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Outfitting COVID-19: An Effective Therapeutic Approach

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ABSTRACT

Use of antisense oligonucleotides of the type 3'-(N)x-AAAUUUG-(N)x-5' against slippery sequence and polynucleotides against pseudoknots forming sequences of SARS-CoV-2 RNA would block the first translation of ORF1a and ORF1b and hence dwindle the virus replication. It is easy to synthesize and deliver the antisense oligonucleotides to the target by directly injecting the nano formulation into the blood.

Keywords: Oligonucleotides, Nanoparticle, Slippery sequence, Pseudoknots

1 Introduction

COVID-19 public-health emergency is caused by the outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). While it is important to investing time to search the origins of the pandemic (1-3) it is the prime need of the moment to find out remedy against the outbreak and it is the molecular and biochemical mechanisms of multiplication of the virus would help us better than any other ways.

2 Current Therapeutic Approaches

Angiotensin-converting enzyme II (ACE2) expression is enhanced by SARS-CoV-2 infection (4). Repression of ACE2 gene by EZH2-mediated H3K27me3 modifications of ACE2 promoter could be a targeted for prevention and adjuvant therapy of COVID-19. ACE inhibitors or angiotensin receptor blockers would be helpful for patients to fight COVD-19 (4, 5). Non-specific medicines, including antimalarial and broad spectrum antibiotics are being used in many clinics. That the convalescent plasma (CP) would benefit COVID-19 patients along with antivirals is emerging. However, without control subject it is not clear yet although within three days of CP therapy patients exhibited improved clinical symptoms CP was given within two weeks of symptom onset (6-8).

3 Mechanism of Synthesis of Virus Subparticles

Prevailing knowledge based on studies with viral replication processes is; after the entry into the host cell, translation of ORF 1a and 1b into polyproteins Pp1a (4382 aas) and Pp1ab (7073 aas) is the primary function of the guest genome. Both the polyproteins then cleaved into fifteen non-structural proteins (nsps), which assemble and form the replication-transcription complex (RTC). ORF1b is translated by ribosome shifting one nucleotide in the −1 direction, from the ORF1a reading frame into ORF1b reading frame (reviewed in Tan et al.; 9). This mechanism of repositioning is facilitated by two RNA **elements; (i) a 5'-UUUAAAC-3' heptanucleotide** slippery sequence (10), and (ii) RNA pseudoknot structure (see figures S1 and S2; 11). After the formation of RTC the full-length positive strand of genomic RNA transcribes to form a full-length negative-strand template for the synthesis of new genomic RNAs and overlapping subgenomic negative-strand templates. These subgenomic mRNAs are then transcribed and translated to produce the structural and accessory proteins. Several heterologous nuclear ribonucleoproteins (hnRNA) family members (hnRNPA1, PTB, SYN-CRYP) have been found to be essential for efficient RNA replication (9, 11, 12).

Formation of the cap structure of eukaryotic host and virus mRNAs basically requires three successive enzymatic reactions (13). Removal of the the γ-phosphate group from the 5'-triphosphate end (pppN) of the newly transcribed mRNA chain to generate the diphosphate 5'-ppN by RNA 5'-triphosphatase (RTP) is initial reaction. Then, RNA-guanylyltransferase transfers a GMP to the 5'-diphosphate end to produce the cap core structure (GpppN). Finally, guanine-N7 methyltransferase (GNMT) methylates the attached GMP (capping) at the N7 position to produce a cap-0 structure (me7GpppN). The 2'-O of ribose of the first and second nucleotides of the mRNA in higher eukaryotes and their viruses are additionally methylated. The responsible enzyme is ribose 2'-O-MT to form cap-1 and cap-2 structures, respectively (14, 15; see the supplementary figure S1). Ribose 2'-O-methylation of viral RNA cap provides a mechanism for viruses to escape host immune recognition (16-18).

Cap-0 reaction is mediated by the C-terminal domain of CoV nsp14 (19) using S-Adenosylmethionine (SAM) as the methyl group donor (see supplementary figure S1). Conversion of cap-0 to cap-1 structures involves nsp16 that acts as a 2'-O-MT and forms a complex with nsp10 that appears to be required for efficient binding to SAM and the RNA substrate. Interestingly, SARS-CoV nsp10 plays an essential role in the specific binding of nsp16 to m7GpppA-capped RNA (first nucleotide is adenine). The crystal structure of the heterodimer of nsp16/nsp10 with bound methyl donor SAM showed that nsp10 may stabilize the SAM-binding pocket and extend the RNA-binding groove of nsp16 (20, 21). Thus, blocking the translation of the virus SARS-CoV-2 mRNA definitely serves the purpose. Use of non-specific drugs would complicate the disease keeping the virus in dormant stage with a chance to relapse by re-activation of the pathogen and outbreak of the disease. Efficacy of Chloroquine (CQ) and a less toxic derivative of CQ, Hydroxychloroquine (HCQ) against malaria provoked some research groups around the globe and in many clinics, it has been in use against COVID-19 (see supplementary manuscript/letter that I wrote to WHO).

4 Recommendation for Target Specific Convenient Therapy

In view of this, and to obtain toxicity free therapy of COVID-19 patients my recommendation is to conjointly use: (i) A oligonucleotide against the 5'-UUUAAAC-3' heptanucleotide slippery sequence, and (ii) dismantling the pseudoknot preventing the pairing of matching sequences/RNA dimerization (22). Oligonucleotide of the antisense-type 3'-(N)x-AAAUUUG-(N)x-5' [where N is any one of the A, U, G, C and x = 8 to 10] will block the slippery sequence and antisense oligonucleotides against pseudoknots forming sequences (22-24) may stop the translation of the viral RNA by ribosome shifting. The polynucleotides can easily be design synthesize and delivered by well-tailored nanoparticle carriers. Ligands like EGF/folate attached with nanoparticles surface (oligonucleotides loaded) would easily be delivered to the affected tissues by ligand-receptor complex formation where there is overexpression of the cognate receptors, including EGFR/CD44 and subsequent endocytosis will bring them into the sites of action (25, 26). See figure 1 for the overall procedure in brief.

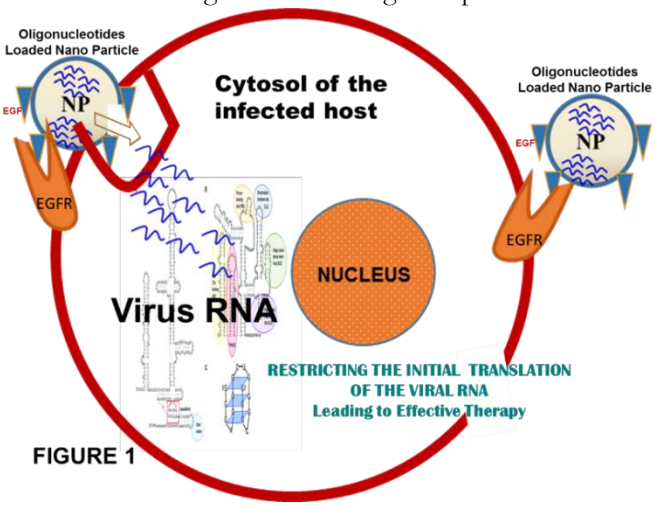

Figure 1: Source of the viral RNA pseudoknots:
https://www.researchgate.net/publication/327750579_RNA_Structure-A_Neglected_Puppet_Master_for_the_Evolution_of_Virus_and_Host_Immunity/figures?lo=1

Legends to the figure: Schematic image depicts the procedure of possible antisense oligonucleotide (ON) therapy of COVID-19. ON encapsulated NPs with surface tagged ligand (EGF) may directly injected (intravenous) to the circulation. It will come to the lung and bind to cells by EGF-EGFR complex formation. Endocytosis of the NPs will release the ONs and specific binding will block the virus RNA translation.

It could be tested within seven days against lung cancer cell line or animal model.

5 Caution and Secondary Benefit

Biodegradable nanoparticles from a variety of materials, including proteins, polysaccharides and synthetic biodegradable polymers should be used. If the proteins for synthesizing nanoparticles would mimic the virus spike protein (S); the system may develop antibody against S.

6 Declarations

6.1 Acknowledgement

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6.2 Competing Interests

I declare that there is no conflict of interest.

7 Supplementary File

Supplementary information along with figures (S1, S2…) can be accessed at the following URL- https://preprints.aijr.org/index.php/ap/preprint/view/41/29

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