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CRISPR-Cas9: a weapon against COVID-19

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Abstract

In current pandemic circumstances novel coronavirus is a salutary challenge for all over the world and coronavirus used the host cell for replication. Coronavirus usually use the host cellular products to perform their basic functions. Various specific target sites also present in coronavirus proteins for target specific therapy such as small inhibitor molecule for viral polymerase or prevent the attachment of viruses to the receptor sites for vaccination purpose. The virus attaches to ACE2 receptors and uses enzyme to cleave translated products which encodes for various enzymes like RNA polymerase, helicase etc. The system needs some processes which lead for the disturbance and make the virus unable to replicate. The recombinant DNA technology makes a great advancement in every field of life with a number of importance in agriculture, industries, and clinics. It is used to manipulate the genetic material of living organism for the purpose of producing desirable products such as disease resistant crops, treatment of cancer, genetic disease and viral disease. Thus, for the purpose of antiviral strategies, the specific technique called CRISPR/Cas9 is used, and this technique has the capability to target specific nucleotide sequence inside the genome of coronavirus.

Introduction

Coronavirus disease 2019 (COVID-19) is a worldwide pandemic caused by sever acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The SARS-CoV-2 has a diameter of 60–140 nm and a positive-sense, single-stranded 29,891 bp RNA genome [2, 3]. Genome sequence alignment has revealed a 79.5% sequence identity between SARS-CoV-2 and SARS-CoV [4-6]. In Wuhan China, SARS-CoV-2 was observed to cause COVID-19 in human [7, 8]. Gradually, COVID-19 spread all over the world and almost 29 million cases were observed led to high mortality rate [9]. SARS-CoV-2 was observed in the individuals of seafood market of Wuhan [5]. The incubation period of SARS-Cov-2 is 14-15 days (**Fig. 1**) [10].

COVID-19 genome Organization

Under electron microscope, coronavirus have a crown like structure [11, 12]. The coronaviruses genome is 27 to 32kb among the RNA viruses [7, 13], however SARS-CoV-2 genome size varies from 29 kb to 32.2 kb [14, 15]. The major symptoms of COVID-19 are pneumonia, mild cough, flu, chest pain and fever [16, 17]. The condition of COVID-19 patients varies from severe symptoms to minor depends upon the immunity of the patients [18]. There are many factors that contribute to the susceptibility of infection and severe condition of COVID-19 [19].

Coronavirus envelop has an extra layer of glycoprotein and derived from host cell membrane [20]. The spike protein is made of glycoprotein and helps to attach with ACE2 receptor of host cell [21, 22]. The viral genome protected by the capsid called as nucleocapsid and made of proteinous subunit called capsomeres. The two third of genome from its 5` ends contain ORF1a and ORF1b encoded the replicase polyprotein, while one third of genome of SARS-CoV-2 from its 3` ends encode the structural protein include spike, envelop, membrane, nucleocapsid and non-structural polyprotein [23]. The genome of SARS-CoV-2 comprise short untranslated portion on both 5` and 3` end. The protease cleaved the translational products of ORF1ab and encoded 16 naps enzymes including RNA polymerase, papain-like protease, helicase and chymotrypsin-like protease [24, 25].

Overview of Virology

SARS-CoV-2 belongs to *Nidovirales* order, family *Coronaviridae* and genus Coronavirus [26]. Coronaviruses contain RNA as the genomic material and use nested RNA set for their replication. Additionally, the sub-family of coronavirus has four different genera such as alpha-coronavirus, beta-coronavirus, gamma-coronavirus and delta-coronavirus [5, 27]. The alpha and beta-coronavirus are two genera of coronavirus that contribute as the major infectious agents in humans. The two alpha-coronavirus strains are HCoV-229E and HCoV-NL63, and two beta-coronavirus strains are HCoV-HKU1 and HCoV-OC43 mostly infect the humans [28].

Genome Editing Techniques

The recombinant DNA technology makes a great advancement in every field of life with a number of importance in agriculture, industries and clinics [29]. It is used to manipulate the genetic material of living organism for the purpose of producing desirable products such as disease resistant crops, treatment of cancer, genetic disease and viral disease [30]. Thus, the specific technique called CRISPR/Cas9 is capable to mark specific sequence of nucleotide within the genome of virus to degrade the genome of virus. There are some other genomes editing techniques are also use for this purpose. These genome editing techniques are categories into three main classes [31] as ZNF (Zinc Finger Nuclease System), TALEN (Transcription Activator like Effector Nuclease System), CRISPR-Cas9 (Clustered Regulatory Interspaced Short Palindromic Repeats- Associated 9).

Zinc Finger Nucleases (ZNF) System

ZFNs are amassed by melding a non-sequence-specific cleavage domain to a site-specific DNA binding domain that is laden in the zinc finger. The zinc-finger protein with site specific binding DNA properties is mainly determine in 1985, as part of transcription factor IIIa in Xenopus oocytes [32]. The zinc finger domain designated by functional specificity and contained an array of Cys_2-His_2 zinc finger, through the highly conserved interaction of their zinc-finger domain with homologous DNA sequences. Usually, an individual Cys_2His_2 zinc finger contains of nearly 30 amino acids, which

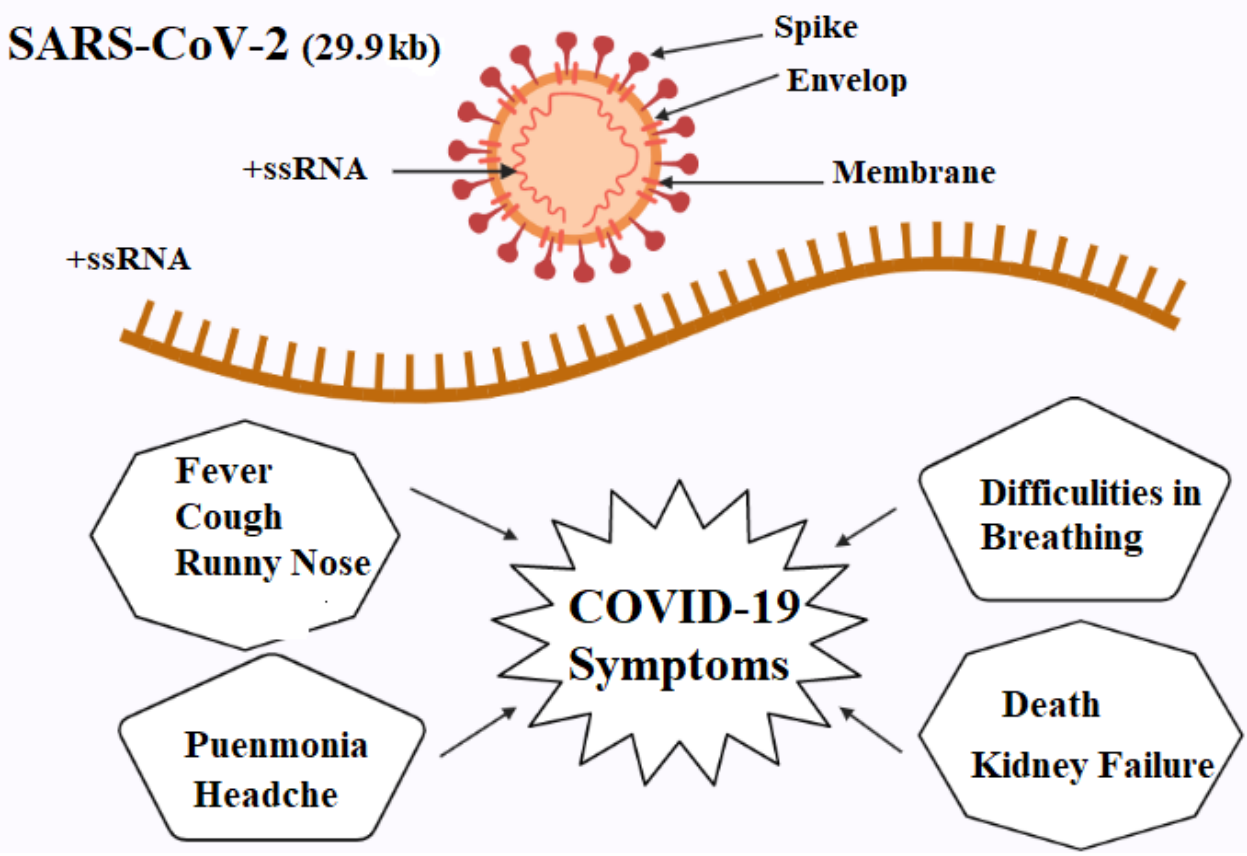

Fig. 1: SARS-CoV-2 and its Symptoms: the size of SARS-CoV is 29.9 bp and is a single stranded, positive sense RNA virus. The symptoms of SARS-CoV-2 include fever, cough and pneumonia.

found two anti-parallel β sheets disparate an α-helix. Cys_2-His_2-ZF is an modifiable deoxyribonucleic acid recognition domain and is measured to be the utmost general form of deoxyribonucleic acid -binding motif in eukaryotic transcription factors [33]. The first class is zinc-finger nucleases, the endonuclease is a synthetic restriction enzyme generated by combining a zinc finger to a DNA cleavage domain of the Folkl restriction endonuclease with custom planned Cys2-His2-Zinc finger proteins, which bind to specific complementary sequence within the host cell [34, 35]. The double strand disruption by repair machinery of host DNA is used for repairing.

Transcription Activator like Effector Nuclease (TALEN) System

The second class is TALEN that is known as the transcription activator like effector nuclease. It is protein-based DNA targeting system. TALEN have better efficiency and specificity than ZFNs. TALEN contains a common DNA cleavage domain that fuses with a sequence-specific DNA-binding domain to create a DSB. This DNA-binding domain comprises of a vastly conserved reiteration sequence of transcriptional activator-like effector (TALE), a protein formerly found in the phytopathogenic bacterium Xanthomonas that obviously changes gene transcription in host plant cells [36]. The binding of transcriptional activator-like effector to deoxyribonucleic acid is arbitrated by a central region with a 33- to 35- amino acid sequence motif. The sequence of each amino acid repeats are structurally similar excluding the two hypervariable amino acid (The repeating variable di-residues or RVD) at positions 12 and 13 [37].Transcription activator like effector nuclease (TALEN) has endonuclease restriction enzyme that can be engineered by combining a TAL-effector DNA joining domain to cut specific sequence of DNA [38]. TAL-effector protein and an endonuclease restriction enzyme are used to engineer TALEN to produce double stranded break at specific DNA sequence. High fragment deletion at the specific target site can generated through this process.

CRISPR/Cas9 System

Clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9) is considered as the most powerful system for genome editing. CRISPR-Cas9 system can edit the genome from target specific sites (Figure 2) [39, 40].

The CRISPR-Cas9 system is simple, very easy to use, specific, and powerful and continues to improve rapidly [41]. Basically, the CRISPR-Cas9 system is a natural defense system present in archaea and bacteria [42]. It is used by archaea and bacteria to protect from the attack of virus. CAS protein and CRISPR loci were identified through the genome sequencing of bacteria and archaea. The CAS restriction protein and CRISPR loci was found in approximately 50% of bacterial genome, while 90% in archaea genome or in their residential plasmid. Today, this naturally occurring defense system is used to direct the degradation of specific target bases sequence into the DNA. The CRISPR-Cas9 contains two distinct components such as a guide RNA (gRNA) and an endonuclease (Cas9).

The gRNA and Cas9 in cell are co-expressed lead to disrupt the DNA. The gRNA is a complementary guided sequence of 20 base pair designed in laboratories. The guided sequence is used to recruit the gRNA and Cas9 endonuclease composite to its mark site by follow the base pairing scheme of Watson-Crick (Figure 3) [43].

The gRNA and Cas9 complex are successfully bind to the complementary target sequence, to disrupt the target sequence by subsequent endonuclease require precise Protospacer Adjacent Motif (PAM) sequence is a trinucleotide sequence. The PAM sequence immediately follows the target sequence. The endonuclease (Cas9) cleaves two RNA or DNA strands to produce the breakage of DNA or RNA double strand and this two or single strand break is called double or single strand break (DSB or SSB). The DSB happen at three to four bases upstream of the sequence of PAM [41].

The repairing machinery of DNA repairs the DSB following the NHEJ pathway of DNA repair. In result of DSB, some portions of DNA/RNA delete which can produce frameshifts mutation or may produce stop codon. In this way, ORF1ab of the target gene can be successfully deleted.

Notably, restriction endonuclease Cas9 system has significance to accomplish of the target specificity and offers nearly high-class on-target breakage of genome. The surveyor assay can be assayed the off-target breakage, which notices mis-matched base pairs follow-on from NHEJ and sequencing of whole genome. However, CRISPR-Cas9 method endures to develop and amended with admiration to genome excision proficiency and reduces the possibility of off-target effects [44-46]. The off-target action can be reduced 50-1500 folds in the cell lines by emerging paired Cas9 nickase scheme [47, 48] and also enhance the site specific DSB induction [49, 50].

CRISPR-Cas9 system should be appropriate to DNA virus extinction from cell. Viruses those causes diseases of human has been freshly treated with CRISPR-Cas9 system and use as a defense against viruses [51].

Applications of CRISPR-Csa9

Today CRISPR/Cas9 technology is more widely used in many fields, including drug development, biofuel production, food production, plant development, genome manipulation technology. SARS-CoV-2 virus belongs to *Nidovirales* family that has main effects on world economy. Recently, an epidemic has ascended in Wuhan city at the end of 2019, triggering a severe death ratio of 35%. In peak of COVID-19 improved patients, it has been detected that extreme immune response leads to long-lasting lung destruction and functional frailty that short the life duration. COVID-19 adulteration is associated with cytokine storm [52]. Still, there is no actual antiviral drug existing in marketplace against COVID-19 contamination. However, vaccine improvement against COVID-19 will necessitate many years [53]. Advance genome editing technology such as CRISPR/Cas9 is require, at this level to control viruses containing complex RNA. The genome of Coronaviruses varies from 26 to 34 kb, with compound mechanism for the translation of non-structural proteins (NSPs). The viral production is motivated by replication and translation complexes (RTCs) that are gathered from NSPs, in complex with the host factors. It is supposed that endoplasmic reticulum-derived membrane layer (ERDM) and double-membrane vesicles are associated with these RTCs. The recurrence of Nidovirus is reliant on conveyance across the membrane, signaling pathway of host or host cell factors and cycles [54]. The CypA is a cytosolic protein, which is involved in the replication of RNA viruses [55] and also involve in knockdown contribute to the replication of nidoviruses like human coronavirus (HCoV)-NL63[56], Feline coronavirus, HCoV-229E [57].

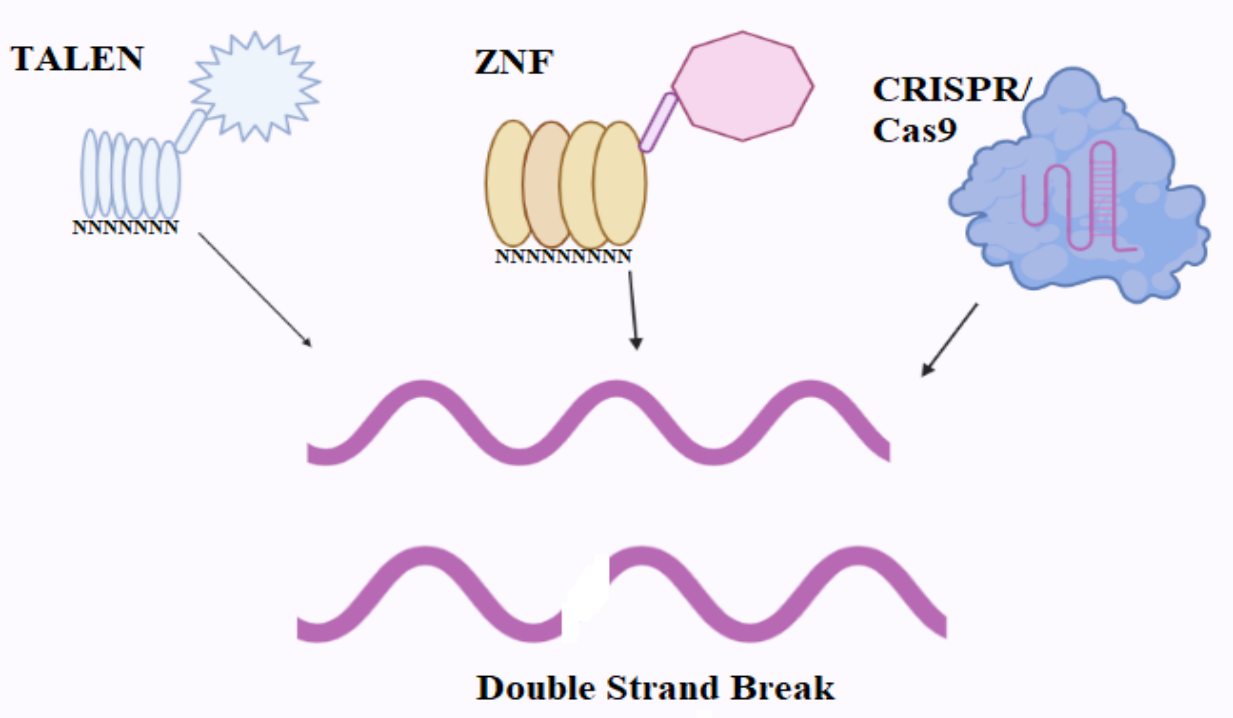

Fig. 2: Genome Editing Technology and their working Mechanism: Above diagram show working principal of TALEN, ZNF and CRISPR/Cas9. These are all techniques produce double strand break at specific site and use to degrade the viral attack.

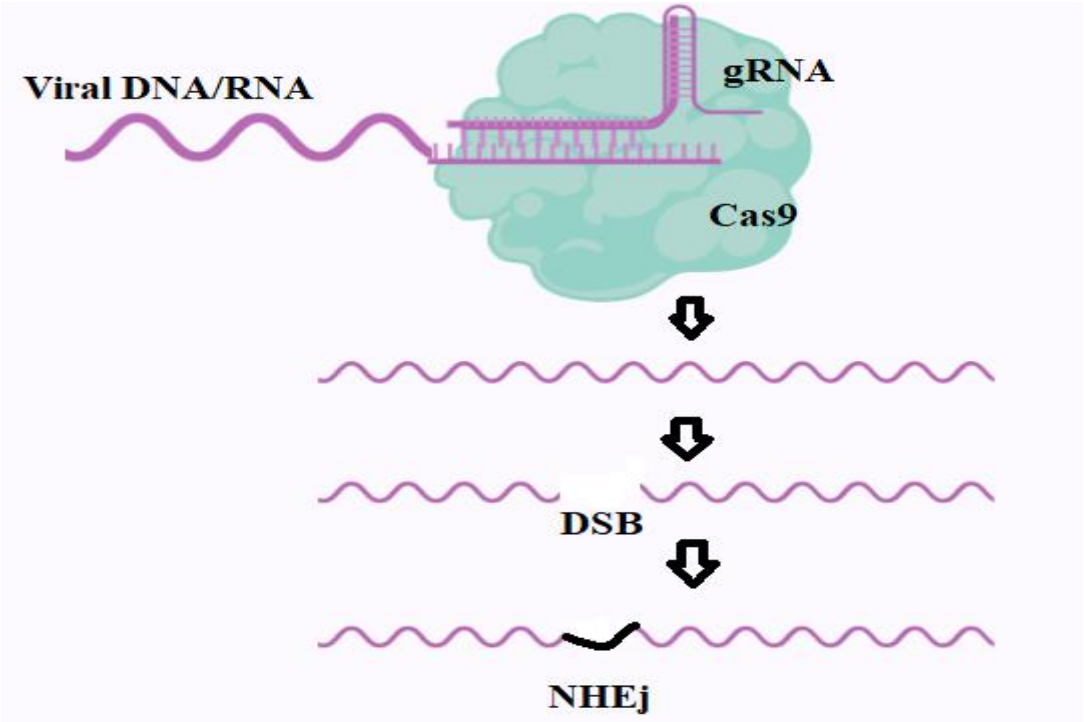

Fig. 3: working mechanism of CRISPR/Cas9: the CRISPR/Cas9 contains a gRNA and a restriction endonuclease. The gRNA helps the Cas9 to reach its target site then Cas9 cleave the target sequence and produce double stranded break repaired through the cell repairing machinery by non-homologous end joining method (NHEJ).

Employed the CRISPR/Cas9 method to examine three nidoviruses in the same cell line i.e., Huh7 and determined that their replication procedure is reliant on CypA knockout. The CRISPR-Cas tool has been used for the regulation of epigenetic mechanism in eukaryotic cells. It is also used to help RNA/DNA for fitting genetic faults and improving genetic traits.

Conclusion

The CRISPR-Cas9 is a significant technique that can easy to use and have less off-target activity. This tool is use to degrade the viral RNA/DNA into the host cells. For this purpose, gRNA and Cas9 protein can be used. gRNA and Cas9 are combine with target complementary sequence lead to produce Cas9 breakage. The genome of viruses into the cells of human can be degraded.

Conflict of interest

The authors declare no conflict of interest.

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