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Review

Targeting Crucial Host Factors of SARS-CoV-2

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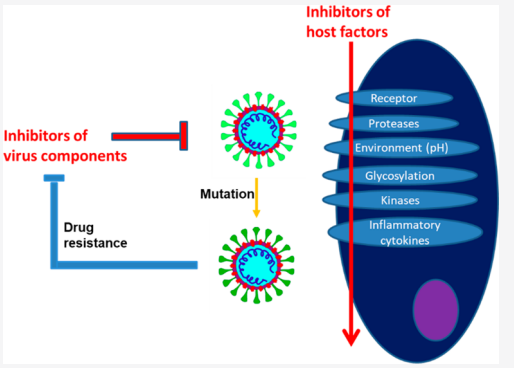
ABSTRACT: Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread worldwide since its first incidence in Wuhan, China, in December 2019. Although the case fatality rate of COVID-19 appears to be lower than that of SARS and Middle East respiratory syndrome (MERS), the higher transmissibility of SARS-CoV-2 has caused the total fatality to surpass other viral diseases, reaching more than 1 million globally as of October 6, 2020. The rate at which the disease is spreading calls for a therapy that is useful for treating a large population. Multiple intersecting viral and host factor targets involved in the life cycle of the virus are being explored. Because of the frequent mutations, many coronaviruses gain zoonotic potential, which is dependent on the presence of cell receptors and proteases, and therefore the targeting of the viral proteins has some drawbacks, as strain-specific drug resistance can occur. Moreover, the limited number of proteins in a virus makes the number of available targets small. Although SARS-CoV and SARS-CoV-2 share common mechanisms of entry and replication, there are substantial differences in viral proteins such as the spike (S) protein. In contrast, targeting cellular factors may result in a broader range of therapies, reducing the chances of developing drug resistance. In this Review, we discuss the role of primary host factors such as the cell receptor angiotensin-converting enzyme 2 (ACE2), cellular proteases of S protein priming, post-translational modifiers, kinases, inflammatory cells, and their pharmacological intervention in the infection of SARS-CoV-2 and related viruses.



KEYWORDS: *SARS-CoV-2, host factors, drug targets, inhibitors, coronavirus, COVID-19*

Influenza viruses and coronaviruses are the cause of many severe disease outbreaks, mainly affecting the respiratory tract. Although the global case fatality rate (CFR) of coronavirus disease 2019 (COVID-19) caused by recently discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) appears to be lower than that of SARS-CoV (9.5%) and the Middle East respiratory syndrome (MERS) (34.4%), the higher rate of person-to-person transmissibility of COVID-19[1−3] has caused the disease to spread globally within a short period. Following the first reported case from Wuhan, China, in December 2019,[4−7] the disease has spread to other parts of China[8] and quickly to other countries. By October 6, 2020, almost 35 million people were infected globally, with nearly 1,039,406 deaths, comprised mainly of older people with underlying comorbidities such as diabetes, hypertension, or cardiovascular disease.[9,10] In the United States of America alone, there are over 7.3 million infected people and almost 208,433 fatalities.[11−13] On January 30, 2020, the World Health Organization (WHO) declared the COVID-19 outbreak as the sixth public health emergency of international concern, following swine flu (H1N1, 2009), polio (Poliovirus, 2014), Ebola (EBOV, 2014), Zika virus disease (ZIKV, 2015−2016), and the Kivu Ebola epidemic (2018−2020).[14] The outbreak of MERS (MERS-CoV, 2012), although not declared as a public health emergency, is still ongoing and has caused 858 deaths since September 2012.[15]

As per the classification by the International Committee on Taxonomy of Viruses (ICTV), the family *Coronaviridae* has two subfamilies, subfamily *Letovirinae* and subfamily *Orthocoronavirinae*. The subfamily *Orthocoronavirinae* has four genera, including genus *Alphacoronavirus*, genus *Betacoronavirus*, genus *Gammacoronavirus*, and genus *Deltacoronavirus*. The *Betacoronavirus* has 5 subgenera, and SARS-related viruses belong to subgenus *Sarbecoviruses*, whereas MERS-related coronaviruses belong to subgenus *Merbicovirus*.[16] The coronaviruses that infect humans include human coronaviruses (HCoV) 229E (HCoV-229E) and HCoV-NL63 in the *Alphacoronaviruses* and HCoV-OC43, HCoV-HKU1, SARS-CoV, SARS-CoV-2, and MERS-CoV in the *Betacoronaviruses*.[17]

SARS-CoV-2 is a single-stranded positive-sense RNA virus with a genome size of 29.8 to 29.9 kbp.[26] SARS-CoV-2 and

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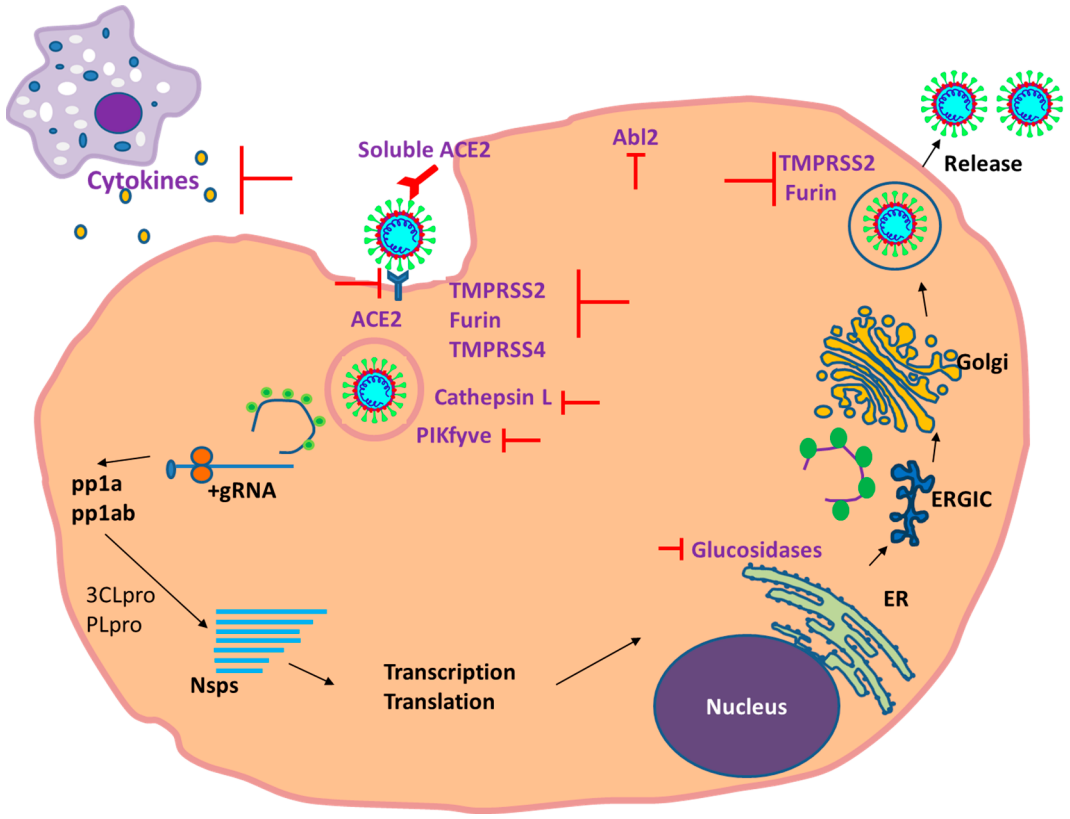

Figure 1. Potential host factors as drug targets in the SARS-CoV-2 life cycle. The studies on SARS-CoV and other coronaviruses have helped to predict the life cycle of SARS-CoV-2. SARS-CoV-2 attaches to the cell surface receptor angiotensin-converting enzyme 2 (ACE2) by its spike protein,[3,18−20] which is cleaved by cell surface proteases such as TMPRSS2, furin, TMPRSS4, and TMPRSS11[21,22] to initiate cell entry. Various host kinases, including Abelson tyrosine kinases (Abl2) and phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase (PIKfyve), are involved in the membrane/intracellular trafficking of SARS-CoV-2.[23,24] When the virus is taken up into endosomes, cathepsin L can also prime the spike protein. In SARS-CoV, upon release of viral RNA into the cytoplasm, ORF1 and ORF2 (open reading frame, ORF) are translated to polyproteins pp1a and pp1ab. These polyproteins are then cleaved by virally encoded chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro) to give 16 nonstructural proteins (Nsps) forming an RNA replicase−transcriptase complex.[25] The copies of negative RNA are produced, and using this as a template, positive RNA is produced in the replication process. Through discontinuous transcription, 7−9 subgenomic RNAs are produced.[25] The viral nucleocapsids are assembled from genomic RNA and N protein in the cytoplasm. Post-translational modifications such as glycosylation happen in the endoplasmic reticulum (ER) and the Golgi complex. After budding of the virions into the lumen of the ER−Golgi intermediate compartment (ERGIC) of infected cells, they are released by exocytosis. The cleavage of the spike protein of the newly released virions also can happen at the cell surface.

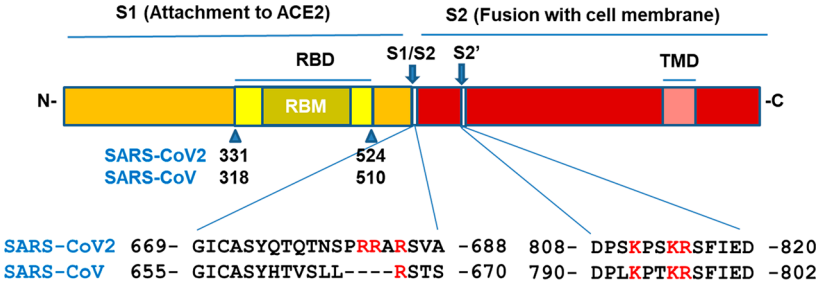

Figure 2. Comparison of the S protein of SARS-CoV and SARS-CoV-2.[21,39] Basic amino acid residues are in red colored text. S1: spike subunit 1; S2: spike subunit 2; RBD: receptor-binding domain; RBM: receptor-binding motif; TMD: transmembrane domain.

SARS-CoV are phylogenetically related, sharing approximately 79.6% genomic sequence identity;[3] their spike (S) proteins also have a high degree of homology.[27,28] Both viruses also use ACE2 as a common cell receptor[21] (Figure 1). ACE2 is a critical host enzyme involved in regulating blood pressure. However, since the enzyme active site and SARS-CoV receptor-binding site on ACE2 are at different positions,[29−31] it may be possible to target the receptor region without compromising enzyme function. Coronaviruses encode a structural S glycoprotein, which is responsible for binding to the host receptor. The S protein has two functional subunits: a receptor-binding domain S1 and a second domain, S2, that contains sequences that mediate fusion of the viral and cell membranes (Figure 2). Host cell proteases are required to cleave the S glycoprotein, leading to exposure of the fusion peptides, which is required for cell entry (Figure 1). The S

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protein has two cleavage sites, S1/S2 and S2′. The cellular proteases transmembrane protease serine 2/epitheliasin (TMPRSS2) and furin are required for activation of SARS-CoV-2 in human airway cells (Calu3).[32] One study employing a pseudovirus also supported the role of furin and TMPRSS2 in TMPRSS2^{+} Calu3 cells and outlined the requirement of a multibasic S1/S2 cleavage site.[21] However, further study is required with primary lung cells and actual SARS-CoV-2, as the expression of other proteases varies with cell type.[33]

The enveloped viruses enter cells in two ways. The virus can fuse with the cell membrane and deliver its genome to the cytosol. Alternatively, using endocytic machinery, the virus may be endocytosed and activated by acidic pH in the endosome or endosomal proteases. Following the fusion of viral and endosomal membranes, the viral genome is released to the cytosol; hence, the endocytic pathway may be pH dependent. The SARS-CoV entry is mediated by clathrin- and caveolae-independent mechanisms.[34] Endosomal protease cathepsin L is also involved in S protein priming, the proteolytic separation process of the S1 and S2 subunits.[22] Specific inhibitors of host proteases can block priming of the S protein and thus prevent entry. Chloroquine and hydroxychloroquine, which interfere with the pH of endosomes, can also inhibit SARS-CoV-2 entry in Vero E6 cells.[35] The coronavirus genome carries multiple open reading frames (ORFs), and all the coronaviruses have two large ORFs (ORF1a and ORF1b) at 5′ occupying two-thirds of the viral genome[36] and encoding nonstructural proteins. The remaining part of the genome encodes structural and other proteins that are expressed from subgenomic mRNAs.[37] Once released into the cytoplasm, the viral RNA is used by host ribosomes to initiate translation of viral polyproteins pp1a and pp1ab. The virus-encoded chymotrypsin-like protease (3CLpro) and papain-like protease (Plpro) cleave these polyproteins into 16 functional nonstructural proteins (Nsps). Several Nsps, such as helicase and RNA-dependent RNA polymerase (RdRp), together with host factors, form a replication−transcription complex that is responsible for the replication of viral RNA.[38]

The β-coronaviruses (MERS-CoV, SARS-CoV) and γ-coronavirus (infectious bronchitis virus) modify ER membranes to facilitate viral replication and RNA synthesis, indicating double-membrane vesicles as a potential drug target in coronavirus infection.[40] During the assembly of coronaviruses, the N protein and RNA form a helical nucleocapsid.[41] The ER bound structural proteins, S, envelope E, and membrane (M), along with helical nucleocapsid, assemble into virions resulting in budding through the membrane[41] and then are transported via vesicles and released out of the cell (Figure 1). Many host enzymes in the ER and Golgi complex, including ER-α glucosidases I and II, are involved in post-translational modifications, including glycosylation. As some of the viral proteins and host cell receptors are glycosylated, blocking of glycosylation by inhibitors can interfere with viral replication and pathogenicity.[42−44] Mature virions are then released from the infected cell through exocytosis.[45]

Detailed information on virus dynamics and host response is necessary for diagnosis, drug design, and therapy. With the availability of real-time PCR and antibody-based tests, there are improvements in the diagnosis process,[11,46] but so far, there is no therapy or vaccine formally approved by the FDA for COVID-19. However, on May 1, 2020, the FDA issued an emergency use authorization for the investigational small-molecule antiviral drug remdesivir as a therapy for COVID-19-confirmed patients hospitalized with severe disease. Recently, the FDA-approved drug dexamethasone has been shown to reduce COVID-19 deaths by nearly 1/3 in extremely ill patients on ventilators in the RECOVERY clinical trial.[47] Although these results are exciting and encouraging, our choices are still very limited, and a complete cure remains a timely challenge. Hence, there is an urgent need for research on the repurposing of previously approved drugs, as developing a new drug might be time-consuming. Several small molecules are being validated in addition to developing antibodies and vaccines. Clinical trials of promising drug candidates are also being conducted to find a quick solution to rapidly spreading COVID-19. Viruses use host machinery at various stages of the viral life cycle, starting from cell attachment to the release of the virion. Many invading viruses subvert host functions. It is essential for advancing our understanding of viral life cycles by identifying these altered functions, which can provide novel drug targets that are less likely to mutate post-therapy. Although SARS-CoV and SARS-CoV-2 are closely related, there is a difference in the receptor-binding domain (RBD) region of their spike proteins.[19,39] Initial studies show possible cross-neutralization between SARS-CoV-2 and SARS-CoV,[39] but studies with patient sera indicate that, although cross-binding of antibodies between SARS-CoV-2 and SARS-CoV occurred, the cross-neutralization was rare and weak.[48,49] However, potent cross-neutralizing (at 100 nM) monoclonal antibodies against SARS-CoV-2 were developed from the memory B cells of survivors of the SARS-CoV outbreak in 2003, and out of nine neutralizing antibodies, eight targeted the RBD region of S protein, while one targeted the N-terminal domain of the S1 region of the S protein.[50] Because a significant portion of non-neutralizing antibodies against the virus is present in the sera of SARS-CoV-2 infected people[49] and as antibody-dependent infection of human macrophages by SARS-CoV has been reported,[51] the risk of antibody-dependent enhancement (ADE) needs to be carefully evaluated during the process of vaccine development.[52−54]

Targeting host factors may result in therapies with a broader range than traditional antivirals, and modern-day bioinformatics tools can help us speed up studies. Out of 29 proteins of SARS-CoV-2, 26 have been expressed in the human cell to identify the host-interacting proteins, and scientists have successfully identified 66 druggable human proteins out of 332 high-confidence SARS-CoV-2-human protein−protein interactions.[55] Upon further screening of these hit molecules with multiple viral assays, two groups of molecules with antiviral activity have been identified, including the inhibitors of mRNA translation and the predicted regulators of the Sigma-1 and Sigma-2 receptors, which are reported to be involved in the early steps of viral RNA replication of some RNA viruses such as HCV and HIV.[56,57]

This Review discusses the recent developments in the identification of critical host factors as drug targets in various stages of the life cycle of SARS-CoV-2 and related viruses. We also discuss the pharmacological intervention of these host factors.

RECEPTOR ACE2

ACE2 is a negative regulator of the renin−angiotensin system and is expressed in the lung, brain, heart, kidney, liver, endothelium, and intestine.[58−60] Alveolar epithelial type II cells of the lungs have abundant ACE2 receptors.[61] Coexpression of

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Table 1. Proteases Known to Play a Role in the SARS-CoV and SARS-CoV-2 Life Cycles

protease	mRNA (kbp)	protein	knockout mouse
TMPRSS2 (epitheliasin)	3.2	Human-492 aa, Mouse-400 aa 54 kDa (70 kDa in SDS-PAGE with post-translational modifications)	no known abnormalities[95]
TMPRSS11D (HAT)	2.8	Human-418 aa, Mouse-417 aa	no known abnormalities[96]
TMPRSS4 (CAP2)	5.5	Human-437 aa, Mouse-435 aa	no known abnormalities[97]
cathepsin L	1.6	Human-333 aa, Mouse-334 aa	abnormal skin and bone development[98]
furin	4.2	Human-794 aa, Mouse-793 aa	furin null mice die[99] no adverse effects with short-term administration of furin-inhibitor hexa-D-arginine[100]

ACE2 and proteases involved in S protein priming might make the cells more susceptible to COVID-19. Coronaviruses HCoV-NL63, SARS-CoV, and SARS-CoV-2 bind to ACE2 by their RBDs of the S protein[3,27,62] and do not use previously reported coronavirus receptors such as aminopeptidase N and dipeptidyl peptidase-4.[3] The RBD of SARS-CoV-2 binds to ACE2 with a 10−20-fold higher affinity compared to that of SARS-CoV.[63] This may contribute to the higher infectivity and transmissibility of SARS-CoV-2 compared to SARS-CoV.[63,64] The S protein of SARS-CoV-2 binds approximately 1000 times more tightly to ACE2 than the bat virus S protein does with dissociation constant (K_d) values of <100 nM and >40 μM, respectively.[65] In addition, a recent bioinformatic analysis speculates that an A321Q mutation from SARS-CoV to SARS-CoV-2 in the endosome-associated-protein-like domain of the nsp2 protein could stabilize the protein and account for a higher contagious ability of SARS-CoV-2 than SARS-CoV.[66] However, the possibility of this difference in a nonstructural protein leading to an increase in SARS-CoV-2 contagious ability needs to be further validated.

The binding sites for HCoV-NL63, SARS-CoV, and SARS-CoV-2 on ACE2 are distinct from the active site of the enzyme.[29−31] Hence, not surprisingly, the treatment of ACE-2-bearing cells with MLN-4760, a potent ACE2 enzyme inhibitor, has no effect on the S−RBD interaction or virus entry.[67] However, it should be noted that the interaction between the S protein of HCoV-NL63 and ACE2 is slightly different from that between SARS-CoV and ACE2.[45,68] Specific mutations in ACE2 that are known to affect the binding of SARS-CoV have not affected the binding of HCoV-NL63.[69] Moreover, unlike SARS-CoV RBD, which is linear, the RBD of the HCoV-NL63S protein is located between residues 232 and 694 and is not linear.[70]

Because the area of interaction between ACE2 and viral S protein is quite large,[63] peptides or antibodies can cover a larger area and have the chemical properties needed to apprehend the virus before it sticks to a cell. Although peptide-based drugs have issues with cell permeability and stability, the success of clinically approved peptide Fuzeon (Roche) to treat human immunodeficiency virus (HIV) by inhibiting fusion suggests that this direction is promising. A 23-mer peptide binder selenium-binding protein 1 (SBP1) derived from the ACE2 α1 helix binds RBD of SARS-CoV-2 with low nanomolar affinity (K_d = 47 nM) in a kinetic binding assay.[71] Recombinant human ACE2 (rhACE2) has been used in clinical studies,[72,73] indicating its safety. With the clinical-grade soluble ACE2, the SARS-CoV-2 recovery from Vero cells is reduced by a factor of 1000−5000, which is further supported by studies on engineered human blood vessel organoids and human kidney organoids.[74] A rhACE2 fused to a fragment crystallizable region (Fc) fragment has also showed binding to RBD with higher affinity and neutralized viruses pseudotyped with S glycoproteins from SARS-CoV and SARS-CoV-2.[75]

Although soluble ACE2, ACE2-Fc, and enzyme-inactive ACE2-Fc fusion proteins can block SARS-CoV-2 and SARS-CoV from infecting cells,[74,76,77] using these proteins as drugs may have a number of adverse effects. As ACE2 is a key enzyme playing a central role in the homeostatic control of cardiorenal actions,[77] the administration of wild-type (WT or native) ACE2 may over-catalyze their natural substrates. Using an inactive ACE2 mutant may solve the problem, but if it binds to the substrate, it can still compete with natural ACE2, leading to under-catalyzation of the substrate. These adverse effects may disturb the host hormone balance. Also, native ACE2 does not have favorable pharmacokinetic (PK) properties.[72] In contrast, although ACE2-Fc-fusion can improve PK, it may lead to an Fc receptor-mediated enhanced infection similar to antibody-dependent enhancement that has been observed for SARS CoV and SARS-CoV-2.[51,78−80] Therefore, additional new approaches are needed to target virus entry.

HOST PROTEASES

There are 588 human proteases listed in the degradome database:[81] 192 metalloproteases, 184 serine proteases, 164 cysteine proteases, 27 threonine, and 21 aspartyl proteases.[82] Transmembrane serine proteases (TTSP) can be classified into three groups on the basis of transmembrane domain structure: Type I, which has a carboxy-terminal transmembrane domain; Type II, which has an amino-terminal transmembrane domain spanning through the cytosol; Type III, which anchors to the membrane by glycosyl-phosphatidylinositol (GPI).[83] The Type II group of serine proteases has 20 proteases that are subdivided into four subfamilies: hepsin/transmembrane protease/serine (TMPRSS), human airway trypsin-like (HAT)/differentially expressed in squamous cell carcinoma (DESC), matriptase, and Corin.[84]

Proteases are druggable, and many small-molecule inhibitors have been approved for clinical use.[85] Aprotinin with 58 aa is a nonspecific protease inhibitor, especially for trypsin, chymotrypsin, plasmin, and kallikrein.[86] It was originally approved by the FDA for preventing blood loss and transfusion during coronary artery bypass graft surgery[87] but was later suspended due to an increased risk of complications or death.[88] Aprotinin attenuates inflammatory, coagulation, and fibrinolytic pathways by inhibiting kallikrein, thrombin, and plasmin,.[89] Aprotinin also inhibits the release of pro-inflammatory cytokines[90] and hence can be studied further in regard to SARS-CoV-2 infection. Inhalable aprotinin is approved in Russia to treat mild-to-moderate forms of influenza and parainfluenza in

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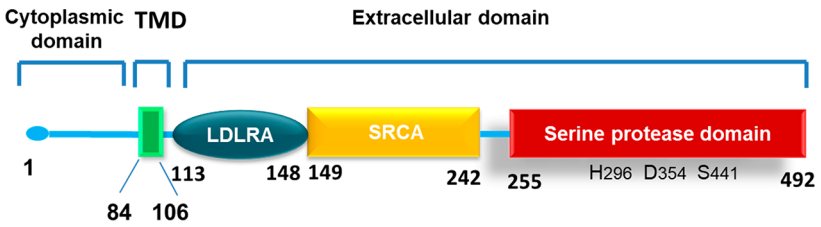

Figure 3. Human TMPRSS2.[102] The extracellular domain includes a serine protease domain (aa 255−492), a scavenger receptor cysteine-rich domain (SRCA, aa 149−242), and LDL receptor class A (LDLRA, aa 113−148) having a binding site for calcium. The figure also shows the transmembrane domain (TMD, aa 84−106) and cytoplasmic domain (aa 1−84). Histidine (H), aspartate (D), and serine (S) are the three catalytic residues; the attached numbers indicate their positions.

humans.[91] Aprotinin inhibits TMPRSS2 in a dose-dependent manner,[92] and in influenza and parainfluenza mouse models, it reduced the mortality rate by 50%.[91]

Coronaviruses use multiple strategies for proteolytic priming of the S protein using proteases such as endosomal cathepsins, cell surface TMPRSS proteases, furin, and trypsin.[93] Coronaviruses enter cells by fusion, either directly at the cell surface or by being internalized by endosomes.[93] In SARS-CoV and some other coronaviruses, the S cleavage occurs at two distinct sites, one at the S1/S2 boundary and another within the SARS-CoV S2 domain (S2′, R797). Also, a furin cleavage site at the S2′ cleavage site within S2 793-KPTKR-797 (S2′) has been shown to allow trypsin-independent cell fusion in this domain, which is increased when another cleavage site is added at the junction of S1 and S2.[94] The important host proteases and their knockout studies are listed in Table 1.

Compared to the classical route of targeting viral components, the inhibition of host factors such as proteases involved in the virus life cycle could be advantageous due to the reduced risk for rapid drug resistance. The selection of target proteases must be made carefully, as these proteases are involved in normal physiology and are structurally similar to other family members. Combination therapies may reduce side effects because of the lower drug dose.[82]

TMPRSS2

TMPRSS2 (transmembrane protease serine S1 member 2; the murine TMPRSS2 orthologue, also known as epitheliasin) is a multidomain Type II transmembrane serine protease. The human and mouse TMRSS2 genes encode proteins of 492 (Figure 3) and 490 amino acids, respectively. The proteins are highly glycosylated, showing a higher molecular mass (70 kDa) than the predicted molecular weight of ∼54 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis.[95] Amino acid sequence similarity between mouse and human TMPRSS2 is 81.4%.[101] TMPRSS2 is expressed in the epithelia of the gastrointestinal, urogenital, and respiratory tracts of the embryo and adult mouse.[101]

The activation of TMPRSS2 requires its cleavage, which is autocatalytic, releasing a 32 kDa serine protease domain.[103] Having trypsin-like specificity, when released into the extracellular space, the active serine protease may interact with other proteins on the cell surface, soluble proteins, matrix components, and proteins on the adjacent cells.[101,103] His_{296}, Asp_{345}, and Ser_{441} are the three residues in the active site of TMPRSS2.[104]

Coexpression of ACE2 and TMPRSS2 is found in human lungs,[105] primary conjunctival and pterygium lines,[106] nasal and bronchial epithelium,[107,108] and gut.[109] Although other host proteases have been described to prime the spike proteins, there is a significant contribution by TMPRSS2 *in vitro*.[19] The expression of TMPRSS2 is increased in prostate cancer cells and regulated by androgens.[110,111] Androgen receptor activity is considered a requirement for transcription of the TMPRSS2 gene.[112] Therefore, androgen may play a role in SARS-CoV-2.[113] Androgen deprivation therapy (ADT) has decreased TMPRSS2.[114] In a population-based study of 4532 men with prostate cancer, there was a lower rate of SARS-CoV-2 infection in men receiving ADT than in patients who did not.[115] Although the study had shortcomings, such as its observational nature, the use of a tumor registry as a comparison group, and the small samples of SARS-CoV-2-positive patients without ADT (n = 114) and with ADT (n = 4), the findings support the hypothesis of a protective role of androgen deprivation in COVID-19.[116] *In vivo* studies in TMPRSS2-knockout (KO) would give more clarity on whether TMPRSS2 is dispensable for SARS-CoV-2 infection and pathogenesis.

The physiological role of TMPRSS2 is still not clear.[95] However, TMPRSS2 is involved in inflammation, tumor growth, and metastasis.[117−119] TMPRSS2-KO mice develop normally and survive to adulthood without any abnormalities in organ histology or function.[95,120] In the TMPRSS2-KO mice model with low pathogenic influenza A viruses (H1N1, H3N2, and H7N9), the cleavage of HA was severely impaired, leading to failed infectivity, whereas the viruses were fully activated proteolytically in $TMPRSS2^{+/+}$ wild-type mice.[120] TMPRSS2 cleaves surface glycoprotein HA of influenza viruses using a monobasic cleavage site, the fusion protein F of the human metapneumovirus, and the S protein of coronaviruses (HCoV-229E, MERS-CoV, SARS-CoV, and SARS-CoV-2). These cleavages are a prerequisite for virus fusion and propagation. The insert sequence SPRR in the S1/S2 protease cleavage site of SARS-CoV-2 enhances spike protein cleavage by TMPRSS2.[21,121] R667 is required for SARS-CoV S cleavage by both trypsin and TMPRSS2, while R797 is dispensable. Conversely, R797 but not R667 is needed for the activation of SARS-CoV S by TMPRSS2.[122]

TMPRSS2 is also involved in many other host activities, including cleavage of ACE2,[123,124] which is the cell surface receptor for SARS-CoV and SARS-CoV-2. Production of the 13 kDa cleaved C-terminal ACE2 fragment was found to be dependent on the enzymatic activities of TMPRSS2, HAT, and hepsin, and this fragment was not generated when cells expressed enzymatically inactive mutants of these proteases, while TMPRSS3, TMPRSS4, and TMPRSS6 did not facilitate ACE2 proteolysis in HEK293 cells.[123] TMPRSS2 expression was previously described in several tumor entities, and hence, TMPRSS2 has emerged as a potential target for drug design. TMPRSS2 also plays a role in the influenza virus life cycle,[125]

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and hence, TMPRSS2-specific inhibitors may act as broad antivirals without causing substantial unwanted side effects.

Camostat mesylate (camostat) is a serine protease inhibitor and thus inhibits TMPRSS2 and other proteases. The drug is approved in Japan to treat chronic pancreatitis and post-operative reflux esophagitis[19] and is known to inhibit the entry of various viruses. However, one study showed that camostat-mediated suppression of SARS-S entry never exceeded 65%, even in the presence of a high concentration of the drug (100 μM), indicating that, despite the presence of TMPRSS2, 35% of the viruses utilized endosomal cathepsins for cell entry.[126]

Nafamostat mesylate (nafamostat), also called as FUT-175 and 6′-amidino-2-naphthyl-4-guanidinobenzoate dihydrochloride, is a broad-spectrum serine protease inhibitor originally synthesized by Fujii and Hitomi.[127] Nafamostat, a strong tryptase inhibitor,[128] is used as a short-acting anticoagulant,[129] and it also has some antiviral and anticancer properties.[130,131] Nafamostat was found to inhibit MERS-S-mediated membrane fusion at an IC_{50} of 0.1 μM, which is 10-fold less than that of camostat mesylate; also, with nafamostat, the reduction in MERS-CoV load was 100-fold more than the camostat.[132] Recently, studies revealed that nafamostat was very potent in inhibiting the membrane fusion and infection of SARS-CoV-2 in human lung cells with potency in the nanomolar range.[133] In clinical trials, nafamostat showed no major adverse effects;[134−136] however, as an anticoagulant, the risk of bleeding is one of the most common adverse effects (>5%).[130,137−139] In one case, a 65 year-old man experienced cardiac arrest following nafamostat treatment.[140] In this regard, clinical trials of nafamostat for COVID-19 should be carefully monitored for safety aspects. Nafamostat is administered by intravenous infusion, whereas camostat is administered orally.

In a substrate-based screening, a TMPRSS2 inhibitor with a K_i value of 0.9 nM was identified that efficiently blocked influenza virus propagation in human airway epithelial cells at 10 and 50 μM.[141] The mechanism of action of TMPRSS2 inhibitors may be by allosteric action or through directly binding to the active site, and directly binding to the active site may be advantageous, as it can inhibit related airway proteases such as TMPRSS4 and HAT.[142] In another screening of 68 640 compounds, bromhexine hydrochloride (BHH), an FDA-approved ingredient in mucolytic cough suppressants, has emerged as a strong inhibitor of TMPRSS2 (IC_{50} 0.75 μM), in addition to other compounds such as 0591-5329, 4401-0077, 4554-5138, and 8008-1235.[112] In addition, 4-(2-aminomethyl) benzenesulfonyl fluoride (AEBSF) and anti-inflammatory protein alpha-1 antitrypsin (A1AT, FDA-approved) are also inhibitors of TMPRSS2.

TMPRSS11D

TMPRSS11D is also known as human airway trypsin-like protease (HAT), a type II transmembrane serine protease coexpressed with ACE2 in bronchial epithelial cells and pneumocytes.[143] TMPRSS11D was first identified in fluid secreted in human airways (trachea and bronchi).[144] It has a catalytic region and membrane anchoring region. The amino acid sequence of the catalytical region of this enzyme reveals high structural homology with other members of human serine protease such as mast cell tryptase, hepsin, or acrosin.[145−148] The protease is involved in several important physiological functions, such as the stimulation of the proliferation of human bronchial fibroblasts[149] engaged in the protease-activated receptor 2.[150]

TMPRSS11D is made up of 2817 bp of mRNA. The gene *TMPRSS11D* is the human ortholog of a long splice variant of the rat airway trypsin-like serine protease 1 (RAT1), also called rat adrenal secretory serine protease (AsP).[152] Human TMPRSS11D protein consists of 418 aa and a predicted molecular mass of ∼46 kDa, having 67% homology in amino acid sequences of RAT1 and 66% with mouse airway trypsin-like protease (MAT1).[151] The activation mechanism of TMPRSS11D has not been studied in detail. The active form of HAT with 27 kDa has been consistently detected as a soluble protein.[144] TMPRSS11D cleaves the S protein in a slightly different way than TMPRSS2. Mutagenesis and mass spectrometry studies revealed that TMPRSS11D cleaved the SARS-CoV S protein at R667 and activated SARS-S for cell−cell fusion in both *cis* and *trans*, whereas TMPRSS2 cleaved SARS-S at multiple sites and activated SARS-S only in *trans*.[153] Also, TMPRSS2 but not TMPRSS11D expression rendered SARS-S-driven virus-cell fusion independent of cathepsin activity.[153] *Tmprss11d*$^{-/-}$ mice did not develop any deformities in embryonic development, health, and long-term survival in the absence of external challenges or additional genetic deficits, and hence, TMPRSS11D appears to be dispensable.[96]

In HEK-293T cells expressing the S protein of SARS-CoV and SARS-CoV-2 and GFP-expressing replicon (eGFP), there was increased syncytia formation in the presence of TMPRSS11D, indicating splicing of an S protein by TMPRSS11D.[22] Substrate analog inhibitors of TMPRSS11D with 4-amidinobenzylamide moiety were checked for activity and selectivity; the incorporation of norvaline led to a potent inhibitor (K_i = 15 nM) with improved selectivity for HAT in comparison to the coagulation proteases thrombin and factor Xa or fibrinolytic plasmin. Additionally, these inhibitors were able to inhibit influenza virus replication in MDCK cells expressing HAT.[154]

TMPRSS4

TMPRSS4/cyclase-associated actin cytoskeleton regulatory protein 2 (CAP2) is another member of type II transmembrane serine proteases, previously referred to as TMPRSS3.[155,156] TMPRSS4 protein is around 48 kDa in size with 437 aa and is known to be involved in cancer.[155] TMPRSS2 and TMPRSS4 knockdown by RNA interference in Caco-2 cells reduced the spread of the influenza virus, whereas treatment with trypsin released a fully infectious virus.[157] In a cell−cell fusion assay with 293T effector cells expressing SARS-CoV S and 293T target cells transfected to express ACE2 or TMPRSS4, SARS-CoV S was activated for cell−cell membrane fusion but failed to cleave SARS-CoV S as determined by Western blot.[158] In addition to TMPRSS2, TMPRSS4 was also involved in the SARS-CoV-2 entry in human small intestinal enterocytes.[159] Mice deficient in TMPRSS4 were viable, fertile, and without any known histological abnormalities.[97]

Screening of a compound library against TMPRSS4 serine protease activity yielded several classes of compounds, including 2-hydroxydiarylamide with an IC_{50} of 6 μM.[160] *N*-(3,5-bis(trifluoromethyl)-phenyl)-5-chloro-2-hydroxybenzamide, a derivative of 2-hydroxydiarylamide, also exhibited relatively potent inhibitory activity (IC_{50} = 11 μM) against TMPRSS4.[160]

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CATHESPIN AND OTHER CYSTEINE PROTEASES

Cathepsins are serine or cysteine proteases, most of which become activated at the low pH found in lysosomes. Cysteine cathepsins are involved in various physiological processes and are especially present at high concentrations in endosomes and lysosomes where they are required for the breakdown of protein and major histocompatibility complex class II-mediated immune responses.[161,162] The human genome has 11 cathepsins that include B, C, F, H, K, L, O, S, V, W, and X.[163] Cathepsins are found in the cytoplasm, cell nucleus, and the extracellular space,[164] and some cathepsins have largely overlapping specificities. Cathepsin has been associated with various diseases, including cancer.[164]

Cathepsin L is a lysosomal cysteine protease that is synthesized as a preproform[165] and processed into a 41 kDa active form in the Golgi apparatus. The active form has two fates: to be targeted to lysosomes and to be secreted out of the cells. Mice deficient in cathepsin L show abnormal skin and bone development and have increased resistance to osteoporosis following ovariectomy.[98]

Some cathepsins have been known to be involved in the pathogenesis of viruses. In 293T cells expressing the porcine deltacoronavirus (PDCoV) S protein, cathepsins L and B in lysosomes primed the S protein for membrane fusion, and similar fusions were also observed with extracellular trypsin in cell cultures; however, pretreatment of cells with bafilomycin-A1, a lysosomal acidification inhibitor, completely inhibited the entry of PDCoV.[166] Additionally, the ablation of cathepsins L and B using siRNA reduced viral infection significantly. The treatment of cells with trypsin-activated PDCoV entry in the absence of the endosomal pathway indicates two independent mechanisms of cell entry.[166] Enveloped viruses such as SARS-CoV, MERS-CoV, ebolavirus (EBOV), hepatitis E virus, and Nipah virus require cathepsin L for their glycoprotein processing and cleavage; for SARS-CoV and EBOV, the cleavage can happen by cathepsin L in the endocytic vesicles.[167,168] For SARS-CoV S protein, the cleavage by cathepsin L supposedly occurs at a postreceptor-binding stage during virus entry.[169]

In HEK293 cells expressing human ACE2, the SARS-CoV-2 pseudovirions entry was inhibited by E64D (inhibitor of cathepsin B, H, and L and calpain) and SID 26681509 (inhibitor of cathepsin L) but not by CA-074 (inhibitor of cathepsin B).[22] Cathepsin B has roles in the life cycle of various viruses. Although cathepsin $B^{-/-}$ macrophages and cathepsin inhibitor CA-074Me-treated A549 cells are able to incorporate influenza A virus virions and permit viral RNA synthesis, they produce less HA protein and progeny virions than wild-type or untreated cells.[170] Compared to TMPRSS2, cathepsin may be less necessary in the coronavirus entry and life cycle. HCoV-229E prefers cell-surface TMPRSS2 to endosomal cathepsins for cell entry,[171] and TMPRSS2 can activate HCoV-229E for cathepsin-independent host cell entry.[172] In BALB/c mouse models, the mouse survivability was 60% with the serine protease inhibitor camostat (30 mg/kg) when mice were challenged with SARS-CoV (MA15), whereas cysteine protease inhibitor SMDC256160 at (50 mg/kg) did not lead to any improvement in survival.[21,124] HCoV-NL63 infection is not dependent on cathepsin L activities.[173]

Due to its increased specificity, the cysteine protease inhibitor E64C (an analog of E64) inhibits infection by the SARS-CoV S glycoprotein within HIV pseudovirions.[168] E64D is a permeable cell derivative of E64C and inhibits calpain and other cysteine proteases, such as papain and cathepsins B and L. However, even at 50 μM, E64D does not inhibit SARS-CoV-2 replication.[32] The addition of E64D to cell cultures should be done in serum-free media, as esterases in serum will cleave the ethyl ester and reduce cell permeability. More potent and specific inhibitors for cathepsin may be required to combat SARS-CoV-2 infection. K11777 is an irreversible cysteine protease inhibitor and was shown to inhibit SARS-CoV replication with an IC_{50} of <0.05 μM for strains in Vero 76 cells, displaying low IC_{90}'s of 0.35 and 1.04 μM against strains Urbani and Toronto-2 of the SARS-CoV, respectively, in a virus reduction assay.[124] K11777 also has good PK profiles in animal models[174] and hence represents a potential molecule for use in developing drugs against SARS-CoV2. The screening of 1000 molecules for cathepsin L inhibitors yielded MDL28170 (also known as calpain inhibitor III or Z-Val-Phe-CHO) as a potent inhibitor of cathepsin L-mediated substrate cleavage with an IC_{50} of 2.5 nM,[168] and it was also able to inhibit SARS-CoV entry in a pseudotype infection assay.[175] MDL28170 also inhibits other cysteine proteases such as calpain. Other inhibitors of calpain, SJA 6017[176] and BLD-2660, are under clinical study for use in COVID-19.[177] Calpain inhibitors also show anti-inflammatory properties.[178,179] Calpain inhibitors I and II also inhibit SARS-CoV-2 3CLpro and hence have potential for inclusion in the design of dual inhibitors.[180]

FURIN

Furin is grouped into the family of highly specific protein convertases (PCs), which are calcium dependent.[181] The type I transmembrane protein furin has a 104 kDa pro-furin precursor and is then converted into a 98 kDa form by an autocatalytic process.[182] Furin has a role in various normal physiological and also pathogenic processes, such as viral propagation, activation of a bacterial toxin, cancer, and metastasis.[183] Furin is also known as a paired basic amino acid cleaving enzyme (PACE). In mammals, the PC family includes nine members, out of which seven members including furin, PC1/3, PC2, PC4, PACE4, PC5/6, and PC7 cleave after multiple basic residues.[184,185] The PCs (PC1/3, PC2), which specialize in peptide-hormone and neuropeptide processing, are also called prohormone convertases. Furin and its analogs are involved in the maturation of a huge number of inactive protein precursors[186,187] and are therefore involved in many normal physiological processes. Protein convertase furin displays embryonic lethality,[99,188] but short-term administration of the furin inhibitor hexa-D-arginine did not show any adverse effects in mice.[100] Also, these proteases are involved in various diseases, such as viral and bacterial infections, neurodegenerative disorders, tumorigenesis, diabetes, and atherosclerosis.[181,183,189]

Furin can activate a glycoprotein of HIV-1,[190] and trafficking of MHV to lysosomes and processing by lysosomal proteases were dispensable when the furin cleavage site was introduced upstream of the fusion peptide in the S protein.[191] The inhibition of furin but not lysosomal protease affected MERS-CoV, which has a minimal furin cleavage site just upstream of the fusion peptide in Huh-7 cells.[191]

On the basis of the structural analysis, SARS-CoV-2 has a furin-like cleavage site, which is absent in SARS-CoV[18,192] as well as SL-CoV-RaTG13, a CoV with the highest nucleotide sequence homology to SARS-CoV-2, which was isolated from

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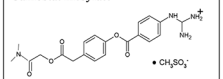
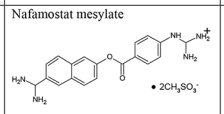
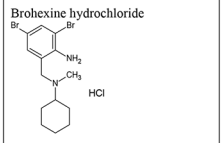
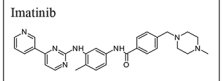
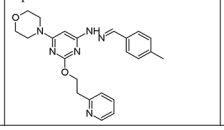
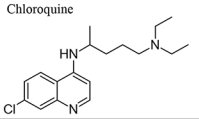
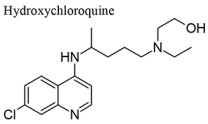
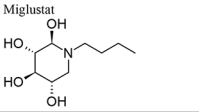
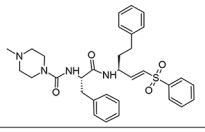
Table 2. Compounds That Have the Potential to Target Host Factors of SARS-CoV-2 and Their Current Status

Sl. No	Compound name and structure	Initial use	Host factors of SARS-CoV-2	*In vitro* and *in vivo* efficacy and clinical trials against Coronaviruses (expected/actual start date for phase study)
1	Camostat mesylate 	Pancreatitis [281]	TMPRSS2 [19] Pro-inflammatory cytokines [281]	**SARS-CoV** in mouse [124] **SARS-CoV-2** in CaCo2, Vero-TMPRSS2 [19] **Phases 1 and 2:** April 4 2020 [286] **Phase 2:** May 31 2020 [287] **Phase 3:** April 11 2020 [288] **Phase 4:** June 1 2020 [289]
2	Nafamostat mesylate 	Anticoagulant [290]	TMPRSS2 [132] Pro-inflammatory cytokines [291]	**MERS-CoV**, Vero-TMPRSS2 and Calu3 cells [132] **SARS-CoV-2**, Calu3 cells [133] **Phases 2 and 3:** April 2020 [292]
3	Brohexine hydrochloride 	Mucolytic agent [293]	TMPRSS2 [112]	**Phase 4:** April 10 2020 [294]
4	Imatinib 	Anti-cancer [295]	Abl2 (membrane fusion?) [296] Pro-inflammatory cytokines [297]	**MERS-CoV**, Vero and MRC5 cells [202] **SARS-CoV**, Vero and Calu3 cells [202] SARS-CoV-2, Vero cells [298] **Case Report** [24] **Phase 2:** Sept 1 2020 [299], April 13 2020 [300] **Phase 3:** June 2 2020 [301], July 29 2020 [302]
5	Apilimod 	Crohn's disease [303] Autoimmune diseases [304]	PIKfyve (endosomal fusion?) [23] Pro-inflammatory cytokines [305]	SARS-CoV-2, Vero cells-[23] iPSC cells [219] **Phase 2:** Jul 15,2020 [306]

Sl. No	Compound name and structure	Initial use	Host factors of SARS-CoV-2	*In vitro* and *in vivo* efficacy and clinical trials against Coronaviruses (expected/actual start date for phase study)
6	Chloroquine 	Anti-malaria [307]	ACE2 Glycosylation [253,256] Anti-inflammation [308]	**SARS-CoV**, Vero-E6 cells [309,310] **SARS-CoV-2**, Vero cells [311] **HCoV-OC43**, newborn C57BL/6 mice [312] **Phase 2:** April 7 2020 [313], April 6, 2020 [314]
7	Hydroxychloroquine 	Anti-malaria, Anti-rheumatoid arthritis, Anti-lupus [315]	Endosomal pH elevation [316] Pro-inflammatory cytokines [317]	**SARS-CoV-2**, Vero cells [311] **Phase 4:** April 28 2020[318]
8	Miglustat 	Gaucher and Niemann-Pick type C diseases [250]	Inhibits β-glucosidase 2 involved in the glycosylation [319,320]	**SARS-CoV**, Vero cells [42] **SARS-CoV-2**, Vero cells [249]
9	K-11777 	Chagas disease [321]	Cathepsins B and L [322]	**SARS-CoV**, Vero cells [124]
10	BLD-2660 (structure not available)	Anti-fibrotic activity in NASH [323]	calpain?	**Phase 2:**-May 4, 2020 [177]
11	DAS181 (recombinant sialidase protein)	Parainfluenza [324]	Removes host sialic acid [325]	**Phases 2 and 3:** May 25, 2020 [263]

a bat in Yunnan in 2013. In Calu3 cells, furin and TMPRSS2 cleave the S protein of SARS-CoV-2 at the S1/S2 site.[193] Hoffmann et al.[21] demonstrated that furin inhibitor dec-RVKR-CMK inhibited SARS-CoV-2 entry. However, dec-RVKR-CMK also inhibits other proteases such as cathepsins L and B, trypsin, papain, and TMPRSS2.[194] Macro and small molecules can be used as inhibitors of PCs. Most of the small molecule inhibitors belong to three groups, including pure peptides, peptide mimetics, or nonpeptidic compounds.[183] Decanoyl-Arg-Val-Lys-Arg-CMK and hexa-D-arginine (D6R) are small synthetic furin inhibitors that are suitable for clinical purposes.[195] Although SARS-CoV-2 replication was inhibited by a furin inhibitor MI-1851 in human airway cells,[32] the nonspecificity and irreversibility of available furin inhibitors are major concerns. Whether furin is an attractive drug target for SARS-CoV-2 will require additional investigation.

KINASES

Viruses hijack many host kinases at various steps of their life cycle.[196,197] Since these kinases are upstream of cellular pathways, they become good targets for broad-spectrum therapy. Following the successful development and approval of kinase inhibitors for cancer[198] and inflammation,[199] kinases appear to be a good choice for repurposing as antivirals. Many of the approved inhibitors of several families of kinases, such as Nak, ErbB, Src, Abl, Cdk, and PI3K/Akt/mTor, have been shown to have antiviral activity.[200] However, the antiviral potential of many more kinase inhibitors remains unexplored, particularly against SARS viruses.

Abl kinases are reversible nonreceptor tyrosine kinases, which are a subgroup of tyrosine kinases that rely on intracellular signals originated by the extracellular receptor;[201] these kinases regulate many cellular pathways such as cell migration, adhesion, and actin reorganization.[202] Mammals have two Abl kinases, Abl1 (Abl in mice) and Abl2 (Arg in mice).[202] Abl pathway inhibitors are known to have antiviral activity in the EBOV, coxsackievirus, and vaccinia virus.[203−206] More recently, small molecules imatinib mesylate and dasatinib have been shown to have antiviral activity toward SARS-CoV and MERS-CoV.[207] Imatinib targets Abl2, which is required for efficient SARS-CoV and MERS-CoV replication,[202] and has an IC_{50} of 9.82 and 17.69 μM toward SARS-CoV1 and MERS-CoV, respectively. The IC_{50} of dasatinib is 2.10 and 5.47 μM in SARS-CoV1 and MERS-CoV, respectively. Similar IC_{50} values were observed for both imatinib and dasatinib against SARS-CoV-2 at nontoxic concentrations.[208] The administration of imatinib to a COVID-19 patient was also shown to improve fever and other laboratory parameters,[24] and clinical trials of imatinib are ongoing (Table 2). The anti-inflammatory activity[209] and possible interference in virus-endosomal membrane fusion[202] might potentiate the efficacy of imatinib in COVID-19 treatment. Apart from imatinib, other kinase inhibitors also have shown activity against SARS-CoV-2 and other related viruses.[210] Abl and Arg kinases have been found in cancer cells and are known to promote the secretion of the endosomal protease cathepsin L,[211] which is involved in SARS-CoV S protein cleavage priming.[168]

The Src family kinases are also known to have a role in coronaviruses. Saracatinib, an inhibitor of Abl/Src, inhibits MERS-CoV with an IC_{50} of 2.9 μM in Huh-7 cells at the initial stages of the MERS-CoV life cycle.[212] AP2-associated protein kinase 1, which promotes endocytosis, and cyclin G-associated kinase, which mediates endocytosis, belong to the Nak family and may be exploited by some viruses, including HCV[213] and DENV.[214] Abemaciclib (CDK4/6 inhibitor) and osimertinib (inhibitor of EGFR) have shown antiviral activity toward SARS-CoV-2 in Vero cells with IC_{50} values of 6.6 and 3.2 μM, respectively.[215] The Janus kinase-2 inhibitor fedratinib also

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suppresses the production of TH17-related cytokines, thus indicating the potential in treating COVID-19 for patients with TH17-related cytokine storms.[216]

Phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase (PIKfyve), an endosomal lipid kinase, is responsible for the production of phosphoinositide PI(3,5)P2, which is involved in endomembrane homeostasis. PIKfyve, a class III lipid kinase having a size of 240 kDa, is found in the cytosolic side of the endosomal membranes.[217,218] Apilimod, a small molecule inhibitor of PIKfyve, has shown inhibition of the infection of a chimeric vesicular stomatitis virus (VSV) bearing the fusion proteins of SARS-CoV-2 by preventing the release of the viral contents from the endosomes in human astroglial SVG-A derived cells as well as in fully infectious SARS-CoV-2 in Vero E6 cells with an IC_{50} of 10−15 nM.[23] Apilimod has also shown 85% inhibition of SARS-CoV-2 in human pluripotent stem cell (iPSC)-derived pneumocyte-like cells.[219] Although apilimod does not inhibit cathepsin B and L activity or alter endosomal pH, there are reports of interference of apilimod in the maturation of endosomes.[220] Clinical trials of apilimod have been conducted for Crohn's disease, rheumatoid arthritis, and common variable immunodeficiency,[221,222] and it has been found to be well tolerated. Other inhibitors of PIKfyve such as Vacuolin-1, YM201636, and the WX8 family of chemicals have been tested for cancer and autoimmune diseases.[223−225] Currently, several clinical trials of kinase inhibitors are ongoing in COVID-19 patients[210] (Table 2). The anti-inflammatory and/or antiviral activity of some of the kinase inhibitors may be beneficial in COVID-19 cases involving cytokine storms. However, the risk factors of fungal and bacterial infection must be considered when treating with kinase inhibitors.[226]

POST-TRANSLATIONAL MODIFIERS

After being translated from mRNA, many proteins undergo chemical modifications before attaining their functions in different cells across the body. Post-translational modification (PTM) is required for the development of their functional diversity, homing, proper folding, and solubility. Various kinds of PTM occur in the ER or Golgi complex by the addition of functional groups (phosphorylation, glycosylation, lipidation (palmitoylation and myristoylation), acetylation, and methylation), cleaving of peptide bonds, or the formation of disulfide bonds.[227] Many viruses make use of PTM processes, such as glycosylation, which plays a role in immune evasion, virulence, and attachment.[228,229] Although there are several forms of glycosylation occurring in nature, N-linked and O-linked glycosylations play significant roles in viral pathology. In N-linked glycosylation, the carbohydrate (also called glycans) attaches to the amide nitrogen of the asparagine residue of the protein. This attachment occurs early in protein synthesis and is followed by trimming and remodeling of the oligosaccharide in the ER and Golgi complex to form glycoproteins with different sizes of oligosacharides.[228]

Glucosidases I and II present in the ER trim the three-terminal glucose moieties on the N-linked glycans attached to nascent glycoproteins[228] Iminosugars are carbohydrate mimetics in which the endocyclic oxygen of the parent carbohydrate is replaced with nitrogen; these are known to inhibit ER-α glucosidases involved in the glycosylation process. Several iminosugars have been explored in the past three decades as antiviral agents[230] in mouse models against viruses such as dengue virus (DENV),[231−236] Japanese encephalitis virus,[237] EBOV, and Marburg virus.[238] Naturally occurring 1-deoxynojirimycin (DNJ) and castanospermine are the sources of many derivatives that are currently being tested. Clinical trials have been conducted in DENV,[239] HIV,[240] and hepatitis C (HCV) patients.[241] In HIV patients, although some effects on viremia were observed with an *n*-butylated form of DNJ (NB-DNJ) and with celgosivir, a prodrug of castanospermine, the maintenance of therapeutic concentrations of the drug in serum and permeability inside the cells was difficult.[242] In dengue patients, celgosivir did not reduce fever or viral burden but reduced TNF-α levels.[239] However, these clinical studies indicate that the iminosugars DNJ and celgosivir are well tolerated and safe and, hence, might be modified for better potency.

In SARS-CoV, there are 23 potential glycosylation sites in the S protein,[41] two of which are in the RBD region (aa 319−515). Mutation in the RBD glycosylation sites N330 or N357 does not affect binding to ACE2, indicating these glycosylation sites may not be necessary for the attachment of SARS-CoV to cells. However, cell surface C-type lectin receptors such as DC-SIGN and L-SIGN bind to glycosylated ligands of many viruses, augmenting the virus entry.[243,244] In one study, the SARS-CoV S protein bound to 293T cells overexpressing DC-SIGN with lower efficiency than cells overexpressing both DC-SIGN and ACE2. Since lectin DC-SIGN binds to carbohydrates, improper glycosylation of the S protein may counter the binding.[243,245] Glycosylation sites N109, N118, N119, N158, N227, N589, and N699 in the S protein have been found to be critical for SARS-CoV entry mediated by the DC-SIGN and/or L-SIGN.[246] An interaction of mannose-binding lectin with SARS-CoV S-pseudotyped virus could block the binding of the virus to DC-SIGN, and N-linked glycosylation at N330 is critical for the interaction of mannose-binding lectin with SARS-CoV S protein.[247,248]

Apart from the viral protein, the glycosylation of the host protein also plays a role in viral propagation. Four iminosugars *N*-butyl-DNJ, CM-10-18, IHVR-11029, and IHVR-17028 significantly inhibited the transduction of SARS-CoV and HCoV-NL63 spike glycoprotein-pseudotyped lentiviral particles by altering the N-linked glycan structure of ACE2, resulting in impaired membrane fusion and the reduction of infectious virions.[42] Butyl-DNJ (also known as miglustat) was also found to be effective against SARS-CoV-2 with an IC_{50} of 41 ± 22 μM in a plaque reduction assay.[249] Miglustat is presently in clinical use for Gaucher disease and Niemann-Pick disease type C.[250]

In SARS-CoV, proteins S, E, M, and 8ab are N-glycosylated, whereas 3a is O-glycosylated.[247] The S protein of the SARS-CoV-2 is highly glycosylated with 22 N glycosylation potential sites, and there is O-glycosylation at Thr323 and Ser325 in the RBD region, which ranges from aa 331 to 524.[251] The glycosylations of the S and E proteins are required for proper folding of the proteins and to retain the infectivity of SARS-CoV and SARS-CoV-2.[252] One of the possible mechanisms by which chloroquine exhibits anti-SARS-CoV activity is by interfering in the glycosylation of the ACE2 receptor.[253] Apart from binding to a defined protein receptor, some coronaviruses have a sialic acid-binding activity.[254,255] Chloroquine inhibits quinone reductase 2,[256] which is structurally close to UDP-*N*-acetylglucosamine 2-epimerases involved in sialic acid biosynthesis. Sialic acid was the first defined virus receptor.[257] Beta-CoVs recognize O-acetylated sialic acid and carry a sialyl-*O*-acetyl-esterase that cleaves off the sialic acid

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moieties and thus helps in the release of virus from the infected cells.[257] However, in a clinical study with 821 asymptomatic participants exposed to people with confirmed COVID-19, there was no improvement of illness with hydroxychloroquine treatment started in adults after 4 days of exposure to a SARS-CoV-2-infected individual within 6 ft for over 10 min.[258]

Apart from the glycosylated proteins of the virus itself, the cell receptors of several viruses are also glycosylated proteins. Thus, the inhibition of ER glucosidases can also disrupt viral receptors in addition to affecting viral glycoproteins. The efficient attachment of the SARS-CoV virions to the host cells may require the N-linked glycosylation of SARS-CoV, although it is not required for binding to ACE2.[247]

DAS181 is a recombinant sialidase protein of *Actinomyces viscosus* origin that cleaves sialic acid present on the surface of epithelial cells lining the human airway tract. DAS181 protected mice from lethal avian influenza H5N1 virus infection.[259,260] DAS181 removes sialic acid from the respiratory epithelium[261] and thereby prevents the binding of influenza[259] and parainfluenza[262] viruses. Clinical evaluation of DAS181 for the prevention of COVID-19 is underway.[263]

Disulfide bond formation is another type of post-translational modification. When dithiothreitol was added to murine coronavirus mouse hepatitis (MHV)-infected cells, the produced S protein was reduced and did not bind to a monoclonal antibody, indicating the role of disulfide bonding in the folding of S proteins[265]; however, the reduction of recombinant SARS-S protein did not affect binding to ACE2.[264]

Palmitoylation happens when fatty acids such as palmitic acid attach to cysteine, serine, or threonine. Palmitoylation of the coronavirus S protein was initially identified following the incorporation of ^{3}H-palmitate to the S protein of the MHV-A59[266] and treatment with 2-bromopalmitate, an inhibitor of palmitoyl acyltransferase that reduced palmitoylation of the MHV S protein and the infectivity of MHV *in vitro*.[267] The mutational analysis of cysteine-rich clusters of the cytoplasmic portion of the SARS-CoV S protein indicates the role of palmitoylation for the fusogenic activity of the SARS-CoV S protein.[268]

In summary, glycosylation, disulfide bond formation, and palmitoylation are important post-translational modifications with respect to viruses. Current studies are mainly focused on the pharmacological interference of glycosylation.

PRO-INFLAMMATORY CYTOKINES

Another important host factor that is associated with the severity of the SARS-CoV-2 infection is unregulated inflammation reported to cause cytokine storms, leading to organ failure and death.[269,270] Although the infection rate is lower in children, the multisystem inflammatory syndrome has been reported.[271] Higher levels of cytokines such as IL-1β, IFN-γ, IP-10, MCP-1, IL-4, and IL-10 are reported in COVID-19 patients.[5] Moreover, higher plasma levels of IL-2, IL-7, IL-10, GCSF, IP-10, MCP-1, MIP-1A, and TNF-α were reported in ICU COVID-19 patients with severe disease.[5,272] SARS-CoV-2 infection activates an inflammatory response that plays an antiviral role, but overproduction of cytokine occurs when there is a loss of negative feedback. This unbalanced feedback recruits more immune cells to the site of infection, leading to organ damage. Suppressing cytokine storms is essential for preventing disease deterioration in patients with COVID-19, especially in critically ill patients.

The acute lung injury due to SARS-CoV infection-mediated aggressive inflammation[273] can lead to fatality. In a mouse model, ACE2 is known to give protection from severe acute lung injury by acid aspiration or sepsis, and downregulation of ACE2 reduces the protective effect to lung injury.[274] In macrophages, ACE2 controls the expression of several pro-inflammatory cytokines, including TNF-α and IL-6 *in vitro*.[275] SARS-CoV tends to downregulate ACE2, thus increasing the expression of cytokines.[61,76] A detailed study of the effect of ACE2 downregulation and its effect on the cytokine storm in COVID-19 is required.[20,276,277] Taken together, these data call for clinical validation of a combination of virus life cycle blockers and anti-inflammation therapies to minimize the severity of COVID-19.

Apart from the antiviral activity, many of the front-runners among promising drugs against COVID-19 (Table 2), including camostat, nafamostat, imatinib, and hydroxychloroquine, also have anti-inflammatory activity.[278−283] Ambroxol hydrochloride is an active *N*-desmethyl metabolite of bromhexine hydrochloride, also known to be anti-inflammatory.[284] Various anti-inflammatory agents such as tocilizumab, camrelizumab, and thymosin and methylprednisolone are in clinical trials targeting COVID-19.[285] Furthermore, a detailed understanding of immune dysfunction is necessary to select immunomodulators to retain homeostasis.

CONCLUSIONS

Several coronaviruses are responsible for new disease outbreaks around the world, including COVID-19. The combination of high transmissibility of SARS-CoV-2 and unregulated host immune response during infection makes the disease more severe in elderly people and those with previous medical conditions. Apart from the loss of lives, the interruption of daily activities has created global social disruption and economic loss. Hence, there is an unmet medical need for broad antivirals that combat these diseases. The frequent evolution of viruses not only changes the severity of the disease but also contributes to the development of drug resistance. In this regard, modulating the host factors involved in the viral life cycle is a good strategy against viral diseases. There are several ongoing preclinical and clinical studies mainly aimed at repurposing the approved drugs as well as lead candidates (Table 2). Variation in the severity of infection of COVID-19 makes recruitment for clinical trials complicated. Hence, detailed guidelines are needed for conducting and comparing the results across the globe.

Considering the available data, TMPRSS2 seems to be an attractive target, mainly because it is a major host protease that cleaves the SARS-CoV-2 S protein followed by cell fusion. Moreover, TMPRSS2$^{-/-}$ mice do not show any abnormality, and hence, TMPRSS2 is dispensable, although it is involved in many other host processes, including ACE2 activation. More TMPRSS2 inhibitors should be investigated and tested against SARS-CoV-2. Other proteases, including furin, TMPRSS4, TMPR11D/HAT, and cathepsin L, are also involved in the viral entry process, but further studies are required to know to what extent they influence viral entry. Treatment with a combination of protease inhibitors can be beneficial; however, the nonspecificity of the available protease inhibitors hinders their study in wild-type cells. The highly glycosylated S and E proteins of SARS-CoV-2 open the window for testing the molecules that interfere with both the glycan processing of viral proteins and the host proteins involved in the virus

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propagation. Some kinase inhibitors, especially Abl2 inhibitors and PIKfyve, are also promising as they are both antiviral and anti-inflammatory in nature. The cytokine storm, which is one of the main causes of organ failure and death, must be dealt with using anti-inflammatory drugs. Therefore, drugs with both antiviral and anti-inflammatory properties might be beneficial in treating COVID-19. Given the complexity of coronaviruses and the host interaction, continued work is needed to unravel their molecular nature further and to find out compounds that interfere in the host−pathogen interaction.

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Notes

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ABBREVIATIONS

A1At, anti-inflammatory protein alpha-1 antitrypsin; ACE2, angiotensin-converting enzyme; AEBSF, 4-(2-aminomethyl) benzenesulfonyl fluoride; ASP, adrenal secretory serine protease; BHH, bromhexine hydrochloride; CAP2, cyclase-associated actin cytoskeleton regulatory protein 2, also TMPRSS4; CFR, case fatality rate; COVID-19, coronavirus disease 2019; Dec-RVKR-CMK, decanoyl-Arg-Val-Lys-Arg-chloromethyl ketone; DENV, dengue virus; DESC, differentially expressed in squamous cell carcinoma; DFP, diisopropylfluorophosphate; E, envelope; EBOV, Ebola virus; eGFP, green fluorescent protein-expressing replicon; ER, endoplasmic reticulum; Fc, fragment crystallizable region; GPI, glycophosphatidylinositol; HA, hemagglutinin; HAT, human airway trypsin-like protease, also TMPRSS11D; HCoV, human coronavirus; HCV, hepatitis C virus; HEK, *Homo sapiens* embryonic kidney; HIV, human immunodeficiency virus; K_d, dissociation constant; K_i, inhibitor constant; KO, knockout; M, membrane; MAT1, mouse airway trypsin-like protease; MDCK, Madin-Darby canine kidney; MERS-CoV, Middle East respiratory syndrome coronavirus; N, nucleocapsid protein; NSP, nonstructural protein; PCR, polymerase chain reaction; PCs, proprotein convertases; PDCoV, porcine deltacoronavirus; PK, pharmacokinetic; RAT-1, rat airway trypsin-like serine protease 1; RBD, receptor-binding domain; RBM, receptor-binding motif; RdRp, RNA-dependent RNA polymerase; rhACE2, recombinant human ACE2; S, spike protein; SARS-CoV, severe acute respiratory syndrome coronavirus; SPB1, selenium-binding protein 1; TMD, transmembrane domain; TMPRSS, transmembrane protease serine; TMPRSS11D, also HAT; TMPRSS2, TMPRSS S1 member 2, also epitheliasin; TMPRSS4, also CAP2; TSP, transmembrane series proteases; TTSP, Type II TSP; WT, wild-type or native; ZIKV, Zika virus; α1-PDX, α1-antitrypsin Portland; MHV, mouse hepatitis virus (murine coronavirus); AAK1, AP2-associated protein kinase 1; Abl, Abelson murine leukemia viral oncogene homologue kinase; Akt, v-akt murine thymoma viral oncogene; Arg, Abl2; CDK, cyclin-dependent kinase; DAS181, recombinant sialidase protein; EGFR, epidermal growth factor receptor; E-protein, envelope small membrane protein; ErbB, derived from the oncogene encoded by the erythroblastosis virus; GAK, cyclin G-associated kinase; GCSF, granulocyte colony-stimulating factor; H5N1, highly pathogenic Asian avian influenza A; Huh-7, human hepatoma cell line; IC, inhibitory concentration; IFN-γ, interferon gamma; IL, interleukin; MCP-1, monocyte chemoattractant protein-1, also CCL2; MIP-1A, macrophage inflammatory protein 1-alpha; mTor, mechanistic target of rapamycin; Nak, numb associated kinase; P13K, phosphatidylinositol-3-kinase; Src, derived from the gene encoded by the Rous sarcoma virus; TH17, T cells producing IL-17; TNF-α, tumor necrosis factor; PIKfyve, phosphatidylinositol-3-phosphate/phosphatidylinositol 5-kinase; IP-10, IFN-γ-inducible protein; PTM, post-translational modification

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