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Review Article

A Review of SARS-CoV-2 Genetic and Structure: Hot Cellular Targets for Virus Entry

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) uses several molecules such as angiotensin-converting enzyme 2 (ACE2), cluster of differentiation 26 (CD26), Ezrin, and Neuropilin-1 (NRP-1) for viral entry. In this review, the entire structural and genomic combination and the mechanism of virus entry, are discussed. This study might be useful for further drug design studies. SARS-CoV-2 neutralization allows the immune system to fight the virus before its entry. COVID-19 enters the host bloodstream by infecting endothelial cells via a cluster of differentiation 147 (CD147). SARS-CoV-2 not only uses ACE2 for its entry but also affects ACE-2 and its enzymatic activity on Ang II and bradykinin, it also imbalances the RAAS and bradykinin system and elevates the inflammation. High levels of bradykinin, cause nonproductive cough as the result of fluid extravasation and leukocyte recruitment to the lung. Accordingly, we suggest replicase transcriptase complex (RTC) and specific non-structural proteins (Nsps) such as Nsp7,8, Nsp10, Nsp12, and Nsp16 are perfect targets of study because RTC and Nsps are the golden elements in the maintenance of COVID-19 appearance and masking. Base on this evidence COVID-19 uses various receptors for its entry and it might block these receptors' activity to evade the immune system and spread to other cells.

Keywords: COVID-19; SARS-CoV-2; Genetic, Structure; Non-Structural Proteins; Angiotensin-Converting Enzyme 2.

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Introduction

SARS-CoV-2 which is known as COVID-19 causing virus has a positive single-stranded RNA genome and it enters living cells via the mucosa of the respiratory or digestive tract or eyes. SARS-CoV- 2 seems to have the maximum identity with coronavirus in pangolins (1). Severe pneumonia caused by SARS-CoV-2 seems to be associated with the high rate of virus replication, infiltration of inflammatory cells, and cytokine storm that finally leads to acute respiratory syndrome and organ failure. (2). The SARS-CoV-2 structure is made of spike protein (S protein), nucleoproteins, membrane proteins, and non-structural proteins such as RNA polymerase, 3-chymotrypsin-like protease, papain-like protease, helicase, glycoprotein, and accessory proteins (3). The 3-D structure of the receptor-binding domain (RBD) region of the S protein seems to maintain the van der Waals forces in

binding to the angiotensin-converting enzyme 2 (ACE2) receptor of humans in the entry process (4). In this study we discussed the entire structural and genomic combination and the mechanism of virus entry, to highlight the weak points of the virus. This study might support further drug design and therapeutic studies by highlighting these weak points.

SARS-CoV-2 genome structure

The genome of SARS-CoV-2 is 30kb and contains around 14 open reading frames (Orfs). One of the main Orfs is the 5' Orf1a / Orf1b encodes and translates to two polyproteins, pp1a and pp1ab, that encode 16 non-structural proteins (Nsp1-16) and form replicase transcriptase complex (RTC) and includes the papain-like protease (Nsp3), the main protease (Nsp5), the Nsp7-Nsp8 primase complex, the primary RNA-dependent RNA polymerase (Nsp12), a helicase/triphosphatase (Nsp13), an exoribonuclease (Nsp14), an endonuclease (Nsp15), and N7- and 2'O-methyltransferases (Nsp10/Nsp16) (5). ORF2 encodes S protein which is made of two subunits. These two subunits are activated after cleavage, then they mediate the virus entry (6). ORF3b and ORF7a have an antagonizing role in the INF signaling pathway. They play an essential role in evading the immune cells so they protect viral replication and lead to increased production of pro-inflammatory cytokines and lung damage (7, 8). ORF4 encodes a membrane protein (Envelope protein) that is incorporated into virions (9). ORF5 encodes a structural protein called membrane protein (M protein) (10). ORF6 encodes a protein that can antagonize the JAK-STAT signaling pathway and finally decreases type 1 IFN. Additionally, it might be the reason for reduced and functionally exhausted T cells especially in elderly patients (11, 12). ORF8 seems to inhibit Heme metabolism via binding to porphyrin and dissociate the iron atom and inhibit porphyrins via its potentially and probably heme oxidase activity (13). ORF9 encodes Nucleocapsid protein (N protein) (14). ORF10 encodes a short peptide of length 38 residues that are unique proteins and it can be used for SARS-CoV-2 detection (15); See figure 1.

Non-structural proteins (Nsp1-16) and Replication/Transcription Complexes (RTC)

An important section in the SARS-CoV-2 lifecycle is the translation of the replicase gene (16). Nsp1 is a non-structural protein that can increase the virus evade from the immune system via inhibiting type 1 IFN production and STAT1 phosphorylation (17). Interestingly NSP1 blocks 40S of human ribosome so it suppresses human transcription and mediates mRNA lysis and backup viral mRNAs. Nsp2 has papain-like proteinases (PLP) activity and it lyses the N-terminus of the replicas polyprotein and mediates viral membrane assembly, additionally suppress INF production and NF-κB pathway activity (18). Nsp3/5 and Nsp12 RNA-dependent RNA polymerase (RdRp) (replication enzyme) play a protease role, Nsp 3 has a papain-like protease effect and Nsp5 plays the main protease role and they take part in the cleavage of the replicase polyproteins in SARS-CoV-2 (19, 20). Another point about Nsp12 is that it increases the rate of virus replication. It also gives the virus the ability to recombine. Remdesivir seems to be a good choice but in some cases, therapeutic resistance appears due to mutation in Nsp12 (21). Nsp3 and Nsp5 also induce pro-inflammatory cytokine expression and cleavage of viral polyprotein via several mechanisms such as mediating the cleavage of NLRP12 which might be the cause of hyper-inflammatory response linked to severe COVID-19 (22, 23). Many of the Nsps assemble into the replicase-transcriptase complex (RTC) to create an environment for RNA replication and transcription (24). Some of these Nsps such as Nsp4 and Nsp6 Contribute to the rearrangement of the membranes originating from the rough endoplasmic reticulum (RER) into double-membrane vesicles (DMV) in order to protect the RTC from immune defense detection so it is another mechanism of viral evade from the immune system (25, 26). Nsps potentially have more effects in COVID-19, for instance, Nsp1 and 3 block immune responses via targeting IFN in other words they are antagonists of type 1 IFN specially IFN-β (27). Nsp7 and Nsp8 construct primase complex and create a clamp for RNA polymerase by arms hexadecameric complex in another word they construct a unique multimeric RNA polymerase capable of both de novo initiation

and primer extension (28, 29). Nsp13 has enzymatic role of RNA helicase and nucleoside triphosphate hydrolase (NTPase) so it can hydrolyze all types of NTPs and unwind RNA helices base on the presence of NTP, it also is dose-dependent and some bismuth salts can effectively suppress these two enzymatic roles in COVID-19 (30) (figure 1).

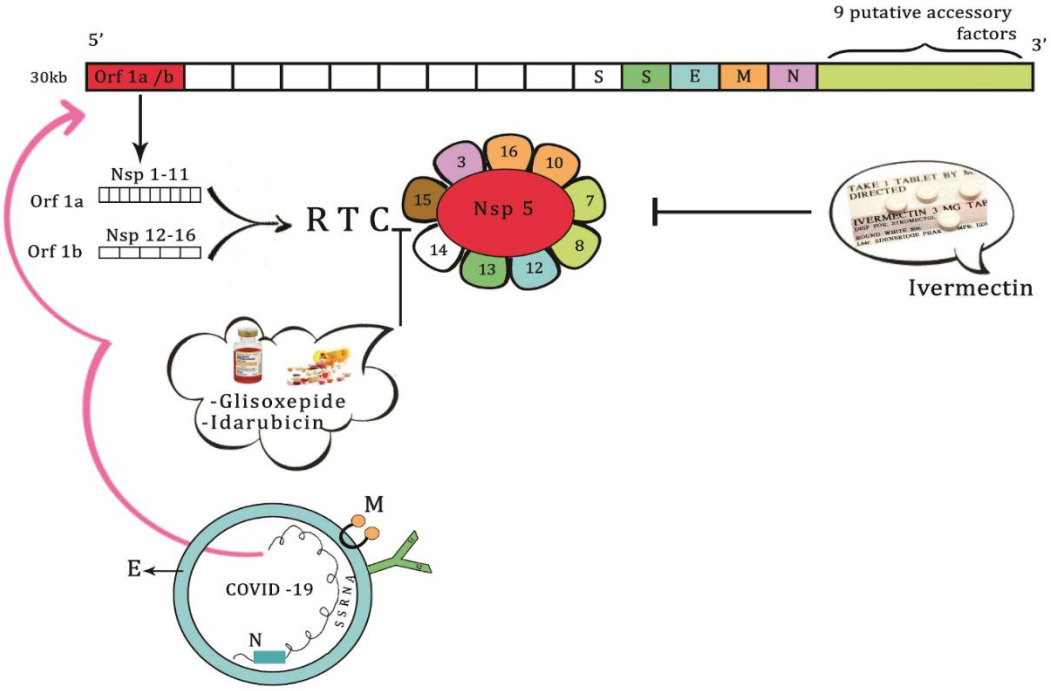

Figure 1: The genome of SARS-CoV-2 is 30kb which encodes around 14 open reading frames (Orfs). One of the main Orfs is the 5' Orf1a / Orf1b which encodes 16 non-structural proteins (Nsp1-16) which form transcriptase complex (RTC) and it includes the papain-like protease (Nsp3), the main protease (Nsp5), the Nsp7-Nsp8 primase complex, the primary RNA-dependent RNA polymerase (Nsp12), a helicase/triphosphatase (Nsp13), an exoribonuclease (Nsp14), an endonuclease (Nsp15), and N7- and 2'O-methyltransferases (Nsp10/Nsp16). Nsp15 is essential in the virus life cycle. Glisoxepide and Idarubicin are two drugs that bind to the active site of Nsp15. Ivermectin is an FDA-approved drug that controls SARS-CoV-2 replication in vitro.

Cellular and viral proteins interaction focusing on SARS-CoV-2 entry

ACE2 receptor and the imbalance of renin-angiotensin-aldosterone system (RAAS)

SARS-CoV-2 uses cellular proteases such as human airway trypsin-like protease (HAT), cathepsins and transmembrane protease serine 2 (TMPRSS2) that notch the S protein and increase penetration in the cell. SARS-CoV-2 probably uses angiotensin-converting enzyme 2 (ACE2) as the main receptor in the entry process (31). Evidence reported that SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) receptor to infect human cells and a serine protease TMPRSS2 is used for S protein priming, so SARS-CoV-2 entry and spread are proteases dependent processes (32, 33). However, studies indicated that ACE2 was highly expressed by monocytes, but t lymphocytes especially T cells have very low levels of ACE2 (34), so other receptors might mediate SARS-CoV-2 entry into T cells. The interaction between ACE2 and trimeric spike protein especially receptor-binding domain (RBD) that is recognized via the extracellular peptidase domain of ACE2 via polar residues and the cleavage of SARS-CoV S protein is facilitated via cathepsin L in endosomes that are called receptor-mediated endocytosis (35). Initially, SARS-CoV-2 cell entry and infection depend on

ACE2/TMPRSS2 co-expressing and ACE2 expression is an interferon (IFN) stimulated gene (ISG) so SARS-CoV-2 might guaranty its cell-cell fusion and entry via upregulating IFN (36). Interestingly a study conducted by Yumiko Imai et al on injecting SARS-CoV spike into mice indicated it decreased ACE2 expression and worsening lung injury in mice and this result reflects the protective effect of cellular ACE2 (figure 2) (37).

Cellular protease Furin and Cathepsins L

Based on studies on S protein, the S1 domain has a receptor binding (RBD) role and is divided into two sub-domains, with the N-terminal subdomain that often binds to sialic acid and the C-terminal subdomain that binds to a specific proteinaceous receptor (38). The S2 plays role in viral membrane fusion (39). Among others, the cellular protease furin cleaves the SARS-CoV-2 S protein at the S1/S2 site containing multiple arginine residues. Cellular protease furin and Cathepsins L were not reported in other animal-related coronaviruses types and seem to be required for effective proteolytic processing of S protein in human cells (40). The specific arginine cleavage sites are essential for SARS-CoV-2 entry into lung cells via S protein fusion and might be associated with viral virulence (figure 2) (31).

Transmembrane protease serine 2 (TMPRSS2)

TMPRSS2 is known to be expressed in the human airway epithelia and alveoli. This protease, localized to 21q22.3 locus, facilitates the trypsin-independent spread of several respiratory viruses including the influenza virus. TMPRSS2 is found to play an essential role in SARS-CoV-1 and SARS-CoV-2 mediated pathology. TMPRSS2 is implicated in S protein priming following viral infection and is known to promote the uncoating of the virus (41-45). More specifically, TMPRSS2 proteolytically processes the S protein and cleaves it into fragments. This inhibits the antibody-mediated viral neutralization of the humoral immunity since secreted antibodies cannot recognize S protein fragments (46).

A Disintegrin and A Metalloproteinase 17 (ADAM17)

ACE2 is a membrane-bound glycoprotein that consists of an active site located at the heavily glycosylated N-terminal extremity, together with a transmembrane and a short C-terminal cytoplasmic sequences (47). ACE2 exists in a catalytically active soluble form due to the action of the A Disintegrin and A Metalloproteinase 17 (ADAM-17) among other members of the family of zinc metalloproteases. Thus, this metalloprotease might be considered a good target for controlling viral entry (48, 49). Some reports suggest that ADAM-17 mediated ACE2 shedding increase viral entry and promotes lung pathogenesis (50). In fact, ADAM-17 stimulates the cleavage of the tumor necrosis factor-α precursor (pro-TNF-α) and thus activates the TNF-α mediated tissue damage. In particular, ADAM-17 flare-up severe T-cell-mediated lung injury via enhancing TNF-α production from cytotoxic T cells (CD8+ lymphocytes) during viral infection. Importantly, ADAM-17 upregulation mediates an inflammatory response as a result of ACE/Ang II/AT1R axis overactivation (51, 52). Recent studies on ADAM17 inhibition suggest its possible protective effect on COVID-19 via cleaving the precursor of tumor necrosis factor α (pro-TNF-α) or ACE2 shedding and interfering SARS-CoV-2 entry (figure 2) (53).

Extracellular matrix metalloproteinase inducer (EMMPRIN)/ CD147

Considering that the SARS-CoV-2 infection is associated with lymphopenia, studies suggest that the virus can also target immune cells (54). However, it appears that a receptor, other than ACE2, mediates the SARS-CoV-2 entry into lymphocytes since ACE2 is highly expressed in monocytes, but not in lymphocytes especially T lymphocytes (34). Interestingly, T cells appear to be more permissive to the SARS-CoV-2 infection, as a result of the extracellular matrix metalloproteinase inducer (EMMPRIN)/CD147 expression on their surface. CD147 plays an essential role in lymphocyte development, regulation of the immune system response, and extracellular matrix deposition. Its expression is up-regulated upon T cell activation (55, 56). Therefore, it appears that the SARS-CoV-2 S protein mediates potent

infectivity, even on cells expressing low ACE2 levels, via other receptors. This fact can probably explain its high transmission rate. On the other side, a study suggests that CD147 is upregulated during hypoxia (55), which is a very common condition in COVID-19. Moreover, high levels of CD147 induce glycosylation and membrane shedding (figure 2)(57).

CD26

CD26 or dipeptidyl peptidase IV(DPPIV) is a surface protease involved in T-cell activation (58). Studies on transgenic mice indicated CD26 expression induces an age-related reduction of thymus cellularity and impairment of lectin-related thymocyte proliferation. Also, the peripheral blood T-cell decreases in CD26 transgenic mice as a result of the increase of the apoptotic rate of CD4+ and CD8+ and these data suggest CD26 plays a role transduction pathway during T cells maturation (59). Inhibiting the enzymatic activity of CD26 suppresses T-cell proliferation and antibody production in the mice model (60). Studies on a docked complex model of SARS-CoV-2 spike glycoprotein and CD26 indicated a possible tight interaction between the S1 domain and the CD26 surface (61). SARS-CoV-2 uses CD26 for its entry just like ACE2 and interestingly, both CD26 and ACE2 seem to be associated with senescence which generates large amounts of inflammatory cytokines, as a result of the senescence-associated secretory phenotype (SASP) (figure 2)(62).

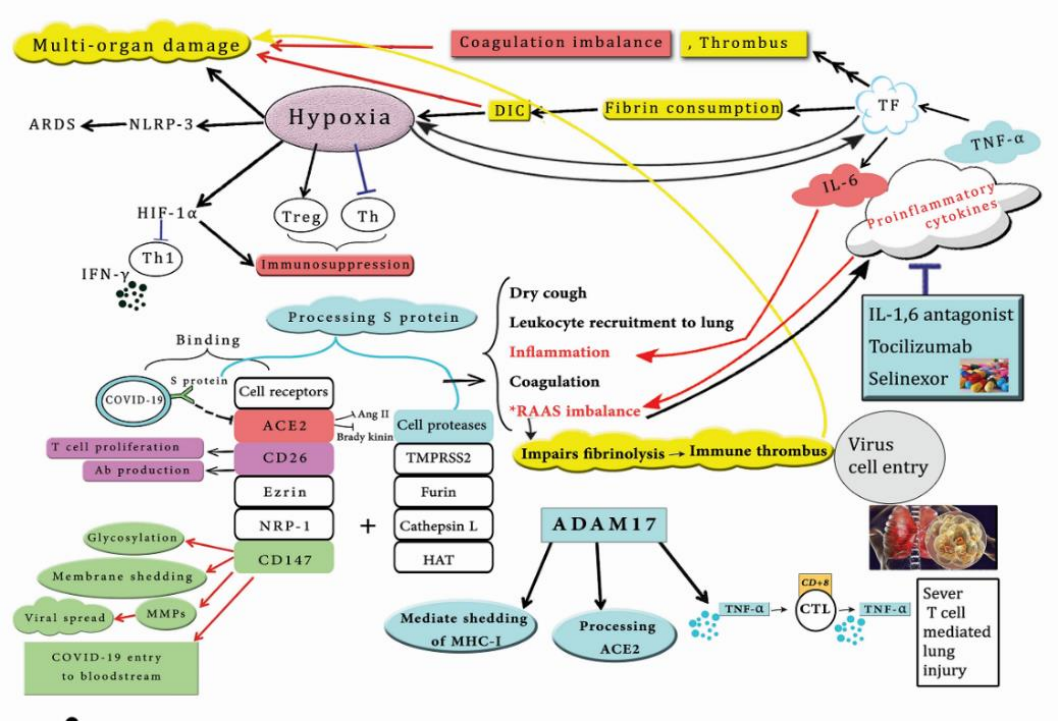

Figure 2: SARS-CoV-2 uses cell receptors such as ACE2, CD26, Ezrin, NRP-1, CD147 for its entry. Various proteases such as TMPRSS2, Furin, Cathepsin L, HAT for S protein processing. ADAM17 is another protease that mediates ACE2 processing and plays important role in viral spread, shedding of MHC-I, and inducing TNF-α production. Using IL-1 and 6 antagonists and Tocilizumab and Selinexor are suggested. TNF-α also activates the coagulation system via activating TF. TF induces several processes such as inflammation, thrombus, and DIC; these processes lead to hypoxia which finally ends in multi-organ failure. Hypoxia activates NLRP3 and leads to ARDS. It also induces HIF-1α production which got multiple effects such as immunosuppression. According to the effect of the proinflammatory cytokines on the coagulation system, thrombus and DIC management should be highlighted in COVID-19 management to decrease mortality.

Ezrin: Ezrin is a membrane–actin, in another word it is a cytoskeleton organizer (63). Ezrin, play an essential role in epithelial cell morphogenesis. It is inactivated via its interaction with Phosphatidylinositol 4,5-bisphosphate (PIP_2) and it is activated via phosphorylation of threonine 567 (T567) (64). This protein interacts with the S protein of SARS-CoV-2 and it seems to be associated with virus entry. Ezrin mediates SARS-CoV-2 entry like ACE2 and CD147. The S1 domain of the S protein attaches to the host cell membrane receptors such as Ezrin, (65-67).

Conclusion and Future Perspective

It should be noted that there is a wide range of items in facing COVID-19, because it is much complicated than we think. Accordingly, we suggest targeting RTC and specific Nsps such as Nsp7,8 ,Nsp10, Nsp12, and Nsp16 are perfect targets because they are the golden elements in the maintenance of SARS-CoV-2 appearance and masking, so the immune system can trace the virus via stabilizing its appearance and controlling the immune system hijacking.

Remdesivir seems to be ineffective in some cases and this therapeutic resistance appears to be due to mutation in Nsp12. Based on recent studies, SARS-CoV-2 not only uses ACE2 for its entry but also has an inhibitory effect on ACE-2. Base on this study SARS-CoV-2 uses various receptors for its entry and it is possible that blocking these receptors activity may cause to evade the immune system and spread to other cells.

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Ethical Considerations

This article does not contain any studies with human participants or animals performed by any of the authors.

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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Conflict of Interest

The authors declared that they have no conflict of interest.

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