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The biogenesis of SARS-CoV-2 spike glycoprotein: multiple targets for host-directed antiviral therapy

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19 (coronavirus disease-19), represents a far more serious threat to public health than SARS and MERS coronaviruses, due to its ability to spread more efficiently than its predecessors. Currently, there is no worldwide-approved effective treatment for COVID-19, urging the scientific community to intense efforts to accelerate the discovery and development of prophylactic and therapeutic solutions against SARS-CoV-2 infection. In particular, effective antiviral drugs are urgently needed. With few exceptions, therapeutic approaches to combat viral infections have traditionally focused on targeting unique viral components or enzymes; however, it has now become evident that this strategy often fails due to the rapid emergence of drug-resistant viruses. Targeting host factors that are essential for the virus life cycle, but are dispensable for the host, has recently received increasing attention. The spike glycoprotein, a component of the viral envelope that decorates the virion surface as a distinctive crown (“corona”) and is essential for SARS-CoV-2 entry into host cells, represents a key target for developing therapeutics capable of blocking virus invasion. This review highlights aspects of the SARS-CoV-2 spike biogenesis that may be amenable to host-directed antiviral targeting.

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1. Introduction

SARS-CoV-2 coronavirus entered human history near the end of 2019. Starting from China, SARS-CoV-2 rapidly spread globally, with more than 40 million people testing positive worldwide, and causing more than 1,100,000 deaths as of October 20, 2020 (https://coronavirus.jhu.edu/map.html).

SARS-CoV-2 is a member of the Coronaviridae family that comprises a large number of enveloped, positive-sense single-stranded RNA viruses causing respiratory, enteric and neurological diseases of varying severity in domestic and wild animals, as well as in humans [1].

On the basis of their phylogenetic relationships and genomic structures, coronaviruses (CoV) are subdivided in four genera: alpha-, beta-, gamma- and delta-coronavirus; among these, only alpha- and beta-CoVs can infect humans [1,2]. Human coronaviruses (HCoV) include four globally distributed viruses (HCoV-NL63, HCoV-229E, HCoV-OC43 and HCoV-HKU1) that generally cause mild upper respiratory tract diseases in immunocompetent hosts, and three highly pathogenic (HP) viruses that have emerged since the beginning of this century [1–4]. HP-HCoV include, in addition to SARS-CoV-2, the lineage B alpha-CoV SARS coronavirus that emerged in China and Hong Kong in 2002–2003, causing more than 8,000 cases worldwide with a death rate of approximately 10% (https://www.who.int/csr/sars/country/table2004_04_21/en/), and the lineage C beta-CoV MERS (Middle East Respiratory Syndrome) coronavirus that emerged in 2012 in the Arabian Peninsula, causing over 2,500 confirmed cases and a case-fatality rate higher than 34% (https://www.who.int/csr/don/24-february-2020-mers-saudi-arabia/en/).

SARS-CoV and MERS-CoV infections can result in acute respiratory distress syndrome (ARDS), which may lead to long-term reduction in lung function and death [5]; infection with SARS-CoV-2 in humans manifests as coronavirus disease-2019 (COVID-19), a spectrum of diseases ranging from asymptomatic infection to respiratory symptoms that, in a subset of patients, may progress to pneumonia, ARDS, multi organ dysfunction and death [6,7].

Given the sudden appearance and rapid spread of SARS-CoV-2,
there is no current validated vaccine or SARS-CoV-2-specific targeting therapy that is clinically approved, although steroids, heparin and statins look promising for lowering fatality rates, and the antiviral remdesivir has been proven to reduce the duration of symptomatic disease presentation [8,9].

Despite the extraordinary worldwide commitment, efforts to defeat COVID-19 are hampered by lack of information on several important aspects of this new coronavirus, ranging from SARS-CoV-2 biology to its interaction with the host response.

At the genomic level SARS-CoV-2 is highly similar (nearly 80% identical) to SARS-CoV [10,11] and to RaTG13-CoV (>95% identical) circulating in bats, the natural reservoir host for multiple coronaviruses [10–13]. Similarly to other coronaviruses, it harbors a large (~30 kb) nonsegmented RNA genome, with the replicase-transcriptase gene encoded within the 5’-end and the structural proteins encoded in the 3'-end, following the CoV invariant gene order: 5’- S (spike) - E (envelope) - M (membrane) - N (nucleocapsid)-3’. Numerous small open reading frames, encoding accessory proteins, are distributed among the structural genes [2,14].

The spike protein, together with the M and E proteins, is anchored into the viral envelope, decorating the virion surface as a distinctive crown (“corona”) (Fig. 1A), and is essential for viral entry into target cells [15,16]. As it induces neutralizing antibody responses, S-protein is also an important target for vaccine development [17,18], and therefore it has been studied extensively [19–21].

1.1. The SARS-CoV-2 spike glycoprotein

The SARS-CoV-2 spike is a trimeric class I fusion protein. As most viral fusion proteins, each monomer is synthesized as a fusogenically-inactive precursor of about 180 kDa, which assembles into an inactive homotrimer and is endoproteolytically cleaved by cellular proteases, giving rise to a metastable complex of two functional subunits: S1 (bulb) responsible for receptor binding and the membrane-anchored S2 (stalk) that contains the fusion machinery (Fig. 1A).

The structure of the SARS-CoV-2 S precursor has been characterized and it consists of a signal peptide (SP) located at the N-terminus, followed by the S1 and S2 subunits (Fig. 1B). The S1 subunit contains the N-terminal domain (ND) and the receptor-binding domain (RBD), with the receptor-binding motif (RBM), responsible for recognition and attachment to the host angiotensin-converting enzyme 2 (ACE2) receptor (Fig. 1B) [20]. The attachment process starts with the recognition of the RBD 394 glutamine residue by the lysine 31 residue on the human ACE2 [22]. Interestingly, the SARS-CoV-2 S protein has a 10- to 20-fold higher affinity for ACE2 than the S protein of SARS-CoV-19 [19].

S2 harbors the fusion peptide (FP), a short segment of 15–20 conserved mainly hydrophobic amino acids, which anchors to target membranes and plays an essential role in mediating membrane fusion by disrupting and connecting lipid bilayers of the host cell. The FP is followed by two heptapeptide repeat sequences HR1 and HR2, the transmembrane anchor domain (TM), and a short cytoplasmic tail (CT) (Fig. 1B) [20]. Similarly to other viral fusion proteins, when the S1 subunit recognizes its receptor on human cells, the HR1 and HR2 domains are exposed to interact with each other to form a six-helical bundle (6-HB), consequently bringing viral and cellular membranes into close proximity to permit lipid bilayer fusion [23]. Fusion inhibitors derived from the SARS-CoV-2 HR2 domain and pan-CoV fusion inhibitors have been developed that are able to bind to S-HR1 trimers forming heterologous 6-HBs, thus inhibiting viral/cell membrane fusion [23].

As most viral glycoproteins that become incorporated into the envelope membrane bilayer, SARS-CoV-2 S protein is extensively decorated with N-linked glycans at both the S1 and S2 subunits, possessing a total of 22 N-linked glycan sites (as compared with 23 on SARS) (Fig. 1B) [20,24,25]. Glycosylation plays an essential role in establishing viral spike proteins bioactive conformation and stability, for shaping viral tropism and has effects on virus antigenic properties, receptor binding and fusion activity [26–28]. Using a site-specific mass spectrometric analysis, Watanabe et al. have recently revealed the glycan structures on a recombinant SARS-CoV-2 S immunogen, mapping the glycan-processing states across the trimeric viral spike [24].

There is still little information on the molecular processes involved in SARS-CoV-2 spike protein glycosylation and maturation; however, due to the similarity with other CoV spike proteins, such as the SARS-CoV S, the knowledge obtained on these viruses can be used to identify elements of the secretory pathway essential for virus morphogenesis that could represent appealing targets for novel anti-coronavirus drugs [3,29,30].

1.2. Coronavirus spike glycosylation

Like other enveloped viruses, coronaviruses exploit the ability of the host cell to produce, properly fold and transport glycoproteins to the correct cellular location [29,31]. For SARS-CoV-2 and other CoVs, these steps take place in organelles of the early secretory pathway, the endoplasmic reticulum (ER), the ER-Golgi intermediate compartment (ERGIC), and the Golgi apparatus, and efficient virus spread critically depends on hijacking the host secretory machinery (Fig. 2) [29,31].

Co-translational N-glycosylation was shown to be essential for CoV spike proteins to fold properly and to exit the ER. Interestingly the S protein of some coronaviruses, including S proteins of transmissible gastroenteritis virus (TGEV) [32,33] and SARS-CoV-2 (Santoro et al., unpublished results), was shown to aggregate when glycosylation was inhibited; furthermore growth of coronaviruses in the presence of the N-glycosylation inhibitor tunicamycin resulted in the production of spikeless, noninfectious virions [34–36].

In addition, tunicamycin and α-glucosidases I and II inhibitors, which hamper the removal of terminal glucose residues from N-glycan chains attached to the nascent glycoprotein in the ER, such as N-methyl-1-deoxynojirimycin and castanospermine, were shown to influence the intracellular transport as well as the antigenic properties of MHV (mouse hepatitis virus) coronavirus spike glycoprotein [37], and to cause both a delay in the surface expression of the glycoprotein and a drastic reduction in progeny virion formation. Castanospermine and the α-glucosidase inhibitor N-butyl-deoxynojirimycin (NB-DNJ) were also found to alter SARS S protein maturation and to decrease incorporation of the spike into virus particles [38,39].

Potential use of α-glucosidase inhibitors, including imino sugars, as host-directed antiviral agents have been largely investigated [40], and potential candidates for treatment of COVID-19, including miglitol, celgosivir and miglustat, have been recently reviewed [41]. It should be noted that, in the case of N-linked glycans, more than 30 enzymes, located in the cytosol, ER and Golgi apparatus, are required to generate, attach and process oligosaccharides, and may then represent potential targets for S protein biogenesis inhibitors; however, the molecular events participating in the protein glycosylation process have been largely described [29,40,42] and will not be discussed here.

1.3. CoV spike glycoprotein folding and disulfide bond formation

Like most viral glycoproteins, during its biosynthesis the S polypeptide, while it is co-translationally N-glycosylated in the ER,
interacts with the cellular molecular chaperones calnexin and/or calreticulin, after which the carbohydrates are processed in the ER and in the Golgi apparatus \[27,43\]. In the case of the SARS coronavirus it has been shown that the binding of the S protein to calnexin is essential for correct folding of the glycosylated spike protein, and that this ER chaperone plays a critical role in the infectious ability of progeny viruses and consequently on SARS-CoV infection \[39\].

In addition to the calnexin/calreticulum folding machine, post-translational formation of disulfide bonds plays a decisive role in
the generation of the final glycoprotein architecture. As an example, disulfide bond formation was shown to be essential for the correct folding, trafficking and trimerization of the spike protein of the MHV coronavirus [44]. In the case of SARS-CoV-2 S protein, nine cysteine residues were found in the S1 receptor binding domain, eight of which form four pairs of disulfide bonds [45]. Among these four pairs, three (Cys336-Cys361, Cys379-Cys432 and Cys391-Cys525) were found to stabilize the β sheet structure, whereas the fourth (Cys480-Cys488) connects the loops in the distal end of the RBM [45]. These intra-molecular disulfide bonds are believed to contribute to the stereospecific orientations of the ACE2-interacting amino acid residues of the spike protein, and therefore to play a relevant role in the binding of the RBM to the receptor. It has been hypothesized that the perturbation of the spike functionally active conformation through the reduction of accessible disulfide bonds may be a feasible strategy to disintegrate the spike protein from the ACE2 receptor, preventing infection [46]. In addition, mutations in SARS-CoV-2 spike that allow the production of thermostable, disulfide-bonded S-protein trimers that are trapped in the closed, prefusion state, have been recently described, and potential applications of a designed, thermostable S trimer as a reagent for serology/virology and as an immunogen have been recently suggested [47].

A critical role for the correct dynamics of disulfide bond formation of viral glycoproteins, such as the influenza hemagglutinin (HA) and the parainfluenza fusion (F) protein in the early secretory pathway is played by the calnexin/calreticulin associated co-chaperone ERp57 [48]. ERp57, also known as GRP58 (glucose-regulated protein-58) is a member of the protein disulphide-isomerase (PDI) family coded by the PDIA3 gene, mostly, but not exclusively, localized in the ER [48,49]. Like other PDIS, ERp57 is characterized by the presence of two catalytically active thioredoxin-like domains (TLD) containing a cys-gly-his-cys sequence, termed a and a', which provide ERp57 with its redox properties, and two inactive TLD, termed b and b'. Crystallography studies demonstrate that the four TLD form a twisted "U"-shape structure [50].

ERp57 is a multifunctional protein acting both as oxidoreductase

**Fig. 2. Schematic representation of SARS-CoV-2 spike glycoprotein biogenesis.** Different steps of SARS-CoV-2 replication cycle are illustrated in the cartoon, including binding to the ACE2 receptor (blue), virus entry, viral RNA replication, sub-genomic RNA transcription and translation. RdRp, RNA-dependent RNA polymerase; E, envelope; M, membrane; N, nucleoprotein; S, spike; CNX, Calnexin; ER, endoplasmic reticulum; ERGIC, ER-Golgi intermediate compartment. Gray text boxes highlight host-cell processes implicated in SARS-CoV-2 spike biogenesis that might represent potential targets for host-directed antiviral drugs.
and isomerase, helping ER-glycoproteins to obtain native disulfides by rearranging nonnative linkages. The catalytically-active domains are located at the C- and N-termini at the top of the U, while the two noncatalytic domains are localized to the inside surface providing the binding sites for calnexin/calreticulin; in particular, ERp57-b' needs to associate with calreticulin/calnexin that position lectin-bound misfolded glycoproteins allowing ERp57-mediated catalysis [50,51]. Interestingly ERp57 was recently shown to be the target of the broad-spectrum anti-infective agent nitazoxanide [52].

Nitazoxanide, a thiazolide originally developed as an anti-protozoal agent and used in clinical practice for the treatment of infectious gastroenteritis [53,54], has recently emerged as broad-spectrum antiviral drug [55,56]. We and others have previously reported that nitazoxanide and its active circulating-metabolite tizoxanide are effective against a broad range of RNA viruses, including influenza and paramyxovirus influenza, hepatitis C and rotavirus infection in vitro as well as in clinical studies [52,57–61].

Rather than affecting viral targets, thiazolides act through a cell-mediated mechanism. In the case of influenza and paramyxovirus viruses nitazoxanide was found to act by a novel mechanism, impairing terminal glycosylation and intracellular trafficking of class-I viral fusion glycoproteins influenza HA and paramyxovirus F spike proteins [52,57], an effect mediated by the inhibition of ERp57 [52].

Interestingly nitazoxanide was found to also be effective against different animal and human coronaviruses in vitro [55,62,63], as well as in clinical studies (Rossignol et al., submitted). Recent studies have also reported the antiviral activity of nitazoxanide against SARS-CoV-2 in cell culture assays [64,65]. We are currently investigating the possibility that, as for the influenza HA and paramyxovirus F glycoproteins, nitazoxanide may act by affecting the biogenesis of SARS-CoV-2 spike protein, impairing its correct folding, maturation and fusion activity (Santoro et al., unpublished results).

1.4. Furin and SARS-CoV-2 spike cleavage

Previous studies on different coronaviruses have shown that activation of the CoV spike protein is a complex process involving several host proteases and multiple cleavage events at distinct sites of the glycoprotein [66]. A variety of host proteases, including cell surface TMPRSS2 (transmembrane serine protease 2) proteases, trypsin, furin, and endosomal cathepsins, have been shown to direct S protein cleavage during CoVs entry or viral protein biogenesis, depending on their distribution in host cells. In addition to controlling virus entry, S protein cleavage may also influence host tropism and pathogenesis of CoV infection [16,67–69].

The SARS-CoV-2 spike is cleaved at the boundary between the S1 and S2 subunits that remain non-covalently bound in the pre-fusion conformation [70–73]. Similarly to other viral fusion glycoproteins, including influenza hemagglutinin and paramyxovirus fusion F-protein, S cleavage at the S1/S2 boundary is essential for membrane-fusion activity [71–73]. As for all CoVs, a second cleavage by host proteases occurring at a different site termed S2' located immediately upstream of the fusion peptide has been proposed to activate the protein for membrane fusion via extensive conformational changes [67,70,74–76].

In the case of SARS-CoV-2 early characterizations of the genome revealed the existence of an exposed loop at the S1/S2 site harboring a polybasic furin-like cleavage (FLC) site containing multiple arginine residues (RRAR) [Fig. 1B] [15,19,77,78]; a similar multibasic cleavage site was previously found in MERS-CoV, but not in other lineage B beta-CoVs, including SARS-CoV and SARS-related coronaviruses (SARs-CoV) [77].

Furin, a protease belonging to the proprotein convertase (PC) family, is ubiquitously expressed in the Golgi apparatus of all cells, with some cell types showing enhanced expression or altered intracellular distribution [79,80]. To date, furin and/or PC-mediated cleavage is considered to represent a key event in activating fusion activity of envelope glycoproteins of several evolutionarily diverse virus families, including, besides Coronaviridae, Flaviviridae, Filoviridae, Retroviridae, Orthomyxoviridae and Paramyxoviridae; however, both subcellular localization and timing of furin-mediated activation may vary considerably depending on the virus family [80,81].

Interestingly, in the case of SARS-CoV-2, the FLC site is processed during S protein biogenesis; in fact, differently from SARS-CoV, the spike of SARS-CoV-2 is mainly detected as the cleaved S1/S2 subunits when exogenously expressed in different types of cells (Fig. 1B–D) [15,77].

The furin-like cleavage site, promoting S-protein “priming” intracellularly during morphogenesis, facilitates virus progeny entry into the target cells and may be responsible for SARS-CoV-2 high infectivity and transmissibility, providing a gain-of-function to the new coronavirus for efficient spreading in the human population compared to other lineages B beta-CoVs [15,22]. More importantly, the acquisition of similar polybasic cleavage sites is associated with increased pathogenicity in different viruses, including avian influenza A viruses (AIVs) [82,83]. In the case of AIVs, a multibasic cleavage site in the virus surface glycoprotein, the hemagglutinin (HA), is considered a critical virulence factor [82,83]: the HA cleavage site motif of low-pathogenicity avian influenza viruses (LPAIV) typically contains one or two basic amino acid residues that are cleaved by trypsin and trypsin-like proteases with monobasic specificity, confining LPAIV replication to trypsin-expressing epithelial cells of the respiratory and gastrointestinal tracts. On the other hand, the HA cleavage site motif of highly pathogenic viruses (HPAIV) contains multiple basic amino acids, facilitating cleavage by ubiquitously expressed proteases with polybasic specificity, most notably furin, thus allowing HPAIV to replicate in multiple tissues, including the vascular endothelium. Therefore, the FLC site may be implicated in the ability of SARS-CoV-2 to invade different tissues and organs, not restricting virus replication to the respiratory and gastrointestinal tracts. In fact, although SARS-CoV-2 infection predominantly causes substantial respiratory pathology, it can also result in several extrapulmonary manifestations, including, beside gastrointestinal symptoms, myocardial dysfunction and arrhythmia, thrombotic complications, kidney and hepatocellular injury, neurologic illnesses and ocular symptoms [84].

As indicated above, similarly to SARS-CoV, the cellular receptor of SARS-CoV-2 is the angiotensin-converting enzyme 2. ACE2 is a transmembrane protein acting as a carboxypeptidase negatively regulating the renin–angiotensin system [85]. In addition to the respiratory system, ACE2 expression was observed is several human organs and cell types, including enterocytes, cardiomyocytes and renal tubules [86]. Since ACE2 is expressed in multiple extrapulmonary tissues, direct viral tissue damage is considered a possible mechanism of injury [84].

On this basis, in addition of the well studied TMPRSS2 inhibitors [16,67], furin inhibitors might be considered as a treatment option for COVID-19. Since furin, unlike TMPRSS2, is required for normal cell function [87], blocking this enzyme for prolonged time periods might cause unwanted toxic effects; however, short treatments might be well tolerated and lead to a therapeutic benefit [88,89]. In recent years, several small molecule and peptide-based inhibitors targeting furin/PCs have been developed as putative antiviral agents, which may inhibit maturation and fusion activity of viral envelope glycoproteins [80,81,90]. Among these, the peptide
inhibitor decanoyl-RVKR-chloromethylketone (CMK) has proven to be particularly effective in inhibiting SARS-CoV-2 spike cleavage during biogenesis in different types of cells (Fig. 1D).

Interestingly, it was recently reported that the loss of the furin cleavage site in the spike of a SARS-CoV-2 mutant (ΔPRRA) resulted in reduced infection in human respiratory cells (but not in Vero E6 cells) and ablated disease in a hamster pathogenesis model of SARS-CoV-2, demonstrating a critical role for the FLC in SARS-CoV-2 replication and pathogenesis [91].

1.5. Coronavirus spike protein S-acylation

Protein S-acylation, a post-translational modification that involves linkage of a fatty acid chain predominantly to a cysteine amino acid in a thioester bond, was first described by Schmidt and Schlesinger in the VSV (Vesicular Stomatitis Virus) glycoprotein G [92], and subsequently found to be ubiquitous and highly conserved from yeast to human [93,94]. Since the fatty acid molecule is predominantly palmitate, the term ‘palmitoylation’ is also used, but other fatty acids both saturated (e.g., myristic and stearic) and unsaturated (e.g., oleic and arachidonic) can also form modifications.

S-acylation affects protein trafficking, protein-protein and protein-membrane interactions, and, being coupled to membrane fusion or virus assembly, is known to influence viral replication and pathogenesis.

Coronavirus S protein palmitoylation was initially identified in cells infected with MHV coronavirus [43]. Treatment of palmitoyl acyltransferase inhibitor 2-bromopalmitate was found to decrease MHV S protein palmitoylation, and caused a significant reduction in MHV infectivity [95–97]. Reduction of S palmitoylation was also found to impair S association with the M protein with subsequent exclusion of the spike protein from virions. In addition, MHV mutants harboring mutations in the putative palmitoylation sites exhibited reduced infectivity, further supporting the importance of palmitoylation in virion assembly and infectivity [95–97].

Alpha-coronavirus TGEV S protein is also modified by palmitoylation, and inhibition of palmitoylation by 2-bromopalmitate treatment reduced TGEV replication in cell culture [98].

In the case of SARS-CoV, the cytoplasmic portion of the spike protein contains four cysteine-rich clusters, two of which (clusters I and II) were found to be modified by palmitoylation; S-mediated cell fusion was markedly reduced by mutations in these cysteine clusters as compared with wild-type protein, suggesting that palmitoylation in the endodomain may be required for the fusogenic activity of SARS-CoV S protein [99]. In a different study, using a recombinant nonpalmitoylated SARS-CoV S protein mutant, it was shown that similarly to MHV S protein, palmitoylation of the SARS-CoV S protein was required for its partitioning into membranes and for cell–cell fusion [100]. However, differently from MHV S protein, SARS-CoV spike palmitoylation was not required for S–M interaction [100]. Finally, treatment with nitric oxide or its derivatives was used, but other fatty acids both saturated (e.g., myristic and stearic) and unsaturated (e.g., oleic and arachidonic) can also form modifications.

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Very little is currently known about palmitoylation of the SARS-CoV-2 spike protein. Analysis of the alignment of SARS-CoV and SARS-CoV-2 S proteins has revealed that all 9 putative palmitoylation sites in SARS-CoV are conserved in SARS-CoV-2, and it has been speculated that palmitoylation could contribute to nascent SARS-CoV-2 spikes targeting to GM1 lipid rafts in the producing cells [102]. Characterizing SARS-CoV-2 spike glycoprotein S-acylation, and understanding the molecular mechanisms involved may uncover new targets for antiviral therapy during S protein biogenesis.

1.6. Perspectives

As obligatory parasites, viruses are directly dependent upon their host cell environment for replication, protein expression and assembly of progeny particles. With few exceptions, therapeutic approaches to combat viral infections have traditionally focused on targeting unique viral components or enzymes; however, it has now become evident that this strategy often fails due to the rapid emergence of drug-resistant viruses. Targeting host factors that are essential for the virus life cycle, rather than pathogen components directly, has recently received increasing attention. Genome-wide approaches have been used successfully to identify cellular factors that are critical for virus replication, but are dispensable for the host, and can thus serve as novel targets for antiviral drug development [103].

The SARS-CoV-2 spike, which is essential for virus invasion, represents a key target for developing therapeutics capable of blocking viral entry and inhibiting membrane fusion. Characterizing the components of the molecular machines operating in the biogenesis of SARS-CoV-2 spike protein, together with their tissue specificity and redundancy, will provide opportunities in the search for unique targets for novel host-directed antiviral drugs for therapeutic intervention in SARS-CoV-2 infections.

Declaration of competing interest

All authors declare no conflict of interest.

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