



# Molecular mechanisms for understanding the association between TMPRSS2 and beta coronaviruses SARS-CoV-2, SARS-CoV and MERS-CoV infection: scoping review

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## Abstract

The aim of this scoping review was to identify knowledge gaps and to describe the current state of the research on the association between TMPRSS2 and the essential beta coronaviruses (Beta-CoVs) infection and the molecular mechanisms for this association. We searched MEDLINE (OVID), EMBASE, and the Cochrane Central Register of Controlled Trials (CENTRAL). We included 13 studies. Evidence shows an essential role of TMPRSS2 in Spike protein activation, entry, and spread into host cells. Co-expression of TMPRSS2 with cell surface receptors (ACE2 or DPP4) increased virus entry. This serine protease is involved in the formation of large syncytia between infected cells. TMPRSS2 cleaved the Spike protein of SARS-CoV, SARS-CoV-2, and MERS-CoV, and increased virus propagation. Accumulating evidence suggests that TMPRSS2 is an essential protease for virus replication. We highlighted its critical molecular role in membrane fusion and the impact in viral mRNA replication, then promoting/driving pathogenesis and resistance.

**Keywords** TMPRSS2 · Gene · Betacoronavirus · SARS-CoV-2 · SARS-CoV · MERS-CoV

## Introduction

Emerging coronaviruses can constitute a severe threat to human health. The subfamily Coronavirinae within the family Coronaviridae comprises viruses that cause respiratory, neurological, and intestinal symptoms in mammals and birds (Wang et al. 2020a, b). The severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in 2002 in Southern China. The Middle East respiratory syndrome coronavirus (MERS-CoV), which emerged in Saudi Arabia, was the pathogen responsible for severe respiratory disease outbreaks in 2012 (Huang et al. 2020).

On 11 March 2020, WHO declared COVID-19 infection a pandemic, and the number of individuals infected and deaths quickly raised. Then, it is essential to monitor

viral evolution, infectivity, transmissibility, and pathogenicity (Wang et al. 2020a, b; Huang et al. 2020; WHO 2020). The disease affected patients with underlying chronic illness, but documented infections in health care workers indicated human-to-human transmission. Now, it is very established that SARS-CoV-2 is capable of widespread human-to-human transmission (Wang et al. 2020a, b). Acute respiratory distress syndrome (ARDS) and shock associate with pulmonary inflammation and extensive lung damage in patients (Wang et al. 2020a, b; Huang et al. 2020). However, nowadays, we do not entirely understand the pathophysiology of SARS-CoV-2 infection and SARS-CoV and MERS-CoV (Zhu et al. 2020).

TMPRSS2 gene encodes a self-membrane protein of 492 amino acids, which anchors to the plasma membrane. It converts to its form through autocatalytic cleavage between Arg255 and Ile256 (Shulla et al. 2011). After cleavage, the mature proteases are mostly membrane-bound, yet a noticeable portion of them can liberate into the extracellular milieu. TMPRSS2 predominantly expresses in the prostate, lungs, colon, liver, kidneys, and pancreas. Also, it activates protease-activated receptor 2 (PAR-2), a G-protein-coupled receptor, that causes the upregulation of matrix metalloproteinase-2 (MMP-2) and MMP-9, which are critical proteases

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in the metastasis of tumor cells. Some reports have shown how this TMPRSS2-mediated pathway allows the spread and pathogenesis of SARS-CoV, demonstrating that infection in the presence of this protease could induce higher concentrations of pro-inflammatory cytokines and cytopathic effects (Shulla et al. 2011; Wang et al. 2020a, b). Moreover, this enzyme may have an activating effect on the receptor to which some coronaviruses bind, such as human ACE2, which gives it a replication potential in human cells (Wang et al. 2020a, b; Huang et al. 2020).

This study aimed to identify knowledge gaps and to describe the current state of the research on the association between TMPRSS2 and the essential beta coronaviruses (Beta-CoVs) infection and the molecular mechanisms for this association. Also, to make specific recommendations for future research.

## Methods

We performed this scoping review according to the recommendations of the Joanna Briggs Institute (Peters et al. 2017).

### Eligibility criteria

#### Participants

We included theoretical information, *in vitro* or *in vivo* studies, studies in animals, or *in silico* studies assessing the molecular mechanism to determine the association between TMPRSS2 and the betacoronavirus SARS-CoV-2, SARS-CoV, and MERS-CoV infection.

#### Concept

We focused on the molecular mechanisms that explain the association between this serine protease and the infection of this virus.

#### Context

We did not limit for language or setting.

#### Exclusion criteria

Studies that did not include information about TMPRSS2 and Spike protein of coronaviruses.

#### Information sources

We included studies (human, animal, reviews, systematic reviews, and primary studies) to respond to the two

objectives. We searched the literature following medical subject headings (MeSh), Emtree language, Decs, and text words related. We searched MEDLINE (OVID), EMBASE, and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception to nowadays. To ensure literature saturation, we scanned references from relevant articles identified through the search, conferences, thesis databases, Open Grey, Google scholar, and clinicaltrials.gov.

### Data collection

Two researchers reviewed each reference by title and abstract. Then they scanned full-texts of relevant studies, applied pre-specified inclusion and exclusion criteria, and extracted the data. Disagreements were resolved by consensus. Two trained reviewers using a standardized form independently extracted the following information from each article: author, publication year, study design, geographic location (origin), authors' names, title, objectives, methods, virus species, cleavage site, outcomes, funding source, and other key findings.

### Synthesis of results

We descriptively showed the results, trying to respond to the two objectives. Results were also classified under main conceptual categories to facilitate comprehension.

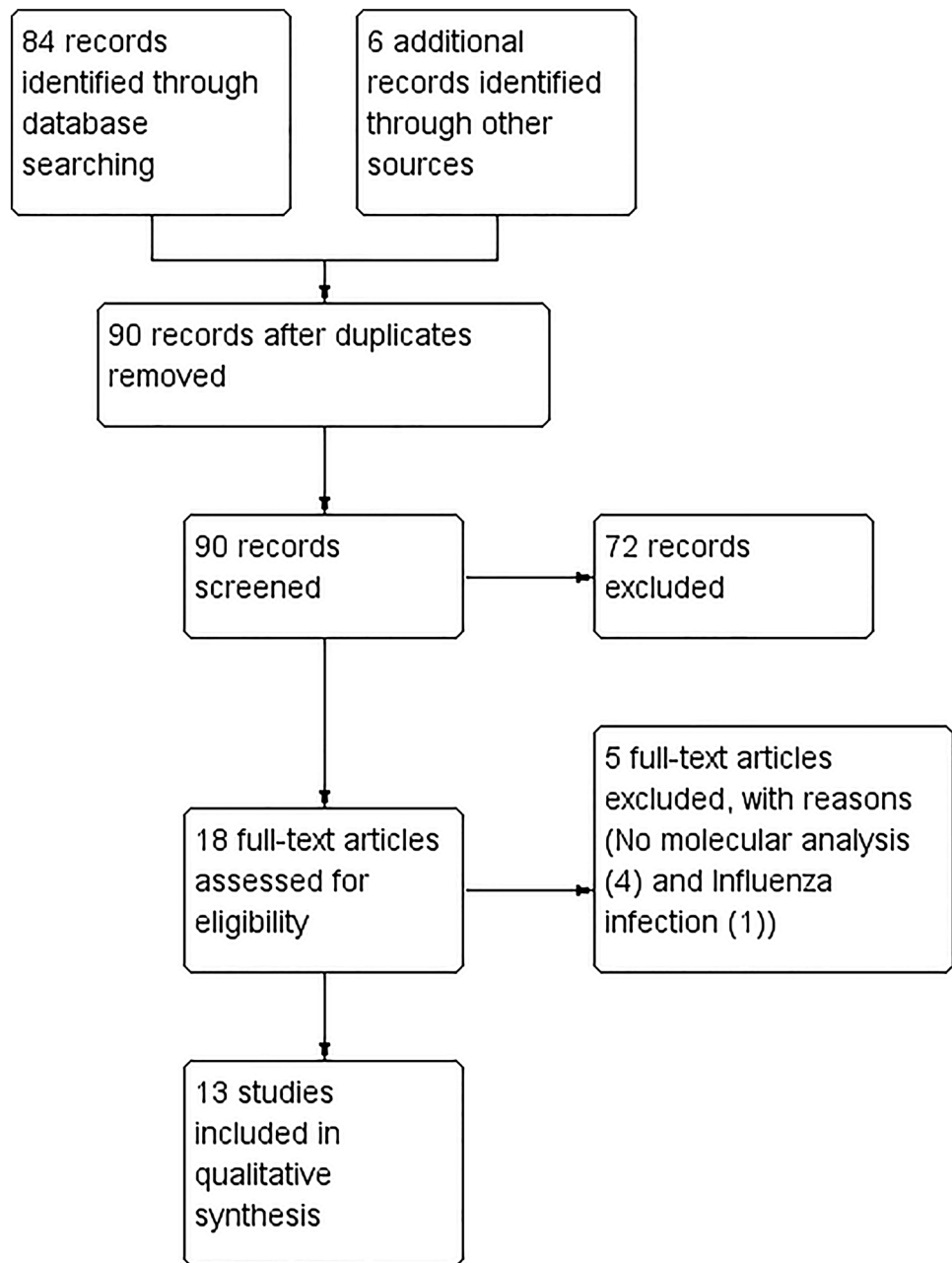
## Results

### Study selection

We found 84 studies with the search strategies and three with other sources. After exclusions, we finally included 13 studies (Matsuyama et al. 2010; Matsuyama et al. 2020; Glowacka et al. 2011; Shulla et al. 2011; Shirato et al. 2013; Heurich et al. 2014; Shen et al. 2017; Reinke et al. 2017; Kleine-weber et al. 2018; Iwata-Yoshikawa et al. 2019; Bilinska et al. 2020; Zang et al. 2020; Hoffmann et al. 2020) in the qualitative analysis (Fig. 1).

### Characteristics of included studies

Five studies evaluated SARS-CoV (Glowacka et al. 2011; Shulla et al. 2011; Heurich et al. 2014; Reinke et al. 2017; Matsuyama et al. 2020), two evaluated MERS-CoV (Shirato et al. 2013; Kleine-weber et al. 2018), two studies evaluated both (Shen et al. 2017; Iwata-Yoshikawa et al. 2019), and three others evaluated SARS-CoV-2 (Hoffmann et al. 2020; Matsuyama et al. 2020). Multiple essays were reporting the expression of TMPRSS2 in different cell lines. The authors frequently used 293 T cells, Vero, Vero E6, Caco-2,

**Fig. 1** Flowchart of selected studies

or Calu-3 cells. For infecting the cells, six studies used a lentiviral vector as plasmids. Also, they used plasmids encoding TMPRSS2, ACE2, or DPP4. For analysis of antigens expression, detection of proteins, quantification of messenger-RNA (mRNA), studies performed western blot and/or Real Time-Polymerase Chain Reaction (RT-qPCR). The authors also used other techniques such as immunohistochemistry, electron microscopy, and next-generation sequence (Table 1).

### Primary outcome: molecular mechanisms

We described the molecular mechanisms for the association between TMPRSS2 and beta-CoV infection in the following

paragraphs. We described some additional information regarding this topic in Table 2.

### Cleavage and activation of spike protein

The SARS-CoV spike protein (SARS-S) incorporates into the viral envelope and mediates viral entry into target cells. For this, the surface unit (S1) of SARS-S binds to the cellular receptor angiotensin-converting enzyme 2 (ACE2) and the transmembrane unit (S2), then fuses the viral membrane with a host cell membrane (Reinke et al. 2017; Iwata-Yoshikawa et al. 2019).

**Table 1** Characteristics of included studies

Study	Country	Virus	Reference	Cells	Molecular template	Cluster
Reinke et al. (2017)	Germany	SARS-CoV	Plasmids pCAGGS	Human embryonal kidney 293 T cells and African green monkey derived COS-7 cells were grown in Dulbecco's modified Eagle's medium	Western blot	Trypsin, TMPRSS2, cathepsin B/L
Heurich et al. (2014)	Germany	SARS-CoV	Plasmids pCAGGS and pcDNA3.1 zeo, lentiviral vector pNL-Luc-E-R	293 T and Cos-7 cells were maintained in Dulbecco's modified Eagle medium (DMEM; Gibco, Invitrogen)	Western blot	TMPRSS2, HAT, ADAM17
Glowacka et al. (2011)	Germany	SARS-CoV	Plasmids pCAGGS, pcDNA3.1zeo, pNL4-3 E-R-Luc	Vero E6 and 293 T cells	Western blot, qRT-PCR, Immunohistochemistry	TMPRSS2, TMPRSS4, mouse matriptase-3
Shulla et al. (2011)	United States	SARS-CoV	Plasmids pCAGGS, MCS (TMPRSS2 and TMPRSS11d), pcDNA3.1 (SARS S and ACEC9), pNL4.3-Luc R-E-, pCDM8-NL63 S	293 T and hACE2-293 cells	Western blot, Immunoprecipitation	TMPRSS2, HAT, TMPRSS11a
Matsuyama et al. (2010)	Japan	SARS-CoV	SARS-CoV Frankfurt 1 strain, recombinant vaccinia virus containing the gene encoding SARS-CoV-S	Vero E6, Vero and Vero-TMPRSS2 cells, lung tissue samples from SARS-CoV-infected cynomolgus monkeys	Western blot, qRT-PCR	TMPRSS2
Shirato et al. (2013)	Japan	MERS-CoV	N/A	HeLa cells constitutively expressing TMPRSS2 (HeLa-TMPRSS2 cells), Vero cells, and Vero cells constitutively expressing TMPRSS2 (Vero-TMPRSS2 cells)	Western blot, qRT-PCR	TMPRSS2
Kleine-Weber et al. (2018)	Germany	MERS-CoV	pCAGGS-based plasmids encoding VSV-G, wtMERS-S or cleavage site mutants, expression plasmids for hTMPRSS2 and hDPP4	293 T, Vero E6 cells, human colorectal adenocarcinoma cell line Caco-2	Western blot, quantitative PCR	TMPRSS2, Furin, Cathepsin L
Shen et al. (2017)	China/Japan	SARS-CoV, MERS-CoV	N/A	N/A	N/A	TMPRSS2, cathepsin
Iwata-Yoshikawa et al. (2019)	Japan	SARS-CoV, MERS-CoV	Frankfurt 1 isolate of SARS-CoV, MERS-CoV (HCoV-EMC 2012 strain)	Vero E6 cells, TMPRSS2-KO mice, hDPP4-Tg mice	Immunohistochemistry, Panel for inflammatory cytokines and chemokines, qRT-PCR	TMPRSS2

Table 1 (continued)

Study	Country	Virus	Reference	Cells	Molecular template	Cluster
Hoffmann et al. (2020)	Germany	SARS-CoV-2, SARS-CoV	Plasmids, Retroviral transduction and blasticidin-based selection, Transfection by calcium phosphate precipitation	293T, BHK-21, Huh-7, LLC-PK1, MRC-5, MyDauLu/47.1, NIH/3T3, RhiLu/1.1, Vero, Calu-3, Caco-2, MDBK, MDCKII, A549, BEAS-2B and NCI-H1299 cells	Western blot, quantitative qRT-PCR	TMPRSS2
Matsuyama et al. (2020)	Japan	SARS-CoV-2	N/A	VeroE6/TMPRSS2, VeroE6, Vero, Calu-3, and A549 cells	Next generation sequence, electron microscopy, qRT-PCR	TMPRSS2
Bilinska et al. (2020)	Poland	SARS-CoV-2	N/A	Murine olfactory epithelium cells	RNAseq, qRT-PCR, in situ hybridization, Western blot, immunocytochemistry	TMPRSS2, TMPRSS4
Zang et al. (2020)	USA	SARS-CoV-2	pcDNA3.1/nV5-DEST vector, pLX304 lentiviral vector, pEGFP-N1 and pCMV-TdTomato, VSV	African Green Monkey kidney epithelial cell lines MA104, human embryonic kidney cell line HEK293	qPCR, brightfield and immunofluorescence microscopy, Next generation sequence	TMPRSS2, TMPRSS4

Shulla 2011 slightly evidenced TMPRSS2-specific SARS-S cleavage. The resulting fragments are assumed to represent the activated S proteins operating in pseudovirus entry. It can trigger the uptake of virions into host cell endosomes (Shulla et al. 2011). Spike cleavage by TMPRSS2 takes place in the Golgi or plasma membrane, either during assembly or attachment and release (Shirato et al. 2013). Glowacka et al. demonstrated that TMPRSS2 cleaved SARS S at multiple sites inducing incomplete SARS-S shedding in cells coexpressing TMPRSS2 and SARS-S.

They also found that TMPRSS2 facilitated *trans*-cleavage of SARS-S, indicating that it could activate SARS S for membrane fusion (Glowacka et al. 2011). According to Matsuyama 2010, as they found that TMPRSS2 affected SARS S attached to receptors at the cell surface but not newly synthesized SARS S (Matsuyama et al. 2010). On the other hand, Reinke 2017 found that residue R667 was essential for SARS S cleavage by TMPRSS2 while the same residue was dispensable for S protein activation (Reinke et al. 2017).

Shirato 2013 found that cleavage fragments activity was required for viral spread into the host. Also, differential glycosylation of the cleavage products might reflect differential cellular localization of the corresponding cleavage processes. In contrast, TMPRSS2 might process SARS S early after import in the constitutive secretory pathway and before N-glycans are fully processed (Shirato et al. 2013).

Kleine-Weber et al. described different MERS-CoV spike protein (MERS-S) cleavage sites for each protease as follows: S2' site (amino acids RSAR) for activating proteases (including TMPRSS2); S1/S2 site (amino acids RSVR) for furin; and AFNH motif for endosomal cysteine protease (Cathepsin B/L). When they evaluated the impact of mutations introduced in these sites, they found that it required an intact S2' site. In contrast, the cathepsin L site mutation had no impact on S protein-driven entry, and mutation of the S2' site abrogated MERS S activation by TMPRSS2 (Kleine-weber et al. 2018). Similarly, Shen 2017 described cleavage sites and found that after cleavage of SARS-S, S1 and S2 domains remained noncovalently associated but not disulfide bonds (Shen et al. 2017).

Recently, Hoffmann et al. showed that priming of SARS-CoV-2 spike protein (SARS-2-S) evidenced several arginine residues at the S1/S2 cleavage site of SARS-2-S but not SARS-S. However, the S2 cleavage site of both was similar (Hoffmann et al. 2020).

## Cleavage of ACE2

As mentioned above, studies show that SARS-CoV and SARS-CoV-2 bind to cellular receptor ACE2. Matsuyama 2010 studied the expression of ACE2 using specific antibodies, and they weakly detected ACE2 antigens in uninfected

**Table 2** Synthesis of results

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Reinke et al. (2017)	R667; R797; 543/ R544; R563/ K566	Residue R667 is required for SARS S processing by TMPRSS2 and trypsin. Residues N-terminal of R667 might impact proteolytic processing of SARS S	Activation of SARS S by a cellular protease is a prerequisite to membrane fusion and protease choice determines at which cellular localization membrane fusion occur	N/A	A serine protease inhibitor active against TMPRSS2 but not a cysteine protease inhibitor for active against cathepsin B/L proteolytic rodents from SARS-CoV-induced pathogenesis	Binding of SARS-CoV to ACE2 can trigger uptake of virions into host cell endosomes, where cathepsin B/L cleave and activate the S protein	N/A	Coexpression of high amounts of TMPRSS2 and SARS S results in the production of several C-terminal cleavage fragments
Heurich et al. (2014)	R697; R716	Binding of SARS-S to ACE2 triggers subtle conformational rearrangements in SARS-S, which are believed to increase the sensitivity of the S protein to proteolytic digest at the border between the S1 and S2 subunits	Catalytic domain of ACE2 binds to SARS-S with high affinity. Arginine and lysine residues within ACE2 amino acids 697 to 716 are essential for ACE2 cleavage by TMPRSS2 and HAT and that ACE2 processing is required for augmentation of SARS-S-driven entry but not for SARS-S activation	N/A	Virions can be taken up into endosomes, where SARS-S is cleaved and activated	If no SARS-S-activating proteases (i.e., TMPRSS2 with ACE2) are expressed at the cell surface, virions are taken up into endosomes, where SARS-S is cleaved and activated by the pH-dependent cysteine protease cathepsin L	N/A	TMPRSS2 facilitates SARS-CoV infection via two independent mechanisms: cleavage of ACE2, which might promote viral uptake, and cleavage of SARS-S, which activates the S protein for membrane fusion
Glowacka et al. (2011)	N/A	TMPRSS2 cleaved SARS-S at multiple sites, generating fragments of 150, 110, 85, 55, and 45 kDa A fraction of uncleaved SARS-S produced in TMPRSS2-expressing cells was incorporated into particles TMPRSS2 facilitated trans-cleavage of SARS-S when mixing SARS-S and TMPRSS2-expressing cells, and S protein fragments were detected in culture supernatants	N/A	TMPRSS2 on target cells allowed efficient SARS S-dependent cell-cell fusion Large syncytia were frequently detected in TMPRSS2-expressing Vero E6 cells infected with SARS-CoV	N/A	Cathepsin inhibitor reduced infection by SARS-S pseudotypes, and it was not rescued by SARS-S processing by TMPRSS2 TMPRSS2 on target cells allowed efficient SARS S-driven virus-cell fusion in the presence of a lysosomotropic agent and a cathepsin inhibitor	Reduction of neutralizing activity of serum from immunized mice and less reduction of viral infectivity in TMPRSS2-expressing cells Cleavage by TMPRSS2 reduced SARS-S susceptibility to inhibition by neutralizing antibodies	mRNA was low or absent in brain and heart tissue It was detected in samples from pancreas, kidney, and lung Immunostaining for TMPRSS2 and ACE2 demonstrated strong positive staining of type II pneumocytes and alveolar macrophages

Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Shulla et al. (2011)	N/A	There was evidence of TMPRSS2-specific SARS-S cleavage indicated by the presence of C-terminal 120- and 85-kDa S fragments	Cultures with gradually increasing levels of TMPRSS2 revealed doses ultimately eliminating full-length ACE2. When TMPRSS2 was scarce, ACE2 localized to TMPRSS2-containing regions. TMPRSS2 and ACE2 coimmunoprecipitated and enzymatic activity was required for TMPRSS2 association with ACE2. The expression of these two proteins on separate cells did not foster virus entry at a level above that observed when only ACE2 was expressed. ACE2 was not proteolyzed when TMPRSS2 was on separate cells	A coculture of effector and target cells indicated a tenfold increase in membrane fusion	TMPRSS2 enhanced SARS S-mediated pseudovirus entry and exhibited restricted S-specific enhancing effects. Cultures with undetectable complete ACE2 were 30-fold more susceptible to HIV-S transduction than controls. Viral RNAs were translated to generate significantly more N proteins in TMPRSS2+ cells. There was significantly more S protein in TMPRSS2+ cells	Inhibitors of endosomal acidification potentially suppressed S mediated transductions, but it was eliminated by TMPRSS2, which fully activated SARS-S-mediated entry (1,000-fold)	N/A	N/A

Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Matsuyama et al. (2010)	N/A	S proteins synthesized in either Vero or Vero-TMPRSS2 cells were not cleaved TMPRSS2 affects viral S protein attached to receptors at the cell surface but not newly synthesized S proteins	N/A	Large syncytia were observed in Vero-TMPRSS2 cells but not in Vero cells Infection with vaccinia virus encoding the SARS-CoV S gene and plasmid-based expression of S protein induced syncytia Inhibitor of serine and cysteine proteases, suppressed S-protein-induced cell-cell fusion and syncytia formation Producer cells are refractory to self-fusion due to a lack of receptors at the cell surface	N/A	Treatment with a cathepsin inhibitor caused a decrease of SARS-CoV entry into Vero cells but not into Vero-TMPRSS2 cells	N/A	TMPRSS2 antigens were detected in type I pneumocytes Weak staining of ACE2 antigens was detected in enlarged type II pneumocytes Mild lesions: type I pneumocytes resembled TMPRSS2-expressing cells, more than ACE2-expressing cells, SARS-CoV antigens were detected in the cytoplasm Severe lesions: marked immunostaining of TMPRSS2 and ACE2 antigens was detected in the cytoplasm of enlarged type II pneumocytes. The presence of SARS-CoV antigens did not correlate with the presence of either ACE2 or TMPRSS2 antigens



Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Shirato et al. (2013)	N/A	Lysine residues at the cleavage site in the viral S protein are a target for trypsin	N/A	The syncytia were more pronounced in Vero-TMPRSS2 cells than in the exogenous protease treated parental Vero cells	Protein on the MERS-CoV particle is sufficient for the induction of cell-cell fusion in the absence of protein synthesis	The virus titer in the Vero-TMPRSS2 cell medium was 1 or 2 log units higher than that in the parental Vero cell MERS-CoV employs both the cell surface and the endosomal pathway to infect Vero-TMPRSS2 cells	Cell-cell fusion mediated cytotoxicity may potentially cause the immune system to develop severe inflammation in response to viral infection	N/A
Kleine-Weber et al. (2018)	S2' site (amino acids RSAR) for activating pro-teases; S1/S2 site (amino acids RSVR) for furin; AFNH motif for endosomal cysteine protease (Cathepsin L)	Alteration of the S1/S2 site markedly reduced entry into Caco-2 but not Vero E6 cells An intact S2' site was universally required for S protein driven entry None of the mutations introduced into the S1/S2 site markedly reduced entry into Vero E6 cells The second but not the first arginine within the S2' site was sufficient for efficient S protein-driven entry into Caco-2 cells	N/A	N/A	Transduction mediated by wildtype MERS-S was comparable for Vero E6 and Caco-2 cells, and generally higher as compared to untransfected 293 T cells 293 T cells previously transfected with expression plasmid for DPP4, transduction levels were as high as for Vero E6 and Caco-2 cells Preincubation of Caco-2 cells with protease inhibitors showed that entry driven by S proteins lacking an intact S1/S2 site was dependent on TMPRSS2 but not cathepsin L activity	Mutation of the cathepsin L site had no impact on S protein-driven entry Entry driven by MERS-S WT and S protein variants with mutations in the S1/S2 site was inhibited by Cathepsin L inhibitor treatment S protein mutants with inactivated S1/S2 site was rescued by TMPRSS2 with reduced efficiency as compared to MERS-S WT	N/A	Appreciable levels of TMPRSS2 mRNA were only detected in Caco-2 cells and lung tissue

Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Shen et al. (2017)	N/A	Cleavage site is located at the S1/S2 boundary and another is within S2 upstream of the putative fusion peptide (S2') After cleavage of spike, S1 and S2 domains remain associated by noncovalently, but not disulfide bonds	N/A	N/A	SARS-CoV entry increased 2.6-fold in the presence of TMPRSS2 siRNA targeting TMPRSS2 caused a five-fold decrease in SARS-CoV entry into Calu-3 cells The levels of SARS-CoV RNA are nine-fold higher in cells expressing active TMPRSS2 than in cells expressing enzymatically inactive TMPRSS2	N/A	N/A	N/A

Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Iwata-Yoshikawa et al. (2019) (SARS-CoV)	N/A	N/A	N/A	N/A	Strongly antigen-positive cells were observed in the bronchiolar epithelium of WT mice infected with SARS-CoV Some antigen positive cells were seen in alveoli from both WT and TMPRSS2-KO mice	N/A	WT mice showed clear loss of body weight, it was not observed in TMPRSS2-KO mice (day 2: $p < 0.05$ ; days 3 and 4: $p < 0.0001$ ) Concentrations of FGF-basic (day 3: $p < 0.0001$ ), keratinocyte-derived chemokine/CXCL1 (day 2: $p < 0.01$ ), IL-12 (p40/p70) (day 2: $p < 0.01$ ), IL-4 (day 3: $p < 0.05$ ), and IL-10 (day 3: $p < 0.05$ ) were significantly lower than those in WT mice A transient increase in TLR3 expression in the lungs of TMPRSS2-KO mice was observed at 6 h, but not in WT mice ( $p < 0.05$ ) IFN-alpha4 (day 3: $p < 0.01$ ) and IFN-beta (day 3: $p < 0.01$ ) mRNA expression levels were higher in WT mice than in TMPRSS2-KO mice	N/A

Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Iwata-Yoshikawa et al. (2019) (MERS-CoV)	N/A	N/A	N/A	N/A	<p>On day 1, many viral antigen-positive cells were observed in the bronchi and alveolar areas of hDPP4-Tg mice; there were none in the bronchi and only a few in the alveoli of TMPRSS2-KO Tg mice</p> <p>On day 3, many viral antigen-positive cells were present in these areas in hDPP4-Tg mice, but there were fewer in TMPRSS2-KO Tg mice</p>	N/A	<p>hDPP4-Tg mice showed a temporary and mild loss of body weight; however, only very slight changes were observed in TMPRSS2-KO Tg mice (days 6 and 7; <math>p &lt; 0.001</math>)</p> <p>Titers of neutralizing antibodies in sera from TMPRSS2-KO Tg mice were significantly lower than those in sera from hDPP4-Tg mice (<math>p &lt; 0.05</math>)</p> <p>Concentrations of FGF-basic (day 7: <math>p &lt; 0.01</math>), GM-CSF (day 5: <math>p &lt; 0.05</math>), MIG/CXCL9 (day 5: <math>p &lt; 0.05</math>), IL-17 (day 5: <math>p &lt; 0.05</math>), and TNF-<math>\alpha</math> (day 5: <math>p &lt; 0.05</math>) in the lungs of TMPRSS2-KO Tg mice were lower than those in the lungs of hDPP4-Tg mice</p> <p>Expression levels of IL-6 (day 7: <math>p &lt; 0.05</math>) were higher in TMPRSS2-KO Tg mice than in hDPP4-Tg mice</p> <p>IFN-<math>\alpha</math>4 (day 2: <math>p &lt; 0.05</math>) and IFN-<math>\beta</math> (day 2: <math>p &lt; 0.001</math>, day 3: <math>p &lt; 0.01</math>) mRNA expression levels were higher in hDPP4-Tg mice than in TMPRSS2-KO Tg mice</p>	N/A

Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Hoffmann et al. (2020)	SARS S: amino acids 662 to 670 for S1/S2, and 793 to 800 for S2' SARS-2-S: amino acids 676 to 688 for S1/S2, and 811 to 818 for S2'	Efficient proteolytic processing of SARS-2-S in human cells evidenced the presence of several arginine residues at the S1/S2 cleavage site of SARS-2-S but not SARS-S The S2' cleavage site of SARS-2-S was similar to that of SARS-S	Most amino acid residues essential for ACE2 binding by SARS-S were conserved in SARS-2-S SARS-CoV-2 infected BHK-21 cells expressing ACE2 but not parental cells with high efficiency after 16 h ( $p < 0.01$ ) Directed expression of human and bat ACE2 allowed SARS-2-S- and SARS-S-driven entry into otherwise non-susceptible BHK-21 cells ( $p < 0.001$ ) Antiserum against human ACE2 blocked SARS-S- and SARS-2-S-driven entry ( $< 0.0001$ )	N/A	Most human cell lines and the animal cell lines Vero and MDCKII were susceptible to entry driven by SARS-S and SARS-2-S facilitated entry into an identical spectrum of cell lines Camostat reduced MERS-S-, SARS-S-, and SARS-2-S- but not VSV-G-driven entry into the lung cell line Calu-3 ( $p < 0.001$ ). It also reduced Calu-3 infection with authentic SARS-CoV-2 ( $p < 0.001$ ), and inhibited SARS-S- and SARS-2-S- but not VSV-G-driven entry into primary human lung cells ( $p < 0.05$ )	When using a cathepsin B/L blocker, inhibition of entry into TMPRSS2 + Caco-2 cells was less efficient compared to 293 T cells Camostat mesylate did not interfere with SARS-2-S-driven entry into the TMPRSS2-cell lines 293 T and Vero ( $p < 0.001$ ) Directed expression of TMPRSS2 rescued SARS-2-S-driven entry from inhibition by cathepsin B/L blocker ( $p < 0.001$ ) Ammonium chloride (endosome acidification blocker) strongly inhibited SARS-2-S- and SARS-S-driven entry into TMPRSS2-293 T cells ( $p < 0.001$ ) Camostat partially blocked SARS-2-S-driven entry into Caco-2 and Vero-TMPRSS2 cells ( $p < 0.001$ ) Full inhibition was attained with camostat and an inhibitor of Cathepsin B/L ( $p < 0.001$ )	Four sera obtained from three convalescent SARS patients inhibited SARS-S-driven entry in a concentration-dependent manner, and these sera also reduced SARS-2-S-driven entry, although with lower efficiency compared to SARS-S	N/A

Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Matsuyama et al. (2020)	N/A	N/A	N/A	In five cases among seven, clear cytopathic effect with detachment/ floating and syncytium formation developed at 2 or 3 days	The amount of SARS-CoV-2 RNAs in the culture supernatants of Vero, Calu-3, and A549 cells after 48 h was low and was measurably higher when VeroE6 cells were used. Viral RNA copies in the VeroE6/TMPRSS2 cell culture supernatants were > 100 times greater than those from VeroE6 cells. VeroE6/TMPRSS2 displayed ~tenfold greater number of SARS-CoV-2-infected cells than the parental VeroE6 cells	N/A	N/A	The messenger RNA expression level of TMPRSS2 in VeroE6/TMPRSS2 cells is ~tenfold higher than in normal human lung tissue and other human cell lines
Bilinska et al. (2020)	N/A	N/A	Expression profiling data for murine OE indicate that ACE2 is mainly expressed in non-neuronal cells. Expression of ACE2 was significantly lower in the brain as compared to OE	N/A	N/A	N/A	SARS-CoV-2 virus accumulates in sustentacular cells first and, by interfering with their metabolism, affects the function of olfactory receptor neurons	TMPRSS2 is widely expressed in both neuronal and non-neuronal cells, higher levels in non-neuronal cells

Table 2 (continued)

Study	Domain	S protein cleavage	ACE2 cleavage	Syncytia formation	Virus replication and spread	Cathepsin L-independent entry	Immune response	TMPRSS2 expression
Zang et al. (2020)	N/A	TMPRSS serine proteases facilitate virus infection by inducing S cleavage and exposing the fusion peptide for efficient viral entry	N/A	SARS-CoV infection was associated with cell fusion causing syncytia formation between primary intestinal epithelial cells (IECs). The cell fusion may lead to subsequent cytopathic effect and a breach of the intestinal epithelium integrity	They showed that SARS-CoV is rapidly inactivated by simulated human colonic fluid and a limited number of viral RNA-positive fecal samples did not contain infectious virus The large quantities of viral RNA that transit through the GI tract and shed into the feces may not carry substantial infectious risk	N/A	N/A	TMPRSS2 was primarily expressed in ACE2-secreting IECs

type II pneumocytes. However, the authors detected immunostaining of TMPRSS2 and ACE2 antigens in severe lesions (Matsuyama et al. 2010). Glowacka et al. also detected intense positive staining of both proteins in type II pneumocytes and alveolar macrophages (Glowacka et al. 2011). In Shulla et al. (2011), TMPRSS2 eliminated full-length ACE2 in a dose-dependent manner, but ACE2 colocalized to TMPRSS2-containing regions when TMPRSS2 scarce and showed that it required an enzymatic activity of TMPRSS2 for this association.

Interestingly, when these two proteins were expressed in separate cells, SARS-CoV entry into host cells was not higher than those expressing one of them. It indicates that the priming of ACE2 by TMPRSS2 was necessary for virus entry (Shulla et al. 2011). Heurich 2013 concluded that TMPRSS2 facilitates SARS-CoV infection through cleavage of ACE2, which might promote viral uptake (Heurich et al. 2014).

The catalytic domain of ACE2 binds to SARS-S with high affinity, and it triggers conformational rearrangements that could increase proteolysis sensitivity. In that sense, arginine and lysine residues within ACE2 amino acids 697–716 are essential for ACE2 cleavage by TMPRSS2.

Augmentation of SARS-S-driven entry requires the processing of ACE2 but not for its activation (Heurich et al. 2014). It is noteworthy that, according to Hoffmann 2020, most amino acid residues essential for ACE2 binding by SARS-S were conserved in SARS-2-S. In this study, authentic SARS-CoV-2 infected cells expressing ACE2 with high efficiency but not cells without this receptor ( $p < 0.01$ ) and antiserum against human ACE2 blocked SARS-S- and SARS-2-S-driven entry ( $p < 0.001$ ) (Hoffmann et al. 2020).

### Syncytia formation

Some studies show that syncytia formation is more frequent and pronounced between SARS-CoV infected cells (or even SARS-S expressing cells) expressing TMPRSS2 and between MERS-CoV infected cells expressing TMPRSS2 (Matsuyama et al. 2010; Glowacka et al. 2011; Shulla et al. 2011; Shirato et al. 2013). Interestingly, outcomes from Matsuyama et al. (2010) indicate that TMPRSS2 expression must oppose that of SARS S to induce membrane fusion. Syncytia formation was not induced in cells that did not express TMPRSS2, nor between TMPRSS2 and SARS-S and cells without TMPRSS2. However, large syncytia formed when TMPRSS2 was expressed either in target cells or both in the target and producer cells (Matsuyama et al. 2010). In that sense, SARS S can be activated for virus-cell and cell–cell fusion when TMPRSS2 is expressed on viral target cells (Glowacka et al. 2011; Shulla et al. 2011). Syncytia formation is frequent in cells expressing TMPRSS2 infected with SARS-CoV-2 (Matsuyama et al. 2020).

## Virus replication and spread

In general, cells expressing TMPRSS2 were more susceptible to virus entry and spread into host cells. Also, when compared to other serine proteases, TMPRSS2 was more effective in enhancing SARS-S-mediated entry (Glowacka et al. 2011; Shulla et al. 2011; Heurich et al. 2014; Reinke et al. 2017). TMPRSS2 expression was also associated with increased amounts of viral RNA. Shulla et al. found that SARS N RNA was ninefold more in TMPRSS2-expressing cells, and this RNA translated to generate significantly more N proteins ( $p < 0.0005$ ) (Shulla et al. 2011). Viruses spread by the increased expression of virus antigens in cells expressing TMPRSS2. Iwata-Yoshikawa et al. observed only very weak SARS-S antigen positivity in TMPRSS2-Knock Out (KO) mice than wild-type mice at day one postinfection. Lungs of TMPRSS2-KO mice showed lower viral replication with no significant differences in the titers of neutralizing antibodies in serum samples from either group (Iwata-Yoshikawa et al. 2019). According to Shen et al., SARS-CoV entry increased in the presence of TMPRSS2, and levels of SARS-CoV RNA were higher in cells expressing active TMPRSS2 than in cells expressing enzymatically inactive TMPRSS2 (Shen et al. 2017).

We found similar results with MERS-S. Shirato et al. evidenced higher virus titer in the Vero-TMPRSS2 cell medium than in the parental Vero cell medium (Shirato et al. 2013). MERS-CoV replicated more slowly, and antigen positivity in TMPRSS2-KO mice was fewer or absent at the first and third postinfection days (Iwata-Yoshikawa et al. 2019).

For the recent SARS-CoV-2, Matsuyama et al. evidenced that viral RNA copies in specimens with cytopathic effects developed within two days were more significant than those in the other specimens. They used Vero E6/TMPRSS2 cells and found more than 100 times higher viral RNA copies than Vero E6, which also showed higher amounts compared to other cell types used (Matsuyama et al. 2020). In gut epithelial cells, Zang et al. demonstrated that TMPRSS2 alone did not mediate viral infection. However, co-expression of TMPRSS2 with ACE2 resulted in enhanced infectivity, inducing S protein cleavage and exposing the fusion peptide for efficient viral entry (Zang et al. 2020).

Further analyses using protease inhibitors support these results. In the study performed by Kleine-Weber et al. (2018), preincubation of Caco-2 cells with protease inhibitors showed that entry driven by MERS S lacking an intact S1/S2 site was dependent on TMPRSS2, indicating a dominant role of TMPRSS2 in MERS-S-driven entry. Reinke et al., also used a serine protease inhibitor and found that it protected rodents from SARS-CoV-induced pathogenesis, while a cysteine protease inhibitor active against cathepsin B/L did not (Reinke et al. 2017). Finally, Hoffmann et al. used camostat mesylate treatment (a drug active against TMPRSS2) and found a

significant reduction in SARS-S- and SARS-2-S-mediated entry into Calu-3 and human lung cells. It also reduced the infection by authentic SARS-CoV-2 into Calu-3 cells. On the other hand, treatment of other cell lines (Caco-2 and Vero-TMPRSS2) partially blocked SARS-2-S-driven entry, but when they added an inhibitor of Cathepsin B/L, complete inhibition was attained (Hoffmann et al. 2020).

Bilinska et al. used a mouse model and determined whether cells in the olfactory epithelium (OE) express the obligatory receptors for entry of the SARS-CoV-2 virus by using RNAseq, RT-PCR, in situ hybridization, Western blot, and immunocytochemistry. Their mouse model showed that, with older age, amounts of ACE2 protein increased in the OE, as did gene expression of TMPRSS2. Sustentacular cells were identified as the cell type that expressed both SARS-CoV-2 host receptors required for cell entry. Results suggest that the SARS-CoV-2 virus accumulates in sustentacular cells first and, by interfering with their metabolism, affects the function of olfactory receptor neurons (Bilinska et al., 2020).

## Cathepsin L-independent entry and spread

As mentioned above, some studies have used Cathepsin B/L inhibitors to study Spike protein-mediated entry under these conditions or even evaluate cleavage by this cysteine protease. Spike protein cleavage and activation by pH-dependent cathepsin B/L occurs in the endosome (Reinke et al. 2017). Studies have shown that inhibition of Cathepsin L decreased virus entry into cells with no expression of TMPRSS2 on the cell surface. Expression of TMPRSS2 could even activate Spike protein and enhance SARS-S-mediated entry (Matsuyama et al. 2010; Glowacka et al. 2011; Shulla et al. 2011). Kleine-Weber et al. found similar results for MERS S in which they introduced mutations into different cleavage sites. They found that mutation of a single arginine within S1/S2 reduced entry into Caco-2 cells by 4.5 to 29.4-fold ( $p < 0.001$ ). Mutation of any single arginine at the S2' site had only a minor, statistically significant, effect on S protein-mediated transduction of Vero E6 cells ( $p < 0.001$ ), and mutation of S2' abrogated MERS-S activation by TMPRSS2. However, entry mediated by the S protein mutants with inactivated S1/S2 site was rescued by TMPRSS2 in cells previously treated with Cathepsin L inhibitor (Kleine-weber et al. 2018). For SARS-CoV-2, Hoffmann et al. also evidenced that expression of TMPRSS2 rescued SARS-2-S-driven entry from inhibition of cathepsin B/L. It is noteworthy that this study also reported a residual Spike protein priming by cathepsin B/L in a cell line when camostat mesylate was used. However, S protein priming by TMPRSS2 but not cathepsin B/L is still essential for viral entry into primary target cells and viral spread in the infected host (Hoffmann et al. 2020).



## Discussion

Members of the betacoronavirus genus such as SARS-CoV and MERS-CoV have caused significant outbreaks of respiratory disease. It motivates the rapid understanding of virus interactions with human cells and immunopathology. With the novel coronavirus (SARS-CoV-2) outbreak, findings allowed to establish an essential similarity between this and previous Beta-CoVs. In that sense, SARS-S and SARS-2-S share approximately 76% amino acid identity, which makes it essential to know the similarities and differences between the mechanisms of virus entry and spread into host cells (Hoffmann et al. 2020).

SARS-CoV and SARS-CoV-2 bind to the cell surface receptor ACE2, while MERS-CoV binds to DPP4 (Hoffmann et al. 2020; Zhou et al. 2020). Studies described two critical mechanisms for host cell entry, depending on the availability of cellular proteases. When TMPRSS2 coexpresses with the target cell's surface receptors, Spike protein can be activated and then induce virus-cell membrane fusion. When cell surface proteases are not expressed, Spike protein binds to the cell surface's receptor, resulting in virions uptake into endosomes. In this case, Spike is cleaved and activated by pH-dependent cysteine protease Cathepsin B/L, initiating virus-endosome membrane fusion and later release of the viral genetic material into the cytosol (Heurich et al. 2014; Shen et al. 2017).

Immunostaining for TMPRSS2 and ACE2 demonstrated intense positive staining of type II pneumocytes and alveolar macrophages. Similar results were found in severe lesions of lung tissue, and mRNA levels of TMPRSS2 have been detected in lung tissue (Matsuyama et al. 2010, 2020; Glowacka et al. 2011; Kleine-weber et al. 2018). These results show that TMPRSS2 facilitates SARS-CoV infection by cleavage of ACE2, which might promote viral uptake, and cleavage of SARS-S, which activates the S protein for membrane fusion (Heurich et al. 2014).

On the other hand, the expression of receptors and proteases requires a specific spatial orientation to allow S protein activation, S protein-mediated entry and spread into cells. If TMPRSS2 is coexpressed with SARS-S in the same cell, cleavage results in SARS-S shedding into the supernatants, where the S protein fragments could function as antibody decoys. If TMPRSS2 expresses on viral target cells, it can activate SARS-S for virus-cell and cell-cell fusion. Also, relevant targets correspond to cells in which both the ACE2 and TMPRSS2 entry factors are present in the same cells simultaneously (Glowacka et al. 2011; Shulla et al. 2011). The results of the study performed by Bilinska et al., in which older mice expressed increasing levels of ACE2 and TMPRSS2, may explain why animals

(and humans) are more susceptible to COVID-19 infection when they reach old age (Bilinska et al. 2020).

Evidence shows that TMPRSS2 is essential for virus spread into host cells as more significant amounts of viral RNA and/or S protein have been detected in cells expressing this serine protease compared to TMPRSS2-negative cells and large syncytia forms when TMPRSS2 is present. This process indicates a significant cytopathic effect. Cell-cell mediated cytotoxicity and viral replication may cause the immune system to develop severe inflammation in response to viral infection (Shirato et al. 2013). However, outcomes assessing immune response and the role of TMPRSS2 in immunopathology are unclear.

Iwata-Yoshikawa et al. evaluated the immune response in lung cells and the expression of TMPRSS2. TMPRSS2 shows the proliferation of Th2 profile and contributed to inflammatory reactions after Toll-Like Receptor 3 (TLR3) stimulation without the control of endogenous promoter transcription mRNA of chemokines. It also induced the expression of interleukins and interferons (Iwata-Yoshikawa et al. 2019). Its expression results in a focal inflammatory infiltration around the bronchi and the alveoli. In contrast, the expression TLR3 showed lower viral replication. The fragments produced by the TMPRSS2 do not demonstrate a relationship with long-term isotypes in the adaptative immune response. Also, chemokine, IFN-alpha4, IFN-beta, IL-12, IL-4, and IL-10 and viral mRNA, showed increasing levels daily postinfection with a significant association with the inflammatory pathogenesis (Glowacka et al. 2011; Iwata-Yoshikawa et al. 2019).

Finally, using drugs to inhibit TMPRSS2 or other proteases such as Cathepsin B/L has allowed determining alternative mechanisms for S protein activation. In that sense, studies demonstrate that virus spread depends on TMPRSS2 activity, but there could be a residual effect of Cathepsin B/L in S protein priming when TMPRSS2 inhibits. This reaction could be explained by using alternative sites for S protein activation by Cathepsin L but not by TMPRSS2 (Matsuyama et al. 2010; Glowacka et al. 2011; Shulla et al. 2011; Reinke et al. 2017; Kleine-weber et al. 2018; Iwata-Yoshikawa et al. 2019; Hoffmann et al. 2020). Candidate drugs for post-exposure prophylaxis of SARS-CoV-2 infection could be clinically proven drugs such as camostat mesilate, preventing virus-host cell entry by inhibiting TMPRSS2. In addition, nafamostat mesilate may also inhibit cell entry of SARS-CoV-2 due to the amino acid sequence homology between Spike proteins of MERS-Cov and SARS-CoV-2. Cell culture experiments with simian Vero E6 cells infected with SARS-CoV-2, was shown to be inhibitive against SARS-CoV-2 infection, suggesting that

nafamostat mesilate could prevent SARS-CoV-2 infection (Wang et al. 2020a, b; Mckee et al. 2020).

## Conclusions

Emerging coronaviruses can constitute a severe threat to human health. Accumulating evidence suggests that TMPRSS2 is an essential protease for virus replication. However, there is a need for further research and many knowledge gaps exist. The expression of TMPRSS2 and the human proteases on Spike protein plays a critical role in initiating and propagating the virus, the transmissibility, and viral tropism and pathogenesis in the lung.

This review highlighted its critical molecular role in membrane fusion and the impact on viral mRNA replication, then promoting/driving pathogenesis and resistance. Many proteases have been discovered, and maybe an intrinsic mechanism leading to the rapid progress of ARDS in patients.

Identifying molecular mechanisms, advancements in virus genomics, and understanding their consequences will be crucial for developing novel therapeutic strategies to overcome the infection in a precision medicine era.

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