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Review Article

Current Applications and Future Perspective of CRISPR/Cas9 in the Diagnosis and Treatment of COVID 19: A Review

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ABSTRACT

Since the outbreak of COVID-19, scientists have applied various techniques to diagnose and treat the viral disease. However, due to the limitations of other methods, they deployed Clustered-Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) protein (CRISPR/Cas) system that not just successfully diagnosed but also facilitated the therapeutic treatment of the COVID-19. CRISPR-Cas9 was first identified in the bacteria E. coli, which has a unique immune system for cutting the nucleic structures of invasive species. Scientists studied the bacterial system that led to the development of an identical model, generally called the CRISPR-Cas9 genome editing system. It has a guide RNA (gRNA) and Cas9 proteins; gRNA identifies and leads cas9 protein to cleave the specific sequence. This technique has dynamic applications, such as the ability to correct mutations by cleaving the mutant cells and to detect and develop optimal treatments for viral diseases like severe acute respiratory syndrome coronavirus-2 (SARS-CoV2). Apart from the extensive advantages of CRISPR-Cas technology, there are serious concerns regarding the commercialization of this technique. A rational suggestion would be to use it to resist a pandemic like COVID-19 rather than triggering another human race of genome enhancement. This article is aimed to review the background of CRISPR-Cas9, its mechanism as a diagnostic and therapeutic tool for COVID-19, whereas its limitations, future aspects, and ethical boundaries are discussed subsequently.

INTRODUCTION

The outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV-2) has contracted to almost 80 million people around the globe while nearly 2 million people have died since December 2019 when it was first discovered in Wuhan province of China. The disease was spreading so rapidly that millions were getting infected in a single day and the R0 Number (number of new individuals infected by the already infected individual) of coronavirus was estimated to be three [1]. Therefore, there was much need for authentic diagnostic and therapeutic tools to control the disease, reduce the risk of transmission and save the lives of infected people through medications or vaccinations [2]. Hitherto, various conventional diagnostics techniques such as sequencing based methods, immunological methods and PCR based techniques were deployed to detect the novel coronavirus, yet, due to limitations of these methods and low accuracy, it was critical to find methods that give higher accuracy and quick results. In this regard, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-Associated System CAS – a versatile gene editing tool is deployed to facilitate diagnosis and treatment of

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SARS-CoV-2. CRISPR-Cas structures were first found in E.coli bacteria back in the 1980s that guard cells from the foreign invasion species [3]. Since the invention of CRISPR-CAS9 genome editing technology biomedical research has been revolutionized [4]. The technology can perform gene edits of three critical categories which includes disruption, deletion and insertion or correction to treat diseases. This novel genetic editing system targets the abnormal proteins in the DNA, cuts or modifies the abnormal proteins and lets them repair by the natural DNA repairing mechanism [3]. The lexicon of CRISPR technology is that it comprises two parts – an associated enzyme called 'Cas9' and a 'Guide RNA'. Both combine to form a complex known as Cas9/Guide RNA complex – while Cas9 acting as 'molecular scissors' to cut the DNA and guide RNA leads Cas9 to target specific locations in DNA (Figure 1).

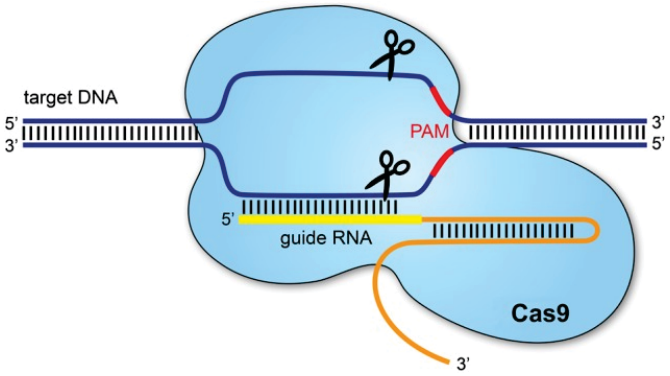

Figure 1: CRISPR-Cas9[5]

The technology has brought promising results in diagnosis and treatment of viral diseases – hepatitis virus infections, HIV, rabies, Dengue etc. Not just infectious diseases, it has provided accuracy in determining hereditary diseases like β-thalassemia, hematologic diseases like sickle cell anemia (SCA). On the other hand, it has been applied in a variety of other species including drosophila, mouse, rat, bacteria and yeast [6]. Due to its high efficiency and low cost, CRISPR-Cas systems have immense applications ranging from disease research and diagnosis, genome-scale screening to gene therapies. 1 However, this review article is primarily focused on the applications of the CRISPR-Cas systems for the diagnosis and therapeutics of SARS-CoV-2, the challenges and limitations associated with the system.

CRISPR-Cas9 as a diagnostic tool for COVID-19

Prevention and rapid treatment of the disease needs quick detection of disease-causing agents. For rapid diagnostic purposes, traditional and conventional techniques including antigen testing, restriction enzymes based, PCR-based methods, and isothermal amplification-based, sequencing-based techniques were previously used. Though these techniques have limitations that make them unable to meet the modern era sensitivity needs of pathogen detection, for instance, detection of different viruses like dengue and SARS-CoV-2 [7]. Compared to these techniques, CRISPR-CAS is applied for pathogen detection which is a highly specific, precise and sensitive technique. It is a next generation technique that can detect the disease-causing pathogens even highly variant viruses in comparatively less time and with better efficiency. Several studies and research have shown the potential of the CRISPR-CAS method as a diagnostic tool for SARS-CoV-2 (COVID 19) virus and various bacterial infections (4). It is one of the best genomes editing tools, consisting of two main classes based on presence of Cas protein i-e, CRISPR Cas Class 1 (types I/cas3, III/cas10, and IV) and CRISPR Cas class 2 (II/Cas9, V/Cas12, and VI/Cas13). CRISPR-CAS9 that facilitated the development of the antiviral strategies for SAR-CoV-2 belongs to class 1 [8]. It works with single subunit Cas protein unlike the first category, which uses multiple subunit proteins for cleavage and targeting the specific sequence. Cas 9 and 12 type target double stranded DNA and Cas 13 targets single stranded RNA.

Mechanism

After determining the desired sequence in DNA, nuclease activity of Cas protein comes forward to take action and targets the point in the sample. These nucleases mediated degradation is tagged with fluorophore dye. The dye produces fluorescent signals that are used as indicators for the determination of a specific sequence which is the main target. Different techniques based on CRISPR Cas 9 mainly Cas 9 and Cas 13 are applied for SARS-CoV-2 detection including SHERLOCK assay (CRISPR Cas 13 based), FELUDA assay (Cas 9 based), detector assay (Cas 12a based), CONAN assay (Cas 3), VaNGuard assay (Cas 12a enzyme-based) etc. SHERLOCK (Specific High-sensitivity Enzymatic Reporter Unlocking), however, is specially developed for the detection of COVID-19 [1]. CRISPR Cas 9 based enzymatic technique for the detection of COVID is FELUDA assay which is considered as a common technique for detection of viral infections. Due to its magnificent diagnostic capability of COVID virus, it is named as FnCas9 or FELUDA assay. This technique consists of basically three steps. First, the RNA sample is taken from a specimen. As COVID virus is RNA virus, so RNA sample is considered. This RNA is converted to cDNA by the activity of reverse transcriptase and biotin-labeled primer is amplified for detection. Then FnCas 9 complex is added to it which is prepared by combination of FnCas enzyme, FAM labeled transcRNA and sgRNA. Ribonucleoprotein (RNP) is bound to the target sequence that activates the FnCas complex. This will result in FAM labeled transcRNA cleavage. In the last, gold nanoparticles conjugated with

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RAM antibodies are added for precise detection (Figure 2) [6].

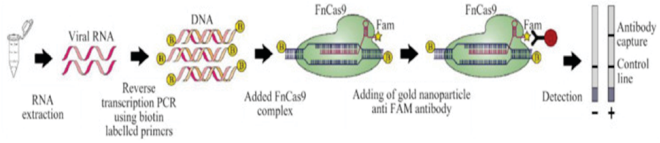

Figure 2: Schematic layout of CRISPR Cas 9 based COVID 19 detection[6]

CRISPR-Cas9 as a therapeutic tool for COVID-19

In a short period since its discovery, CRISPR has successfully built a massive impact on the field of scientific research [9]. Its genome editing function grasps the attention of scientists to find significant applications in numerous disciplines like biodefense, best-quality food production, and fetal medicine production. It can be effectively used to treat infectious viral and bacterial diseases. Comprehensive research has been done and thousands of publications have been written on its incredible efficiency – how it can diagnose diseases in early-stages and treat various infectious diseases like cancer and tuberculosis (TB) [10]. During the COVID-19 outbreak, CRISPR-Cas systems came as a potential diagnostic and therapeutic tool – mainly its class Cas-9 and Cas-13 are considered a preferred option to treat and diagnose SARS-CoV-2 [11]. With the help of a guide-RNA array, its small size makes it appropriate for an "all-in-one" AAV (Adeno-associated virus) to target COVID RNA viruses with high specificity. It cuts the complementary RNA sequence, which is the main target. Unlike Cas 13, Cas 9 needs PAM (protospacer motif) to target the single-stranded RNA sequence (ssRNA). Once the target point is recognized, this COVID incorporated segment is cleaved and changed with the healthy DNA variant [12].

Mechanism of CRISPR-Cas9

CRISPR-Cas9 system includes CRISPR-associated proteins or Cas proteins and gRNA (guide RNA). Cas 9 protein, also known as a genomic scissor, is extracted from the bacterium (Streptococcus pyogenes) and is responsible for the cleavage of DNA strands [13]. Furthermore, two lobes, including the NUC (nuclease) lobe and REC (recognition) lobe, are two regions of the Cas-9 protein. Similarly, crRNA (CRISPR RNA) and transcRNA (trans-activating CRISPR RNA) are two parts of gRNA. It is primarily a three steps process; recognition, breakage, and repair. Through crRNA complementary base pair sequence, Cas 9 protein recognizes the gene of interest (infected area by SARS-CoV-2) directed by sgRNA [14]. Cas 9 protein cannot work until and unless sgRNA is present. It then breaks the double-stranded helix at the upstream region of PAM. DNA starts melting, followed by DNA-RNA hybrid formation and RuvC domains cut the complementary and non-complementary strands, respectively. The infected region is cleaved, followed by a DNA repairing mechanism to make it a healthy variant. NHEJ (Non-Homologous End Joining) and HDR (Homology-directed Repair) are commonly used techniques for repairing processes in the CRISPR Cas 9 system (Figure 3) [15].

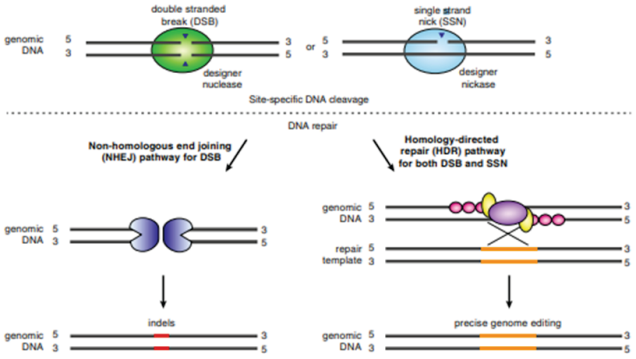

Figure 3: Mechanism of CRSIPR Cas 9 to treat infectious disease like COVID 19 [16].

Limitations

Besides numerous applications of precision, sensitivity, better genome editing tool, working in less time, and role in saving millions of lives, CRSIPR-Cas 9 has some limitations as well which opens the window of the latest CRSIPR systems. It can cause genomic loss while working at target sequence as it is not 100 percent accurate. It is not flexible and specific, i.e., cannot be applied in any sequence of viral genome. It requires PAM to target the sequence at the editing site and only cleave RNA directly. It can cleave DNA after the activity of reverse transcriptase enzymes [12]. CRSIPR Cas technique is efficient, inexpensive and exact but it requires highly experienced staff and strong legislation to be practical. Ethical decisions are made to apply this technology to evaluate potential risk-benefit ratio [17]. So that benefits are maximized and risks are minimized. It can edit the genome of gametes or germ line cells in addition to somatic cells (germline editing). It not only modifies the individual but also his progeny and that should be kept under limits and ethics. Besides curing different abnormalities and mutations, it can enhance the desirable features. So scientists and researchers abide by this technique under moratorium and official policies made for human genome editing [10].

Future Aspects and Ethics

It is clear from the above discussion that the CRSIPR-Cas system possesses the potential to correct mutations, hereditary diseases, fight viral diseases and more. The adoption of this tool has brought beneficial results for the scientific community and has facilitated modern biotechnology [18]. It is because of such advancements

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that has led biotechnology to genetically engineer plants, microorganisms and animals in a much easier way. Regarding the future of CRSIPR, it is suggested that the government, scientific community and researchers must pay attention to its availability. On the other hand, the world is also witnessing the rise of pharmacogenomics. Further studies found that CRSIPR-Cas9 can directly penetrate the genomic sequence of the cell by employing 'cell-penetrating peptides' or 'nucleofection' that will allow quick editing. This genome editing technology will pave the way forward for designing such drugs that could treat a vast range of diseases and mutations – and certainly equip the scientists, healthcare workers, and researchers to prepare for pandemics like COVID-19 beforehand [19]. Besides, its potential power to treat diseases – there is a serious impediment in the progress and implementation of CRSIPR which is 'gene enhancement' or 'gene editing' of human beings. Or more simply put it can be used for unethical purposes, thereby introduction of it in clinical practice would certainly raise social concerns. In the past scientists have made attempts on human embryos using CRSIPR technology to knock out abnormal genes such as the CCR5 gene responsible for HIV virus which resulted in criticism from the scientific communities [20]. The fact that this technology can bring heritable genetic traits can cause a race of 'designer babies' or even worse such as wild or lawless modifications of human beings that can lead to extinction of entire species [21]. Not only humans, it can induce variations in other animals as well and as we have learned from the COVID-19 outbreak – a little mutation can bring the whole world on its knees. For that reason, it is mandatory that scientific communities, governments and legislative bodies must set or define its boundaries – there must be a clear distinction between the genetic treatment and genetic enhancement. Henceforth, techniques like CRSIPR could not get involved in heinous misdeeds and the field of science can progress [10].

CONCLUSIONS

CRSIPR genome editing technology undoubtedly holds a promising future for treating and diagnosing diseases. The two components of CRSIPR-cas9, i.e., Cas9 effector proteins and Guide RNA, are crucial for the future of genome engineering as they allow the modification of DNA sequences. Furthermore, there is room for improvement in delivering Cas9 and its associated guide RNA to target specific sequences of cells. The newly evolving Cas9 enzymes from the bacteria, such as the *Streptococcus pyogenes* type II CRSIPR system, will significantly enhance the delivery method to cleave desired sequences. CRSIPR-Cas9 has played a substantial role in fighting the recent COVID-19 pandemic. It has proved its ability to diagnose, genetically edit and treat viral diseases, genetic disorders, and tumors. On the other hand, this system's powerful genome editing ability has raised social and ethical concerns for its commercialization. The applications of this technology can trigger a race for an enhanced genome that will lead to a superhuman race or even worse. Consequently, scientists and society at large have to address the commercial applications of this technique so that maximum benefits could reap out of it while minimizing the risk factors.

Conflicts of Interest

The authors declare no conflict of interest.

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