Molecular Therapy
Nucleic Acids
Editorial

Small interfering RNA: a tailored approach to explore the therapeutic potential in COVID-19

The novel coronavirus disease 2019 (COVID-19) that has been gripping the world in recent times belongs to a myriad of single-stranded RNA that caused the Middle East Respiratory syndrome (MERS) in 2012 and severe acute respiratory syndrome (SARS) in 2003, both of which were transmitted to humans from animal origin.[1]

Small interfering RNA (siRNA) functions with a high degree of specificity by degrading the mRNA in a sequence-specific manner (Watson and Crick base pairing) at the post-transcriptional level through a phenomenon known as post-transcriptional gene silencing (PTGS).[2] Here, we emphasize that it could be a promising approach in managing the current outbreak of COVID-19.

At present, there is no specific treatment option available for advanced COVID-19. While emerging vaccines should limit the propagation of COVID-19 and eventually control the disease, they may not be of use in those who have already developed the symptoms or in immunodeficient individuals and might become outdated with the advent of a new strain. Targeted approaches using the small molecules have the drawback that it takes several years to optimize the lead molecule that has an acceptable level of biological function with the ability to reach the target site.[3,4] Also, the small molecules, such as protease inhibitors/nucleoside analogs, and vaccines need to know the structural and functional aspects of the virus.[5] The constraints posed by the above options can be surpassed by oligonucleotides (siRNA), which are less toxic and can target unreachable sites,[6,7] with an additional advantage of the ability to be rapidly and precisely designed using advanced bioinformatic tools and the machine learning method/artificial neural network, such as BIOPREDSI-DSIR (software), since the key criterion necessary is the sequence of the target.[2]

Ever since the first study, conducted in 2001, to evaluate the gene-specific silencing potential in RNA viruses,[8] the RNAi tool has been sufficiently explored in treating several viral infections.[6] A study conducted by Rota et al.[9] characterized the genomic sequence of SARS, which made the designing of siRNA feasible. A study conducted by Wu et al.[10] demonstrated the use of a siRNA, which achieved success in inhibiting the SARS-CoV replication by targeting the coding sequence of the spike protein and 3′ UTR.

Genome sequencing studies of SARS-CoV-2 revealed that it is closely related to bat-SL-CoVZXC21 and bat-SL-CoVZC45. The bat-SLCoVZXC21 and bat-SL-CoVZC45 are bat-derived SARS-like coronaviruses that were isolated in China's Zhoushan in the year 2018 (accession numbers for bat-SLCoVZXC21 and bat-SL-CoVZC45 are GenBank: MG772934.1 and MG772933.1, respectively).[11] Specifically, the receptor binding domain of SARS-CoV-2 organized under lineage B showed closeness to SARS-CoV through phylogenetic analysis.[12] The spike (S) protein mediates receptor binding and membrane fusion, essential in tropism and transmission.[12] The spike protein is classified into two subtypes: S1 and S2. The S1 subunit is majorly responsible for receptor binding, whereas the S2 domain is essential for membrane fusion. When the composition of amino acid was identified in the spike protein S1, which is essential for receptor binding, it was found that at least 50 conserved amino acid sequences were present in common. A modeling study proved that the receptor binding of SARS-CoV occurs through Angiotensin-converting enzyme 2 (ACE2), which might be similar in the case of COVID-19 as the external subdomain of the receptor binding domain is similar in both of these viruses.[12] Because at least 50 amino acid sequences are conserved in SARS-CoV and SARS-CoV-2 in the spike S1 region, strategies that were employed earlier in the designing of siRNA that target the spike gene for SARS might also have probability in regulating COVID-19 infection and transmission.[12] Another essential target is envelope E protein, which is encoded by the *envelope E* gene, which is essential in the formation of viral envelope and viral assembly. Meng et al.[13] designed siRNAs to block the expression of the *envelope E* gene of the SARS virus. They targeted two siRNAs against different positions on the gene (siRNA1: 157 to 177; siRNA2: 206 to 227) and successfully blocked the expression by approximately 90%.[13] In a recent study that characterized the COVID-19 genome, it was identified that the *E* gene shares its identity up to 98.7% with closely related viruses of subgenus Sarbecovirus origin.[12] Meng et al.[13] also evaluated the RNA-dependent RNA polymerase (*RdRp*) gene, which is also an excellent therapeutic target since it is necessary for the replication process, inhibiting the expression of this gene and thereby preventing the replication.[13] A study published in February 2020 explored the genomic and proteomic comparisons of SARS-CoV and SARS-CoV-2 (COVID-19) and concluded that the identity of the protein sequence of RdRp is 96.35% (SARS-CoV NCBI: NP_828869.1 and SARS-CoV-2 NP_828869.1: YP_009725307.1).[14] Another study targeting the *replicase 1a* region of SARS-CoV with multiple siRNAs (pooled siRNAs) proved that replication can be inhibited by drastically reducing the cytopathic effects in various strains of SARS coronaviruses.[15] In an *in vitro* study, Zheng et al.[16] demonstrated a sustained prophylactic effect with significant inhibition of SARS-CoV infection and replication in fetal Rhesus kidney cells (FRhK-4) when administered a cocktail of siRNAs prior to the infection. Moving this study forward to the next level in a non-human primate model, Li et al.[17] evaluated this phenomenon in the Rhesus macaque through intra-tracheal

administration and successfully established the prophylactic effect of siRNA much better in comparison to co-delivery and post exposure with no adverse effects. Since the US Food and Drug Administration (FDA) is considering the combination of three siRNAs (cocktail) as a single entity, it could be a beneficial element for circumventing the mutational escape since viruses are prone to undergoing mutagenesis.[18] All these *Spike*, *Envelope E*, *RdRp* and *replicase 1a* genes can be good targeting sites.

In a recent study, it was observed that the SARS-CoV-2 genome is 85% similar to SARS-CoV-like viruses.[14] The siRNA designing approaches utilized to target the SARS-CoV genes can also be used to downregulate the genes of SARS-CoV-2. At present, there are more advanced strategies and better algorithms available to make siRNA more potent and highly functional.[2] Additionally, a few more genes of SARS-CoV-2-encoding proteins, such as M, N, and helicase, share a similarity of more than 90% with SARS-CoV.[14] All of these targets could be explored therapeutically using siRNA strategy.

The functionality of siRNA depends majorly on the ability to ameliorate the off-target effects and immune stimulation. Off targeting can be prevented by refraining homology to the non-specific genes, following the asymmetric principle to eliminate the passenger strand considered as a guide strand, maintaining low melting temperature (Tm) at the seed region and high GC content in the non-seed region.[2] The chemical modification of siRNA is also necessary as it evades immune activation in the lung and inhibits the target gene knockdown to improve the potency.[19] For example, 2′-O-methyl or 2′-fluoro-modification can eliminate TLR7 binding by mitigating off-target effects or backbone modification using phosphorothioate and boranophosphate replacing the phosphodiester bond can significantly enhance the potency of siRNA.[2]

Even after satisfying the above-mentioned criteria, carrier and delivery of siRNA is still a hurdle that can be transcended to make it a suitable therapeutic option. A carrier plays an important role in delivering siRNA specifically to the site of action with maximal payload without compromising the functionality of the siRNA. Additionally, the carrier should not be toxic or stimulate unwanted immune responses to achieve its maximum potency. There are several types of siRNA carriers, such as polymer-based carriers (poly lactic-co-glycolic acid (PLGA), chitosan, polyethylenimine (PEI), poly [2 (dimethylamino) ethyl methacrylate] (pDMAEMA), etc.), lipoplexes, and peptides such as peptide transduction domains (PTDs) and cell penetrating peptides (CPPs).[20] Each formulation has distinct advantages and disadvantages. For instance, CPPs have the advantage of enhancing the cell permeability, whereas liposome-loaded siRNA, when administered through the inhalation route, has a disadvantage of undergoing structural modifications that cause early release of the siRNA. Therefore, it is crucial to characterize the physical (size, shape, and density), chemical, and pharmacokinetic properties of each delivery system to ensure the compatibility of the carrier with the siRNA.[21] Escaping the endosomal pathway is also a critical parameter and rate-limiting step for successful siRNA development.[2] Another key criterion for a successfully developed and optimized siRNA formulation is to have long-term stability with the additional requirement for repeated usability.[22]

After an appropriate formulation, choosing a suitable delivery route is essential. siRNA can be delivered in two ways: systemic or local administration. When administered through the systemic (parenteral) route, siRNAs undergo rapid clearance and involve significant exposure to other organs. Also, siRNAs can stimulate innate immune responses, which, in this case, might help in tackling the infection. A local route of administration can ensure a better deposition onto the epithelial cells of the lung. It can also bypass serum nucleases and has a rapid and better uptake rate compared to the systemic route, making it an ideal route for COVID-19 infection. However, the intratracheal route is invasive and less feasible, and the intranasal delivery mode does not have the ability to reach the deep lung tissues. Hence, the delivery through inhalation is considered superior over other modes of local delivery. Aerosolized siRNA delivered using a microsprayer (high pressure syringe) developed by Penn-Century may also hold promise for future clinical applications. The inhalation route is simple and advantageous, but the molecule size is one of the important factors affecting drug delivery using inhalation. Particles smaller than 1 μm can potentially agglomerate, while particles greater than 5 μm have a chance to be deposited in the oropharynx, leading to ineffective delivery of the inhaled particles.[21] The strategic choice of the route of delivery also depends on the clinical condition of the patient. The local route is challenging in patients with cough and excessive mucous secretions or infected/inflamed lung tissue; in such cases, the systemic route may be the preferred choice.[22]

Although vaccination is the mainstay in combating viral infections such as COVID-19, the development of newer therapies (RNAi) could provide alternative therapeutic strategies to contain rapidly disseminating disease. Typically, the development of a new drug from bench to bedside is a tedious and time-consuming process. However, in the current emergency situation, the FDA is intending to fast track the development of new drugs (RNAi) upon encouraging results in clinical trials.[23] Although there are no active clinical trials with respect to siRNA drugs, some are in the pre-clinical stages of development, and future prospects show development of inhalant siRNA with features such as better half maximal effective concentration (EC_{50}), active until it reaches the site of action, and no or less off-target effects.[24]

Several pharma companies are collaborating to develop some potential siRNA molecules; for instance, Alnylam, which has RNAi expertise, and Vir, with infectious disease expertise, are developing RNAi therapeutics targeting COVID-19 on a large scale in a commercially viable manner. Sequence selection was based on siRNA potency, specificity, and manufacturability.[25] A promising potential inhalable siRNA drug under their development is VIR2703 (ALN-COV), which is planned to be filed as an investigational new drug (IND) by the end of the year. It demonstrated decreased replication in the *in vitro* studies, with 99.9% reactivity to 4,300 SARS-CoV-2-like genomes.[25]

Similarly, Agilent has also invested in oligonucleotide therapeutics. OliX pharmaceuticals and Sirnaomics are the other pharmaceutical companies exploring pathways on inhalational siRNAs for COVID-19. siRNA has the potential to be more effective for treating COVID-19.[4]

There is a probability of occurrence of future pandemics where the situation may be more catastrophic than that of the present. In such a scenario, targeting the conserved regions will help in using the existing, validated siRNAs for future pandemics.[4]

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https://doi.org/10.1016/j.omtn.2020.12.009

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