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Review

The role of cell surface sialic acids for SARS-CoV-2 infection

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Abstract

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a new virus that has higher contagious capacity than any other previous human coronaviruses (HCoVs) and causes the current coronavirus disease 2019 pandemic. Sialic acids are a group of nine-carbon acidic α-keto sugars, usually located at the end of glycans of cell surface glycoconjugates and serve as attachment sites for previous HCoVs. It is therefore speculated that sialic acids on the host cell surface could serve as co-receptors or attachment factors for SARS-CoV-2 cell entry as well. Recent in silico modeling, molecular modeling predictions and microscopy studies indicate potential sialic acid binding by SARS-CoV-2 upon cell entry. In particular, a flat sialic acid-binding domain was proposed at the N-terminal domain of the spike protein, which may lead to the initial contact and interaction of the virus on the epithelium followed by higher affinity binding to angiotensin-converting enzyme 2 (ACE2) receptor, likely a two-step attachment fashion. However, recent in vitro and ex vivo studies of sialic acids on ACE2 receptor confirmed an opposite role for SARS-CoV-2 binding. In particular, neuraminidase treatment of epithelial cells and ACE2-expressing 293T cells increased SARS-CoV-2 binding. Furthermore, the ACE2 glycosylation inhibition studies indicate that sialic acids on ACE2 receptor prevent ACE2–spike protein interaction. On the other hand, a most recent study indicates that gangliosides could serve as ligands for receptor-binding domain of SARS-CoV-2 spike protein. This mini-review discusses what has been predicted and known so far about the role of sialic acid for SARS-CoV-2 infection and future research perspective.

Key words: ACE2, coronavirus, ganglioside, SARS-CoV-2, sialic acid

Graphical Abstract
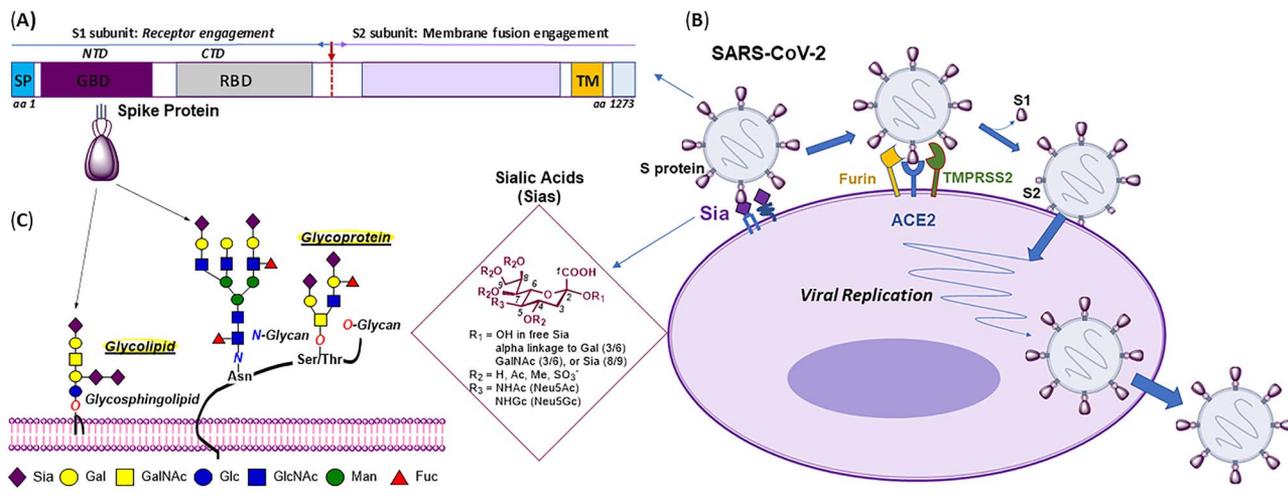
COVID-19 and SARS-CoV-2 virion

Coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has swept across the world, producing devastating effects not only on human health but also on the global economy (Lu et al. 2020; Zhu et al. 2020). SARS-CoV-2 belongs to the coronaviridae family, which comprises four genera: *Alpha-*, *Beta-*, *Gamma-* and *Deltacoronavirus* according to a current proposal to the International Committee of Taxonomy of Viruses. Among them, seven coronaviruses (CoVs) (CoV-229E, CoV-NL63, OC43-CoV, HKU1-CoV, SARS-CoV, MERS-CoV and SARS-CoV-2) infect humans, which are all thought to be of zoonotic origin (Table I) (Mittal et al. 2020). SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2003 and 2012, respectively. SARS-CoV-2 has emerged in late 2019 and holds a contagious capacity greater than any other previous human CoVs. Tremendous progress has been made, aimed at understanding infection and transmission mechanisms and pathogenesis and developing potential therapeutic, preventive and diagnostic strategies. It has been known that SARS-CoV-2 and SARS-CoV spike (S) protein share the same functional host cell receptor, angiotensin-converting enzyme 2 (ACE2). Recent studies confirmed the structural similarities of the receptor interaction between the S protein of SARS-CoV and SARS-CoV-2, but also identified some key divergences (Mittal et al. 2020; Shang et al. 2020). A surface plasmon resonance (SPR)-based kinetic study demonstrated that SARS-CoV-2 S protein has higher affinity ($K_D = \sim 15$ nM) on binding to ACE2, which is about 10- to 20-fold higher than SARS-CoV S protein's binding affinity to ACE2 (Wrapp et al. 2020). Several mechanisms that make SARS-CoV-2 transmission more efficient and aggressive than the previous CoVs have been proposed; however, many molecular details are still lacking (Elrashdy et al. 2020).

SARS-CoV-2 and S protein

The SARS-CoV-2 virion is a spherical single-stranded RNA virus, which is enveloped with an average envelope diameter between 65 and 97 nm (Yao et al. 2020). Like other CoV, it contains S protein trimers protruding from the envelope surface, which mediates SARS-CoV-2 infection through binding to host cell surface receptor. The trimeric S protein contains 1273 amino acids and consists of S1

and S2 subunits (Fig. 1A) (Yao et al. 2020; Casalino et al. 2020). The S1 subunit can be further divided into the N-terminal domain (NTD) and the C-terminal domain (CTD) (Casalino et al. 2020). The NTD contains glycan-binding domain (GBD) that interacts with glycoproteins and glycolipids on the host cell surface in most CoVs (Tortorici et al. 2019). The CTD contains the receptor-binding domain (RBD) that binds to the ACE2 receptor, which is largely recognized as the main entry route for SARS-CoV-2 and other CoVs into host cells (Yan et al. 2020).

SARS-COV-2 receptors and host enzymes

Host cell receptor is the key for SARS-CoV-2 tropism, transmission and pathogenesis. Recent studies confirmed that host cellular ACE2 serves as the viral receptor and mediates the process of SARS-CoV-2 infection in human cells. In particular, the virus S protein binds to the cellular ACE2 receptor that is distributed all over the surface of a large diversity of cell types from the upper airways and lungs (Lan et al. 2020; Ou et al. 2020; Wang et al. 2020). In addition, host cell surface enzymes have also been recognized as important players in the infectious process, such as the transmembrane protein TMPRSS2 (Hoffmann et al. 2020a) and furin (Örd et al. 2020) (Fig. 1B). TMPRSS2, the primary serine protease in many epithelial cells, has been reported to promote cleavage of S protein to induce SARS-CoV-2 invasion (Glowacka et al. 2011; Benton et al. 2020; Matsuyama et al. 2020; Sternberg et al. 2020; Hoffmann et al. 2020b). Specifically, after binding to the ACE2 receptor, the S protein is cleaved by TMPRSS2 into two subunits, S1 and S2; subsequently, the remaining screw-like S2 starts work by fusing with the cell membrane and allowing the virus to enter the cell (Benton et al. 2020). Furthermore, furin, a specialized serine endoprotease that cleaves the multibasic PRRAR motifs in the S protein, is not present in any CoV species up to date (Örd et al. 2020). This is another contributing factor in the replication and virulence of SARS-CoV-2. It is clear now that cleavages of SARS-CoV-2 S protein by both TMPRSS2 and furin contribute to the high pathogenicity of the novel SARS-CoV-2.

Potential co-receptors/attachment factors for SARS-CoV-2 infection

Despite the central role of ACE2 in the virus–human cell interaction, other host cell surface molecules are proposed as potential

Table I. Classification of coronaviruses and their ACE2 and sialic acid bindings

	Coronavirus												
Genus:	*Alphacoronavirus*				*Betacoronavirus*					*Gamacoronavirus*		*Deltacoronavirus*	
Lineage:					A					B		C	D
Virus:	HCoV-229E	HCoV-NL63	PEDV	TGEV	BCoV	HCoV-HKU1	HCoV-OC43	MHV	PHE	SARS-CoV (2003)	SARS-CoV-2 (2019)	MERS-CoV (2012)	IBV
ACE2-binding:	-	+	-	-	-	-	-	-	-	+	+	-	-
Sia-binding:	-	-	+	+	+	+	+	+	+	-	?	+	+

Major coronaviruses that bind the host receptor ACE2 and sialic acid (Sia) are listed. HCoVs are shown in red. BCoV, bovine coronavirus; HCoVs, human CoVs; IBV, infectious bronchitis virus; MERS-CoV, Middle East respiratory syndrome coronavirus; MHV, mouse hepatitis virus; PEDV, porcine epidemic diarrhea virus; PHEV, porcine hemagglutinating encephalomyelitis virus; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TGEV, transmissible gastroenteritis virus. The table is inspired from the report by Mittal et al. (2020).
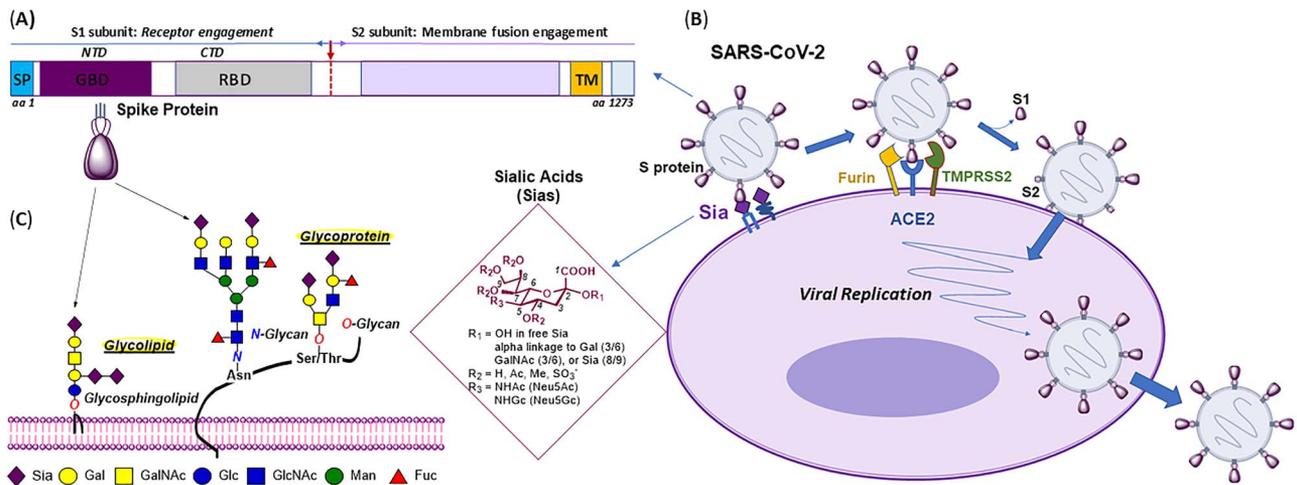
Fig. 1. Predicted sialic acid-dependent attachment of SARS-CoV-2 and its cellular entry pathway: (**A**) Spike protein; (**B**) SARS-CoV-2 spike protein interacts with both cell surface sialic acids and ACE2 for virus cell entry; (**C**) sialic acids (Sias) and their linkages on cell surface glycolipid and glycoprotein. CTD, C-terminal domain; GBD, ganglioside binding domain, NTD, N-terminal domain; RBD, receptor-binding domain; SP, signal peptide; SR, serine–arginine-rich; TM, transmembrane domain.

co-receptors/attachment factors for ACE2-dependent SARS-CoV-2 entry, such as neuropilin, heparan sulfate and sialic acid (Zamorano Cuervo and Grandvaux 2020; Seyran et al. 2020). In particular, it is predicted that SARS-CoV-2 S protein interacts with both cell surface ACE2 and sialic acids through its RBD and a sialic acid-binding domain, which is close to the RBD. These co-receptors/attachment factors are thought to contribute to SARS-CoV-2's overwhelming contagious capacity over other HCoVs (Fig. 1B) (Zamorano Cuervo and Grandvaux 2020; Seyran et al. 2020). This mini-review discusses what has been predicted and known so far about the host cell surface sialic acid's role for of SARS-CoV-2 infection and future research perspective.

Sialic acid bindings of CoVs and two-step attachment mechanism

Numerous viruses recognize host cell surface sialic acids, a family of nine-carbon sugar neuraminic acid (5-amino-3,5-dideoxy-D-*glycero*-D-galactononulsonic acid) derivatives present at the terminal of the glycans of both glycolipid and glycoprotein on all vertebrate cells (Fig. 1C) (Matrosovich et al. 2015; Park 2019; Wasik et al. 2016). Overall, sialic acids occur in an extraordinary structural diversity, which arises from the composition and complexity of the glycan chain, differences in the glycosidic linkage through which the sialic acid is joined to the adjacent sugar residue. In addition, differential modifications of sialic acid exist at C5 [either *N*-acetyl moiety (*N*-acetylneuraminic acid (Neu5Ac)) or *N*-glycolyl moiety (*N*-glycolylneuraminic (Neu5Gc))] in combination with modifications of the hydroxyl groups at C4, C7, C8 and C9 by acetyl, methyl and sulfate groups (Fig. 1C) (Varki 2008).

Sialic acids are highly expressed on epithelial cells, including those in the lungs and the oral cavity (Cross and Ruhl 2018), and are often used as receptors for lung viral infection. Sialic acid binding activity has been confirmed for many CoVs (Table I). For example, the alphacoronavirus transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus recognize host cell surface sialic acids (Vlasak 1988; Schultze et al. 1996). Many betacoronaviruses from lineage A such as bovine coronavirus (BCoV), porcine hemagglutinating encephalomyelitis virus (PHEV), HCoV-OC43, HCoV-HKU1, the JHM strain of mouse hepatitis virus and lineage C MERS-CoV

have also been shown to interact with sialic acid moieties for cell entry processes (Kremplet al. 1997; Schwegmann-Weßels and Herrler 2006; Peng et al. 2012; Huang et al. 2015; Liu et al. 2015; Lim et al. 2016; Hulswit et al. 2019; Park et al. 2019; Tortorici et al. 2019; Widagdo et al. 2019; Qing et al. 2020). In addition, gammacoronavirus infectious bronchitis virus also recognizes sialic acids by its S protein (Promkuntod et al. 2014; Winter et al. 2016). However, since the outbreak in 2003, there has been no report that SARS-CoV can bind sialic acids yet.

It has been known that 9-O-acetylated sialic acids (9-O-Ac-Sias) are recognized by binding of the NTD of S protein of many betacoronaviruses. Structural analysis of the PHEV S protein revealed a site within the NTD formed by two hydrophobic pockets compatible with 9-O-Ac-Sias binding (Hulswit et al. 2019). Further, a recent cryo-electron microscopy (cryo-EM) analysis of HCoV-OC43 S protein revealed in unprecedented detail of the binding pocket in the NTD that allows interaction with 9-O-Ac-Sias (Tortorici et al. 2019). Sialic acid is joined to the adjacent sugar residue in different glycosidic linkages. It was found that both TGEV (Krempl et al. 1997) and IBU (Winter et al. 2016) preferentially recognize α2,3-linked sialic acid. In addition, MERS-CoV S protein also preferentially binds to α2,3-linked sialic acid over α2,6-linked sialic acid receptors, which are abundant in the major sites of replication in lower respiratory tracts, particularly the alveoli of the human lung, which likely explains the tropism and transmission (Widagdo et al. 2019). Another cryo-EM and crystallography study showed that MERS-CoV S protein binds sialic acids in a groove within the NTD near to the binding site to the dipeptidyl peptidase 4 entry receptor, supporting a two-step attachment mechanism (Park et al. 2019). A most recent study showed a similar mechanism that sialic acids play distinct role for MERS-CoV infection (Qing et al. 2020). This study illustrated the flexible nature of coronavirus S proteins with an NTD capable of dual-binding modalities enabling attachment to both sialic acids and protein (mCEACAM1a) receptors. Consequently, viral attachment likely occurs in a two-step fashion, the first low affinity binding to sialic acid-containing molecules followed by higher affinity binding to the protein receptor nearby, together leading to an efficient cell entry process (Qing et al. 2020).

In silico and microscopy studies of sialic acids for SARS-CoV-2 Binding

The observations for sialic acid binding by other CoVs led to the assumption of the potential sialic acids binding during SARS-CoV-2 infection (Milanetti et al. 2020; Petrosilloet al. 2020; Vandelli et al. 2020). Several in silico predictions and modeling and microscopy studies indicated sialic acid binding possibility of SARS-CoV-2, which could contribute to the overwhelming contagious capacity of SARS-CoV-2 over other HCoVs. A recent study on structure-based sequence comparison of the NTD of S protein of SARS-CoV-2 with MERS-CoV and SARS-CoV showed three divergent loop regions in SARS-CoV-2, which are similar with MERS-CoV sialoside-binding pockets (Awasthi et al. 2020). Also, comparative binding analysis with host sialosides revealed conformational flexibility of SARS-CoV-2 divergent loop regions to accommodate diverse glycan-rich sialosides. These similarities with MERS-CoV and differences with SARS-CoV suggest an evolutionary adaptation of SARS-CoV-2 S protein, which facilitates its interaction with host cell surface sialic acids and then viral infection with host cells with wide tissue tropism (Vandelli et al. 2020). In addition, in silico analyses through molecular docking
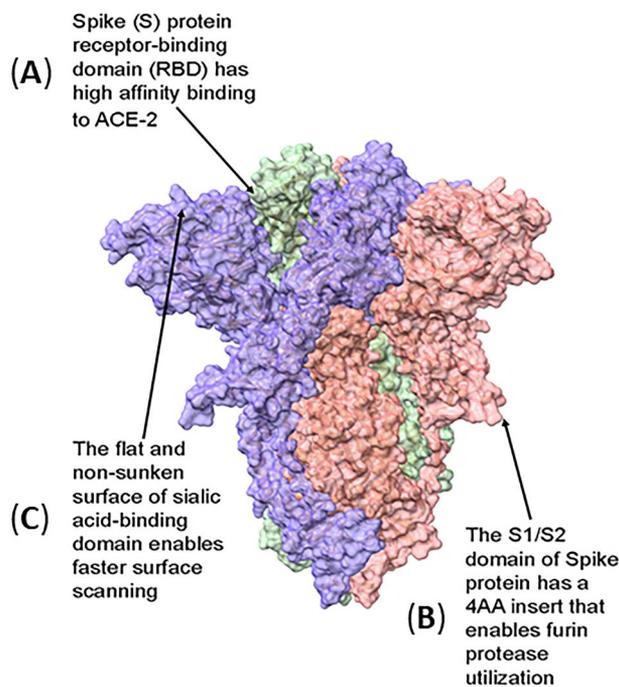
Fig. 2. Protein structure model of SARS-CoV-2 S protein showing (**A**) the RBD for ACE2 binding, (**B**) the S1/S2 domain with a four-amino acid insert for furin cleavage and (**C**) the flat sialic acid-binding domain for host cell surface sialic acid binding (Seyran et al. 2020).

simulations and electronic density mapping surface also predicted the existence of a sialic acid-binding site in SARS-CoV-2 NTD domain similar to that in MERS-CoV (Awasthi et al. 2020; Milanetti et al. 2020).

Cryo-EM is a powerful tool to determining the high-resolution structures of many viral assemblies as well as those of assembly intermediates (Luque and Castón 2020). A recent cryo-EM study of SARS-CoV-2 S protein revealed much more extended loops in the NTD region as previously reported (Wrobel et al. 2020). The comparison of the overall structure of SARS-CoV-2 NTD with that of BCoV NTD indicated its binding of 9-O-acetylated sialic acids. Intriguingly, SARS-CoV-2 NTD appears to retain core structural features of the NTD of BCoV and other members of the *Betacoronavirus* genus. This further indicates the sialic acid-binding possibility of SARS-CoV-2.

It has been known that many HCoV S proteins interacts with host cell surface sialic acids through weak and reversible hydrogen bond interactions that promote viral surfing over host cell surface (Burckhardt and Greber 2009). For SARS-CoV-2 S protein, a flat and nonsunken sialic acid-binding domain has been proposed (Fig. 2) (Seyran et al. 2020). Specifically, a flat surface of the 290 amino acid residue-long NTD of SARS-CoV-2 S protein was proposed to facilitate S protein's sialic acid-binding capacity and thereby promotes viral surfing on the host cell surface like other HCoVs (Caldas et al. 2020; Milanetti et al. 2020; Seyran et al. 2020). Indeed, a more effective cell entry was proposed through the dual and even triple binding of SARS-CoV-2 to ACE-2 receptor and gangliosides present over lipid rafts of host cells, forming a trimolecular complex (Milanetti et al. 2020).

Recent molecular dynamic simulation studies of SARS-CoV-2 S protein interaction with a model ganglioside GM1, a glycosphingolipid containing one sialic acid residue, indicated the formation of a trimolecular complex between a glycan binding domain (GBD)
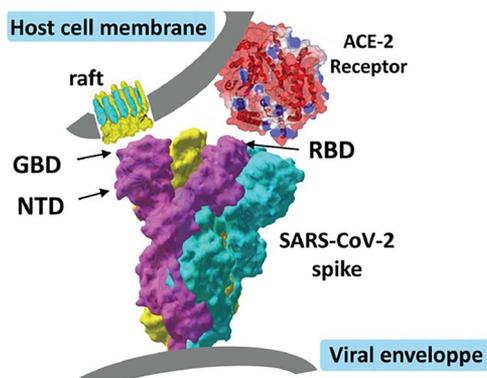
Fig. 3. Proposed dual recognition of ACE2 and gangliosides by SARS-CoV-2 S protein. The S protein displays two distinct domains, the tips of which are available for distinct types of interactions. RBD binds to the ACE2 receptor, and the GBD in NTD binds to the ganglioside-rich domain of the plasma membrane. Lipid rafts, which are membrane domains enriched in gangliosides (in yellow) and cholesterol (in blue), provide an attractive interface for adequately positioning the viral S protein at the first step of the viral infection process (Fantini et al. 2020).

(111–162 aa) and two GM1 molecules (Fig. 3) (Fantini et al. 2020; Sántha et al. 2020). These studies proposed that SARS-CoV-2 S protein could bind to ganglioside regions exposed at the cell membrane potentially favoring the subsequent interaction of the RBD with ACE2. Also, the GBD of the SARS-CoV-2 S protein consists in a flat electropositive surface at the tip of the NTD. This GBD may allow a functional interaction of the virus with lipid rafts of the plasma membrane independently of the RBD. Lipid rafts are lipid microdomains enriched in cholesterol and glycosphingolipids. The proposed lipid rafts in SARS-CoV-2 infection are consistent with the reported role of lipid rafts in the infection cycle of other CoVs (Glende et al. 2008; Lu et al. 2008; Radenkovic et al. 2020). In particular, lipid rafts coalescence may lead to the recruitment of the ACE2 receptor, which also exists in the lipid rafts (Glende et al. 2008). The lipid raft-initiated concentration of virus particles has been reported for HIV-1 fusion process (Hammache et al. 1998; Fantini et al. 2021). Actually, ganglioside expression is higher in epithelial intestinal and brain cells, both of which are infected by SARS-CoV-2 (Engin et al. 2020; Fenrich et al. 2020). In light of what is currently predicted regarding CoV NTD-sialic acid binding, the question whether SARS-CoV-2 can bind to and functionally use sialic acids/ganglioside for cell entry still remains to be more fully investigated in vitro and in vivo as well.

Microarray studies of sialic acids for SARS-CoV-2 binding

Glycan microarray is a useful tool for screening specific glycan-protein interaction. Sialoglycan microarray reveals the interactions of sialic acids with proteins and viruses (Song et al. 2011). Sialoglycan microarray can be used to screen S protein's sialic acid binding event. However, a recent sialoglycan microarray study did not detect significant fluorescent signals when recombinant SARS-CoV-2 S protein was incubated with sialic acid-containing oligosaccharides on array chip (Hao et al. 2020). This result could not define if SARS-CoV-2 S protein binds sialic acid or not. It is important to note that the immobilized sialic acids may not fully mimic the native sialic acid presentation on the cell surface in vivo, where sialic acid-containing molecules are present in the flexible plasma membrane environment

and can form a cluster of sialic acids. A great challenge for using microarray to study viral infection mechanism is the production of artificial systems that are able to mimic the molecular recognitions in living systems. If this is the case, multidimensional membrane mimetic glycan array might be useful (Narla et al. 2015). In this perspective, a formation of sialic acid-containing membrane cluster may be required for SARS-CoV-2 S protein binding in vivo.

Recently, a polymer-stabilized gold nanoparticle bearing sialic acids was used to detect SARS-COV-2 S protein binding by Baker et al. (2020), which demonstrated a selective binding toward recombinant SARS-COV-2 S protein over SARS-COV S protein and a panel of lectins. They further confirmed the sialic acid binding with virus mimicking particle bearing SARSCOV-2 S1 and SARS-COV-2 S protein-presenting pseudotyped lentivirus, respectively. Interestingly, this study preliminarily confirmed that single sialic acid showed stronger binding to recombinant SARS-COV-2 S protein over α2,3-sialyllactose and α2,6-sialyllactose present on the polymer-stabilized gold nanoparticles, indicating that only the sialic acid unit, not the lactose, is engaged in binding. This indicates that the binding pocket of the SARS-COV-2 S protein may be shallow. Further investigations are highly needed to support this observation.

In vitro and ex vivo studies of sialic acids on ACE2 receptor for SARS-CoV-2 binding

ACE2 serves as receptor for SARS-CoV-2 infectious process. ACE2 receptor is extensively glycosylated, with both N- and O-glycans, which contain sialic acids (Shajahan et al. 2020). Therefore, in addition to sialic acids on the proposed gangliosides, the sialic acids on ACE2 receptor are potential additional attachment factors for the virus anchoring to host cells as well (Radzikowska et al. 2020). A recent study by Neelamegham and co-workers investigated the role of N- and O-glycans and sialic acids on ACE2 receptor expressed on HEK293T cells for SARS-CoV-2 viral entry (Yang et al. 2020). In this study, the sugar structures displayed by the ACE2 receptor were modified either genetically or chemically, using a small molecule that disrupts the formation of the glycans. They found that N- and O-glycans had only minor contribution to ACE2-S protein binding. Interestingly, sialidase (*Arthrobacter ureafaciens* α2–3,6,8,9-neuraminidase) treatment of ACE2 expressed on HEK293T cells, increased recombinant RBD-Fc and S1-Fc binding by 26 and 56%, respectively. Furthermore, the ACE2 sialic acids modestly shield pseudovirus binding as well. These results indicate a precluding role of ACE2 receptor sialic acids for SARS-CoV-2 binding.

A most recent study by Yuen and colleagues using neuraminidase (NA) treatment of human lung epithelial cells and ex vivo human lung tissues demonstrated a different role for sialic acids on MERS-CoV, SARS-CoV and SARS-CoV-2 infection (Chu et al. 2021). In this study, they pre-treated epithelial Calu3 and Caco2 cells with NA from *Arthrobacter ureafaciens* to remove cell surface sialic acids, followed by incubation with MERS-CoV, SARS-CoV and SARS-CoV-2, respectively, and quantified virus production in the cell lysates and supernatants at 24 h post infection. They found that NA treatment of Calu-3 human airway cells reduced MERS-CoV entry by 86% compared with mock treatment. This is consistent with the fact that MERS-CoV uses sialic acids as co-receptors. However, instead of ACE2, MERS-CoV uses dipeptidyl-peptidase 4 as entry receptor (Park et al. 2019). In contrast, NA treatment did not reduce but significantly increased SARS-CoV infection (492%). Interestingly, NA treatment did not reduce but just modestly increased SARS-CoV-2 infection (80.3%). Furthermore, ACE2 glycosylation mutants

study demonstrated that the sialic acid present on ACE2 receptor prevents SARS-CoV-2 S–ACE2 interaction, which is consistent with previous study that NA treatment of ACE2-expressing HEK293T cells increased SARS-CoV-2 S protein binding (Yang et al. 2020). In addition, they treated ex vivo human lung tissues with NA, followed by SARS-CoV-2 and SARS-CoV challenge. They found that in comparison with SARS-CoV, infection of SARS-CoV-2 to human lung tissues is less affected by sialic acid-mediated restriction. It was speculated that the capacity of SARS-CoV-2 to overcome sialic acid-mediated restriction may contribute to its efficient person to person transmission in comparison with SARS-CoV. It should be pointed out that due to the NA's substrate specificity, only certain cell surface sialic acids can be released. However, there was no detailed desialylation of the cells and lung tissues confirmed in this study. Therefore, it is an area that calls for further in vitro and in vivo investigation. Further, it is necessary to profile the sialoforms of ACE2 in detail and define the specific sialoform for precluding S protein binding in vivo.

Another most recent study by Crispin and co-workers investigated whether the glycosylation state of ACE2 receptor impacts the interaction with SARS-CoV-2 S protein (Allen et al. 2021). They generated a panel of glycan modified ACE2 variants. Using a mass spectrometric approach, they determined the site-specific glycan structures present on ACE2 both with and without glycan engineering. Then, they used SPR to determine binding affinities between SARS-CoV-2 S protein and ACE2 variants. They found that that when ACE2 glycans are hypersialylated, or when all glycans were converted to oligomannose type, there was a modest decrease in affinity. However, when the sialic acid residues were removed, a statistically significant but modest increase in affinity was observed. This result is consistent with previous two studies that NA treatment of ACE2-expressing HEK293T cells (Yang et al. 2020) and epithelial Calu3 and Caco2 cells (Chu et al. 2021) increased SARS-CoV-2 S protein binding, indicating a different role of sialic acids on ACE2 for SARS-CoV-2 binding and infection.

In vitro studies of sialic acids on glycolipids for SARS-CoV-2 binding

As discussed above, molecular dynamic simulation studies suggest that SARS-CoV-2 S protein could bind gangliosides for viral entry (Fantini et al. 2020). A most recent study led by Macauley and Klassen revealed that the RBD of SARS-CoV-2 S protein recognizes monosialylated gangliosides (Nguyen et al. 2021). They found that the pentasaccharide of ganglioside GM1 was the preferred ligand of RBD in their initial CaR-ESI-MS screening of their glycan libraries. Then, they used an artificial membrane embedded within gangliosides to confirm the specific binding of the RBD. They found that GM1, GM2 and GM3 gangliosides were recognized by the RBD and the monomeric affinities ($KD = 100–200$ µM) of gangliosides for the RBD are similar to heparan sulfate, which are proposed as a negatively charged glycan ligand of the RBD. Next, they used sialyltransferase inhibition, genetic knockout of sialic acid biosynthesis and NA treatment of ACE2-expressing HEK293 cells to confirm the RBD binding and infection of SARS-CoV-2 pseudotyped lentivirus to the ACE2-expressing cells, all which showed decreased binding and infection upon depleting cell surface sialic acid level. Together, these results suggest that gangliosides facilitate viral entry of SARS-CoV-2, which are agree with molecular dynamic simulation studies of SARS-CoV-2 S protein with ganglioside GM1 (Fantini et al. 2020).

Summary and future perspective

SARS-CoV-2 is a highly contagious virus that uses multiple ways for rapid and aggressive transmission. It has become clear that the highly transmission nature of SARS-CoV-2 is based on the unique structural features of its S protein, which can bind not only ACE2 receptor but also other host cell molecules for its cell entry. It is common for many CoVs that use S protein to bind host cell surface sialic acids as receptor or co-receptors for their cell entry. Several in silico predictions modeling and microscopy studies indicate that SARS-CoV-2 can bind cell surface sialic acids through the S protein NTD that displays a flat ganglioside binding site and enables the virus to bind lipid rafts of the plasma membrane, where the ACE2 receptor also resides. As predicted in part by the in silico modeling, this ganglioside-binding domain consists of a large flat interface enriched in aromatic and basic amino acid residues.

Gangliosides are a family of glycosphingolipids bearing one or more sialic acid residues. Gangliosides present on cell surfaces in all mammalian in conjugation with other lipids and cholesterol to form membrane microdomain organization like lipid rafts, which are involved in many biological processes in human physiology and pathology like viral infections (Aerts et al. 2019). A most recent study revealed that monosialylated gangliosides could serve as the ligands for the RBD of SARS-CoV-2 S protein (Nguyen et al. 2021). Several functional roles that gangliosides can play in virus infection have been proposed so far (Fantini et al. 2021). First, due to the negative sialic acid, cell surface gangliosides can provide a negatively charged flat surface that attracts the electropositive tip of virus envelope proteins (Fantini et al. 2015). Second, they can facilitate the recruitment of virus protein receptors that exist in the same lipid rafts (Hammache et al. 1998; Rawat et al. 2006; Lu et al. 2008). Third, they are closely associated with cholesterol to form the lipid rafts that enhance the membrane fusion process (Rawat et al. 2006; Fantini et al. 2019). Finally, they can modify the conformation of the bound viral proteins through the membrane chaperone properties and thus activate the viral proteins (Fantini et al. 2002, 2015). All these factors contribute to virus fusion with host cell membranes (Fantini et al. 2019) as confirmed with several pathogenic viruses including HIV-1 (Fantini et al. 2000, 2019). Overall, except GM1, there are many gangliosides in the lipid rafts, and their compositions are sensitive to the cellular environments and stimuli (Aerts et al. 2019). Therefore, profiling the specific gangliosides and lipid rafts in the host cells related to SARS-CoV-2 infection deserves a deeper exploration. Finally, while sialic acids may function as co-receptors or attachment factors to simply tether a virus to the host cell membrane, whether sialic acid binding can play a role in SARS-CoV-2 cell entry process, such as plasma membrane fusion entry or endosomal pathway, which also deserves a higher consideration and detailed exploration. If all these molecular mechanisms are confirmed, they will provide tremendous opportunities for developing effective therapeutic, preventive and diagnostic tools for the COVID-19 pandemic.

On the other hand, recent in vitro and ex vivo studies of sialic acid on ACE2 receptor indicated an opposite role for SARS-CoV-2 binding. In particular, NA treatment of epithelial cells (Chu et al. 2021) and ACE2-expressing 293T cells (Yang et al. 2020) increased SARS-CoV-2 binding. Removal of sialic acid by NA (desialylation) could modulate the functionality of the Sia-containing molecules, which is often involved in signal transduction in either physiological or pathological processes (Pshezhetsky and Ashmarina 2013). Therefore, NA's involvement in SARS-CoV-2 infection deserves a full investigation. In particular, both exogenous and endogenous

sialidases can contribute the desialylation of cell surface receptors and thus sialidases can be drug targets for COVID-19 treatment. Overall, the cell surface sialic acids have been recognized and explored for ARS-CoV-2 infection. The coming years should reveal the details of the role of sialic acids in SARS-CoV-2 infection.

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Conflict of interest statement

The author declares no competing financial interest.

Abbreviations

ACE2, angiotensin-converting enzyme 2; BCoV, bovine coronavirus; COVID-19, coronavirus disease 2019; cryo-EM, cryo-electron microscopy; CTD, C-terminal domain; human CoVs (HCoVs); IBV, infectious bronchitis virus; MERS-CoV, Middle East respiratory syndrome coronavirus; MHV, mouse hepatitis virus; NA, neuraminidase; NTD, N-terminal domain; PHEV, porcine hemagglutinating encephalomyelitis virus; RBD, receptor-binding domain; SARS-CoV-1, severe acute respiratory syndrome coronavirus 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SPR, surface plasmon resonance; TGEV, transmissible gastroenteritis virus; TMPRSS2, transmembrane protein transmembrane protease serine type 2.

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