SARS-CoV-2 cell entry beyond the ACE2 receptor

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Abstract

Background Angiotensin-converting enzyme 2 (ACE2) is known as the major viral entry site for SARS-CoV-2. However, viral tissue tropism and high rate of infectivity do not directly correspond with the level of ACE2 expression in the organs. It may suggest involvement of other receptors or accessory membrane proteins in SARS-CoV-2 cell entry.

Methods and Results A systematic search was carried out in PubMed/Medline, EMBASE, and Cochrane Library for studies reporting SARS-CoV-2 cell entry. We used a group of the MeSH terms including “cell entry”, “surface receptor”, “ACE2”, and “SARS-CoV-2”. We reviewed all selected papers published in English up to end of February 2022. We found several receptors or auxiliary membrane proteins (including CD147, NRP-1, CD26, AGTR2, Band3, KREMEN1, ASGR1, ANP, TMEM30A, CLEC4G, and LDLRAD3) along with ACE2 that facilitate virus entry and transmission. Expression of Band3 protein on the surface of erythrocytes and evidence of binding with S protein of SARS-CoV-2 may explain asymptomatic hypoxemia during COVID19 infection. The variants of SARS-CoV-2 including the B.1.1.7 (Alpha), B.1.617.1 (Kappa), B.1.617.2 (Delta), B.1.617.2+ (Delta+), and B.1.1.529 (Omicron) may have different potency to bond with these receptors.

Conclusions The high rate of infectivity of SARS-CoV-2 may be due to its ability to enter the host cell through a group of cell surface receptors. These receptors are potential targets to develop novel therapeutic agents for SARS-CoV-2.

Graphical abstract

Keywords SARS-CoV-2 · ACE2 · CD147 · Neuropilin · CD26
Introduction

The Coronavirus family has caused three major pandemics in the last two decades: severe acute respiratory syndrome coronavirus (SARS-CoV-1), the middle east respiratory syndrome coronavirus (MERS-CoV), and the coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2. Of which, COVID-19 has been the deadliest pandemic, causing more than 3 million deaths worldwide [1].

SARS-CoV-2 shares approximately 86.22% genomic homology with SARS-CoV-1 [2], and about 50% genomic homology with MERS-CoV [3].

Receptor recognition is the first important step for virus-cell interaction. It determines viral cellular tropism, host susceptibility, pathogenicity, and cross-species transmission [4]. It has been demonstrated that similar to SARS-CoV-1, SARS-CoV-2 utilizes the angiotensin-converting enzyme 2 (ACE2) for cell entry [5]. ACE2 is distributed across tissues involving many organ systems including the cardiovascular, gastrointestinal, pulmonary, renal, and nervous systems. Its widespread expression may be responsible for the pathological effects and multi-organ system disease manifestations in patients with severe clinical outcomes [6].

However, organs with low ACE2 expression have still shown severe tissue damage secondary to SARS-CoV-2, suggesting potential involvement of other receptors or accessory membrane proteins in viral entry [7]. For example, ACE2 expression in the lungs is relatively low, and it is also not expressed in blood cells [8] indicating potential reliance on other receptors for cellular entry. Moreover, ACE2 expression in humans decreases with age while severity of COVID-19 illness increases with age [9], further indicating the possible role of other cell entry pathways.

In addition, higher infectivity rates of the new variants of the SARS-CoV-2 may be explained by the higher affinity to a wider range of host cell entry receptors. We will discuss the potential entry receptors beyond ACE2 for SARS-CoV-2 and their secondary effects on virus replication.

ACE2

ACE2 is a critical component of the renin–angiotensin system (RAS). ACE2 is a carboxy-monoepitopeidase that cleaves angiotensin (ANG) II into ANG (1–7) [10]. The ACE2 receptor is mostly anchored to cell membranes and its extracellular domain is proposed to make a bound with the spike (S) membrane protein of the coronavirus 1 and 2 [11]. Although the main route of SARS-CoV-2 cell entry in different organs has not been fully elucidated, there is a general acceptance in the scientific community that ACE2 is a common receptor for SARS-CoV-2 invasion [11].

Several studies support the core hypothesis that the SARS-CoV-2 utilizes ACE2 for cell entry. It has been shown that anti-ACE2 antibodies suppress SARS-CoV-1 infection in Vero E6 cells (African green monkey epithelial cells). Vero E6 cells constitute a highly used cell line for ACEs studies due to a high expression of endogenous ACE2 [12]. A high-affinity binding between the spike protein of the SARS-CoV-1 and ACE2 was demonstrated in this cell line previously [13].

The baby hamster kidney fibroblasts (BHk) cells that normally cannot be infected with SARS-CoV-2 [14] have become susceptible to infection after transfection with human ACE2 [15]. Viral entry using membrane ACE2 (mACE2) causes a reduction in mACE2 and subsequently impaired ANG II balance. This process is suggested to be the main trigger of the acute inflammation, thrombotic processes, and tissue injuries observed in severe COVID-19 [16].

Viral entry using the ACE2 receptor also leads to secondary dysregulation of ACE2 related pathways (because of the receptor usage), potentially contributing to the severe pathogenicity of the virus. ACE2 in the heart and kidneys has a critical role in the balance of blood pressure and its dysregulation leads to heart failure and/or chronic kidney damage [17].

CD147

CD147, also known as basigin (Bsg) or extracellular matrix metalloproteinase, is a member of immunoglobulin superfamily and was first described as a T lymphocyte activation-associated antigen [18] (Fig. 1). This molecule is mainly expressed in the heart, kidneys and lungs and has a diverse range of functions. In addition to its metalloproteinase-inducing ability, CD147 plays a role in lymphocyte signaling and neurological pathways [19]. Furthermore, it is an essential factor in the monocarboxylate transporters (MCTs) system. Importantly, the inflammatory molecules such as cyclophilins A and B, S100A9, and E-selectin act as activating ligands for CD147 [19]. As a pleiotropic functional molecule, any dysregulation in CD147 expression or alteration of its functionality may lead to a pathological status in affected organs [19].

CD147 mediates the invasion process of a few pathogens including measles, HIV virus, malaria, and bacterial agents [20] and facilitates infection of Vero E6 cells by SARS-CoV-1 which is inhibitable by CD147-antagonistic peptide-9 [21].

Furthermore, CD147 interacts with RH5 from Plasmodium falciparum (PRH5) on the RBC surface which results in sticky infected red blood cells (RBCs). Interaction of the viral spike to CD147 receptors on RBCs may similarly induce an adhesive phenotype in the RBC membrane and
lead to varying degrees of hypoxemia and myocardial damage from abnormal interaction with the vascular endothelium [22].

In the structure of the complex of CD147 with PfRH5 protein (PDB id: 4U0Q), PfRH5 binds to the cleft between the two extracellular domains of CD147 [23] (Additional file 1, Fig. S3).

Our in silico analysis with docking web servers predicted that the RBD region of spike SARS-CoV-2 can similarly bind to a region between the two extracellular domains of CD147 [23] (Additional file 1, table S1, 2, 3; Fig. S2, 3, 4). Moreover, in silico analysis by Helal et al. also suggested that the CD147 receptor can interact with the RBD region of SARS-CoV-2 spike protein [24].

Recently, Wang et al. and others have examined the potential role of CD147 as an entry site for SARS-CoV-2 [25]. They demonstrated that CD147 had a higher value of expression in Vero E6 cells in comparison to ACE2. They also observed that CD147 can bind to the RBD region of viral spike protein with high affinity. Meplazumab, an anti-CD147 humanized antibody, significantly decreased the virus entry in these cells. They have also observed that BHK-21 cells became susceptible to SARS-CoV-1 upon transfection with CD147 [25]. Beside in vitro studies, the localization of CD147 and the spike protein have been demonstrated in the kidney and lung tissues from a patient with severe COVID-19 [25].

Lymphopenia is one of the most characteristic clinical features in severe COVID-19 patients, and T-cells are highly affected in severe disease [26]. The SARS-CoV-2 virions were detected in lymphocytes located in the lung of a COVID-19 patient [25]. Given that ACE2 expression is very low in lymphocytes it is speculated that an alternative receptor might be involved in T-cell infection. CD147 is highly expressed by activated T lymphocytes which further suggest that CD147 as a potential receptor for SARS-CoV-2 entry to T-cells.

Wang et al. concluded that SARS-CoV-2 internalized to the host cells through CD147-mediated endocytosis due to co-localization of CD147, spike, and Rab5 in the infected BHK-21 cells and lung tissues from COVID-19 patients [25].

Cyclophilin A (CypA) is a highly conserved protein and has peptidyl-prolyl cis/trans isomerase activity. CypA is known as a ligand of CD147. As a host factor, CypA has a critical role in the life cycle of human immunodeficiency virus type 1 (HIV-1) and many of coronaviruses including HCoV-229E, HCoV-NL63 and SARS-CoV-1 [27, 28] through interaction with CD147.

In the process of infection by HIV-1, the host CyPA binds to viral N protein after invasion and relocates to the surface of the virus during the viral maturation process.
NRP1 possesses a large extracellular and a small transmembrane domain with a short cytoplasmic portion. The extracellular domain contains three subdomains: A, B, and C. The B subdomain involving b1 and b2 subdomains is located in the middle of the extracellular domain and is known as the binding site for vascular endothelial growth factors (VEGF) (Fig. 2) [37]. NRP1 lacks the cytosolic protein kinase domain. It plays a role as a co-receptor for a broad range of ligands including vascular endothelial growth factor (VEGF) [38] which mediates angiogenesis, cell growth, vascular permeability, semaphorin-based axon guidance, and nervous system development [37].

A soluble form of NRP1s (sNRPs) may inhibit NRP-mediated activities through trapping of receptor ligands [39]. This form of NRP is produced by alternative RNA splicing resulting in ectodomain shedding and lack of the cytoplasmic and transmembrane domains [40].

The transmembrane form of NRP-1 acts as an entry site for a few viruses such as Epstein–Barr virus (EBV) [41] and Human T lymphotropic virus type 1 (HTLV-1) [42]. NRP1 binds to the furin-cleaved substrates through their CendR motifs [43].

The SARS-CoV-2 spike protein contains an insertion of a four polybasic amino acid residue “Arg-Arg-Ala-Arg (RRAR)” which introduces a furin-cleavage site at the viral spike protein [4] (Fig. 2).

Cleavage of the spike protein by host furin exposes a conserved C-terminal motif in the S protein named C-end terminal rule, C-end rule (CendR). Through this motif, the spike protein binds to the CendR binding pocket on the extracellular b1b2 domain of NRP1 [44] (Fig. 2). Daly et al. co-crystallized and determined the structure of NRP1 b1 domain in complex with SARS-CoV-2 S1 CendR peptide (PDB id:7JJC) and have shown that R685 of the CendR peptide is the key residue in this interaction and along with R682 mediates binding of S1 to NRP1 [44] (Fig. 2). Neuropilins commonly internalize CendR ligands through endocytosis [9] and monoclonal antibodies against NRP1 significantly decrease the viral load [45].

NRP1 is broadly expressed by the endothelial and epithelial cells of the respiratory and olfactory systems [46], olfactory neuronal progenitors [47] and in vagal [48] and other sensory neurons, while ACE2 is hardly detectable in these cells [45]. A high expression of NRP1 was found in the bronchoalveolar lavage (BAL) cells from the patients with severe COVID-19 in comparison to healthy controls [45]. Furthermore, the infected olfactory epithelial cells in COVID-19 patients had a high expression of NRP1. This may suggest that the virus directly infected these cells via NRP1 leading to anosmia in COVID-19 patients [45, 48]. In fact, NRP-1 inborn error has been associated with Kallmann syndrome that is characterized by loss of smell [49].
The neurotropism of \textit{SARS-CoV-2} for NRP1 receptors in the CNS may explain possible persistence of the virus in the CNS leading to immediate and latent complications \cite{50}. For example, neurotropism allows increased permeability of the blood brain barrier, neurologic dysfunction, and stroke \cite{44}.

NRP1 dysregulation upon viral infection may further contribute to the multi-organ failure in severe COVID-19 cases. The binding site for the CendR motif of S protein on NRP1 is the same as the one for VEGF. Binding the virus with NRP1 could potentially inhibit VEGF-NRP1 interaction and alter the normal signaling pathways in the host cell.

NRP-1 is abundantly expressed on vascular endothelial cells and plays a critical role in controlling the adhesion and permeability of these cells. Its engagement during viral infection may lead to severe vascular related injuries such as sepsis or disseminated intravascular coagulation.

**CD26 (DPP4)**

CD26 is a serine exopeptidase that is widely expressed in lungs, kidneys, and leukocytes. CD26 is also known as dipeptidyl peptidase IV (DPP4) and belongs to type II transmembrane membrane glycoproteins.

The structure of CD26 contains a small cytoplasmic tail with six residues, a transmembrane section with 22 amino acids, and a large extracellular portion containing 738 residues. The transmembrane region is responsible for the enzyme activity and contributes to protein dimerization. The extracellular region that mediates viral interactions contains a flexible stalk, a glycosylated area, a cysteine-rich region as well as a C-terminal catalytic site (Fig. 3).

CD26 has an important role in proteolytic cleavage and activation of a broad range of substrates. This is essential in regulation of immune responses and activation of T cells \cite{51}. CD26 is used by MERS to infect a wide range of human cells. Since MERS is so genetically similar to \textit{SARS-CoV-2}, CD26 has been considered as a potential entry site.

A computational analysis using model-based selective docking predicts a large interface and a tight interaction between S1 domain of \textit{SARS-CoV-2} and CD26 surface. This interaction involves the residue (K267, T288, A289, A291, L294, I295, R317, Y322 and D542) from CD26 that binds to RBD region of S1 in \textit{SARS-CoV-2} \cite{52}. Previous studies have shown similar binding between CD26 and the MERS spike protein \cite{53}. Another computational study using molecular docking simulations predicted substantially weakened interactions in the \textit{SARS-CoV-2}-CD26 complex \cite{54}. Moreover, Tai et al. observed that the spike protein of \textit{SARS-CoV-2} did not bind to 293 T-cells expressing human CD26 \cite{55}. The role of CD26 in \textit{SARS-CoV-2} needs to be further elucidated.

CD26 plays a critical role in glucose and insulin metabolism and CD26 inhibitors (known as Dipeptidyl peptidase 4 inhibitors) are a new therapeutic class in diabetes \cite{56}. Interestingly, in \textit{MERS-CoV} infection, individuals with
type 2 diabetes had shown a higher rate of mortality and complications associated with CD26 dysregulation and impaired immune response [57]. CD26 has an important role in maintaining lymphocyte function, T cell activation and proliferation, and memory T cell generation. It is dysregulated in inflammation, obesity, and diabetes [58]. Given that the diabetic patients with SARS-CoV-2 experience a more severe disease, the potential relation between SARS-CoV-2 and CD26 should be further investigated.

Other receptors

Angiotensin II receptor type 2 (AGTR2) is an important mediator of blood pressure which acts via interaction with ACE2 and is highly expressed in the lungs. Cui et al. showed that AGTR2 interacts with the spike protein with a higher affinity than ACE2 and suggested it could be a potential receptor for SARS-CoV-2 [59].

One of the clinical presentations of COVID-19 is asymptomatic hypoxemia associated with poor outcomes. A biophysical resonant recognition model (RRM) showed that SARS-CoV-2 may potentially interact with the Band3 protein on the surface of erythrocytes (RBC). This may be responsible for the unexpected drops in blood oxygen levels secondary to alteration in the RBC’s membrane [60]. Band 3, also named as anion exchanger 1 (AE1), is involved in the exchange of chloride and bicarbonate across the RBC’s membrane and transport of CO2 during respiration [61]. Band three protein is the most abundant transmembrane protein in the RBCs and is estimated to comprise 25% of red blood cell membrane proteins [62].

Integrins are suggested as potential receptors for SARS-CoV-2 cell entry. Integrins are transmembrane proteins involved in various cellular functions ranging from the cell adhesion and migration process to signaling pathways. Integrins are entry receptors for several viruses including adenovirus, herpes simplex virus-2, and human papillomavirus-16 [63, 64]. The exact mechanism of integrin-mediated viral internalization is not clear. However, integrin’s ability to adhere to solid surfaces may promote viral internalization [65].

It has been reported that SARS-CoV-2 harbors one of the most common integrin binding motifs known as RGD on the RBD of spike protein [66]. RGD is a small motif that contains the minimal residue Arg-Gly-Asp for binding to integrin and plays an important role in infectivity of human pathogenic viruses including Adenovirus [67] or cytomegalovirus (HHV-5) [65, 68]. Viral proteins with RGD motifs can trigger phosphatidylinositol-3 kinase (PI3-K) and/or mitogen-activated protein kinase (MAPK) pathways and stimulate infection [69]. Computational analysis predicted that SARS-CoV-2 needs a distinct conformational change of the receptor binding domain to expose the RGD motif [66]. Future experimental evaluations are needed to determine the functional significance of integrins as SARS-CoV-2 receptors.

A single-cell transcriptome profile of COVID-19 patients has shown that the expression of three receptors (ACE2, KREMEN1 and ASGR1) is highly correlated with the level of cell infectivity. Moreover, the expression of ACE2/KREMEN1/ASGR1 (ASK) together was correlated with increased susceptibility of the cell to infection [70]. Kremen1 is involved in tuning Wnt/β-catenin signaling pathways and is known as an entry receptor for a group of enteroviruses including coxsackievirus A10 (CV-A10) [71]. Kremen1 can be internalized from the cell surface through a clathrin-mediated endocytosis [72] and can thus mediate cellular virus entrance.

ASGR1 (CLEC4H1) is an endocytic recycling receptor, expressed mainly in the liver, and is considered a viral entry cofactor for the hepatitis C virus. While ACE2 has very low expression in the liver, the high expression of ASGRI in the liver could promote hepatitis during COVID-19 infection [73]. Qi et al. found that ANPEP, ENPEP and CD26 have the highest correlation with ACE2 expression by screening the co-expression patterns [8].

ANPEP encoded alanyl aminopeptidase (ANP) enzyme is mainly expressed in enterocytes. ANP is suggested as a viral entrance receptor for a subset of coronaviruses including HCoV-22944 [74] and mediates virus entry by binding to the envelope of the spike glycoprotein [75].

The ENPEP gene encodes Glutamyl Aminopeptidase which is a zinc-containing endopeptidase that regulates blood pressure by degradation of vasoconstricting angiotensin II into angiotensin III [75]. The involvement of this protein in viral infections had not been reported previously. However, it possesses an extracellular zinc-binding domain which may potentiate this receptor as a binding site for SARS-CoV-2 [76].

Zhu et al. performed a genome-wide barcoded-CRISPR activation screen and found that three membrane proteins: LDLRAD3, TMEM30A and CLEC4G could bind to the N-terminal domain (NTD) of the viral spike thus mediating viral entry in a different manner than ACE2 [77].

Transmembrane protein 30A (TMEM30A) mediates the flippase-mediated translocation of phospholipids in the cellular bilayer lipid [78]. TMEM30A may also regulate the trafficking of amyloid-β precursor protein (APP) in endosomes. Dysregulation of endosomal trafficking and aggregation of amyloid-β peptide (Aβ) are the main pathologic characteristics of Alzheimer’s disease (AD) [79].

The LDLRAD3 (Low-density lipoprotein receptor class A domain-containing protein 3) gene encodes a transmembrane protein belonging to the low density lipoprotein (LDL) receptor family that is involved in the processing of neuronal amyloid precursor proteins (APP). It was
previously introduced as a receptor for Venezuelan equine encephalitis virus (VEEV) that is a neurotropic alphavirus [80]. LDLRAD3 may be responsible for the neurologic consequences exhibited during and after COVID-19 disease.

Brockbank et al. conducted a multivariate analysis of SARS-CoV-2 spike interaction with human cell surface receptors. They observed that ASGR1, CLEC4M, NID1, CNTN1 and APOA4 interact specifically with SARS-CoV-2 spike protein [81].

APOA4 is a lipoprotein mainly expressed in the intestine and intestinal enterocytes [82]. APOA4 has been found to have an important role in hepatitis C virus infection [83]. Given that the intestine is one of the target organs for SARS-CoV-2, a potential link between APOA4 and gastrointestinal tract infection should be further explored.

CLEC4 genes encode the proteins belonging to the C-Type lectin family which have high avidity for glycoproteins that are found in viral envelopes [84].

The upper respiratory tract is highly protected by a saliva-rich environment containing a wide range of glycans. Several viruses can exploit these glycans for their cell entrance. SARS-CoV-2 possesses unique O-linked glycans which are absent in SARS-CoV-1, and its glycan shield of the spike protein is heavily sialylated with N- and O-linked glycans [85]. It has been suggested that the presence of the O-linked and the N-linked glycans on the outer envelope of SARS-CoV-2 may introduce potential effective binding sites and enable the virus to use the host glycans and sialic acids as entry factors [86].

The role of the potential viral receptors as protagonist in COVID-19

SARS-CoV-2 has potential to use multiple receptors in cellular entry. Once in the cell, the virus leads to clinical manifestations primarily through direct cell injury and death. However, in addition to subsequent direct viral damage to host cells after entry, SARS-CoV-2 can alter the role of these receptors in healthy physiologic pathways. Functional receptor dysregulation secondary to viral entry may have a protagonist role in the disease morbidity and mortality observed in COVID-19. For example, triggering and dysregulation of ACE2 leads to acute inflammation, thrombosis, and hypercoagulation. CD147 usage by the virus may be responsible for the myocarditis and heart failure via interference of CD147-CypA signaling. Engagement of the RBC CD147 receptors by the virus may be responsible for hypoxemia. Acute coronary syndrome and acute carditis are considered as two major reasons for mortality in COVID-19 patients. Lastly, NRP-1 usage by the virus has a protagonist role in the infection of CNS via the olfactory epithelial cells and the neurological manifestations in COVID-19.

Cell entrance receptors and viral infectivity in the recent variants of SARS-CoV-2

Massive transmission of the SARS-CoV-2 has been caused by the emergence of several variants including the B.1.1.7 (Alpha), B.1.617.1 (Kappa), B.1.617.2 (Delta), B.1.617.2+ (Delta+), and lately B.1.1.529 (Omicron) [87].

In the earlier isolates such as Kappa and Delta, the RBD domain binds to the ACE2 with an affinity comparable to the wild type of SARS-CoV-2, while the Delta+ isolates have shown a significantly reduced affinity [88]. In the meantime, preliminary studies have suggested an increased S1–S2 cleavage and viral infectivity in the Alpha and Delta variants [108]. In the wild-type virus, the host-mediated O-glycosylation S by certain GALNT family enzymes leads to a decrease in furin cleavage of S and the viral infectivity. However, the presence of a P681 mutation in the highly transmissible Alpha and Delta variants revokes the O-glycosylation in this site which leads to the enhanced furin cleavage and viral infectivity in these variants [89].

The recent SARS-CoV-2 variant, Omicron, was first reported in South Africa in November 2021. Omicron harbors numerous mutations in its receptor-binding domain (RBD), which heavily change its infectivity and transmission ability. This variant replicated in human primary nasal epithelial cultures much faster than the Delta variant [90] and other known variants [91]. Peacock et al. observed that among the main SARS-CoV-2 receptors including ACE2, APN, and DPP4, Omicron used ACE2 as the main receptor and its spike binds to human ACE2 with a higher affinity than both the Alpha and Delta variants [90]. This higher affinity may be due to the simultaneous presence of N501Y and Q498R mutations in the Omicron spike [92].

Omicron can use an extended range of host ACE2 which may give it the potential to establish infectivity in animal reservoirs and increase the risk of the emergence of future SARS-CoV-2 variants.

The previous variants of SARS-CoV-2 were strongly dependent on using the host TMPRSS2 protease which limits their tropism. Omicron can use the endosomal pathways and enter the cells in a TMPRSS2-independent manner. This ability permits the Omicron to infect a broad range of cells in the lung epithelia [90].

Furthermore, the cell entrance via endosomal routes in the previous variants was strongly limited by endosomal restriction factors such as interferon-induced transmembrane (IFITM) protein (Table 1). Omicron acquired the ability to avoid this restriction, which makes it possible for Omicron to infect a broad range of ACE2-expressing cells in the airways [90].
Therapy methods based on the viral host receptor

Currently, there are few antivirals for COVID-19 treatment including Remdesivir [93], Ritonavir-boosted nirmatrelvir [94], and Molnupiravir [95]. Initial interaction of the virus with host cell receptors is a determinant step in viral pathogenesis. Targeting the SARS-CoV-2 interaction with host receptors is one of the most attractive issues in treatment of COVID-19 (Table 2).

Therapy methods using ACE2 inhibitors have raised concerns because ACE2 inhibitors may impair RAS function in vital organs such as the heart, kidneys, or vascular system. In addition, a recent study suggested that ACE inhibitors showed no effect on the severity of COVID-19 [96]. An in vitro study shows that human recombinant soluble ACE2 (hrsACE2) has some potential effect in blocking SARS-CoV-2 infection. HrsACE2 is being tested for safety and efficacy for the treatment of COVID-19 [97].

The refractory hypoxemia and myocardial injury in severe patients may be due to the infection of RBC via their CD147 receptors. It has been suggested that melatonin may be a potential therapeutic intervention for the attenuation of hemoglobinopathies, refractory hypoxemia, and myocardial injury [22]. Melatonin is known as a practical agent to preserve the structure and function of RBCs and protect erythrocytes from oxidative hemolysis through a radical-scavenging activity [98].

A humanized anti-CD147 antibody, meplazumab is now being examined in an open-label clinical trial for the patients with COVID-19 and has shown impressive results in these patients [118]. Meplazumab facilitated viral clearance and improved levels of lymphocyte count and reduced level of C-reactive protein (CRP) in severe COVID-19 cases [99].

Introduction of NRP-1 as a receptor for SARS-CoV-2 offers potential for targeting NRP-1. Using traditional antagonists against the b1 domain of NRP-1 for inhibition of ligand binding may be considerable to reduce SARS-CoV-2 infectivity and could control vasculature and coagulation during viral infection.

Monoclonal antibodies against the binding site of the CendR motif in NRP-1 can alleviate infection with SARS-CoV-2. EG00229, EG01377 and EG01377-derived fluorescent molecules [124] are the common NRP-1 inhibitors that bind to the NRP-1 binding pocket through their end Arg-like component and carboxyl groups [100].

Soluble NRPs are also promising candidates for modulation of NRP1-related pathways. They act as trap receptors and suppress NRP-mediated activities, including virus entrance [101].

CD26 inhibitors are also proposed for COVID-19 treatment. The common CD26 inhibitors (also known as DPP4 inhibitors) are unable to block viral-receptor interaction because they bind to a region outside the viral spike binding pocket on CD26.

| Table 2 | Therapy methods based on the viral host receptor |
|---|---|---|---|---|
| **Receptor** | **Suggested treatment** | **Clinical trial** | **References** |
| 1 | ACE2 | APN01 | Phase 2 | [97] |
| 2 | NRP1 | EG00229 | NCT03177668 (Phase 1, 2) | [104] |
| 3 | CD26 | YS110 | NCT04275245 (Phase 1, 2) | [99] |
| 4 | CD147 | Meplazumab | NCT04586153 (Phase 2, 3) | [106] |
| | | Azithromycin | NCT04359316 (Phase 4) | |
| | | | NCT04332107 | |
In an animal model of acute respiratory distress syndrome (ARDS), Sitagliptin, which is a CD26 inhibitor, relieved the LPS-induced lung damage and inhibited proinflammatory cytokines such as IL-1β, TNFα, and IL-6 [102]. ARDS is the main cause of death in severe COVID-19. Moreover, knockout of the CD26 gene exerted an anti-fibrotic effect in mice models of bleomycin-induced pulmonary fibrosis [103].

YS110 is an anti-CD26 human monoclonal antibody that significantly suppressed MERS-CoV infection without affecting the immune function or enzyme activity of the receptor. YS110 is currently being examined in clinical trials as an anti-cancer drug in mesothelioma patients [104]. Further investigation is needed to understand YS110’s role in COVID-19.

Conclusion

The high rate of infectivity of SARS-CoV-2 may be due to its ability to enter the host cell through a group of cell surface receptors. Host genetic factors may influence the viral life cycle within the human body. Furthermore, the triggering of multiple receptors in the process of infection of the host cell may enhance downstream cellular pathogenicity.

Besides the direct viral-induced cellular damage, engaging the host multifunctional receptors may interfere with their baseline function. Functional receptor dysregulation may be responsible for amplification of host proinflammatory cascades, multiorgan dysfunction, and cerebral vascular injury, which are the main causes of death in patients with severe COVID-19.

Currently, the development of effective treatments based on viral entry factors has received significant attention and several such treatments are being tested in clinical trials. Considering the fast spread and rapid emergence of new variants, emergence of translational research detailing alternate viral entry pathways needs to be translated into clinical practice in order to control the pandemic.

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References

1. World Health Organization, Corona virus disease (COVID-19) pandemic. Available at https://www.who.int/emergencies/diseases/novel-coronavirus-2019, 2022.
2. Lu R et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395(10224):565–574
3. Wu F et al (2020) A new coronavirus associated with human respiratory disease in China. Nature 579(7798):265–269
4. Alipoor SD et al (2021) COVID-19: molecular and cellular response. Front Cell Infect Microbiol. https://doi.org/10.3389/fcimb.2021.563085
5. Verdecchia P et al (2020) The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur J Intern Med 76:14–20
6. Zou X et al (2020) Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med 14(2):185–192
7. Kirtipal N et al (2022) Understanding on the possible routes for SARS CoV-2 invasion via ACE2 in the host linked with multiple organs damage. Infect, Genet Evolut 99:105254
8. Qi F et al (2020) Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. Biochem Biophys Res Commun 526(1):135–140
9. Jobe A, Vijayan R (2021) Neuropilins: C-end rule peptides and their association with nociception and COVID-19. Comput Struct Biotechnol J 19:1889–1895
10. Scialo F et al (2020) ACE2: the major cell entry receptor for SARS-CoV-2. Lung 6:867–877
11. Kai H, Kai M (2020) Interactions of coronaviruses with ACE2, angiotensin II, and RAS inhibitors—lessons from available evidence and insights into COVID-19. Hypertens Res 43(7):648–654
12. Ksiazek TG et al (2003) A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 348(20):1953–1966
13. Li W et al (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426(6965):450–454
14. Hoffmann M et al (2020) SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 181(2):271–280.e8
15. Walls AC et al (2020) Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181(2):281-292.e6
16. Fagyas M et al (2021) Level of the SARS-CoV-2 receptor ACE2 activity is highly elevated in old-aged patients with aortic stenosis: implications for ACE2 as a biomarker for the severity of COVID-19. Geroscience 43(1):19–29
17. Imai Y et al. (2005) Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 436(7047):112–116
18. Yu X-L et al. (2008) Crystal structure of HAb18G/CD147: implications for immunoglobulin superfamily homophilic adhesion. J Biol Chem 283(26):18056–18065
19. Yurchenko V et al. (2010) Cyclophilin–CD147 interactions: a new target for anti-inflammatory therapeutics. Clin Exp Immunol 160(3):305–317
20. Pushkarsky T et al. (2001) CD147 facilitates HIV-1 infection by interacting with virus-associated cyclophilin A. Proc Natl Acad Sci USA 98(11):6360–6365
21. Chen Z et al. (2005) Function of HAb18G/CD147 in invasion of host cells by severe acute respiratory syndrome coronavirus. J Infect Dis 191(5):755–760
22. Loh D (2020) The potential of melatonin in the prevention and attenuation of oxidative hemolysis and myocardial injury from cd147 SARS-CoV-2 spike protein receptor binding. Melatonin Res 3(3):380–416
23. Wright KE et al. (2014) Structure of malaria invasion protein RH5 with erythrocyte basigin and blocking antibodies. Nature 515(7527):427–430
24. Helal MA et al. (2020) Molecular basis of the potential interaction of SARS-CoV-2 spike protein to CD147 in COVID-19-associated lymphopenia. J Biомol Struct Dyn 16:1–11
25. Wang K et al. (2020) CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. Signal Transduct Target Ther 5(1):1–10
26. Li Tan QW et al. (2020) Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther. https://doi.org/10.1038/s41392-020-0159-1
27. Carbajo-Lozoya J et al. (2014) Human coronavirus NL63 replication is cyclophilin A-dependent and inhibited by non-immunosuppressive cyclosporine A derivatives including alisporivir. Virus Res 184:44–53
28. Luo C et al. (2004) Nucleocapsid protein of SARS coronavirus tightly binds to human cyclophilin A. Biochem Biophys Res Commun 321(3):557–565
29. Saphire A, Bobardt MD, Gallay PA (2000) Human immunodeficiency virus type 1 hijacks host cyclophilin A for its attachment to target cells. Immunol Res 21(2):211–217
30. Fenizia C et al. (2021) SARS-CoV-2 entry: at the crossroads of CD147 and ACE2. Cells 10(6):1434
31. Dawar FU et al. (2017) Updates in understanding the role of cyclophilin A in leukocyte chemotaxis. J Leukoc Biol 101(4):823–826
32. Pennings GJ et al. (2014) CD147 in cardiovascular disease and thrombosis. Seminars in thrombosis and hemostasis. Thieme Medical Publishers, New York, pp 747–755
33. Cao M et al. (2019) Role of CyPA in cardiac hypertrophy and remodeling. Biosci Rep. https://doi.org/10.1042/BSR20193190
34. Nieto-Torres JL et al. (2015) Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. Virology 485:330–339
35. Faghhi H (2020) CD147 as an alternative binding site for the spike protein on the surface of SARS-CoV-2. Eur Rev Med Pharmacol Sci 24(23):11992–11994
36. Shilits J et al. (2021) No evidence for basigin/CD147 as a direct SARS-CoV-2 spike binding receptor. Sci Rep 11(1):1–10
37. Guo HF et al. (2015) Neutropilin function as an essential cell surface receptor. J Biol Chem 290(49):29120–29126
38. Roy S et al. (2017) Multifaceted role of neutrophilins in the immune system: potential targets for immunotherapy. Front Immunol 8:1228
39. Alipoor SD et al. (2021) The immunopathogenesis of neuroinvasive lesions of SARS-CoV-2 infection in COVID-19 patients. Front Neurol. https://doi.org/10.3389/fneur.2021.697079
40. Cackowski FC et al. (2004) Shi-Yuan Cheng, identification of two alternatively spliced neuropilin-1 isoforms. Genomics 84(1):82–94
41. Wang H-B et al. (2015) Neurupilin 1 is an entry factor that promotes EBV infection of nasopharyngeal epithelial cells. Nat Commun 6(1):1–13
42. Zhang L-L et al. (2017) Human T-cell lymphotropic virus type 1 and its oncogenesis. Acta Pharmacol Sin 38(8):1093–1103
43. Teesalu T et al. (2009) C-end rule peptides mediate neurophilin-1-dependent cell, vascular, and tissue penetration. Proc Natl Acad Sci USA 106(38):16157–16162
44. Daly JL et al. (2020) Neurupilin-1 is a host factor for SARS-CoV-2 infection. Science 370(6518):861–865
45. Cantuti-Castelvetri L et al. (2020) Neurupilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science 370(6518):856–860
46. Mayi BS et al. (2021) The role of Neurupilin-1 in COVID-19. PLoS Pathog 17(1):e1009153
47. Wang R et al. (2018) Efficacy of inospor isomer of CendR peptide on tumor tissue penetration. Acta Pharm Sin B 8(5):825–832
48. Davies J et al. (2020) Neurupilin-1 as a new potential SARS-CoV-2 infection mediator implicated in the neurologic features and central nervous system involvement of COVID-19. Mol Med Rep 22(5):4221–4226
49. Hanchate NK et al. (2012) SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with kallmann syndrome. PLoS Genet 8(8):e1002896
50. Garrigues E et al. (2020) Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19. J Infect 81(6):e4–e6
51. Fleischer B (1994) CD26: a surface protease involved in T-cell activation. Immunol Today 15(4):180–184
52. Vankadari N, Wilce JA (2020) Emerging COVID-19 coronavirus: glycans shield and structure prediction of spike glycoprotein and its interaction with human CD26. Emerg Microbes Infect 9(1):601–604
53. Wang Q et al. (2014) Bat origins of MERS-CoV supported by bat coronavirus HKU4 usage of human receptor CD26. Cell Host Microbe 16(3):328–337
54. Cameron K, Rozano L, Falasca M, Mancera RL (2021) Does DPP4 interact with the DPP4 (CD26) receptor? a molecular docking study. Int J Mol Sci 22(13):7001
55. Tai W et al. (2020) Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol 17(6):613–620
56. Deacon CF (2019) Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. Front Endocrinol. https://doi.org/10.3389/fendo.2019.00080
57. Iacobellis G (2020) COVID-19 and diabetes: can DPP4 inhibition play a role? Diabetes Res Clin Pract 162:108125
58. Yurchenko V et al. (2010) Cyclophilin–CD147 interactions: a new target for anti-inflammatory therapeutics. Clin Exp Immunol 160(3):305–317
59. Cui C et al. (2021) AGTR2, one possible novel key gene for the structural context. Curr Opin Hematol 25(3):163–170
62. Aoki T (2017) A comprehensive review of our current understanding of red blood cell (RBC) glycoproteins. Membranes 7(4):56
63. Miller N, Hutt-Fletcher LM (1992) Epstein-barr virus enters B cells and epithelial cells by different routes. J Virol 66(6):3409–3414
64. Akula SM et al (2002) Integrin α3β1 (CD 49c/29) is a cellular receptor for Kaposi’s sarcoma-associated herpesvirus (KSHV/HHV-8) entry into the target cells. Cell 108(3):407–419
65. Hussein HA et al (2015) Beyond RGD: virus interactions with integrins. Adv Virol 160(11):2669–2681
66. Sigrist CJ, Bridge A, Le Mercier P (2020) A potential role for integrins in host cell entry by SARS-CoV-2. Antivir Res 177:104759
67. Wickham TJ et al (1994) Integrin alpha v beta 5 selectively promotes adenovirus mediated cell membrane permeabilization. J Cell Biol 127(1):257–264
68. Feire AL, Koss H, Compton T (2004) Cellular integrins function as entry receptors for human cytomegalovirus via a highly conserved disintegrin-like domain. Proc Natl Acad Sci USA 101(43):15470–15475
69. Philpott NJ et al (2004) Adenovirus-induced maturation of dendritic cells through a PI3 kinase-mediated TNF-α induction pathway. Proc Natl Acad Sci USA 101(16):6200–6205
70. Gu Y et al (2022) Receptome profiling identifies KREMEN1 and ASGR1 as alternative functional receptors of SARS-CoV-2. Cell Res 32(1):24–37
71. Staring J et al (2018) KREMEN1 is a host entry receptor for a major group of enteroviruses. Cell host microbe 23(5):636-643. e5
72. Mishra SK et al (2012) High-affinity Dkk1 receptor kremlin1 is internalized by clathrin-mediated endocytosis. PLoS ONE 7(12):e52190
73. Saunier B et al (2003) Role of the asialoglycoprotein receptor in binding and entry of hepatitis C virus structural proteins in cultured human hepatocytes. J Virol 77(1):546–559
74. Yeager CL et al (1992) Human aminopeptidase N is a receptor for human coronavirus 229E. Nature 357(6377):420–422
75. Regueru J et al (2012) Structural bases of coronavirus attachment to host aminopeptidase N and its inhibition by neutralizing antibodies. PLoS Pathog 8(8):e1002859
76. Holmes RS et al (2017) Aminopeptidase genes (ENPEP) and proteins: comparative studies of a major contributor to arterial hypertension. J Data Min Genomics Proteomics. https://doi.org/10.4172/2153-0602.e100221
77. Zhu S et al (2022) Genome-wide CRISPR activation screen identifies candidate receptors for SARS-CoV-2 entry. Sci China Life Sci 65(4):701–717
78. Hiraizumi M et al (2019) Cryo-EM structures capture the transport cycle of the P4-ATPase flippase. Science 365(6458):1149–1155
79. Takasugi N et al (2018) TMEM30A is a candidate interacting partner for the β-carboxyl-terminal fragment of amyloid-β precursor protein in endosomes. PLoS ONE 13(8):e0200988
80. Ma H et al (2020) LDLRAD3 is a receptor for Venezuelan equine encephalitis virus. Nature 588(7837):308–314
81. Beaudoin CA et al (2021) Predicted structural mimicry of spike receptor-binding motifs from highly pathogenic human coronaviruses. Comput Struct Biotechnol J 19:3938–3953
82. Hamming IT et al (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 203:631–637
83. Zeisel MB, Felmlée DJ, Baumert TF (2013) Hepatitis C virus entry. Hepatitis C virus. Springer, Berlin, pp 87–112
84. Helenius A (2018) Virus entry: looking back and moving forward. J Mol Biol 430:1853–1862
85. Shajahan A et al (2020) Deducing the N-and O-glycosylation profile of the spike protein of novel coronavirus SARS-CoV-2. Glycobiology 30(12):981–988
86. Prumboom L (2021) SARS-CoV-2: possible alternative virus receptors and pathophysiological determinants. Med Hypotheses 146:110368
87. Kumar V, Singh J, Hasnain SE, Sundar D (2021) Possible link between higher transmissibility of alpha, kappa and delta variants of SARS-CoV-2 and increased structural stability of its spike protein and hACE2 affinity. Int J Mol Sci 22(17):9131
88. McCallum M et al (2021) Molecular basis of immune evasion by the delta and kappa SARS-CoV-2 variants. Science 374(6575):1621–1626
89. Lubinski B et al (2022) Functional evaluation of the P681H mutation on the proteolytic activation of the SARS-CoV-2 variant B. 1.1. 7 (Alpha) spike. Iscience 25(1):103589
90. Pia L, Rowland-Jones S (2022) Omicron entry route. Nat Rev Immunol 22(3):144–144
91. Mlochova P et al (2021) SARS-CoV-2 B. 1.617. 2 delta variant replication and immune evasion. Nature 599:114–119
92. Kumar S et al (2021) Omicron and delta variant of SARS-CoV-2: a comparative computational study of spike protein. J Med Virol 17(1):96
93. Gottlieb RL et al (2022) Early remdesivir to prevent progression to severe Covid-19 in outpatients. N Engl J Med 386(4):305–315
94. Owen DR et al (2021) An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19. Science 374(6575):1586–1593
95. Jayk Bernal A et al (2022) Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients. N Engl J Med 386(6):509–520
96. Triposkiadis F et al (2020) Fallacies in medical practice: renin-angiotensin-aldosterone system inhibition and COVID-19 as a paradigm. Hell J Cardiol 62(3):185–189
97. Zoufaly A et al (2020) Human recombinant soluble ACE2 in severe COVID-19. Lancet Respir Med 8(11):1154–1158
98. Tesoriere L et al (1999) Melatonin protects human red blood cells from oxidative hemolysis: new insights into the radical-scavenging activity. J Pineal Res 27(2):95–105
99. Watsahi K, Shimotohno K (2007) Cyclophilin and viruses: cyclophilin as a cofactor for viral infection and possible anti-viral target. Drug target insights 2:9–18
100. Kawasaki T et al (2018) DPP4 inhibition by sitagliptin attenuates LPS-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol 315(5):L834–L845
101. Jayk Bernal A et al (2022) Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients. N Engl J Med 386(6):509–520
102. Kawasaki T et al (2018) DPP4 inhibition by sitagliptin attenuates LPS-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol 315(5):L834–L845
103. Soare A et al (2020) Dipeditylpeptidase 4 as a marker of activated fibroblasts and a potential target for the treatment of fibrosis in systemic sclerosis. Arthr Rheumatol 72(1):137–149
104. Ohnuma K et al (2013) Inhibition of middle east respiratory syndrome coronavirus infection by anti-CD26 monoclonal antibody. J Virol 87(13):6899–6909
105. Conole D et al (2020) Discovery of a novel fluorescent chemical probe suitable for evaluation of neuropilin-1 binding of small molecules. Drug Dev Res 81(4):491–500
106. Sciences, S.B.U.o.M. Azithromycin in Hospitalized COVID-19 Patients. 2020; Available at https://clinicaltrials.gov/ct2/show/NCT04359316.

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