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Therapeutic Modalities for SARS-CoV-2 (COVID-19): Current Status and Role of Protease Inhibitors to Block Viral Entry Into Host Cells

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Abstract

An acute respiratory disease (SARS-CoV-2, also recognized as COVID-19/2019-nCoV), caused by nCoV created a worldwide emergency. The World Health Organization declared the SARS-CoV-2 as epidemic of international concern on January 2020. After SARS-CoV in 2002 and MERS-CoV in 2012, the emergence of SARS-CoV-2 is marked as third highly pathogenic coronavirus of 21st century. Till now, various researches have been conducted, highlighting SARS-CoV-2 as β-coronavirus with high phylogenetic and genomic similarity with bat-CoV, indicating bats as natural reservoir of coronaviruses. It has also been confirmed that SARS-CoV-2 uses the same (ACE2) receptor for host cellular entry as of SARS-CoV, and primarily spread through respiratory pathway. Evidences shows continuous human-to-human viral transfer, with numerous worldwide exported cases. Currently, there is no specific approved drug available for the treatment of SARS-CoV-2, but various anti-parasitic and anti-viral drugs are being investigated. In this review, we have described several possible therapeutic modalities for SARS-CoV-2 infection based on (i) host protease inhibitors to block viral entry into the cell; (ii) gene silencing using siRNA-based RNAi and (iii) type I interferons (IFN1)-based therapeutics have been discussed in detail. Background knowledge on these strategies highlight them as potential therapeutic targets, which could be evaluated on urgent basis to combat COVID-19 epidemic.

Keywords: SARS-CoV-2, COVID-19, Origin, Therapeutics, protease inhibitors, gene silencing (siRNA), interferons type I (IFN-1)

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INTRODUCTION

SARS-CoV-2 is an enveloped, positive-sense RNA virus with 120–160 nm in diameter, belongs to family Coronaviridae and genus Betacoronavirus. High genetic similarity between SARS-CoV and a bat-CoV (sub-genus Sarbecovirus) has been confirmed by the scientists[1]. They named as corona due to presence of prominent spikes or projections like petal in envelop. It is also their differential feature distinguished them from Orthomyxoviruses[2]. Among four-CoV genera (α, β, γ, δ), the α and β viruses are capable to infect animals, while δ and γ are found to infect avian population. Formerly, six coronavirus strains have been recognized as capable to infect humans. From which, four α-CoV spp. showed mild pathogenicity and respiratory symptoms while two β-CoV spp. (SARS-CoV and MERS-CoV) caused fatal respiratory infections[3]. Genome sequence analysis of SARS-CoV-2 was found to be highly identical (96.2%) with bat-CoV (RaTG13), whereas, the similarity was comparatively less (79.5%) with SARS-CoV. Based on evolutionary and viral genome sequence analyses, bats have been considered as primary carriers of SARS-CoV-2 and the infection might be transferred via intermediate animals (secondary hosts) to reach human population (Fig.1). It is found that ACE2 receptor is necessary for SARS-CoV-2 to infect humans, same as SARS-CoV[4].

Transmission of SARS-CoV-2

The epidemic of SARS-CoV-2 was initially break out in China at the end of 2019, possibly due to seafood market. Various researches indicated that bats could be the natural reservoirs of SARS-related coronaviruses[2,5] however, this fact still lacks potential evidences. Rather, bats serve as reservoir for variety of different CoVs including SARS-CoV and MERS-CoV[6]. Genome sequencing analysis showed that SARS-CoV-2 was 96.2% similar to bat-CoV[5], which indicate that SARS-CoV-2 and bat-CoV might belong to the same ancestral specie, though bats are not sold in that marketplace[7]. Phylogenetic analysis and protein sequence alignment[8] also showed different species (e.g. pangolin, civet cats, snakes and turtles) present similar residues of receptors, which put them also in the suspect category. Disease (COVID-19) transmission between human-to-human primarily occurs between friends and family who have close contact with infected persons or virus carrier person. Direct contact with infected animals or their utilization as foodstuff was assumed to be the major transmission pathway of SARS-CoV-2 infection. However, the transmission route(s) and the source(s) of infection of COVID-19 remains elusive (Fig.1).

Therapeutic modalities for SARS-CoV-2 infection Protease inhibitors to block viral entry into host cells

An attractive therapeutic strategy for SARS-CoV-2 infection, could be the blockage of viral entry into the cell by selectively targeting host factors (Fig.2). The viral spike (S)-glycoproteins are produced as inactive precursors which are
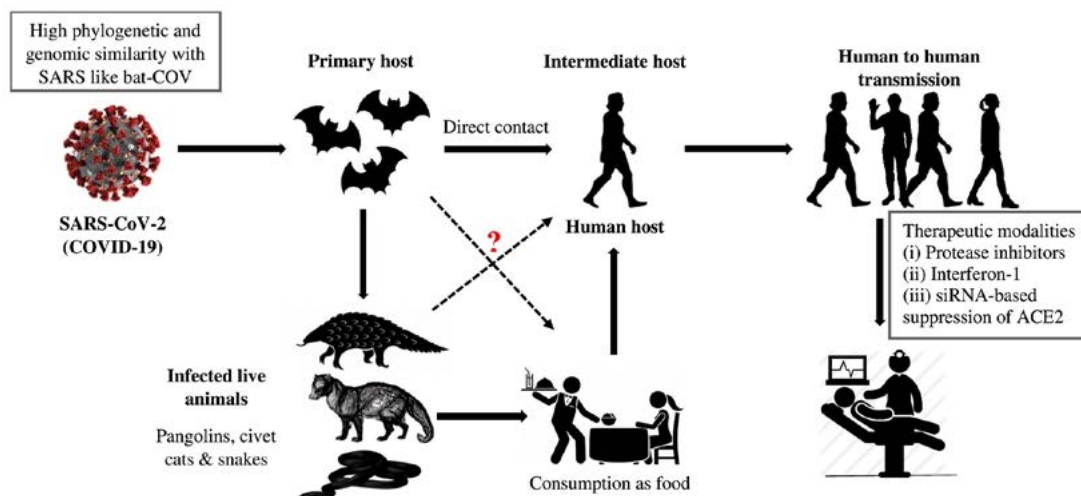

Fig.1. Source of infection and transmission of SARS-related coronaviruses to humans via intermediate hosts.

converted into active form by host cell proteases (proteolytic cleavage – priming). The S-glycoprotein of SARS-CoV-2 attaches the angiotensin-converting enzyme 2 (ACE2), which allow the entry of coronavirus into the host cell[9]. Viral entrance also requires the cleavage of S-glycoproteins by host proteases, which consequences in a non-reversible conformational modification to S-glycoprotein, which allow the endocytosis of viral particle by the host cell[9]. Cleavage of S-glycoprotein (priming), can use host proteases i.e., cysteine proteases [cathepsin B/L (CatB/L)] and/or serine proteases [transmembrane protease (TMPRSS2)][10,11]. Ziegler and coworkers described single-cell RNA-sequencing of non-human and human tissues and discovered the co-expression of three major cell types i.e., (i) enterocytes in terminal ileum; (ii) pneumocytes – type II, in lungs and (iii) goblet mucus-forming cells in nasal cavity[12]. A recent preprint also highlighted several other proteins which interact with 26/29 COVID-19 proteins[13]. Therefore, the respective proteolytic enzymes could be the potential targets for the development of broad-spectrum anti-viral therapeutics (Fig.2).

The development of inhibitors for Cat-B, Cat-L, and other relevant proteases would be an amazing starting point towards the designing of broad-spectrum anti-viral therapeutics[11,14]. The designing of S-glycoprotein-based vaccines for SARS-CoV-2 may depend on the fact that the viral entry depends on ACE2 receptor binding. Cell-lines that enable viral multiplication in the existence of ACE2 may serve as the most effective in the development of large-scale vaccine[4]. The attachment sites between SARS-CoV and ACE2 has been recognized at atomic level. Hence, therapeutic approaches could also target this attachment site with smaller molecules or antibodies[4]. Recently, Hoffmann et al.[11], described the priming of viral S-proteins by TMPRSS2, which
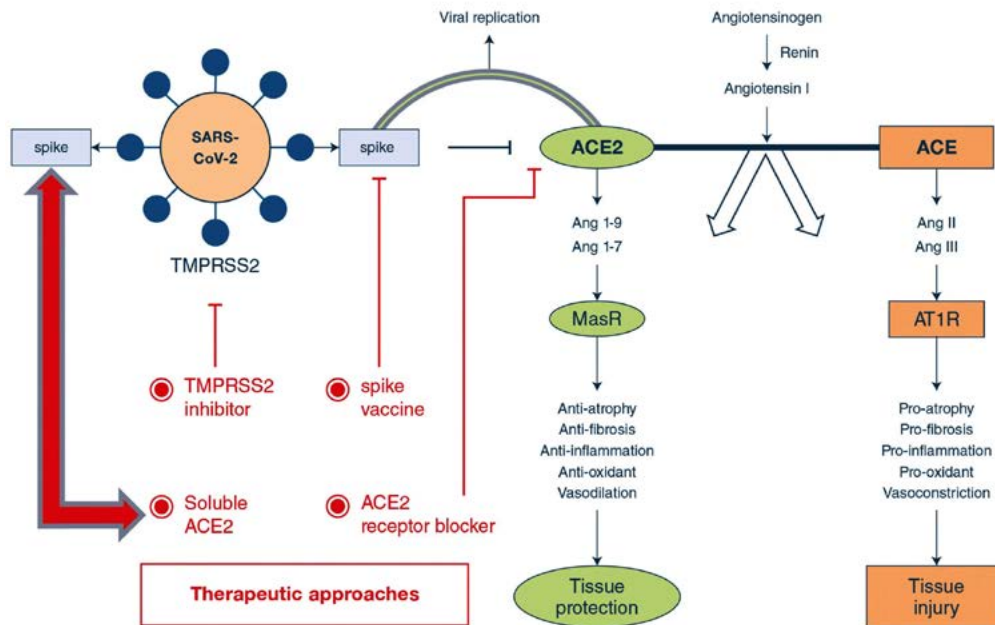

Fig. 2. Potential therapeutic targets to address ACE2-dependent SARS-CoV-2 infection. Both coronaviruses (SARS-CoV & SARS-CoV-2) use ACE2 for their transmission pathogenesis into the host cell. SARS-associate coronaviruses induce down-regulation of the ACE2 by selective binding of S-glycoprotein with ACE2 rather than ACE receptor. This causes viral transmission into host cell and its replication, as well as severe pulmonary infection. Therapeutics may comprise (i) S-glycoprotein-based vaccines; (ii) TMPRSS2 inhibitor to block the S-protein priming; (iii) ACE2 receptor blockage giving anti-ACE2 peptides or antibodies or (iv) slowdown viral entry by soluble form of ACE2, which competitively bind with SARS-CoV-2 and reduce viral spreading ultimately preventing pulmonary infection using exclusive enzyme function. Adapted from Zhang et al.[4]. Article distributed under creative common attribution (CC-BY) license.

is essential for viral entry into human cell types, and the spread of SARS-CoV-2 through binding with specific receptor (ACE2). The protease inhibitors such as 'camostat mesylate' has been studied to block TMPRSS2 activity, which has been approved in Japan[14], which can be an interesting candidate for therapeutic purpose of SARS-CoV-2 infection.

It has been demonstrated that SARS-CoV down-regulates ACE2, by specifically binding with viral S-glycoprotein, contributing to severe pulmonary infection[15]. Following this, it could be suggested that excessive ACE2 may competitively conjugate with SARS-CoV-2 to counterbalance the viral particles, as well as to rescue ACE2 activity, which negatively regulates the RAS activity, and protect from pulmonary infection[16]. Indeed, decreased ACE2 availability and increased ACE activity contribute to pulmonary infection[16,17]. Treatment using soluble type of ACE2 may exert dual functionalities; (i) slowdown viral spreading into host cells[18] and (ii) protecting from pulmonary infection[17,19].

Suppression of ACE2 using siRNA

RNA-dependent gene silencing comprises post-transcriptional gene silencing in plants and RNA interference (RNAi) in mammalian/animal cells. Transfection of siRNA into animal cells causes degradation of target mRNA and consequently suppression of its expression[20]. siRNA-based RNAi may provide a new therapeutic approach for viral diseases (Fig.3). RNAi has been used against HCV and HIV to suppress the replication of viruses in cultured human cells[21-24]. SARS-CoV-2 is a positive-sense, enveloped RNA virus[25], which predominantly requires the attachment of its S-glycoproteins with ACE2 receptor for its entry into host cells as described previously. That S-glycoprotein is almost identical in all SARS-associated coronaviruses, though bit differences in SARS-CoV-2 as compared with other related coronaviruses. Moreover, according to Diao et al.[26], SARS-CoV-2 manage to clearly decrease the population of immune CD8+ and CD4+ cells causing enhanced viral spread inside the host's body. According to Wang et al.[27], SARS-CoV-2 also attaches with CD147 receptor.

Organs with high expression of ACE2 are highly affected by the SARS-CoV-2, as it binds with the receptor for its entry. Following the fact, reducing the availability of those receptors should reduce viral capability to enter into the cell, ultimately reducing the SARS-CoV-2 infection. Blood pressure (BP) regulating hormone (angiotensin II), which is a part of renin-angiotensin system (RAS) is mediated by ACE2 receptor[28]. Therefore, observed distribution of SARS-CoV-2 indicate a pattern of BP variability among population[29]. Also, diabetes, hypertension, viral pneumonia and cardiovascular disorders
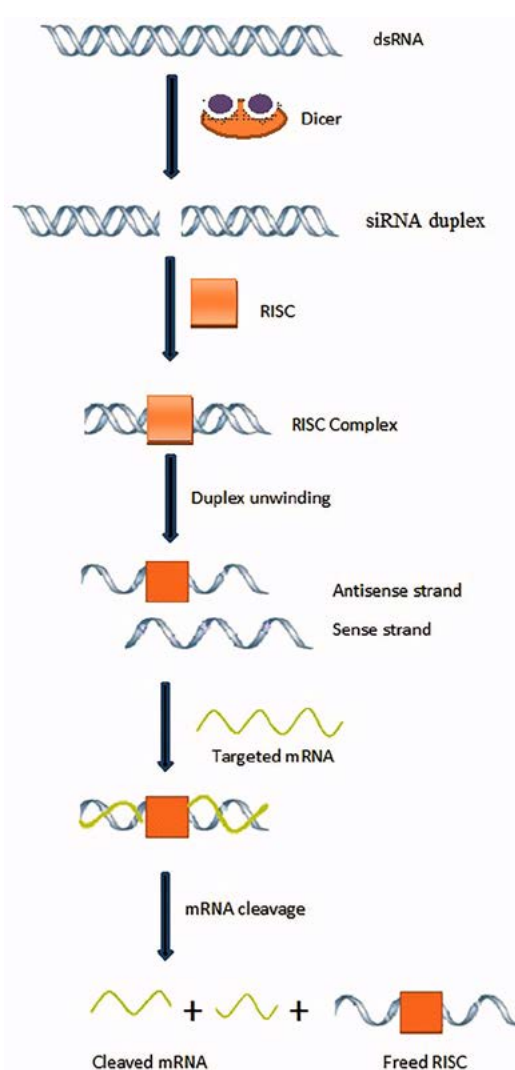

Fig.3 Mechanism of gene silencing using siRNA-based RNAi. Adapted from Alam et al., [31]. Article distributed under creative common attribution (CC-BY) license.

are the common comorbidities increasing the organism's susceptibility to SARS-CoV-2, which are primarily related to BP regulatory mechanism of the body[14]. Hence, the medications used for the treatment of hypertension are being studied for their effectiveness in the treatment of SARS-CoV-2. However, their side effects and metabolic pathways limit their utilization as anti-hypertensive medications[30].

As described previously, viral S-glycoprotein specifically attaches with ACE2 receptor. It has been shown that S-protein of SARS-CoV-2 is highly similar to that of SARS-CoV[32], which caused epidemic in 2002/2003. Researches using mutagenic deletion of ACE2 suggested that the cytoplasmic tail of receptor is essential for shedding. Thus, siRNA-based suppression of ACE2 impaired viral capability to infect host cells[33]. Alam et al.[31], reported various successful experiments using nanocarriers-mediated delivery of siRNA as a therapeutic strategy for hypertension. Tambe et al.[34], described the gene silencing using surface engineered dendrimers with siRNA loading. The presented therapeutic techniques indicate the effectiveness of siRNA as a potential tool to reshape RAS-system. Following that, Merril[35], proposed therapy based on temporarily silencing ACE2 expression by designing specific siRNA molecules, this would effectively block the viral entry by modulating immune system to efficiently prevent from viral infection.

There could be two possible carriers able for targeted delivery such as liposomes (stable nucleic acid lipid particles – SNALP) and the virus itself. It has been described that SNALP can efficiently deliver siRNA[36]. The use of inactivated virus as carrier of siRNA could be effective in targeted delivery to cells which are susceptible by live viral infection. The use of S-glycoproteins and viral coat that could deliver siRNA at targeted place could assist two purposes i.e., (i) targeted delivery at places which are more susceptible to SARS-CoV-2 infection and (ii) blocking the attachment sites for live viruses, ultimately reducing the disease severity. Hence, the use of viral envelope would efficiently perform targeted delivery of siRNA.

Interferons type 1 (IFN1)-based therapeutics

IFN-1 designate a group of cytokines including various subtypes such as α and β (further subdivided into various isoforms), ω, κ and ε-subtypes[37]. These are produced by numerous cells in response to a viral infection. However, upon viral component identification by pattern recognition receptor (PRR), plasmacytoid dendritic cells (pDCs) majorly contribute in the production of IFN-1 in the body[38]. Hence, they initiate the first responsive mechanism as a result of viral infection. IFN-1 are recognized by interferon-α/β receptor (IFNAR) existent at the cell membrane of various cells. Their conjugation on IFNAR causes the addition of phosphate group to the transcription factors (phosphorylation) e.g., STAT1 and its relocalization, ultimately switching interferon-stimulating genes (ISG), which are mostly involved in immunomodulation, signaling and inflammation[39]. They inhibit viral multiplication and initiate various responsive mechanisms e.g., reduction in cellular metabolic activity or discharge of cytokines which ultimately activates the host's adaptive immunity. ISGs comprise PRRs, which enhance their sensitivity towards pathogens, preventing membrane fusion or viral egress, proteins which reduce membrane fluidity, and antivirals which particularly inhibit single stage of viral replication[40,41]. IFN-1, due to their immunomodulatory characteristics, has been used in the treatment of various disorders e.g., subcutaneous injection of IFNβ is being successfully used against multiple sclerosis[42].

SARS-CoV and MERS-CoV exhibit closely related characteristics to COVID-19 coronaviruses, despite variances in their pathology, several proteins, and their epidemiology[43]. Treatment via IFN-1 against MERS-CoV and SARS-CoV has already been described by various in vivo and in vitro studies[44], with and/or without lopinavir/ritonavir as a combinatorial medication[45,46]. Several other combinatorial drugs have been reported against coronavirus infection such as ribavirin[47-49], chloroquine and remdesivir[43,50]. IFNα and β showed good therapeutic potential in various experimental animals [51] but no significant results were obtained in humans[44]. For instance, Sheahan et al.[45], studied combinatorial effect of IFNβ with ritonavir/lopinavir against 2012 respiratory syndrome (MERS-CoV). The results indicated significant improvement in lung functionality. However, the effect was non-significant against viral replication or lung pathology severity. In

a previous study, combination of ribavirin with IFNα2b showed amazing outcomes in monkeys[52], while unconvincing in humans[53]. The insignificant results in various studies using IFN-1 therapy can be explained by several factors, i.e., (i) the inhibitory mechanism of IFN-signaling used by SARS-CoV and MERS-CoV; (ii) trouble to interpret agents behind the improvements (*viz.* IFN or combinatorial drug) and (iii) The limited number of study subjects. Moreover, results may also vary from study to study because of discrepancies in clinical conditions and experimental settings[44]. It has also been hypothesized that IFN-1 were effective in the population only if they lacked any co-morbid disorders[47].

Following previous clinical investigations, IFNβ1a and IFNβ1b were the most potent types of interferons against MERS-CoV[54], and SARS-CoV[55]. In addition, IFNβ-γ synergistically inhibited the replication of coronaviruses[56]. Cinatl et al.[57], studied the effect of IFNs (both α and β) on SARS-CoV persuaded cytotoxicity in Vero cells while the drugs were added after 1 hour of adsorption period. IFNβ was active against SARS-CoV (FFM-1 strain), whereas, combination of IFNα-β was not. Therefore, IFNβ was the only agent used for the inhibition of coronavirus replication. Vero cells were infected with coronavirus (FFM-1) and treated with various concentrations of IFNs, and viral replication was studied using peroxidase staining with immune serum. *In vivo* clinical experiments suggest that the time of IFN-1 administration plays an important part. The reduce the time of IFN-1 administration after viral infection, the maximum the chances of the cure are and vice versa. In addition, later administration may inhibit viral replication cause severe complications[58].

Following previous knowledge, the utilization of IFN-1 as a therapeutic tool against SARS-CoV-2 infection demands high scientific attention. MERS-CoV and SARS-CoV are capable to interrupt the IFN-signaling mechanism. For instance, karyopherin transportation is disrupted by Orf6 protein of SARS-CoV, and hence, inhibiting the transport of transcriptional factors in the nucleus, ultimately triggering IFN's responsive mechanism. Likewise, the phosphorylation of IRF3 is inhibited by Orf3b protein of SARS-CoV[59] which causes switching on the process of IFN expression. However, Orf3b and Orf6 of novel coronavirus-2 are docked and may have lost their anti-IFN functionalities. This could address the fact that why novel SARS-CoV-2 showed high *in vitro* sensitivity to IFNα[60]. Though the replication was not completely stopped by interferon treatment, several folds decrease in viral titers was definitely a significant finding. SARS-CoV-2 showed much higher sensitivity to IFN-1, which indicates that IFN-1 based therapeutics should be as efficient for the previous than for the latter.

Following previous knowledge, it can be concluded that IFN-1 treatment might be safer and very effective against SARS-CoV-2 infection. The available data on SARS-CoV and MERS-CoV would be potential assets in this regard. For instance, studies suggest that IFN-1 should be administered at earlier stages of infection and IFNβ should be the most effective subtype to avoid complications[58]. Moreover, SARS-CoV-2 pathology, primarily consist in lung injury, exhibits closer properties to interferonopathies. This may indicate that COVID-19 causes excessive interferon-medicate response, ultimately resulting in tissue damage. Treatment via IFN-1 should be given at early stages of infection[61], and inflammatory biomarkers in infected patients are linked with increased mortality[5]. At latter stages of infection, it is probable that anti-interferon medications should be prescribed to normalize the adverse events[17].

CONCLUSIONS

The SARS-CoV-2 outbreak, which was started from city level has become a matter of global concern. Scientists are working to characterize the novel coronavirus strain (CoVID-19) and to explore potential therapeutic targets to overcome the epidemic. In this review, we have summarized the available knowledge related to the source of infection and its human-to-human transmission with possible therapeutic modalities i.e., host protease inhibition, ACE2 suppression using siRNA based RNAi and the IFN-1-based therapeutic, which presented excellent therapeutic potential in 2002 SARS-CoV and 2012 MERS-CoV infection. As, the SARS-CoV-2 shares the same ancestral organism, which high phylogenetic and genomic similarity to other coronaviruses these therapeutic modalities might prove to be the efficient targets to overcome COVID-19 pandemic. However, there

is a need of more urgent researches to explore the in-depth knowledge of this novel virus to make clear evolutionary path and stop this fatal disease. Moreover, to explore molecular mechanisms of viral multiplication and host cellular entry, which provide the basics of future studies to develop targeted vaccines and drugs.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ETHICS STATEMENT
This article does not contain any studies with human participants or animals performed by any of the authors.

DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

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