 detection systems have been reported using the collateral cleavage activity of CRISPR effector proteins Cas12 or Cas13.[70–73] Generally, the collateral cleavage activity of CRISPR effector proteins Cas12 or Cas13 is unleashed on a reporter once viral DNA or RNA is recognized by the corresponding gRNA, and the cleavage outcome can be detected with fluorescence-based or other technologies such as lateral flow systems (Fig. 3).

Unlike CRISPR-Cas12, which targets DNA templates and requires reverse transcription of viral RNA genome to DNA,[72,74] CRISPR-Cas13 directly recognizes RNA templates, allowing the detection of crude samples and accelerating the diagnostic process.[20,75,76] Based on the earliest Cas13a-based SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) that consists of Cas13a protein, crRNA targeting RNA, and RNA sensor,[20,77] a protocol named HUDSON (heating unextracted diagnostic samples to obliterate nucleases) that pairs with SHERLOCK was then developed for RNA viral detection directly from bodily fluids, enabling instrument-free DENV detection directly from patient samples within 2 h, which however requires recombinase polymerase amplification (RPA) and T7 transcription.[75] Jon Arizti-Sanz et al developed a one-step SARS-CoV-2 detection system termed SHINE (Streamlined Highlighting of Infections to Navigate Epidemics) that is able to detect SARS-CoV-2 specifically and sensitively from unextracted samples even in low copies and validated in over 150 clinical nasopharyngeal and throat swab samples in less than one year since the SARS-CoV-2 pandemic began.[71,73] Fozouni et al optimized the process and developed an amplification-free CRISPR-Cas13a platform for directly detecting SARS-CoV-2 from RNA in nasal swabs, which can be read with a mobile phone microscope, allowing for rapid, low-cost, point-of-care screening of SARS-CoV-2.[78] A naked eye-detection system using LwaCas13a and gold nanoparticles called CASCADE (CRISPR/CAS-based Colorimetric nucleic Acid DEtection) was developed later. In this approach, the LwaCas13a protein is used for viral RNA recognition, and ssRNA oligonucleotides reporter was conjugated to gold nanoparticles (AuNPs). Upon target recognition, Cas13a cleaves ssRNA reporter thus inducing their colloidal aggregation, which can be easily visualized. It can detect picomolar concentrations of SARS-CoV-2 RNA in clinical nasopharyngeal swabs samples and even femtomolar and attomolar ranges when coupled to RPA or NASBA isothermal nucleic acid amplification.[79] More recently, Jon Arizti-Sanz

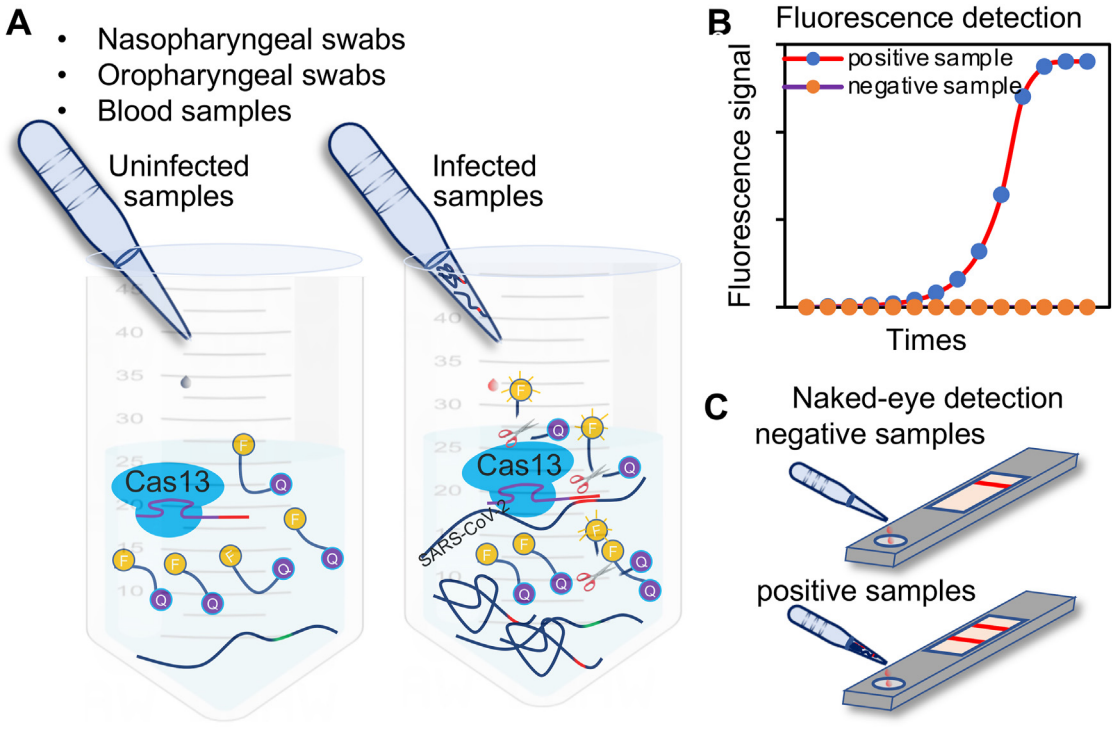

Figure 3 CRISPR-Cas13-based SARS-CoV-2 detection. **(A)** An example of CRISPR-Cas-based detection methods using the collateral activity of CRISPR-Cas13 to cleave the reporter ssDNA/ssRNA after gRNA recognizes and binds to target sequences. Q: quencher, F: fluorophore. **(B, C)** The collateral cleavage outcome can be measured with a fluorescence detection instrument or observed directly when combined with lateral flow assay systems.

et al optimized and simplified their SHINE platform by eliminating heating steps and the need for cold storage of the reagents, and leveraging lyophilized reagents and fast sample inactivation at ambient temperature.[80] These studies demonstrate the specific, sensitive, quick, and straightforward diagnosis of CRISPR-Cas13-based technologies for large-scale SARS-CoV-2 detection and screening.

Emerging strategies for detecting SARS-CoV-2 variants

It is worth noting that the CRISPR-Cas13-based SARS-CoV-2 diagnostic tools may fail to detect emerging variants due to (i) the rapid evaluation and mutation of the SARS-CoV-2 virus[81,82] and (ii) the high specificity of CRISPR-Cas13 due to which a single mismatch may result in the failure of target recognition.[49,54,83] Several strategies and platforms have recently been developed to address this concern. Ackerman et al have reported a cost-effective virus and variant detection platform termed CARMEN, in which samples and Cas13-crRNA complexes are separately confined for barcoding and emulsification before pairwise droplet combinations for detection by fluorescence microscopy allowing for detect 169 human-associated viruses in 8 samples simultaneously.[84] A further optimized tool named mCARMEN was also recently reported from the same laboratory that combines CRISPR-based diagnostics and microfluidics with a streamlined workflow for the clinical testing of SARS-CoV-2 variants, including Delta and Omicron, enabling sensitive and direct RNA detection.[85] Wang et al also reported another strategy, CRISPR-Cas13a cascade-based viral RNA (Cas13C) assay, that can detect SARS-CoV-2 and its mutants in clinical samples utilizing the CRISPR-Cas13a-gRNA and SARS-CoV-2 RNA recognition events to initiate transcription amplification and produce light-up RNA aptamers for output fluorescence signals based on their previous version.[86,87] This allows distinguishing SARS-CoV-2 from other RNA viruses, as well as the viral mutations. Further studies combining CRISPR-Cas13, lateral flow systems, nanoparticles, and advanced gRNA design strategies may help develop a more specific, sensitive, quick, and straightforward diagnostic platform for the detection of SARS-CoV-2 and its emerging variants. Additionally, by taking advantage of the precise target recognition features of the present CRISPR-Cas13 system, future CRISPR-Cas13-based RNA detection tools may also be used to recognize and distinguish emerging SARS-CoV-2 variants.

CRISPR-Cas13 for COVID-19 treatment

CRISPR-Cas13 targets the SARS-CoV-2 genome

CRISPR-Cas13 has been used to target various RNA viruses, including SARS-CoV-2 (Fig. 4), porcine reproductive and respiratory syndrome virus, hepatitis C virus, and dengue virus both *in vitro* and *in vivo*.[88–91] The success of CRISPR-Cas13 in repressing SARS-CoV-2 has been achieved using different models. A CRISPR-Cas13-based strategy termed PAC-MAN (prophylactic antiviral CRISPR in human cells) was developed 5 months after the first COVID-19 case was reported, which is consist of Cas13d protein and crRNA targeting highly conserved regions of SARS-CoV-2 genomes. PAC-MAN can effectively degrade SARS-CoV-2 RNA in a human lung epithelial cell model.[92] Considering the frequent mutations of SARS-CoV-2 that may result in the failure of CRISPR-Cas13 recognition, Wang et al performed a comprehensive set of bioinformatics methods, including sequence alignment, structural comparison, and molecular docking, and identified the conserved SARS-CoV-2-spike(S)-specific segment for CRISPR-Cas13a targeting.[93] Additionally, a CRISPR-Cas13b-based SARS-CoV-2 targeting system has been developed with reprogrammed Cas13b effectors targeting accessible regions of spike and nucleocapsid transcripts and with optimized and multiplexed crRNAs; this system can suppress the replication of SARS-CoV-2 variants, including the variant of concern B.1.1.7 in infected mammalian cells.[94] A recent study reported by Zeng et al showed that the Cas13d system delivered with lipid nanoparticles can effectively treat infections from multiple variants of coronavirus, including Omicron SARS-CoV-2, in human primary airway epithelium air-liquid interface cultures.[47]

In addition to the success of the CRISPR system in repressing virus replication in cell models, recent studies conducted in animal models have shown that CRISPR-Cas13 targeting SARS-CoV-2 sequences prophylactically protect against SARS-CoV-2 infections.[88] A study by Blanchard et al in mice and hamsters revealed that the delivery of polymer-formulated Cas13a mRNA and gRNA recognizing PB1 and highly conserved regions of PB2 of influenza virus and the replicase and nucleocapsid genes of SARS-CoV-2 to the respiratory tract using a nebulizer reduced viral RNA replication and improved symptoms.[88] Combining the recently reported one-step platform for screening the highly efficient and minimal off-target CRISPR-Cas13 crRNAs,[95] with the efficient CRISPR delivery technologies that can specifically and efficiently deliver the CRISPR-Cas13 system to the desired organ or tissues such as lung,[96,97] the CRISPR-Cas13-based RNA interference systems hold great promise for SARS-CoV-2 treatment. Further clinical trials are required to determine the effect of CRISPR-Cas13 on SARS-CoV-2 prevention and treatment.

CRISPR-Cas13 targets the host factors required for COVID-19 infection

Given the rapid mutation and evolution of SARS-CoV-2, researchers are also trying to decrease the viral load by targeting host genes that are essential for SARS-CoV-2 infection and replication (Fig. 4). Using genome-wide CRISPR-Cas9 screening, several host genes, including TMPRSS2, angiotensin-converting enzyme-2 (ACE2), CTSL, RAB7A, and HMGB1, have been proven to be essential for SARS-CoV-2 entry and replication.[98,99] Among these host factors, ACE2 is critical for SARS-CoV-2 infection. However, it is difficult to target ACE2 in therapies because of its essential metabolic role.[100,101] Alternatively, the serine protease TMPRSS2 and cysteine protease CTSL have long been recognized as important proteases mediating virus entry through the virus—host cell surface and endosome membrane fusion, respectively.[102–104] Several CTSL and

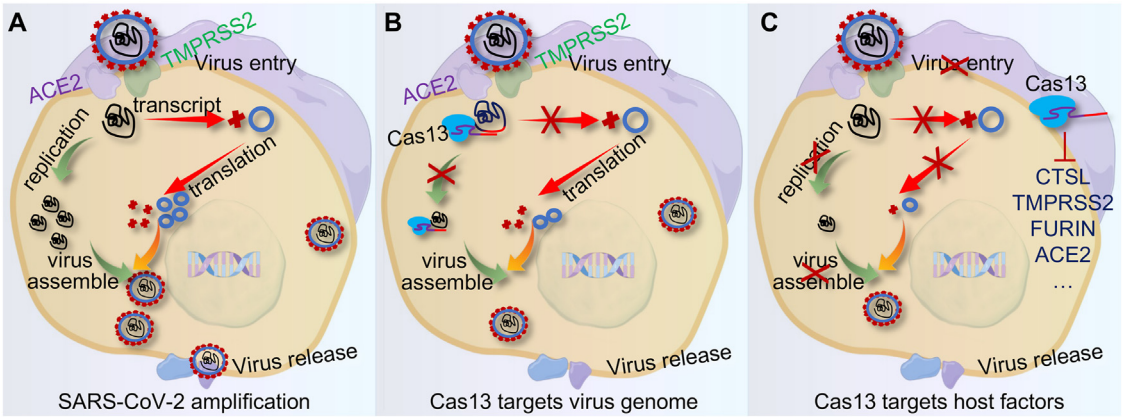

Figure 4 CRISPR-Cas13 targeting strategies for COVID-19 treatment. **(A)** Schematic illustration of SARS-CoV-2 entry, amplification, assembly, and release from host cells. **(B)** CRISPR-Cas13 with gRNA recognizing SARS-CoV-2 genome sequence mediates viral RNA degradation, thereby blocking protein transcription and genome replication. **(C)** CRISPR-Cas13 targeting of host factors interferes in viral entry, genome replication, protein generation, and virus assembly to inhibit SARS-CoV-2 amplification.

TMPRSS2 inhibitors have been reported to block the entry of coronaviruses *in vitro*.[105–109] The administration of N-0385, a TMPRSS2 inhibitor, afforded a high level of prophylactic and therapeutic benefits in a K18-human ACE2 transgenic mouse model of severe COVID-19,[110] However, it is unknown whether targeting CTSL would block authentic SARS-CoV-2 infection *in vivo*. We and our collaborators have recently developed a lung *Ctsl* mRNA-targeted CRISPR-Cas13d-based nanoparticle therapy to curb fatal SARS-CoV-2 infection in a mouse model,[41] and demonstrated that it can decrease lung *Ctsl* expression in normal mice and extend the survival of lethally SARS-CoV-2-infected mice by decreasing lung virus burden, forming a basis for CRISPR-Cas13-based nanotherapy in clinical trials.

Considering the alternative viral entry pathways, especially for emerging mutant variants, including SARS-CoV-2 Omicron sub-lineage BA.5 that may have altered ACE2 binding affinity and CTSL/TMPRSS2 usage,[10,41,111–114] CRISPR-Cas13 based multi-target strategies, such as gRNAs recognizing both CTSL and TMPRSS2 or CTSL and RAB7A, may minimize the mutants' infectivity and treatment resistance.[115] Additionally, mechanistic studies are required to determine other potential host targets for multi-target treatments. More recent studies have revealed that several new host factors such as CFTR, OAS1, and GATA6 are also promising targets for SARS-CoV-2 treatment.[116–118] However, further studies are needed to investigate these possibilities. Notably, other CRISPR systems, such as CRISPR-Cas9 and CRISPR-Cas12, may also potentially detect and treat COVID-19 by recognizing the corresponding DNA sequence.[72,119] Combined therapies with different CRISPR-Cas systems are also worthy of further exploration.

Changes and prospects

Overall, the aforementioned studies have proved the potential of the CRISPR-Cas13 system as an effective diagnostic and treatment tool for COVID-19. However, as described previously and reported by multiple research groups, CRISPR-Cas13 is highly specific and a single-point mutation can significantly decrease its targeting efficiency or even result in recognition failure.[22,83,120] Accumulating data show that SARS-CoV-2 is rapidly evolving, and its emerging mutations may result in the failure of CRISPR-Cas13-based diagnosis and treatment.[121–123] Although several strategies have been reported to predict the conserved structures of SARS-CoV-2 and to design gRNAs with high specificity and minimal off-targets,[95,124] further efforts are warranted to test whether these strategies are sufficient to counteract the rapid viral evolution and mutations and whether multiple-target strategies, such as targeting both virus and host genes such as *CTSL*, are worthy of further investigation to improve treatment efficiency. It is worth noting that CRISPR-Cas13 can also be employed for the diagnoses and treatments of other RNA virus infections, such as SARS-CoV-1 and the Middle East respiratory syndrome coronavirus.[47,125] Developing a reprogrammable RNA detection and degradation CRISPR-Cas system is not only complementary to existing vaccination and antiviral treatment strategies for COVID-19 but also enables the diagnosis and treatment of other RNA virus infectious diseases. In summary, CRISPR-Cas13-based technologies are emerging tools for the diagnosis and treatment of COVID-19 and may also be a promising weapon for future RNA virus pandemics.

Conflict of interests

The authors declare that they have no competing interests.

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