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Review Article

Can Exosome based Therapy Reduces the Severity of COVID-19?

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Abstract

In the last twenty years, several viral epidemics such as severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) has been recorded [1]. In recent times unexplained cases with low respiratory infections detected in Wuhan, China, and poses a big challenge for the whole world [1]. WHO (World Health Organization) formally named this disease as COVID-19 (Corona Virus disease 2019), which is later named by the International Committee on the taxonomy of virus as SARS-CoV-2. Wuhan isolated SARS-CoV-2 belongs to the Sarbecovirus subgenus and Betacoronavirus genus [2]. This virus has been spreading around the world widely. This raises an urgent need for an effective therapeutic approach. Recently different medicines and a combination of drugs have shown promising results. Many companies and researchers are working on finding a suitable vaccine or proper medication for COVID-19. In this review, we aim to provide basic structural information about COVID-19 and possible target to check the growth of these viruses by using exosome-based CRISPR/cas13d technology. Exosome incorporated CRISPR/Cas13d based system may specifically target viral ORF1ab and S RNA of COVID19 to knock down the ability to reproduce. This promising method needs proper investigation and if proven effective that will be an excellent development to fight against the COVID19 pandemic.

Keywords: *Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV); Middle East Respiratory Syndrome Coronavirus (MERS-CoV); COVID-19 (Corona Virus Disease 2019)*

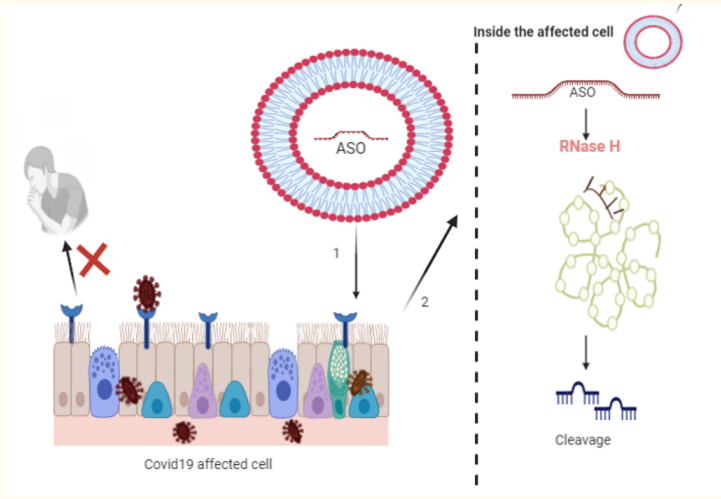

Figure 1: *The S glycoprotein of the virion bind to the ACE2 receptor of target bronchial epithelial cell and get internalize. Exosome incorporated CRISPR/Cas13d therapy can check the expression of ORF1ab and S RNA through RNA interference and inhibit the viral growth.*

Introduction

COVID-19 has spread globally and if proper measures are not done to check its spread then it will infect 25 - 70% of the world population with a mortality rate of 1 - 5 % [4]. The most important problem is that COVID-19 is a new pathogen which imposing a threat to mankind and via mutation is likely to appear a new strain with resistance to previous therapeutic agents in a very short period. An affected person normally suffers from fever, fatigue, dry cough, upper airway congestion, shortness of breath, myalgia or gastrointestinal symptoms [4]. Scientists believe that these changes in the genomic sequence have enhanced the virulence of the virus [3]. There are two circulating strains, one is deadly strain 'L' and one is less virulent or 'S' [3]. The understanding of phenotypic and genotypic structure is very important for the discovery of a specific drug or vaccine. On February 3, 2020, the WHO (World Health Organization) launched a Strategic preparedness and response plan (SPRP) that outlines the support measures to enhance the COVID-19 response and preparedness of UN member countries. Researchers are working on different creative methods to check the virus proliferation, and in this review, we will mainly discuss exosome-based therapy to limit the viral reproducibility by targeting viral RNA using antisense RNA technology.

SARS-COV2 is an enveloped positive-stranded RNA virus whose genome size ranges from 27 - 32 kbp that contains at least six open reading frames [3]. The first ORF is located at the 5′end whereas the other ORF at 3′ end encodes several viral proteins like S (Spike), M (membrane), N (nucleocapsid), E (envelop) [3]. The spike S protein binds to target cell ACE2 (angiotensin-converting enzyme 2) and mediating the entry of the viral particle inside the target cell [5]. The affinity of viral S protein for target cell receptor protein which is 10 - 20 times higher than SARS-COV is very crucial for viral entry [3]. Understanding the mechanism raises an urgent need for the development of a model to restrict the viral entry.

We proposed a flexible CRISPR system to target specific genomic sites of viral RNA. This guide RNA based complex defines the target sequence in the genome where Cas protein will cut [6]. Cas13 is a class2/type VI CRISPR-Cas RNA endonuclease which can induce cleavage and degradation of viral RNA [6]. CRISPR/Cas 13d is an efficient straight forward to disrupt the gene function [7]. We specifically propose to design of guide RNA that can specifically target ORF1ab and S viral RNA that can limit the reproduce ability of COVID19. This may disrupt the viral growth.

For cell-specific delivery, this complex needs a proper delivery vehicle. Cell-derived exosomes are the most suitable choice because of its small size and bio inertness [17]. Cell-specific exosome-based delivery also depends upon the successful incorporation of this CRISPR complex. Different sophisticated methods like electroporation, co-incubation may be utilized for successful incorporation. Moreover, surface engineering can be done to modify the exosome surface that can specifically target the infected lung cell. Finally, mist inhaling device or nebulizer can be a very good option for the successful delivery.

This review presents a comprehensive introduction about the possible application of exosome vesicle to check the COVID-19 viral proliferation by antisense RNA technology. We first discuss the basic description of COVID19 and exosome. We further discuss possible exosome-mediated antisense technology to inhibit the viral gene expression. A brief discussion on future prospective to check viral growth concludes this review.

COVID 19- A new guest

COVID19 belongs to the β coronavirus cluster is a chimeric virus between a bat coronavirus and coronavirus of unknown origin [5]. Recent studies suggest that COVID 19 has genetic sequence homology of around 80% with SARS-COV that strongly supports COVID 19 originally a mutated version of bat coronavirus through an intermediate host of COVID19 where it passes its asexual stages is still unknown [5]. These small zoonotic pathogens can be transmitted to humans through direct contact. Like other coronaviruses COVID19 primarily infect the respiratory tract with a minimum incubation period of 4 - 5 days [9]. COVID19 contains a single-stranded positive-sense RNA genome with six open reading frames (ORF) [3]. RNA expression is mainly translated into four main types of viral proteins- Spike glycoprotein (S), Membrane protein (M), Envelop protein (E), and Nucleocapsid protein (N) [3]. The earlier study suggests that only those

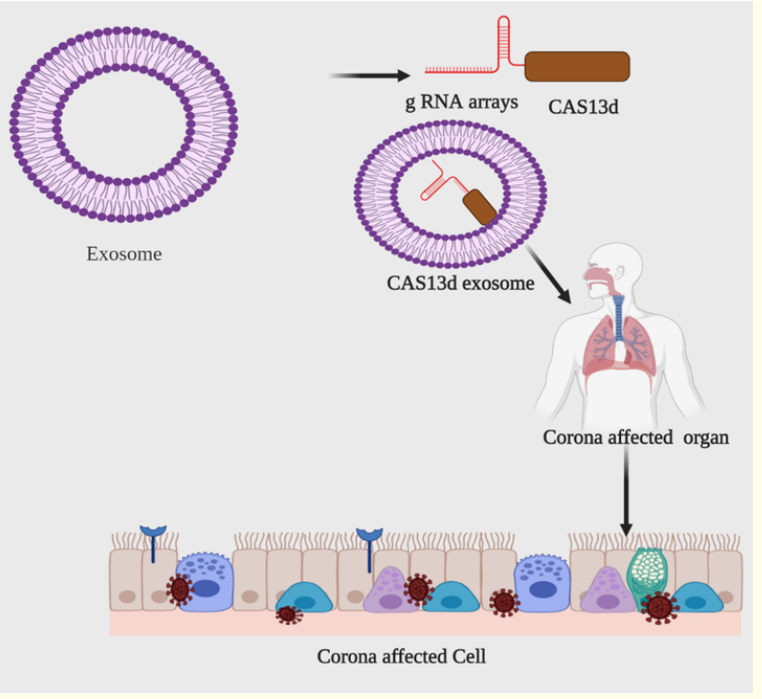

Figure 2: *CRISPR/Cas 13d is an early gene modification technology to induce viral RNA degradation. Exosome enjoy huge immunogenic privilege, that's why can be used as a proper carrier for CRISPR/Cas. Lung is an inaccessible organ, so, mist inhaling device or nebulizer can be a very good option for the successful delivery.*

alveolar epithelial cells that express angiotensin-converting enzyme2 (ACE2) are the principal target of COVID19 [9]. Virion expressed spike protein (S Protein) interacts with the ACE2 receptor and gets internalized [9]. Damage to alveolar cells leads to the activation of different systematic reactions and even cell death [1]. It was found that the receptor-mediated binding ability of SARS-COV2 is 10-12 times higher than SARS-COV [1]. Affected patients were reported to have a higher concentration of (Interleukin) IL-6, IL-10, (Tumour Necrosis Factor) TNF-α, (Macrophage Inflammatory Protein) MIP1α [10]. Activated $CD4^+$ cells induce B cells for the production of virus-specific antibody whereas $CD8^+$ cells can kill the virus affected cells.

In addition to respiratory syndromes, thrombosis and pulmonary embolism have been observed in several cases [10]. Recent examination suggests that there is a difference between the RNA nucleotide sequence of COVID19 isolated from CHINA and USA [7]. A recent report suggested that children, in general, are less susceptible to COVID19 and this suggests designing proper immunotherapy against the virus.

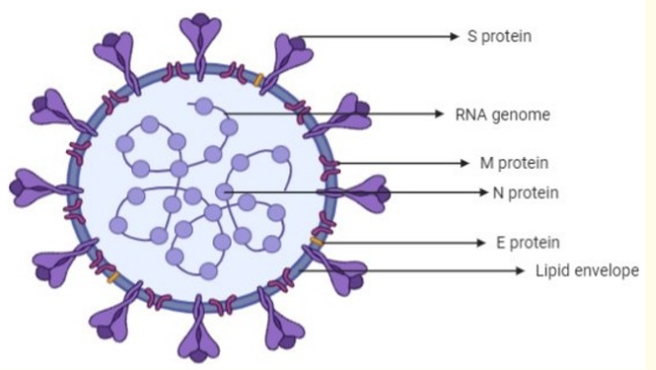

Figure 3: *SARS-COV2 is a beta coronavirus contains four structural protein – envelope (E), spike (S), membrane (M) and nucleocapsid (N). S&M proteins are also transmembrane protein involving in virus assembly during replication. They are enveloped, positive stranded RNA virus. N protein remain associated with RNA and form a nucleocapsid inside the envelope. Polymer of S protein remain associated with nucleocapsid and giving it a crown like appearance, thus the name coronavirus.*

CRISPR/Cas technology- A potential treatment for COVID19

CRISPR-Cas is mainly a prokaryotic adaptive mechanism from bacteriophage and other foreign genetic material [11]. Although it is critical to developing an early gene modification program, the CRISPR/Cas system has recently been applied to induce RNA degradation in the plant, mammalian, or viral cell line [6]. CRISPR/Cas technology composed of two systems where guide RNA specifically targets the effector complex and directed enzyme destroy the invading nucleic acid [11]. Cas9 nucleases first remodeled for biomedical application, where cas9 mainly targets DNA sequences however recently identified cas13 can easily bind to RNA sequences [6]. To inhibit the proliferation of this virus, the CRISPR-Cas13d system is derived from *Ruminococcus flavefaciens,* XPD30002 [D]. Cas13 is a class 2/type VI - Cas RNA endonuclease [6]. Due to small size, high specificity and higher efficacy cas13d is used rather than other cas13 proteins [12].

Very recent research functionally design guide RNAs that specifically target COVID19 replicase transcriptase (ORF1ab) and spike (S) RNAs of the virus to check its expression in translation level [7]. They mainly designed 10,333 guide RNAs to target 10 peptide coding regions of the COVID19 genome without affecting the human RNAs [7]. This target-specific binding form a complex with Cas 13d single effector nucleases can act as a scaffold and RNA cleavage occur outside of the recognition site [7]. For cleavage, cas13d does not require the presence of specific adjacent sequences, and this unique feature advantages for designing guide RNAs to check viral replication that otherwise may escape from traditional drug treatment due to frequent mutation [7].

Advancement in the CRISPR/Cas system in recent years to check RNA virus infection is breath taking. Novel future developments in eliminating COVID19 needs a more effective and target cell-specific therapeutic approach.

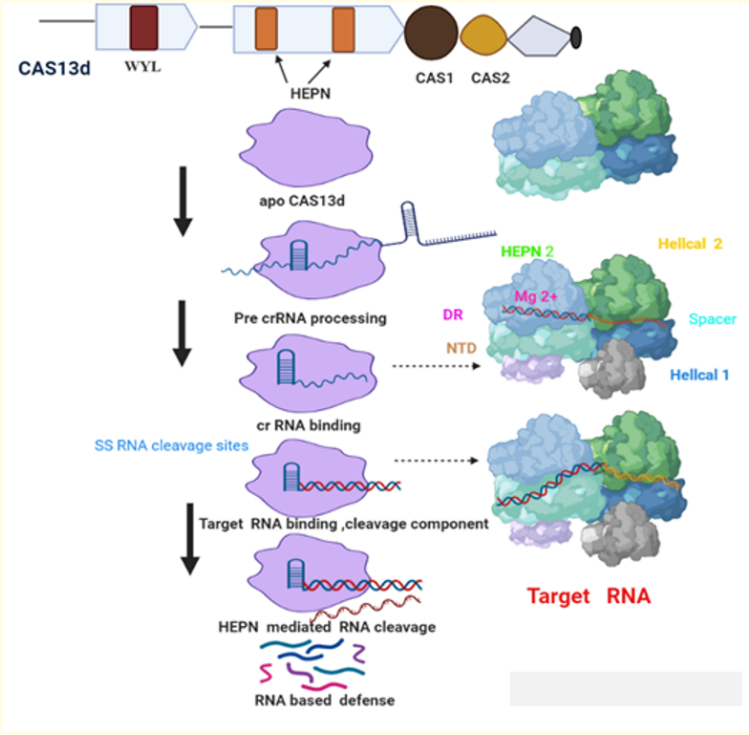

Figure 4: *Schematic of Cas13d effector and three g-RNA array for the for target sequence specific recognition of viral RNA. The specific binding is mediated by crRNA, which forms a complex with the single effector nuclease Cas13d. This leads to cleavage of the viral RNA away from the recognition site and outside of the protein surface. The action of Cas13d doesn't depend on the presence of specific adjacent sequences such as NGG motif. ITR: Inverted Terminal Repeat; HEPN- Higher Eukaryotic and Prokaryotic Nucleotide Binding Domain; NTR: N Terminal Domain.*

Exosome-A nano-therapeutic platform for gene therapy

Over the past decades, there has been broad research carried out on exosomes, which is one type of extracellular vesicle. These membrane-bound bio nanoparticles, size around 40 - 160 nm, density 1.13 - 1.19 g/ml, are ovoid to cup-shaped and developed from a direct fusion of Multivesicular bodies (MVB) or endosomes with the plasma membrane [14,15]. Initially regarded as a garbage bag for the removal of unwanted materials by the cell but recently it is recognized as one of the most important factors in intracellular cell signaling [15]. Exosome interacts with target cells either via direct membrane fusion or through receptor-mediated endocytosis [16]. This results in the release of cargo into the target cell by transcytosis [16].

Naked Nucleic acids contain negative charges on the sugar backbone, will not allow them to cross biological membrane efficiently [17]. Moreover, the lung, the primary infected organ of COVID19 is full of mucus that could interfere with targeting as well as relatively inaccessible organs. Therefore, they must be encapsulated or conjugated in a suitable vehicle to cross the barrier [17]. Cell-derived Bionanoparticles play an important role in the horizontal transfer of different proteins and genetic material such as miRNA, between cells [8]. This quality makes them especially alluring for the delivery of pharmaceutical proteins and nucleic acids, for example, small interfering RNA (siRNA) [8]. Exosomes are the most suitable choice because of their small size which is <100nm and they have immunogenic privilege so they can easily evade rapid clearance by the mononuclear phagocytic system to deliver drug of interest to specific cell [8].

Exosome can serve as a suitable vehicle for the incorporation of cas13d and guide RNA targeting COVID19 infected cells using any mist inhaling device or nebulizer. Co-incubation or electroporation may be used for the incorporation. Further, exosomes can be engineered or surface modified to specifically target infected lung cells, which is the main infected organ by COVID19 and thus can be used for targeted delivery of COVID19.

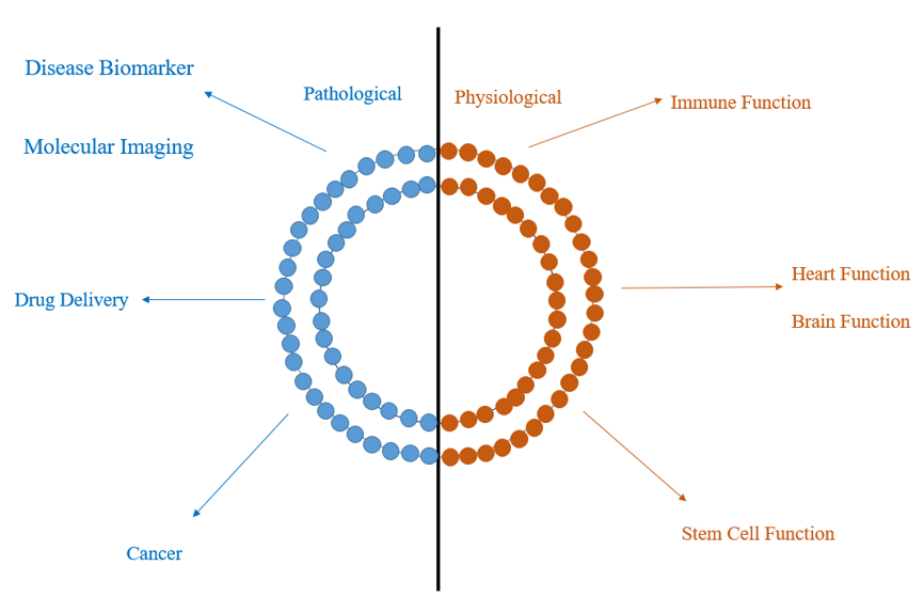

Figure 5: *Exosomes are endosome released cell derived bionanoparticle which plays important role in cell signalling. Exosomes can serve as biomarker for various diseases associated with cancer, genetics, virus or bacteria. This small particles can also be used in molecular imaging or as a Drug delivery vehicle.*

Exosome engineering

Along with being nontoxic and immunologically inert, a suitable delivery vehicle must be target cell-specific. Surface manipulation of cell-derived exosomes can deliver incorporated cargo in a target cell-specific manner. Furthermore, Exosome can also be engineered to incorporate the Cargo of interest for the therapeutic purpose [8,18].

COVID19 surface S protein is a type I transmembrane protein [19]. To generate chimeric plasmid, Seraphin Kuate., *et al*. replaced the coding region of the transmembrane and cytoplasmic domain of S protein in SARS-COV by those of VSV-G (^{GTM}) [04]. The further fusion of S ectodomain with the N terminal of VSV-G leads to the formation of membrane-bound complete hybrid protein [19]. To check the hybrid S protein is functionally active or not they pseudotyped SIV, HIV, MLV based vectors with either wild type S protein or hybrid S protein and similar titer level proved hybrid S protein was bioactive [19]. Cell lysates from 293T cells were transiently transfected with recombinant S plasmid and that increased the expression of S^{GTM} on the exosome surface. Along with S^{GTM}, western blot analyses revealed that HSP90 and CD82 membrane-bound proteins were also found on the exosome surface. To check S^{GTM} is exosome surface-bound sample were ultracentrifuged, it was found that S^{GTM} was found in concentrated supernatant but it was absent in the pellet solution and this confirmed that S^{GTM} seemed to bound on the membrane surface [19].

In this way, S^{GTM} expressing exosomes can be used to incorporate CRISPR/cas13d. Surface engineered exosomes may interact with the ACE2 receptor of the infected cell. This interaction leads to the release of exosome incorporated cargo. CRISPR/cas13d inside the infected cell may halt the expression of ORF1ab and S RNA which might destroy the invading COVID19 nucleic acid and check its replication.

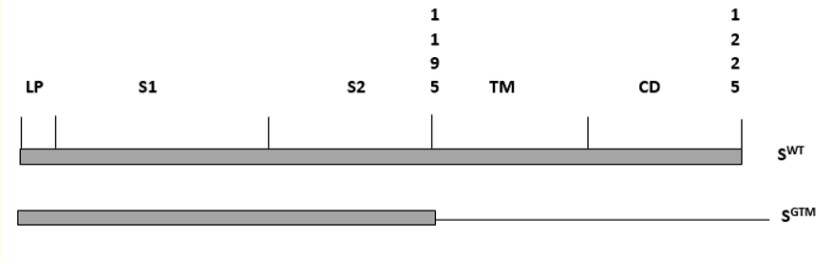

Figure 5: *Schematic of wild type and chimeric S protein. The numbers above the sequence represent the amino acid sequence in the corresponding polypeptide.*

LP: Leader Peptide; TM: Transmembrane Domain; CD: Cytoplasmic Domain; SWT: Wild Type S Protein; SGTM: Hybrid Type S Protein.

Conclusion

The novel coronavirus COVID19 or SARS-COV2 has spread around the world and as of 29 July 2020 there were over 1, 67, 37,842 confirmed cases including 659374 deaths happened around the globe. We propose a novel and straightforward CRISPR/Cas system for treatment and inhibit viral replication. Due to safety and target cell-specific delivery of this system exosome can serve as a potential delivery vehicle. Although we don't know whether this model predicts efficacy against COVID19 and this approach needs further investigation. If proven effective that will be a breath taking development to fight against the COVID19 pandemic.

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