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Mini-review

TMPRSS2: A potential target for treatment of influenza virus and coronavirus infections

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ABSTRACT

Influenza virus and coronavirus epidemics or pandemics have occurred in succession worldwide throughout the early 21st century. These epidemics or pandemics pose a major threat to human health. Here, we outline a critical role of the host cell protease TMPRSS2 in influenza virus and coronavirus infections and highlight an antiviral therapeutic strategy targeting TMPRSS2.

1. Introduction

Pathogenic microorganisms have always been a major threat to human health. The Black Death, etiologically caused by *Yersinia pestis*, is estimated to have killed 30–60% of Europe's total population in the 14th century [1]; the 1918–1920 H1N1 influenza pandemic killed approximately 50 million people worldwide [2]. Today, many pathogenic microorganisms that once posed great threat to human health, such as smallpox and *Yersinia pestis*, have become extinct or are now under control due to advances in medical technology and the public health system [3,4]. For other etiological agents such as influenza virus and coronavirus, however, man have not yet found an effective control method. In the 21st century alone, four large-scale respiratory virus epidemics or pandemics have occurred worldwide: the viruses responsible are SARS-CoV [5], 2009 H1N1 pandemic influenza A virus [6], MERS-CoV [7] and Asian H7N9 influenza A virus [8]. For example, in 2017, H7N9 influenza A virus emerged in China again [9,10]. MERS swept through Saudi Arabia in 2012 and continues to spread there even now, and there have been more than 186 MERS cases in South Korea in 2015 [11–14]. Vaccination has been the most effective means in controlling pandemic, but genetic mutations could make vaccines ineffective via inducing nonprotective responses to newly emerged viruses [15]; this would leave even vaccinated populations highly vulnerable. Antiviral drugs targeting viral proteins will also eventually lose their effectiveness as viral mutation occurs; virus strains resistant to amantadine and oseltamivir have already emerged among 2013 Asian H7N9 influenza virus and 2009 pandemic H1N1 influenza virus [16–18]. The emergence of drug-resistant strains highlights the need for novel antiviral therapeutic approaches. Recently, a great deal of evidence has suggested that a transmembrane protease, serine 2 (TMPRSS2), a type II transmembrane serine protease (TTSP), plays a critical role in SARS and

Abbreviations: ARE, androgen receptor element; AEBSF, 4-(2-Aminomethyl) benzenesulfonyl fluoride hydrochloride; BHH, Bromhexine hydrochloride; CoV, coronavirus; DESC1, serine protease DESC1; EST, (2S,3S)-trans-Epoxysuccinyl-L-leucylamido-3-methylbutane ethyl ester; FDA, Food and Drug Administration; HAT, human airway trypsin-like protease; HAI-2, hepatocyte growth factor activator inhibitor 2; HGF, hepatocyte growth factor; IFITM, Interferon-induced transmembrane protein; MMP-2, matrix metalloproteinase-2; MSPL, transmembrane protease, serine 13; PAI-1, plasminogen activator inhibitor 1; PAR-2, protease activated receptor 2; PPMO, peptide-conjugated phosphorodiamidate morpholino oligomer; RBS, receptor binding subdomain; THE, human tracheal epithelial; TMPRSS2, transmembrane protease, serine 2; TMPRSS4, transmembrane protease serine 4; TTSP, type II transmembrane serine protease; vRNPs, viral ribonucleoproteins.

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MERS coronavirus (CoV) and in 2013 Asian H7N9 influenza virus and several H1N1 subtype influenza A viruses infections, indicating that targeting TMPRSS2 could be a novel antiviral strategy to treat coronavirus and some low pathogenic influenza virus infections [19–28].

2. The structure and physiological function of TMPRSS2

TMPRSS2 gene is located on human chromosome 21: 41, 464, 551-41, 531, 116 (Fig. 1). A significant feature of the TMPRSS2 gene is that several androgen receptor elements (AREs) are located upstream of the transcription start site and the first intron [29,30]. As shown in Fig. 2, the TMPRSS2 gene encodes a predicted protein of 492 amino acids which anchors to the plasma membrane. It converts to its form through autocatalytic cleavage between Arg255 and Ile256. After cleavage, the mature proteases are mostly membrane-bound, yet a noticeable portion of them can be liberated into the extracellular milieu. The protease catalytic domain contains a catalytic triad consisting of the amino acid residues His296, Asp345 and Ser441, corresponding to His57, Asp102 and Ser195 of chymotrypsinogen [29,30]. TMPRSS2 is predominantly expressed in prostate, with relatively lower level of expression in lungs, colon, liver, kidneys and pancreas. In lung cancer cell line A549 and prostate cancer cell line LnCaP, TMPRSS2 is expressed in an androgen-dependent manner [30,31]. It has been demonstrated that TMPRSS2 activates protease activated receptor 2 (PAR-2), a G-protein coupled receptor, and that the activation of PAR-2 causes the upregulation of matrix metalloproteinase-2 (MMP-2) and MMP-9, both of which are key proteases in the metastasis of tumor cells [32]. Furthermore, TMPRSS2-activated hepatocyte growth factor (HGF) promotes c-Met receptor tyrosine kinase signaling and induces a pro-invasive epithelial-mesenchymal transition phenotype in prostate cancer cells [32]. A recent study suggested that TMPRSS2 plays a role as a cell membrane-anchored mediator in cancer pain and pain in general [33]. However, TMPRSS2-deficient mice showed no obvious phenotypic abnormality such as death, infertility or visible sickness, and the exact physiological function of TMPRSS2 in vivo remains unknown. It is speculated that TMPRSS2 may contribute to a specialized but nonvital function that is apparent only under certain conditions [34].

3. TMPRSS2 is involved in proteolytic activation of influenza virus and coronavirus

3.1. Role of viral glycoprotein cleavage for the infectivity of influenza virus and coronavirus

Viral entry is the first step in the viral replication cycle. The entry of enveloped viruses into host cells in most cases requires virions binding to cell surface receptors and fusing to host-cell membrane. Both processes are controlled by viral envelope glycoproteins. Virus entry is a coordinated receptor binding process which involves numerous conformational changes in the viral envelope glycoproteins [35,36].

Influenza virus HA is a class I viral fusion protein which has two functional subunits, HA1 and HA2 (Fig. 3). HA is synthesized as a fusion-inactive precursor HA0. After proper proteolytic cleavage, disulfide bound subunits HA1 and HA2 form the homotrimer. In the structure, the three HA2 chains are seen to form a stem, embraced by the N- and C-terminal segments of HA1. The main central portion of each HA1 chain forms a globular 'head' domain that sits at top the stem. A fusion peptide, consisting mainly of apolar residues, is located in the N-terminus of HA2 and buried at the hydrophobic pocket formed partially by the fusion domain of HA1 [37–42].

Proteolytic cleavage of HA into HA1 and HA2 fragments is a critical step for virus infectivity, because it endows the HA fusion competent. Precursor HA cleavage liberates the fusion peptide, which is inserted into target membranes during fusion. Further, cleavage allows the HA being in a metastable conformation that can be triggered by the acidic pH of endosomes to undergo the structural rearrangements required for fusion. After proteolytic cleavage, the receptor binding subdomain of HA1 attaches to sialic receptors at the target membrane surface of host cells. This triggers internalization of the virion by endocytic pathway. During maturation of the endosome, the acidic environment of the endosome triggers rearrangment of HA1 which further provokes full exposure and release of fusion peptide from inner pocket. Subsequently, low pH-triggered HA2 reconformation causes fusion peptide insertion into the target endosomal membrane, forming an intermediate structure called the prehairpin. Several prehairpins undergo a further structural rearrangements, bending back at a hinge point to drive viral and cellular membranes close proximity, then induces hemifusion, complete fusion, ultimately generates an fusion pore that allows for viral genetic material escaping into the cytosol (Fig. 4) [37–42].

As a class I viral fusion protein, coronavirus spike glycoprotein (S) shares many structural and mechanistic features of influenza virus HA. Coronavirus S protein contains two functional domains, S1 and S2 (Fig. 3). S1 harbors the receptor-binding domain, S2 contains functional elements involved in membrane fusion. A distinctive feature of the coronavirus S protein is that it can harbor more than one proteolytic cleavage site. The first identified cleavage site is located at the S1/S2 boundary and another is within S2 upstream of the putative fusion peptide, which is called S2'. After cleavage of spike glycoprotein, S1 and S2 domains remain associated by noncovalently, but not disulfide bonds. This is an important

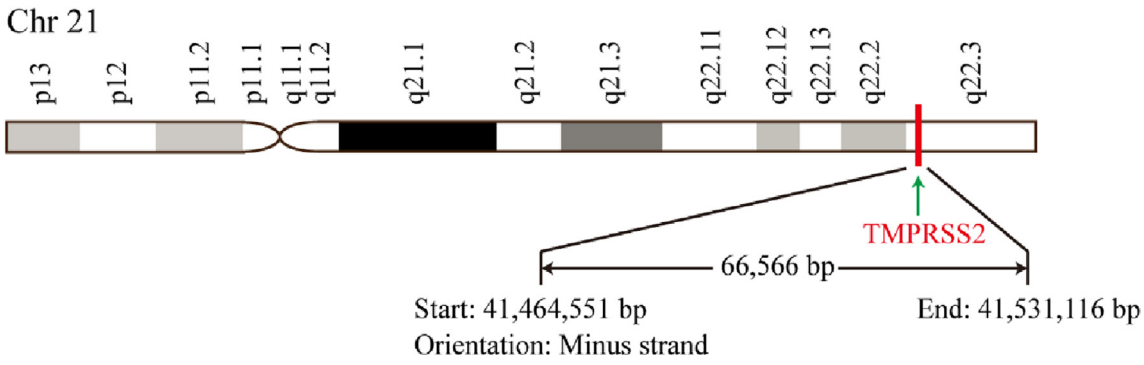

Fig. 1. A schematic diagram of TMPRSS2 genomic location.

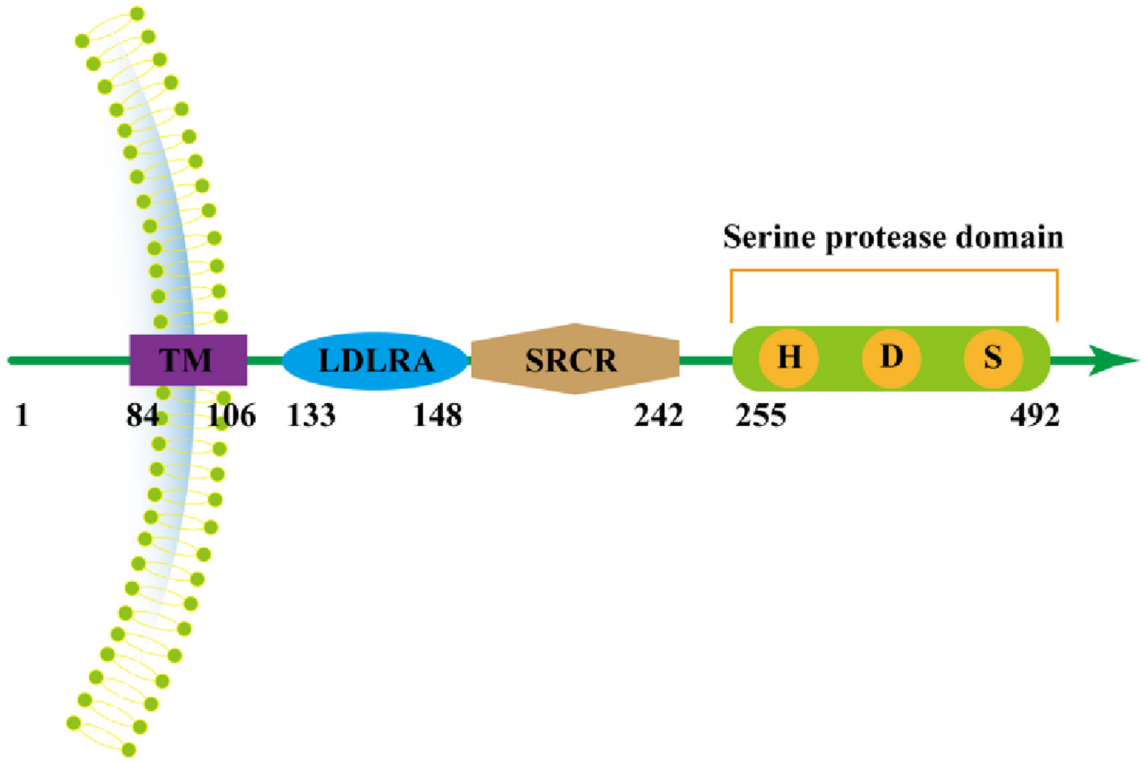

Fig. 2. The location and structure of TMPRSS2 protein. TM: transmembrane domain; LDLRA: low-density lipoprotein receptor domain class A; SRCR: Scavenger receptor cysteine-rich domain; Letters H: histidine; Letters D: aspartate; Letters S: serine.

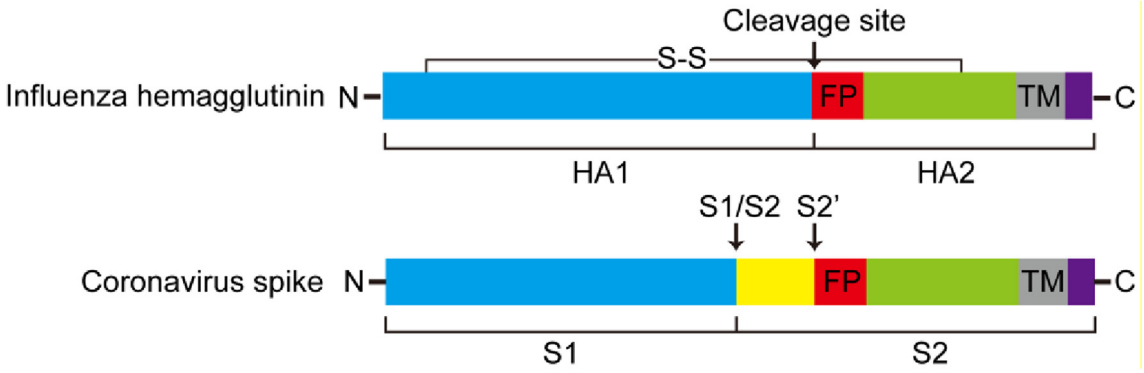

Fig. 3. Structure of influenza hemagglutinin and coronavirus spike protein with cleavage sites. Arrows: cleavage site; FP: putative fusion peptide; TM: transmembrane domain; S-S: disulfide bond.

distinction from influenza virus HA. Since the two domains are not held covalently, the S1 domain may be shed from the S2 stalk domain of the protein [43–45].

3.2. TMPRSS2 plays a critical role in proteolytic activation of some H1N1 subtype influenza A viruses and Asian H7N9 influenza virus

Some studies have suggested that TMPRSS2, HAT and other TTSPs such as transmembrane protease serine 4 (TMPRSS4), *Homo sapiens* serine protease DESC1 (DESC1) and *Homo sapiens* transmembrane protease, serine 13 (MSPL) can cleave human and avian influenza virus HA proteins with an arginine residue in cleavage site [21,22,46–48]. As shown in Fig. 5, HAT cleaves newly synthesized HA during the release of the progeny virions and HA of incoming viruses before they are incorporated into the host cells. TMPRSS2 cleaves only nascent HA within the host cells and is not involved in the proteolytic activation for HA of incoming virions [40,47]. It is speculated that the soluble form of TMPRSS2 possesses only minimal enzyme activity, which is insufficient to support cleavage of HA. In animal models, infection of wild-type mice with H7N9 influenza virus (A/Anhui/1/13) and H1N1 influenza virus (A/PR/8/34) led to severe disease with mortality rates of 100% and 20%, respectively; whereas in TMPRSS2-deficient mice, these viruses were a pathogenic [20]. For example, TMPRSS2 knockout mice were highly tolerant of challenge infection by H7N9 influenza virus (A/Anhui/1/13) and mouse-adapted H1N1 influenza virus A/California/04/09 (maCA04) with ≥1000 50% lethal doses (LD50) for WT mice [19]. These results demonstrate the essential role of TMPRSS2 in the pneumotropism and pathogenicity of H7N9 influenza virus and some H1N1 subtype influenza A viruses in vivo.

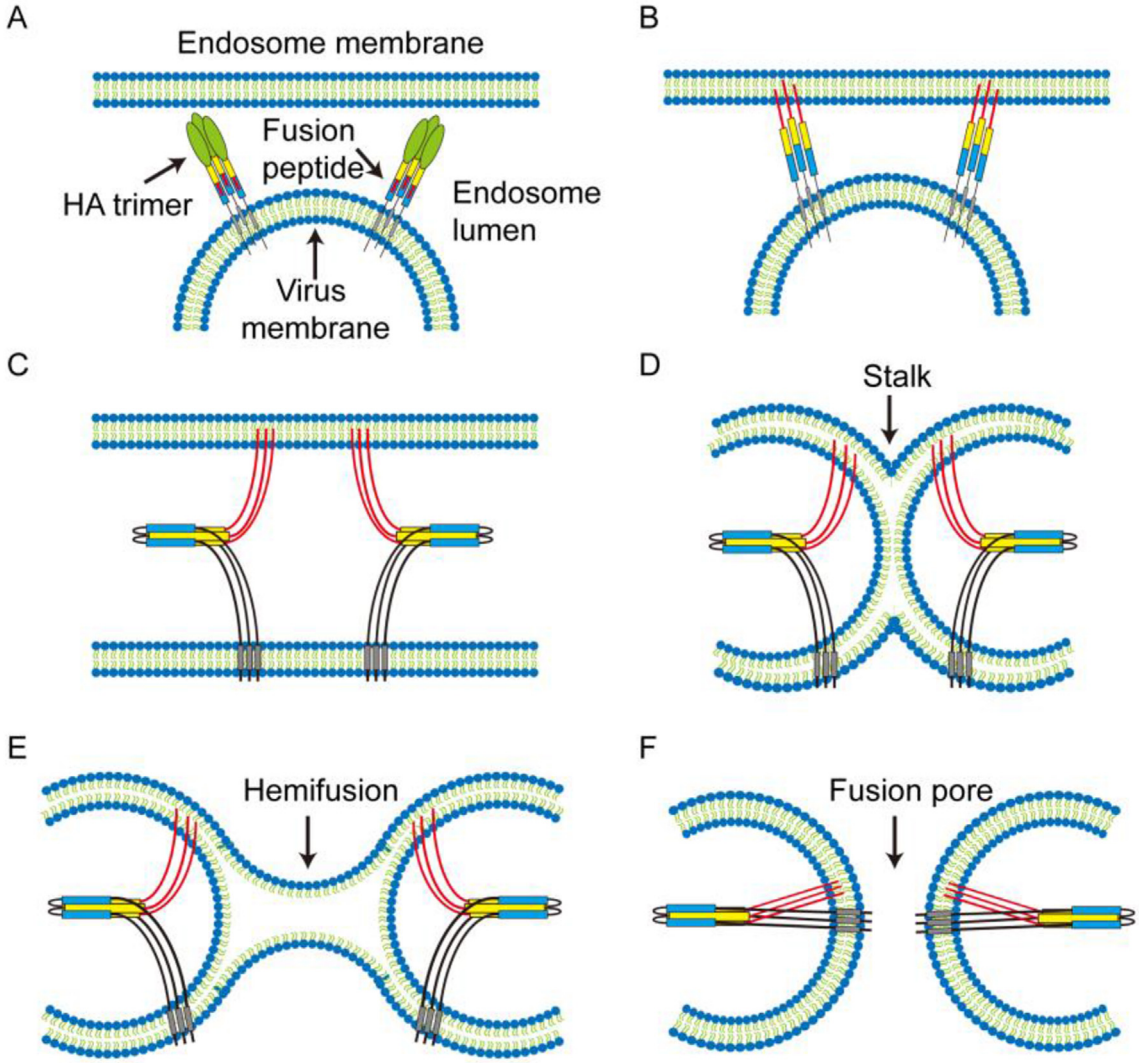

Fig. 4. Membrane fusion mediated by hemagglutinin. A) Viral membrane with two representative cleaved neutral pH HA and endosome membrane. B) The acidic environment of the endosome inducing conformational changes results in fusion peptide exposure and insertion into the target membrane. C) Conformational changes drive the viral and cellular membranes close proximity. D) Formation of pre-fusion stalk intermediate. E) Formation of hemifusion intermediate. F) Formation of fusion pore and viral genome is released into the cytoplasm. The Figure is adapted according to Karen J. Cross [42].

3.3. TMPRSS2 plays a pivotal role in the proteolytic activation of SARS-CoV and MERS-CoV

The SARS-CoV S protein can be cleaved by a wide variety of host proteases, such as TMPRSS2, HAT, MSPL, DESC1, Factor Xa and cathepsin L/B [49–51]. It has been shown that SARS-CoV enters into cells via two distinct pathways: one is mediated by TMPRSS2 at the cell surface and the other done by cathepsin L/B in the endosome (Fig. 6) [43,44,50,51]. The serine protease inhibitor camostat can effectively protect mice infected with the otherwise lethal SARS-CoV from death, but treatment with both serine and cathepsin inhibitors failed to improve survival significantly over that achieved with camostat alone [52], indicating that SARS-CoV propagation and pathogenesis is mediated by TMPRSS2 rather than cathepsin in vivo. Kawase et al. found that SARS-CoV entry increased 2.6-fold in the presence of TMPRSS2; conversely, siRNA targeting TMPRSS2 caused a five-fold decrease in SARS-CoV entry into Calu-3 cells [53]. Moreover, the levels of SARS-CoV RNA are nine-fold higher in cells expressing active TMPRSS2 than in cells expressing enzymatically inactive TMPRSS2 (S441A) [54]. Western blot analysis revealed that SARS-CoV S is cleaved into several fragments upon expression of TMPRSS2 (*cis-cleavage*) in the infected cells as well as upon contact between SARS-CoV S-expressing cells and TMPRSS2-expressing cells (*trans-cleavage*). *Cis-cleavage* results in the release of SARS-CoV S fragments into the cellular supernatant, which may interfere with antibody-mediated neutralization. *Trans-cleavage* activates SARS-CoV S on the target cell, allowing for efficient SARS-CoV S-driven viral fusion [55]. In addition, the activation of SARS-CoV by TMPRSS2 interferes with the inhibition of SARS-CoA S by Interferon-induced transmembrane proteins (IFITMs), a class of interferon-induced host cell proteins that inhibit the entry of several enveloped viruses. Collectively, the obtained evidence suggests that TMPRSS2 plays an important role in SARS-CoV infection [56–58].

The host proteases involved in the priming of MERS-CoV include TMPRSS2 [22], HAT [59], MSPL, DESC1 [46], furin [60] and endosomal cathepsin B/L [44]. Similar to SARS-CoV infection, MERS-CoV infection is largely dependent on the activity of endogenous TTSPs [59–61]. Camostat treatment alone is as effective as camostat plus EST, a cathepsin inhibitor, in the inhibition of virus entry into Calu-3 cells, indicating a dominant role of TMPRSS2 in MERS-CoV entry.

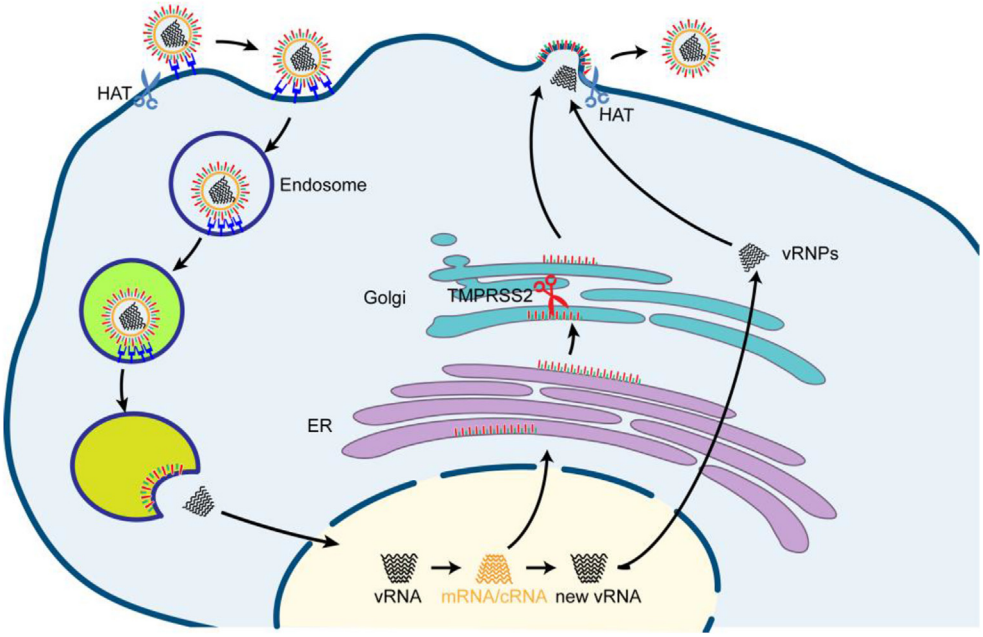

Fig. 5. The replication cycle of influenza virus and the proteolytic cleavage of the host proteases. Influenza virus binds to sialic acid-containing cell surface receptors, the bound virus is then endocytosed. During maturation of the endosome, the pH drops initiates the fusion of the viral envelope with the endosomal membrane and the release of the vRNPs into the cytosol. The vRNPs are imported into the nucleus, then transcription and replication proceed. Translation of viral mRNAs is performed by the cellular machinery. Newly formed viral RNAs are exported to the cytosol, assembled with new virus structural proteins, then packaged together at the plasma membrane, and bud off to release new virions. HA is synthesized as precursor that requires cleavage. HA cleavage by membrane-bound proteases (indicated as scissors) can take place in different part and at different time points during the viral life cycle. HA containing a monobasic cleavage site is cleaved by TMPRSS2 in the Golgi apparatus during assembly or cleaved by HAT on the plasma membrane either during attachment and entry into the cell or during budding of virions.

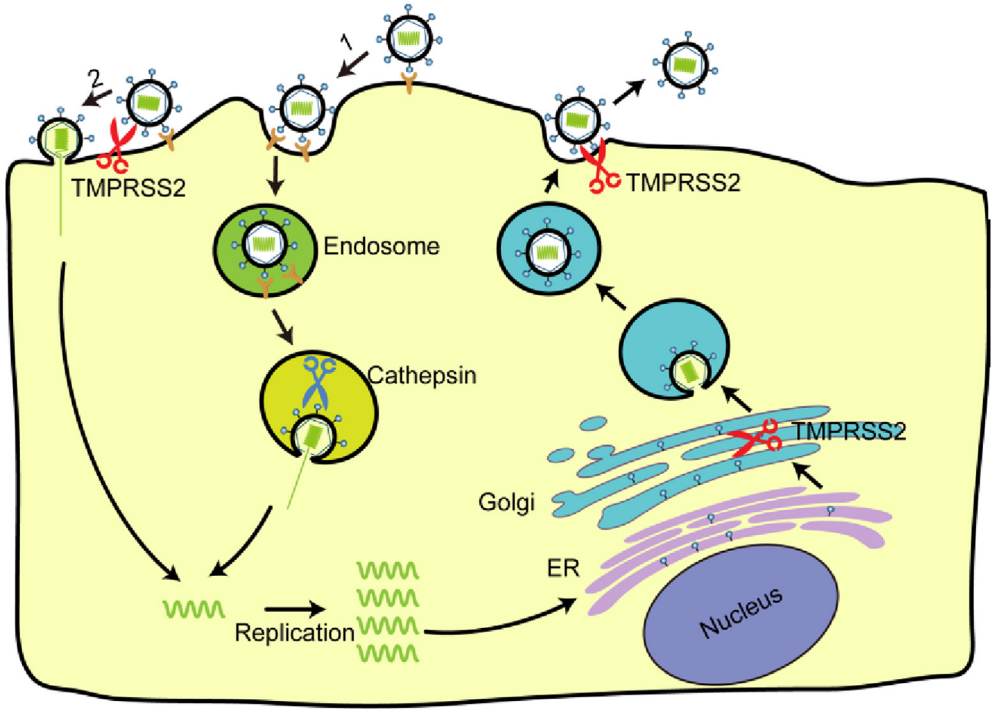

Fig. 6. The replication cycle of coronavirus and the proteolytic cleavage of the host proteases. Coronavirus binds to the cellular receptor, resulting in uptake of virions into endosomes (route 1), where the spike protein is activated by cathepsin. The pH drops in endosome initiates the fusion of the viral envelope with the endosomal membrane and the release of the viral genetic material into the cytosol, then RNA transcription, replication and transcription take place. New viral RNA is transported to the endoplasmic reticulum, Golgi intermediate, the site of assembly. Viral RNA and structural proteins assemble and bud into vesicles. Vesicles are transported to the cell surface and release. Alternatively, the spike protein can be activated at the cell surface, resulting in fusion of the viral membrane with the plasma membrane (route 2). Spike is synthesized as precursor that requires cleavage by host proteases. Spike cleavage (indicated as scissors) can take place in different part and at different time points during the viral life cycle. Spike cleavage by cathepsin occurs in the endosome. Spike cleavage by TMPRSS2 takes place in the Golgi or plasma membrane, either during assembly or attachment and release.

Indeed, TMPRSS2 augments MERS-CoV infection by up to 100-fold in Vero cells [22]. A non-catalytic TMPRSS2 (S441A) significantly abrogated the entry of MERS-CoV compared to normal TMPRSS2 [62]. Although other host proteases such as HAT, MSPL and DESC1 have been suggested to be involved in the priming of MERS-CoV, their expression levels in lung tissue are considerably lower than that of TMPRSS2, indicating a limited role of these proteases in viral propagation in host lung tissue [63,64]. These observations demonstrate that MERS-CoV infection is largely dependent on the activity of endogenous TMPRSS2. The details are shown in Fig. 6.

4. Development of therapeutics targeting TMPRSS2

4.1. Approved drugs for other diseases with TMPRSS2 inhibitory activity

The redundant physiological functions along with its critical role in H7N9 influenza virus (A/Anhui/1/13) and H1N1 influenza virus (A/PR/8/34) and SARS- and MERS-CoV infections suggest that TMPRSS2 can be a promising target for drug development. One possible drug is aprotinin (PDB ID: 1BPI), a polypeptide consisting of 58 amino acid residues purified from bovine lung. This polypeptide can inhibit serine proteases and suppress the cleavage of influenza virus HA, thus limiting the reproduction of the influenza virus [65]. Aerosol inhalation of aprotinin has been observed to have a therapeutic effect against influenza and parainfluenza in mice and humans [66]. A small-particle aerosol of aprotinin has been approved in Russia for the treatment of patients with mild-to-moderate influenza infections [65,66].

Camostat is a serine protease inhibitor used in the treatment of cancer, pancreatitis and liver fibrosis [67–71]. When human tracheal epithelial (HTE) cells are treated with camostat at a concentration of 10 mg/mL, H1N1 influenza virus (A/PR/8/34) titers in the culture supernatant are significantly reduced. Furthermore, camostat administration protects mice from infection with H1N1 influenza virus (A/Taiwan/1/86 and A/PR/8/34) [72,73]. A recent study demonstrated that the inhibition of TMPRSS2 with camostat led to a 10-fold reduction in SARS-CoV titers in Calu-3 cells [54]. The drug is also effective at protecting mice from SARS-CoV infection, with a survival rate of 60% [52]. In addition, camostat at a concentration of 10 μM impairs MERS-CoV entry 15-fold in Vero-TMPRSS2 cells, and the amount of viral RNA in the culture supernatant of Calu-3 cells is reduced 270-fold in the presence of 100 μM camostat at three days post MERS-CoV infection [22]. More recently, nafamostat, a serine protease inhibitor, was reported, to block MERS-CoV infection in vitro via inhabiting the activity of TMPRSS2, and reduced viral entry by 100-fold at a concentration of as low as 1 nM, which is more effective than camostat [74].

In addition, Bromhexine hydrochloride (BHH) is an ingredient in a mucolytic cough suppressant that also effectively attenuates prostate cancer metastasis in mice. Furthermore, it shows specific inhibition of TMPRSS2 (IC50 = 0.75 μM). Given that BHH is an FDA-approved drug with no significant adverse effects, it could be used for treatment of influenza virus and coronavirus infections as an inhibitor of TMPRSS2 [32].

4.2. Other approved serine protease inhibitors and new leading compounds with TMPRSS2 inhibitory activity

Other FDA-approved serine protease inhibitors such as 4-(2-Aminomethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) and leupeptin also exhibited varying degrees of anti-viral activities [74–76]. Pretreatment of C57Bl/6J mice with AEBSF prior to H1N1 influenza virus (A/PR/8/34) infection significantly reduced weight loss and resulted in a faster recovery compared to that seen in untreated mice [73]. Ovomucoid (PDB ID: 2GKT), a trypsin inhibitor, is more effective than aprotinin at suppressing the propagation of H1N1 influenza virus (A/Memphis/14/96) at the concentration of 50 μM [75].

A recent study demonstrated that 3-amidinophenylalanyl-derived compounds could inhibit TMPRSS2 in a nanomolar range. The most potent derivative inhibited TMPRSS2 with a Ki value of 0.9 nM and suppressed H1N1 influenza virus (A/Hamburg/5/2009) propagation in human airway epithelial cells in a dose-dependent manner [77]. It was also reported that three benzamidine-derived peptide mimetic compounds suppressed H1N1 influenza virus (A/Memphis/14/96 and A/Hamburg/5/2009) infection to varying degrees in TMPRSS2- and HAT-expressed cells [47].

4.3. Proteins with TMPRSS2 inhibitory activity

Dittmann et al. reported that plasminogen activator inhibitor 1 (PAI-1, PDB ID: 4U32) efficiently inhibited trypsin- and TMPRSS2-mediated cleavage of HA and suppressed H1N1 influenza virus (A/WSN/33) propagation ex vivo and in vivo [78]. In addition, hepatocyte growth factor activator inhibitor 2 (HAI-2, PDB ID: 3Q02) has been reported to efficiently inhibit HA-cleaving proteases such as TMPRSS2 and TMPRSS4 [64]. HAI-2 has also been shown to inhibit H1N1 influenza virus (A/PR/8/34) infection in vitro and to protect animals from H1N1 influenza virus (A/PR/8/34) infections in a mouse model [79]. These results suggest that localized administration of PAI-1 or HAI-2 in the respiratory tract might be a new therapeutic approach for the treatment of influenza virus, coronavirus or other respiratory virus infections that require host protease-driven maturation.

4.4. PPMO compounds specifically designed for TMPRSS2

It is noteworthy that none of the compounds or proteins described above is a TMPRSS2-specific inhibitor. The non-specific nature of those inhibitors may cause unpredictable side effects. For instance, the Food and Drug Administration (FDA) has found that aprotinin increases the risks of kidney failure, heart disease and stroke, resulting in banned or restricted status of the drug in some countries [80]. Therefore, it is necessary to develop inhibitors that specifically target TMPRSS2. Böttcher-Friebertshäuser et al. have designed and synthesized a single-stranded DNA-like antisense agent with low toxicity called a peptide-conjugated phosphorodiamidate morpholino oligomer (PPMO, Fig. S1), which can readily enter into cells and alter the splicing pattern of pre-mRNA. In cells treated with PPMO, TMPRSS2 was expressed in an incomplete and enzymatically inactive form. Consequently, the cleavage of HA was impaired and H1N1 influenza virus (A/Memphis/14/96) titers can be reduced 100–1000 fold in Calu-3 cells [81].

4.5. A potential drug target for TMPRSS2 inhibition

The male preference of Asian H7N9 influenza A virus [82–85] and 2009 pandemic H1N1 influenza A virus [86–89], SARS-CoV [90–92] and MERS-CoV infections [93–96] and the androgen-dependent expression of TMPRSS2 in a cell line derived from lung tissue (A549) suggest that androgen responsive elements (AREs) are involved in the expression of TMPRSS2 and can be attractive drug targets [32,97]. Devan et al. have developed a polyamide compound which can interact with 5′-WGWWCW-3′ in the consensus ARE in an attempt to inhibit the growth of prostate cancer cells. The study showed that the polyamide moderately inhibited the expression of TMPRSS2 by binding to the ARE in the TMPRSS2 promoter and suppressing RNA transcription [98]. The ARE is most likely to be functionally important in stabilizing juxtaposed enhancer-target

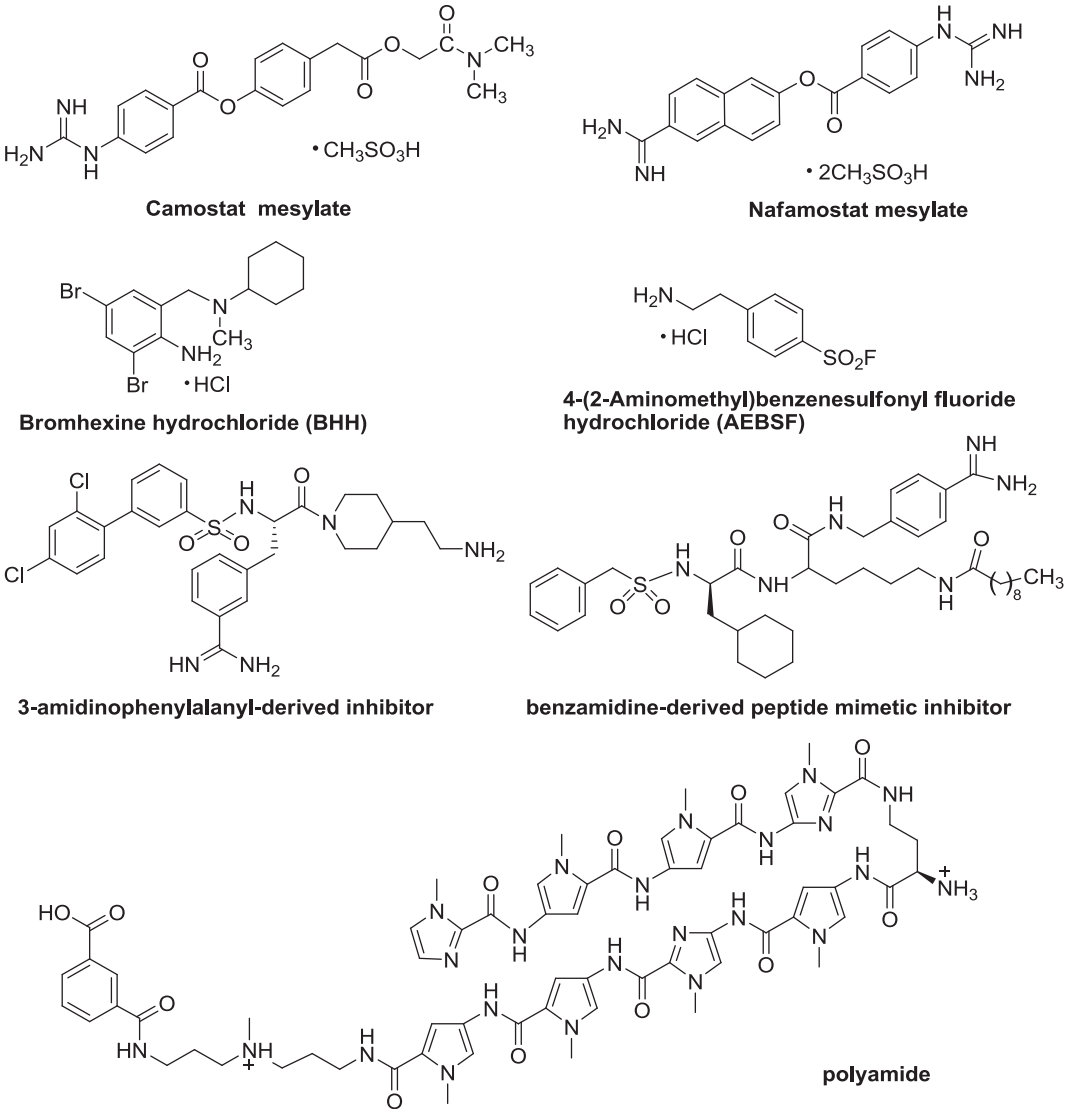

Fig. 7. Structures of some small molecular inhibitors for TMPRSS2 and TMPRSS2-related agents.

gene promoter loops to enable optimal gene expression [99,100]. This indicates that a cell membrane-permeable polyamide can specifically interact with a particular ARE of TMPRSS2 which controls or regulates TMPRSS2 expression. It is thus possible that polyamide compounds targeting to TMPRSS2 would specifically suppress Asian H7N9 influenza A virus and some H1N1 subtype influenza A viruses and SARS and MERS-CoV infections.

Structures of some small molecular inhibitors over-mentioned are summarized in Fig. 7.

5. Conclusion

One of the most direct impact of the recent increase in population mobility and globalization is the spread of infectious disease. Since the turn of the 21st century, influenza and coronavirus epidemics have occurred worldwide. The emergence of drug-resistant viruses and new viruses highlight the need for novel antiviral approaches and strategies. Targeting cellular factors is a relatively new antiviral strategy that may reduce or avoid the emergence of escape mutants. TMPRSS2 is one of the host factors that is essential for pneumotropism and pathogenicity of Asian H7N9 influenza A virus and several H1N1 subtype influenza A viruses infections; this enzyme also plays an important role in SARS-CoV and MERS-CoV infections, indicating that it is a promising drug target for the treatment of these viral infections. Although FDA-approved inhibitors that specifically inhibit TMPRSS2 are not yet available, some drugs such as camostat and nafamostat that have inhibitory activity against a variety of serine proteases have been approved for the treatment of other diseases and also suppress influenza virus and coronavirus infections. Because these drugs inhibit TMPRSS2 in vitro and in vivo, drug repositioning may help in developing novel measures to inhibit efficient viral entry and replication. Taken together, the available evidence suggests that TMPRSS2 is quite likely to be a target for new therapeutics against influenza virus and coronavirus infections.

Conflict of interests

The authors declare that there is no conflict of interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2017.07.016.

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