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Review Article

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Structure of SARS-CoV-2 Spike Glycoprotein for Therapeutic and Preventive Target

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ABSTRACT

The global crisis caused by the coronavirus disease 2019 (COVID-19) led to the most significant economic loss and human deaths after World War II. The pathogen causing this disease is a novel virus called the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of December 2020, there have been 80.2 million confirmed patients, and the mortality rate is known as 2.16% globally. A strategy to protect a host from SARS-CoV-2 is by suppressing intracellular viral replication or preventing viral entry. We focused on the spike glycoprotein that is responsible for the entry of SARS-CoV-2 into the host cell. Recently, the US Food and Drug Administration/EU Medicines Agency authorized a vaccine and antibody to treat COVID-19 patients by emergency use approval in the absence of long-term clinical trials. Both commercial and academic efforts to develop preventive and therapeutic agents continue all over the world. In this review, we present a perspective on current reports about the spike glycoprotein of SARS-CoV-2 as a therapeutic target.

Keywords: COVID-19; SARS-CoV-2; Spike glycoprotein; Angiotensin-converting enzyme 2; Molecular targeted therapy

INTRODUCTION

A novel viral disease, coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in December 2019 in Wuhan, China (1). The virus has been widespread all over the world within a few months, becoming an unprecedented pandemic. The number of infected patients is exploding every second, and currently, that reaches 80.2 million cases and 1.74 million deaths. This number is increasing even when we are writing this article. The mortality

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Conflict of Interest

The authors declare no potential conflicts of interest.

Abbreviations

ACE2, Angiotensin-converting enzyme 2; ADE, Antibody-dependent enhancement; ARDS, Acute respiratory distress syndrome; BH, β-hairpin; CH, central helix; CoV, coronavirus; COVID-19, coronavirus disease 2019; CR, connected region; CT, cytosolic tail; ER, endoplasmic reticulum; ERD, enhanced respiratory disease; FDA, Food and Drug Administration; FIPV, feline infectious peritonitis virus; FP, fusion peptide; HR, heptad repeat; L, loop; MERS-CoV, Middle East respiratory syndrome coronavirus; NSP, non-structural proteins; NTD, N-terminal domain; RBD, receptor binding domain; RBM, receptor binding motif; S, spike; S1, subunit 1; S2, subunit 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, subdomain; SP, Signal sequence; TM, transmembrane; +ssRNA, positive-sense single-stranded RNA.

Authors Contributions

Conceptualization: Jhun H, Choi YO, Bae S, Lee Y, Song CS, Yeom SC, Kim S; Funding acquisition: Hong J, Kim S; Supervision: Kim S; Validation: Kim S; Writing - original draft: Hong J, Jhun H, Choi YO, Kim S; Writing - review & editing: Hong J, Taitt AS, Kim S.

rate is 2.16% globally, but these figures might be over-estimated due to under testing (2). The lockdown is the best strategy in this pandemic situation, although several vaccines and neutralizing antibody therapies were passed emergency use approval in the US and EU (3). The COVID-19 symptoms vary by individual from asymptomatic to severe (4). If the patient is critically ill for acute respiratory distress syndrome (ARDS), oxygen therapy and a mechanical ventilator are required for treatment (1). For now, there is no established treatment for COVID-19 patients.

SARS-CoV-2 is a *Coronaviridae* family member. *Coronaviridae* family has many virulent viruses that infect humans and animals, other than SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) (5). The coronavirus infection initiates with the spike (S) glycoprotein binding to the receptor for cell entry such as aminopeptidase N of HCoV-229E, angiotensin-converting enzyme 2 (ACE2) of HCoV-NL63, SARS-CoV and SARS-CoV-2, and dipeptidyl peptidase 4 of MERS-CoV. The life cycle of coronaviruses, in brief, shows the expression and replication of genomic RNA to generate the full-length copy, which incorporates into the nascent viral particles (6).

The S glycoprotein binds to the cellular receptor after the enzymatic digestion by host factors such as the cell surface serine protease, TMPRSS2, promoting viral uptake and fusion at the cellular membrane. After finishing the entry process, genomic RNA is released and uncoated, followed by the immediate translation of ORF1a and ORF1b. The produced pp1a and pp1ab are processed to the non-structural proteins (NSP), which form the viral replication and transcription complex. Parallelly with the NSP expression, the biogenesis of viral replication organelles such as perinuclear double-membrane vesicles, convoluted membranes, and small open double-membrane spherules build a protective microenvironment for viral genomic RNA replication and transcription of subgenomic mRNAs consisting the nested set of coronavirus mRNAs. After the translation, structural proteins translocate into endoplasmic reticulum (ER) membranes and undergo the ER-to-Golgi intermediate compartment, where they interact with N-encapsid. Newly produced genomic RNA buds into the secretory vesicular lumen. Finally, the viral particles are secreted from the infected cells by exocytosis (7).

Currently, many coronavirus (CoV) vaccines have been developed for domestic animal usages against canine CoV, feline CoV, bovine CoV, porcine epidemic diarrhea virus, transmissible gastroenteritis virus, and infectious bronchitis virus (8). On the other hand, none of the vaccines against human CoV has been tested for a long-term clinical trial other than emergency use authorization. The most similar vaccine candidates close to the full license are two candidates for SARS-CoV and three candidates for MERS-CoV in phase I clinical trials (9). Even though none of the human vaccines has succeeded, previous experiences of human and animal vaccine development for CoVs have suggested brilliant ideas, and the insight develops SARS-CoV-2 vaccines.

The positive-sense single-stranded RNA (+ssRNA) coronavirus, causing severe human infection, was first reported as the SARS-CoV-2 nearly 2 decades ago (10). Including SARS-CoV, beta coronaviruses have caused zoonotic epidemics or pandemics in humans three times after the SARS outbreak. They are SARS in 2002–2003 from China, MERS-CoV in 2012 from the Middle East, and SARS-CoV-2 from China beginning in late 2019. Unlike the previous two, the current COVID-19 outbreak is overwhelmingly contagious and is causing the worst global pandemic in human history (2,4,9).

Understanding the immune correlates of the virus and protection is critical to developing a vaccine against an emerging infectious disease. Much of the immune reactions of SARS-CoV-2 infection is still unclear, but the previous studies demonstrate both humoral and cellular immunity have essential roles in the protection from COVID-19. In non-human primates, vaccine-induced neutralizing antibodies reduced the viral loads very efficiently after SARS-CoV-2 infection (11-14). The treatment and prevention of SARS-CoV-2 infection clinical trials in humans, passive administration of convalescent plasma, purified IgG, and mAbs showed beneficial effects (15-21). A neutralizing antibody was mostly authorized for emergency use as a treatment for COVID-19 by the Food and Drug Administration (FDA) recently (22). For example, the COVID-19 outbreak in a closed space like a fishery vessel, with a high infection rate, showed the protection of neutralizing Abs against SARS-CoV-2 (23).

T cell immunity is another crucial player in protective roles in CoV infections. T cell-deficient mice show viral clearance impairment in SARS-CoV, MERS-CoV, and SARS-CoV-2infections (24-27). In mild SARS-CoV-2 patients, $CD4^+$ and $CD8^+$ T cells specific to the virus were involved in the protective immunity against the virus (28-30). These immune protections show the ideal vaccine should evoke both humoral and cellular immunities for adequate protection. There is a safety concern about the potential SARS-CoV-2 vaccine or therapeutic antibodies, which can potentially enhance the disease. This enhancement of the disease is known as Ab-dependent enhancement (ADE) and the enhanced respiratory disease (ERD) (31). In cases where antibodies interact with the virus but do not neutralize, Fcγ receptor-mediated virus uptake may lead to ADE. The virus uptake can continue to replicate the viral particles, or the antibody-virus complex can stimulate Fc-mediated effector functions (31,32). ADE has been reported in flaviviruses such as the dengue virus and Zika virus (33-35) and observed in CoV infections. A live feline CoV—feline infectious peritonitis virus (FIPV)—challenge showed enhanced mortality when cats were immunized with viral protein-expressing vaccinia virus or passive administration of anti-FIPV antibody (36-39). ADE has also been reported in experimental SARS-CoV and MERS-CoV by animal models (40-45). ERD is another antibody-induced disease enhancement led by Th2 cell-biased immunopathology (46-49). Even without of the preclinical evidence of ADE or ERD in SARS-CoV animal models, safety must be considered when developing SARS-CoV-2 vaccines (41,50,51).

The S glycoprotein of SARS-CoV-2 is the most actively studied region of the virus for its possibility of immunogenicity of vaccines and the treatment target. Considering the likelihood of neutralizing the viral infection to host cells, S glycoprotein is the most rational target for developing vaccines and therapeutics. In this review, we will give insight into the development of vaccines of SARS-CoV-2, avoiding the enhancement of patients' immune responses such as ADE and ERD.

STRUCTURE OF S GLYCOPROTEIN

The S glycoprotein on the envelope of SARS-CoV-2 is consists of 16 subdomains (SDs) with 22 potential glycosylation sites in the extracellular domain, including a single transmembrane (TM) and cytosolic tail (CT) domains (**Fig. 1A and C**). Each S glycoprotein domain was illustrated with a different color bar, and the exact amino acid residue was indicated on the right. The receptor binding domain (RBD) of S glycoprotein in SARS-CoV-2 is studied intensively because the RBD is known for entering SARS-CoV-2 into host cells (52,53). The RBD is 227 amino acid residues shown by the light blue bar and thick red outline, the 4th domain from N-terminus (**Fig. 1C**).

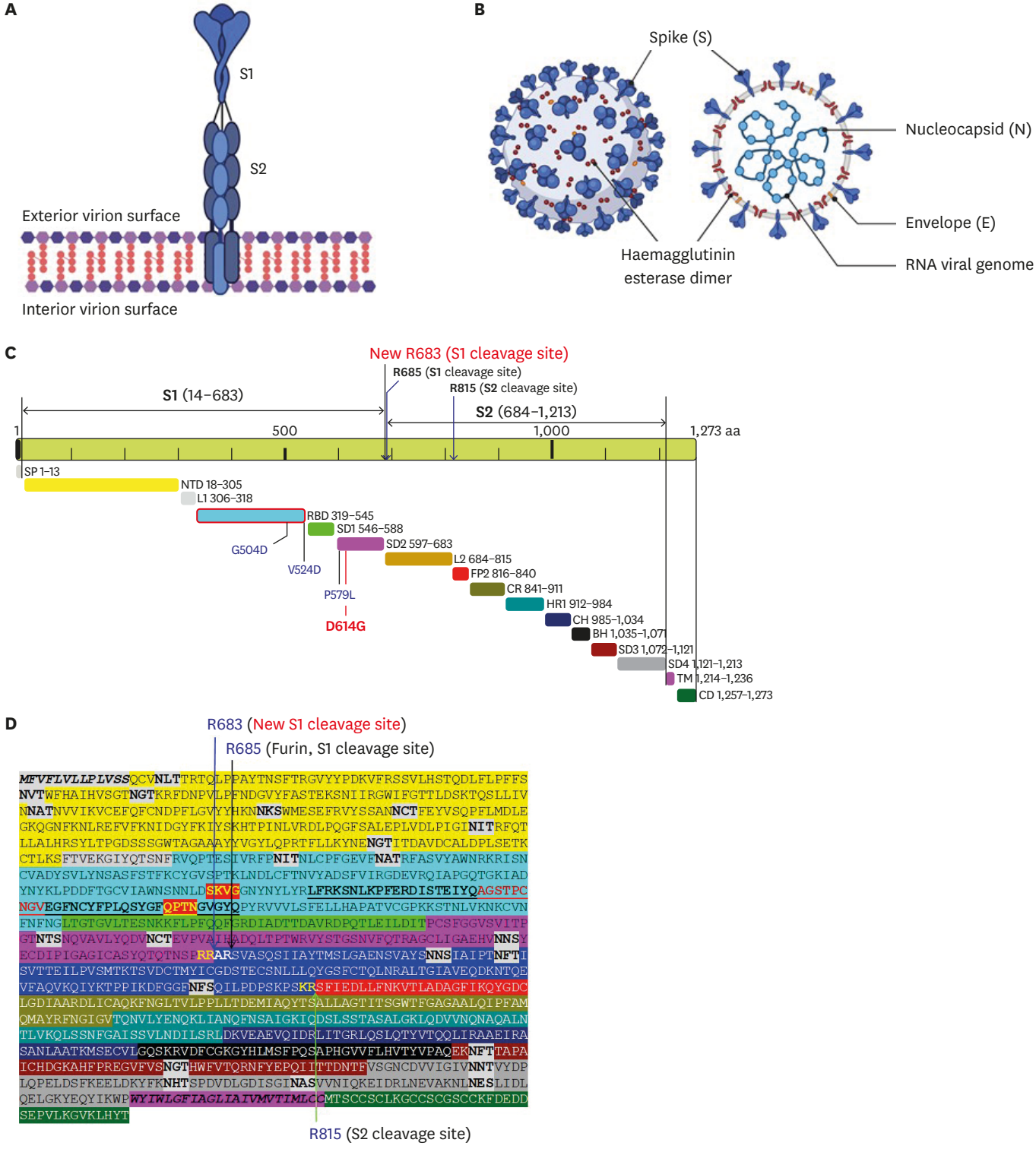

Figure 1. The structure of SARS-CoV-2. Structure of (A) the spike protein and (B) SARS-CoV-2. Created with BioRender.com. (C) Schematic drawing of 16 SDs in SARS-CoV-2 *S* gene. The S glycoprotein is composed of 16 SDs. The known S1 (R685), new S1 (R683), and S2 (R815) cleavage site were indicated at the top. There are 3 new mutation sites in the S1 region from Korean COVID-19 patients noted by blue letters, and aforenamed D614G is indicated by bold red letters. The 16 SDs of spike glycoprotein were illustrated by different colors with specific residues on the right. (D) The amino acid sequence of the *S* gene is divided into 16 SDs. The 16 SDs of S glycoprotein were highlighted by a different color identical to (C). The protease cleavage sites, new S1, furin S1, and S2 were indicated with arrows. The 22 potential glycosylation sites are marked with gray highlight and bold letter (**NxS/T**). Two RBM (SKVG/QPTN) in RBD were indicated by red highlight and yellow letters.

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The amino acid sequence analysis revealed that S glycoprotein has a hydrophobic signal peptide (SP) domain at N-terminus and a single TM domain at C-terminus following a short CT. The SP domain was highlighted by gray color and bold italic letters at N-terminus, while the TM domain was marked by thick pink color and bold italic letters at C-terminus (**Fig. 1D**). The TM domain is a robust hydrophobic stretch of polypeptide penetrating viral envelop or host cell membrane (**Fig. 1A**). The S glycoprotein is like a cytokine or growth factor receptor on the cell membrane, which possesses a single TM structure (54,55). This type of glycoprotein is a classical TM molecule expressed on the surface of viral envelope or host cell membrane (56). The amino acid residues of 16 SDs were highlighted by various colors (**Fig. 1D**) that were indicated by almost identical to the bar color of each domain in **Fig. 1C**.

The S glycoprotein has 22 potential glycosylation sites (**N**x**S**/**T**) marked with gray color and bold letters. The **N** is Asparagine, and **x** is any residue. The **S** is Serine and **T** is Threonine residue. Only three domains, SP, RBD, and TM, are known for precise functions among the 16 domains in the S glycoprotein. The N-terminal SP is involved in maturation, which leads the other extracellular parts of S glycoprotein to the plasma membrane then eventually, the SP is removed by an enzyme (57,58). The TM at the C-terminus function is to anchor the S glycoprotein in the bilayer lipid cell membrane. Therefore, most TM domain is approximately 20 amino acid of hydrophobic sequence, which fits the thickness of cell membrane (59). The third known functional domain is RBD, 227 amino acid residues highlighted by light blue possessing 2 potential glycosylation sites. The most critical site, the 52 amino acid residues responsible for interacting with ACE2, was underlined and highlighted with bold letters (**Fig. 1D**).

AN ENZYME RESPONSIBLE FOR S GLYCOPROTEIN S CLEAVAGE SITE

The S glycoprotein is composed of two large subunits, subunit 1 (S1) and subunit 2 (S2), in the extracellular part (**Fig. 1A and C**). The function of S1 is binding to ACE2, which is known as the receptor of SARS-CoV and SARS-CoV-2, whereas S2 likely plays a role in membrane fusion (60,61). The S1 starts at the residue Glutamine 14 after the SP domain and ends with Arginine 683 at the C-terminal of the S1 cleavage site. The major physiological function of ACE2 is to lower blood pressure by catalyzing the hydrolysis of a vasoconstrictor and angiotensin II into a vasodilator and angiotensin, respectively (62). The blue arrow indicates the suggested S1 cleavage site Arginine 683 in **Fig. 1C and D**. The new S1 cleavage site has two amino acid residues 'AR' shorter than the known S1 cleavage site Arginine 685, which indicated (**Fig. 1C and D**). The S2 starts at the residue Alanine 684 after the S1 cleavage site and ends with Proline 1,213 before the TM domain. The essential 52 amino acid residues SD interacting with ACE2 that was marked by underlined bold letters in RBD (**Fig. 1D**), exposed to ACE2 precise processing remained unclear.

S1 AND S2 SUBUNIT FUNCTION IN INFECTION

The entry of SARS-CoV-2 into the host cell requires cleavage-dependent activation of the S glycoprotein. During the viral infection, including attachment and entry into cells, the S glycoprotein is cleaved into the S1 and S2 subunits, and then the S2 subunit is released (63-65). The entry of SARS-CoV-2 consists of 5 steps: 1-, binding to the cell surface; 2-,

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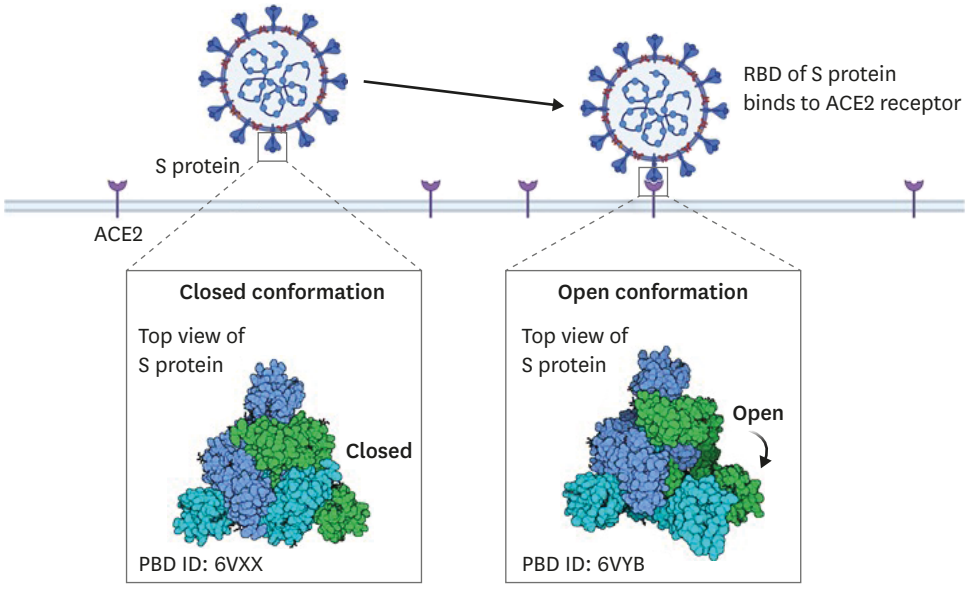

Figure 2. The conformational change of the S protein leading to the interaction with ACE2. The 3D conformational change of the spike protein of SARS-CoV-2 as it binds the human ACE2 receptor. Created with BioRender.com.

conformational change of the S glycoprotein; 3-, cleavage of the S glycoprotein; 4-, the release of S2 subunit; and 5-, S2 mediated fusion of virion followed by endocytosis (60,61).

Intended for the entry of SARS-CoV-2, the RBD of S1 binds to its receptor, ACE2 (63,65-69). After the RBD attachment of S1 to ACE2, the cleavage site of S2 is exposed, followed by the cell entry initiation (66,70). The RBD contains two receptor binding motifs (RBM), 'SKVG' and 'QPTN,' indicated by bold yellow letters and red highlight (**Fig. 1D**), where the peptidase domain of ACE2 binds directly in **Fig. 2**. The RBM of SARS-CoV-2 shares only 50% homology with SARS-CoV (71,72).

The cleavage of the S glycoprotein is processed in 2 steps: 1-, *priming* cleavage between S1 and S2; and 2-, *activation* cleavage at the S2 site (60,61). Although SARS-CoV and SARS-CoV-2 share ACE2 as a common receptor, SARS-CoV-2 utilizes a unique furin cleavage site 'RRAR' at residues 682-685 between S1 and S2 (**Fig. 1C and D**), which does not exist in SARS-CoV. This is one of the reasons why SARS-CoV and SARS-CoV-2 have different virological differences.

MUTATION OF S GLYCOPROTEIN IN INFECTIVITY AND IMMUNOGENICITY

The strategy of neutralizing antibody therapies to interrupt the interaction between virus and host has been questioned since ACE2 is the receptor for the S glycoprotein of SARS-CoV-2. However, the mutation of V417K of RBD revealed that K417 of SARS-CoV-2 binds directly to D30 of ACE2, which leads to more vital interaction than SARS-CoV. This finding suggested that this binding is one of the reasons for higher infectivity of SARS-CoV-2 than SARS-CoV. Additionally, P475A mutation and G482 insertion into the 9 amino acid residues '**AGSTPCNGV**,' indicated by underlined red bold letters (**Fig. 1D**). These 9 amino acid residues are the ring of RBD, which was blocked by the neutralization of SARS-CoV-2, a mAb. This mAb developed against SARS-CoV (73).

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Table 1. Mutations detected in the *S* gene region

Mutation	Location and effect	Mutation	Location and effect
L5F	SP	K458R	RBD
L8V/W	SP	G476S	RBD
H49Y	S1 NTD	E484K	RBD
H69V70/del	S1 NTD	N501Y	RBD
Y145H/del	S1 NTD	G504D	RBD
Q239K	S1 NTD	Y508H	RBD
G321K	RBD	E516Q	RBD
V341I	RBD	H519P	RBD
A344S	RBD	A520S	RBD
A348T	RBD	V524D	RBD
A354T	RBD	P527L	SD2
D364Y	RBD	D614G	SD2 epitope/inter-protomer stabilization
V367F	RBD	V615I/F	SD2 SARS-CoV ADE epitope
K378R	RBD	A831V	FP2 potential fusion protein in S2
R408I	RBD	D839Y/N/E	FP2 S2 subunit
Q409E	RBD	S943P	HR1 fusion core
K417N	RBD	S943P	HR1 fusion core
A435S	RBD	P1263L	CT cytoplasmic tail

As of December 2020, more than 24,000 mutations of SARS-CoV-2 have been reported. Mutations on the *S* gene, in which real expected rates of mutations were 1.21. Structural proteins in CoV have high antigenic variation levels that increase the possibility of immune escape and adaptation to the host. These mutations may prove that SARS-CoV was originated from an animal reservoir and adapted to human hosts. Overall, 22 different point mutation sites in RBD showed including additional 14 mutaion sites in other domains in **Table 1** (74), but those mutations are not overlapped with three new mutations in Korean COVID-19 patients (75,76). A report from the US showed 14 mutations in the S glycoprotein of SARS-CoV-2. This study was focused on the geographic and chronological distribution of the mutation of SARS-CoV-2. The mutations also showed the evolution of SARS-CoV-2 in transmission and evasion from the treatment and the host immune system.

Notably, the D614G mutation in the S glycoprotein is the primary stream of the SARS-CoV-2 mutant strain, although this mutation is located in the SD2 without a specific function (**Fig. 1C and D**). This mutant was first reported in Europe in early February 2020 and then rapidly spread globally, dominating the original virus. Since that time, the D614G mutant has been recombined with the regional strains (77). Other significant mutations in the S glycoprotein are N679K, V772I, and T1238I, but none of these mutants was observed in the RBD (78). Recently, UK and South African variants showed mutations of K417N, E484K, and N501Y in RBD and 69/70 deletion in N-terminal domain (NTD). These variants are spreading rapidly worldwide, affecting changes in several strategies such as vaccines to overcome COVID-19 (79-81).

Our previous study found four mutation sites in Korean COVID-19 patients (75,76). The physiographic map demonstrated three new mutation sites, G504D, V524D, P759L are indicated by blue letters, as well as the aforenamed D614G is characterized by red letters in **Fig. 1C**. The D614G strain showed high infectivity compared to the wild type (77) while the new Korean strain with four mutation sites is under investigation (75,76). Korean COVID-19 samples were obtained in the middle of April 2020. This data suggested that Korean SARS-CoV-2 came from the same origin as the D614G strain and demonstrated how the contagious SARS-CoV-2 spreads so rapidly worldwide.

ATTACHMENT OF S GLYCOPROTEIN

The gene of the S glycoprotein of SARS-CoV-2 presents at the downstream of non-structural polyprotein (82). The S glycoprotein consists of S1 and S2 subunits. S1 is the virus binding region to its receptor, ACE2 on the host, initiating the cell entry (66,70). For this reason, blocking S1-ACE2 binding with small molecules, antibodies, or soluble ACE2 can be useful in the prevention of SARS-CoV-2 viral infection initiation. The 3D structure of the S glycoprotein-ACE2 complex has been identified by cryo-EM and crystallography in several reports (63,65,70,83). Seventeen residues of the RBD—K417, G446, Y449, Y453, L455, F456, A475, F486, N487, Y489, Q493, G496, Q498, T500, N501, G502, Y505—of the S glycoprotein and 20 residues of ACE2—Q24, T27, F28, D30, K31, H34, E35, E37, D38, Y41, Q42, L79, M82, Y83, N330, K353, G354, D355, R357, R393—interact, forming a bridge-like structure. The interaction of the S glycoprotein and ACE2 is conserved in both SARS-CoV and SARS-CoV-2, but SARS-CoV-2 has a higher affinity with ACE2 because it has more interaction sites (63,65,70,83-85).

Targeting RBD-ACE2 interaction is not an easy strategy because the high flexibility and variability of RBD have been predicted (86). Even with the limitation, *in silico* screening of FDA-approved small molecule libraries and natural compounds, some candidates are targeting RBD-ACE2 interaction sites (87,88). Another possible target in RBD is the protomer-protomer interface for discovering new therapeutics to disassemble the trimeric structure of SARS-CoV-2 (89,90). With the advanced *in silico* predictions, more targets may be identified and optimized.

Ab AND SOLUBLE RECEPTOR THERAPY

Some neutralizing antibodies target RBD-ACE2 interaction (91-95). These antibodies antagonize ACE2 to bind to the RBD with high therapeutic and prophylactic efficacy in mice. The other antibody causes the conformation change of RBD by steric hindrance even though it does not bind to the RBD directly (92). Phage-display may be a tool to identify several therapeutic antibody candidates shortly. One of the libraries successfully isolated human mAbs against SARS-CoV-2 (96). REGENERON entered the clinical trial with 2 mAbs in the United Kingdom developed from humanized mice and recovering patients of SARS-CoV-2, which are also targeting the RBD (97). In recent reports, a single amino acid mutation G614 in SD2 of SARS-CoV-2 is more dominant than the original D614 virus, which shows enhanced infectivity *in vitro* and *in vivo* (75,76).

The interaction of ACE2 and SARS-CoV-2 can also be inhibited by using soluble ACE2 that binds the S glycoprotein before the cell entrance. The clinical grade of recombinant ACE2 already showed the inhibition of SARS-CoV-2 infectivity in Vero cells, human vessel- and kidney-organoids (98). ACE2 extracellular domain-human IgG1-Fc fusion protein also showed the inhibition of pseudovirus infectivity *in vitro* (99). However, the fusion protein limitation is 10^3 less potency compared to the REGENERON mAbs (97). Nevertheless, hACE2-Fc fusion protein proved remarkable pharmacological efficacy in the pre-clinical study (99).

THE FUSION OF S GLYCOPROTEIN

Viral entry to the cell initiated by attachment continues to the fusion process. For the fusion process, the cleavages at the S1 then the S2 site R815 (**Fig. 1C and D**) prime the fusion of S

glycoprotein sequentially (66,70,100). Furin-mediated S1/S2 cleavage site is at 'RARR' 685 of the SARS-CoV-2 S glycoprotein during virus trafficking through the secretory pathway (70). Cell membrane protease, TMPRSS2, or the endosomal protease, Cathepsin L, cleaves the S2' cleavage site in SARS-CoV-2 (66,100). So, the virus fusion is available at either the plasma membrane with physiological pH or at the endosomes with acidic pH.

The S2 subunit consists of the loop (L) 2, fusion peptide (FP) 2, connected region (CR), heptad repeat (HR)1, central helix (CH), β-hairpin (BH), SD3, and SD4 in **Fig. 1C**. The fusion process requires a conformational change of the pre-fusion form to the post-fusion form. The interaction between the S glycoprotein and ACE2 receptors turns the pre-fusion trimer unstable, leading to the shedding of the S1 subunit and transitions the S2 subunit to a pre-hairpin intermediate form. The interaction of HR1 and HR2 forms a Hexa-helical bundle fusion core that brings the viral and host cell membrane together to fuse (101).

A STRATEGY FOR TARGETING S1 AND S2 SEPARATELY

Because of the conserved sequence and function, the S2 fusion domain is a more druggable and attractive target than the S1 and RBD. Fusion is a required mechanism for coronavirus entrance into the host cells, so inhibition of the fusion has been focused on as an attractive strategy against pan-coronavirus by way of a broad-spectrum inhibitor. The S2 subunit has 88% and 100% homogeneity of fusion domain and fusion peptide, unlike the 75% and 50% homogeneity of RBD and RBM between SARS and SARS-CoV-2. Additionally, the molecular dynamics simulations showed fusion domain has a higher drug ability than the flexible and variable RBD (86).

The sequence analysis of HR1 and HR2 revealed that the fusion cores show remarkable variation, and this is higher when alpha coronaviruses (NL63, 229E) have large sequence insertions. For these serial reasons, the direction of SARS-CoV-2 treatment should be the generation of specific or pan-coronavirus fusion inhibitors. Specific peptide and lipopeptide of HR2 of SARS-CoV-2 already showed effective inhibition of viral fusion and pseudovirus infection (102,103).

Small molecules can also inhibit the fusion targeting the internal cavity of SARS-CoV-2. A highly conserved homotrimeric cavity exists by HR1, an excellent druggable candidate region (86). The pre-fusion trimer contains an inner cavity targeted to inhibit the post-fusion transition (104,105). Through molecular dynamics simulations and docking screenings from the FDA-approved libraries, some potential HR1 peptide inhibitors and small molecules have been selected targeting this cavity and are under investigation.

Fusion inhibition peptides have a critical defect like protease inhibitor peptides, i.e., short half-life and insufficient oral bioavailability. One strategy to increase the short half-life is lipidation of the peptide. Enfuvirtide and EK1C4 showed increased potency and longer half-life than the non-modified peptides (106,107). The other strategies chosen for Enfuvirtide are PEGylation, glycosylation, and fusion with a human IgG-Fc, and all these increased the potency and half-life of the peptide (108-110).

A recent modification method to increase the half-life of peptides is piggybacking onto the serum albumin by peptide-fatty acid hybrid ligand (111). The downside of Enfuvirtide

is the delivery is by oral route only (112). For infectious diseases of respiratory systems like SARS-CoV-2, the intranasal route can be a safer means of directly controlling the pathogens, bypassing the systemic circulation. Evidence has shown delivering peptides through intranasal delivery in animal models has been effective (113,114).

CONCLUSION

COVID-19 is causing an unprecedented pandemic situation all over the world. The current issue with this infectious disease is the shortage of prevention and treatments for SARS-CoV-2. In recent reports, the relapsed infection of COVID-19 caused skepticism on developing a successful vaccine and therapy. To overcome the current pandemic, both therapy and preventative measures must be prepared for most individuals.

Additionally, the final aim of research and development is to cure the viral infection rather than alleviate symptoms. The vaccination should prevent the virus and prevent the fusion and attachment of SARS-CoV-2 to the receptor. Each viral RdRp, proteases, S glycoprotein-ACE2 binding or fusion, and S protein itself show pros and cons to be the target of vaccines and treatments for pan-coronavirus or SARS-CoV-2 specifically. Understanding the viral genes and proteins will be the critical asset for the current pandemic and for many other infectious agents we will face in the future.

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