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REVIEW ARTICLE

Human cell receptors: potential drug targets to combat COVID-19

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the coronavirus disease 2019 (COVID-19). The World Health Organization (WHO) has announced that COVID-19 is a pandemic having a higher spread rate rather than the mortality. Identification of a potential approach or therapy against COVID-19 is still under consideration. Therefore, it is essential to have an insight into SARS-CoV-2, its interacting partner, and domains for an effective treatment. The present study is divided into three main categories, including SARS-CoV-2 prominent receptor and its expression levels, other interacting partners, and their binding domains. The first section focuses primarily on coronaviruses' general aspects (SARS-CoV-2, SARS-CoV, and the Middle East Respiratory Syndrome Coronaviruses (MERS-CoV)) their structures, similarities, and mode of infections. The second section discusses the host receptors which includes the human targets of coronaviruses like dipeptidyl peptidase 4 (DPP4), CD147, CD209L, Angiotensin-Converting Enzyme 2 (ACE2), and other miscellaneous targets (type-II transmembrane serine proteases (TTSPs), furin, trypsin, cathepsins, thermolysin, elastase, phosphatidylinositol 3-phosphate 5-kinase, two-pore segment channel, and epithelium sodium channel C-α subunit). The human cell receptor, ACE2 plays an essential role in the Renin-Angiotensin system (RAS) pathway and COVID-19. Thus, this section also discusses the ACE2 expression and risk of COVID-19 infectivity in various organs and tissues such as the liver, lungs, intestine, heart, and reproductive system in the human body. Absence of ACE2 protein expression in immune cells could be used for limiting the SARS-CoV-2 infection. The third section covers the current available approaches for COVID-19 treatment. Overall, this review focuses on the critical role of human cell receptors involved in coronavirus pathogenesis, which would likely be used in designing target-specific drugs to combat COVID-19.

Keywords COVID-19 · Coronavirus · SARS-CoV · SARS-CoV-2 · ACE2 · WHO

Abbreviations

$3CL^{pro}$	3C like protease
ACE2	Angiotensin converting enzyme 2
ACEi	Angiotensin converting enzyme inhibitor
ADAM17	A disintegrin and metalloprotease 17
ALI	Acute lung injury
Ang	Angiotensin
ARB	Angiotensin type 1 Receptor Blocker
ARDS	Acute respiratory distress syndrome
ASL	Airway surface liquid
AT1	Alveolar type 1
AT_1R	Angiotensin type 1 receptor
AT2	Alveolar type 2
AT_2R	Angiotensin type 2 receptor
B^0AT1	Sodium-dependent neutral amino acid transporter
CFTR	Cystic fibrosis transmembrane conductance regulator
CoV	Coronavirus
COVID-19	Coronavirus disease 19
CVD	Cardiovascular disease
DPP4	Dipeptidyl peptidase 4
E	Envelope
EMMPRIN	Extracellular Matrix Metalloproteinase Inducer
ENaC	Epithelium sodium channel C- α subunit

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eNOS	Endothelial nitric oxide synthase
ERGIC	Endoplasmic Reticulum-Golgi Intermediate Complex
EST	Trans-epoxysuccinyl-L-leucylamindo3-methylbutane ethyl ester
GCSF	Granulocyte colony-stimulating factor
GI	Gastrointestinal
GMP	Good manufacturing practice
GPCR	G-protein coupled receptor
GSH	Glutathione
GSSG	Oxidized glutathione
HB	Helix bundle
HBV	Hepatitis B virus
HCoV	Human coronavirus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Heptad repeat
ICTV	International Committee on Taxonomy of Viruses
IHC	Immunohistochemistry
IL	Interleukins
IP10	γ-induced protein 10
KO	Knock-out
LPS	Lipopolysaccharide
M	Membrane
MAB	Monoclonal antibody
MCP	Monocyte chemoattractant protein
MDA	Malondialdehyde
MERS	Middle East Respiratory Syndrome Coronavirus
MIP	Macrophage inflammatory protein
MLV	Murine leukemia virus
MMP	Matrix metalloproteases
MOF	Multiple organ failure
M^{pro}	Main protease
MSCs	Mesenchymal stem cells
N	Nucleocapsid
nCoV	Novel coronavirus
nsp	Non-structural proteins
ORF	Open reading frames
PIKFYVE	Phosphatidylinositol 3-phosphate 5-kinase
PL^{pro}	Papain-like proteases
pp	Pesudoparticles
QTL	Quantitative trait locus
RAS	Renin-Angiotensin System
RBD	Receptor binding domain
RdRp	RNA dependent RNA polymerase
RTC	Replicase–Transcriptase complex
S	Spike
SARS	Severe Acute Respiratory Syndrome
scRNA-Seq	Single cell RNA sequencing
SIGN	Special intercellular adhesion molecule-3-grabbing nonintegrin
SOD	Superoxide dismutase
TIMP	Tissue inhibitors of metalloproteinases
TMEM16A	Transmembrane member 16 A
TMPRSS	Transmembrane Protease serine subfamily
TNF	Tumor Necrosis Factor
TPC2	Two pore segment channel
TTSPs	Type 2 transmembrane serine proteases
uMSC	Umbilical cord derived MSC
VSV	Vesicular stomatitis virus
WHO	World Health Organization
WT	Wild type

Introduction

The novel disease COVID-19 caused by SARS-CoV-2 has recently been announced pandemic by WHO (Xu et al. 2020). The virus has been spreading with epidemic features from Wuhan of China to other Asian countries, with cases now reported worldwide (Petrosillo et al. 2020). Initially, the virus was represented as 2019-nCoV. Later, on February 12th, 2020, ICTV (International Committee on Taxonomy of Viruses) formally named it SARS-CoV-2. Initially called disease-X, it was later named COVID-19 by WHO (Liu et al. 2020a). Although the casualty rate of COVID-19 is 2.3%, which is lesser than the SARS-CoV infection that causes SARS (9.5%), and much lower than the MERS-CoV infection that causes MERS (34.4%), while its clinical features are more related to SARS (Petrosillo et al. 2020, 2020). Phylogenetically, the SARS-CoV-2 is more closely related to SARS-CoV (sequence identity 79%) than to MERS-CoV (sequence identity 50%) (Kim et al. 2020; Lu et al. 2020). Both SARS-CoV-2 and SARS-CoV bind to a common human cell receptor, angiotensin-converting enzyme 2 (ACE2), causing their entry into host cell, but MERS-CoV is penetrated by dipeptidyl peptidase 4 (DPP4) or CD26 (Xu et al. 2020; Kim et al. 2020; Zhou et al. 2020a). SARS-CoV-2 infected patients with comorbidities like cardiovascular disease (CVD), hypertension, and diabetes are prone to severe conditions that are fatal (Hanff et al. 2020). Infectivity is provoked when spike protein (S-protein) of SARS-CoV-2 binds to the ACE2 receptor (Walls et al. 2020). Abundant expression of ACE2 was observed in the heart, kidneys, lungs, testis, and gastrointestinal (GI) tract and carried out essential functions in several cells signaling pathways (Turner et al. 2004; Chen et al. 2020). The overexpression of ACE2 facilitates SARS-CoV-2 pathogenicity, entry, and replication (Zheng et al. 2020). Prolonged infection causes Angiotensin (Ang) II/ACE2 regulation imbalance leading to respiratory disease distress and hypoxemia in the lungs, causing Acute Respiratory Distress Syndrome (ARDS), septic shock, metabolic acidosis, and fatality (Peiris et al. 2003; Chen et al. 1998). This severe infection in the

lungs of COVID-19 patients stimulates a drastic elevation of cytokines like interleukins (IL), (IL-2, IL-6, and IL-7), granulocyte colony-stimulating factor (GCSF), γ-induced protein 10 (IP10), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1A (MIP1A), and tumor Necrosis Factor (TNF)-α. Primarily, the level of IL-6 was found very high in COVID-19 patients (Aghai et al. 2007), though this cytokines storm also gets provoked in severe cases of pneumonia (Huang et al. 2020). The cardiovascular system may lead to a cellular inflammatory effect, migration of vascular smooth muscle and endothelial cells, oxidative stress, and might cause atherosclerosis (Xu et al. 2020; Chen et al. 1998). The current study focuses on the prominent expression of ACE2 in various human body tissues that may correspond to infection risk. The genetic and protein expression rate of ACE2 is not constant and differs among cells, tissues, and organs. This study also describes other potential targets and binding sites available for the SARS-CoV-2 infection. These markers can be considered as targets for drugs that could be designed for plausible therapeutics and prophylactics against COVID-19. The current treatment involves the use of ACE inhibitors (ACEi), AT_1R blockers (ARBs), recombinant receptor-binding domain (RBD), mesenchymal stem cells (MSCs), cyclodextrin-soluble ACE2, and serine and cysteine protease inhibitors based therapies. Currently, few drugs are used for the treatment, and few vaccine sets are under trial. However, several receptors and proteins are summarized that require to be elucidated to find targets and domains. Previous studies show that the receptors and their prominent expression could help in the synthesis of novel compounds that could be potential drug candidates in treating coronavirus infection. The coronaviruses' (SARS-CoV, SARS-CoV-2, and MERS-CoV) infection along with possible treatments is represented in Fig. 1.

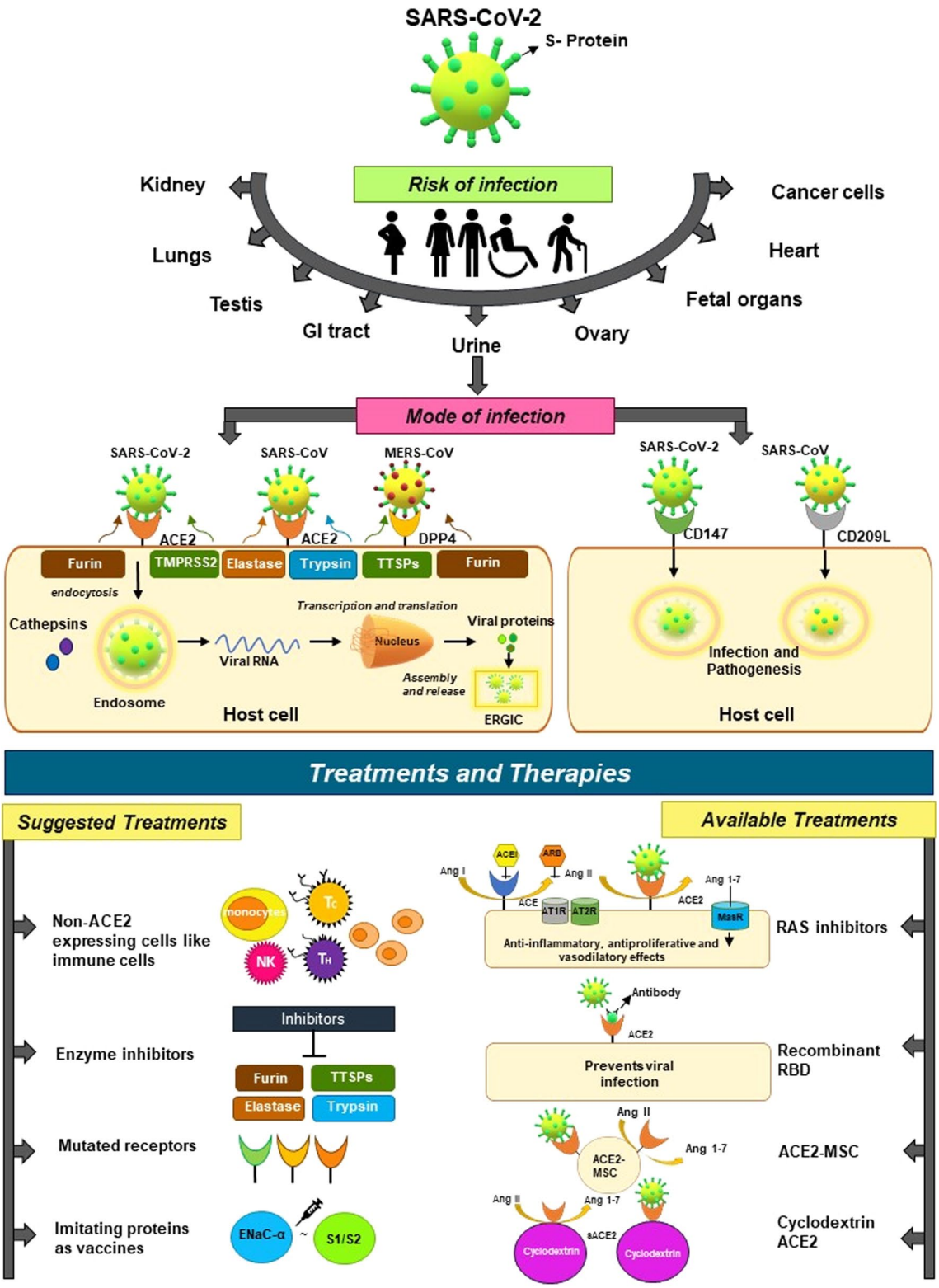
Similarity of SARS-CoV-2 with other coronaviruses

The Coronaviridae family is categorized into four main subgroups: alpha, beta, gamma, and delta. The alpha and beta subgroups represent seven human coronaviruses, wherein five infectious human coronaviruses (OC43, HKU1, SARS-CoV, SARS-CoV-2, and MERS-CoV) belong to betacoronaviruses, while the other two belong to alphacoronaviruses (229E and NL63) (Zhou et al. 2020a). Also, HKU4 and HKU5 are bat coronaviruses belonging to betacoronaviruses (Wang et al. 2014). Another bat SARS-like coronavirus, SL-CoV-RaTG13 (Zhou et al. 2020a) (betacoronavirus (Malaiyan et al. 2020)) is a distinct clade from MERS-CoV (Petrosillo et al. 2020; Zhou et al. 2020a; Malaiyan et al. 2020). Nevertheless, the SARS-CoV-2 shares a close resemblance to SL-CoV-RaTG13 (sequence identity 96%) compared to SARS-CoV (sequence identity 79%) and MERS-CoV (sequence identity 50%) (Kim et al. 2020; Lu et al. 2020; Zhou et al. 2020a). The MERS-CoV gene shows the highest sequence similarity to the bat coronavirus, HKU4, compared to HKU5 (Mann et al. 2008). Understanding the critical differences between the two closely related viruses, SARS-CoV-2 and SL-CoV-RaTG13, would likely find the genetic mutations, alterations in the process of its evolution, and strategically designing target-specific drugs. The inhibitors that would target SL-CoV-RaTG13 may also work effectively on SARS-CoV-2.

Structural similarity between SARS-CoV, SARS-CoV-2, and MERS-CoV with their key domains

The Coronaviridae family has a positive-sense single-stranded RNA with diverse open reading frames (ORFs) that encode for different non-structural, structural, and accessory proteins, which are crucial for viral replication (Fehr and Perlman 2015). The coronaviruses contain several ORFs like 14 in SARS-CoV-2 (Wu et al. 2020), 12–13 in MERS-CoV, and 10 in SARS-CoV (Groneberg et al. 2005; Ramadan and Shaib 2019). The coronaviruses share few common structural proteins that are encoded at the 3′ end of the viral genome, which includes S-protein, an envelope protein (E), membrane protein (M), and nucleocapsid (N) (Fehr and Perlman 2015) (Table 1). The 3′ genome of SARS-CoV-2 also encodes for eight accessory proteins (Wu et al. 2020; Derington et al. 2020). At the 5′ end of the genome of SARS-CoV-2, the ORF1 and ORF2 encode for sixteen non-structural proteins (nsps), which are crucial for viral replication (Wu et al. 2020; Derington et al. 2020). SARS-CoV-2 genome encodes approximately twenty-five proteins, including the S-protein, two proteases involves in cleaving human proteins, polymerases, and endoribonuclease act on RNA, which are responsible for viral infection and replication (Parks and Smith 2020).

The S-protein that associates and binds with cellular receptors for infection are a characteristic feature of the Coronaviridae family. Its nature influences the features related to transmissibility and pathogenesis (Liu et al. 2020b). The S-protein of MERS-CoV, SARS-CoV, and SARS-CoV-2 is a class 1 fusion protein consisting of two subunits (Bosch et al. 2003). The first S1 receptor-binding unit forms the head that contains RBD (Wu et al. 2020; Bosch et al. 2003), whereas the S2 fusion subunit present in the stalk of spike protein of the coronaviruses (Fehr and Perlman 2015). The M-protein is present in large quantities in the virion that provides shape to the developing virus (Fehr and Perlman 2015; Armstrong et al. 1984). E-protein facilitate the virus's assembly and release, and is present in small quantities on the virion surface. Coronaviruses share a typical architecture of E-protein



but with varied structures (Fehr and Perlman 2015; Godet et al. 1992). Also, the SARS-CoV has an ion channel activity, playing an essential role in pathogenesis (Nieto-Torres et al. 2014). The *N* protein has N and C terminal domains, which play a vital role in the in vitro RNA binding through two different mechanisms (Fehr and Perlman 2015; Chang et al. 2006; Hurst et al. 2009). The information of structural proteins of coronavirus is listed in Table 1.

◂**Fig. 1** Illustrates coronavirus infectivity with the target receptor of interaction and other host receptors involved in viral entry and infection. SARS-CoV and SARS-CoV-2 bind to ACE2, it would lead to Ang II's overaccumulation that causes pathophysiological effects in the host cell subsequently to the human system. After binding to the receptor, with the help of host cellular enzymes, the virus would internalize through the endosomal pathway and release the viral RNA that would bind to the host cell genome. This would lead to transcription and translation of viral proteins assembled in the endoplasmic-reticulum-Golgi intermediate complex (ERGIC) and released as small viral particles through exocytosis. Other prominent receptors involved for coronavirus infection are CD147 and CD209L for both SARS-CoV and SARS-CoV-2, while DPP4 is the receptor involved for MERS-CoV infection. Various therapies have been proposed to use RAS inhibitors like ACEi/ARB, recombinant RBD, ACE2-MSC, and cyclodextrin-soluble ACE2 to prevent and reduce the viral load and infection. Along with these possible therapies like the use of enzyme inhibitors, mutated target receptors, non-ACE2 expressing immune cells, and the use of proteins like ENACα as a vaccine candidate that mimics the S1/S2 subunit

Unlike SARS-CoV and SARS-CoV-2, the MERS-CoV entry inside human cells has been confirmed by a crystal structure of complex RBD of MERS-CoV and DPP4 (Petrosillo et al. 2020; Wang et al. 2013, 2014; Tai et al. 2020). Further, the MERS-CoV's RBD has two subdomains: a core that is highly similar to the RBD of SARS-CoV (Wang et al. 2013), and a receptor-binding subdomain, which associates with the DPP4 β-propeller. Although, structural superimposition showed a root mean square deviation (RMSD) of 3.8 Å between S-protein of SARS-CoV-2 and SARS-CoV, both have a binding affinity for ACE2 (Chen et al. 2020; Kumar et al. 2020; Wrapp et al. 2020). The binding affinity of SARS-CoV-2 for ACE2 is 10–20 times higher than of SARS-CoV (Wrapp et al. 2020). Thus, SARS-CoV-2 is highly contagious and transmissible, though having a lower mortality rate (Petrosillo et al. 2020) than SARS-CoV and MERS-CoV, which are chiefly responsible for the nosocomial spread (Malik et al. 2020).

The docking studies of human ACE2 with SARS-CoV-2 S-protein identify the SARS-CoV-2 hotspot residues, Leu441, Phe472, Gln479, Ser480, Asn487, and Tyr491 (Chen et al. 2020), while the X-ray crystallographic three-dimensional (3D) structure (PDB ID: 6M0J) showed interacting residues Lys417, Leu455, Phe486, Gln493, Gln498, Leu455, and Asn501 (Lan et al. 2020). The SARS-CoV-2′s S-protein RBD bind with ACE2 through the interacting residues, RBD: Leu455—ACE2: Asp30, Lys31, and His34; RBD: Lys417—ACE2: Asp30; RBD: Phe486—ACE2: Gln24, Leu79, Met82, and Tyr83; RBD: Gln493—ACE2: Lys31, His34, and Glu35; RBD: Gln498—ACE2: Asp38, Tyr41, Gln42, Leu45, and Lys353; RBD: Asn501—ACE2: Tyr41, Lys353, Gly354, and Asp355 (Lan et al. 2020). The complex 3D structure and interacting residues between the SARS-CoV-2 RBD domain and ACE2 are illustrated in Fig. 2.

Moreover, the mutations in the RBD of SARS-CoV were linked to pathogenic interactions with human ACE2. The SARS-CoV S-protein mutations, C348A, D454A, C467A, C474A, E452A, and D463A, abrogate their association with ACE2 (Wong et al. 2004). Although, mutations like R667S resulted in partial loss of cell–cell fusion and R797N lead to impairment to trypsin mediated membrane fusion (Follis et al. 2006; Belouzard et al. 2009). However, K672S mutation did not affect the cell–cell fusion mechanism (Follis et al. 2006), while C323A and D480A had no effect in binding with ACE2 (Wong et al. 2004). Similar cases of these mutations were also observed in SARS-CoV-2 (Wu et al. 2020; Wan et al. 2020). However, six mutations occur in other regions of RBD, the sub-domain region of the S1 subunit of SARS-CoV-2. The function of these mutations in viral pathogenicity must be further investigated (Petrosillo et al. 2020; Wu et al. 2020; Ge et al. 2013). Among the unsubstituted regions of SARS-CoV-2 concerning SARS-CoV and six mutated regions in the RBD of SARS-CoV-2, there is a possibility that these alterations could be a reason for the stronger affinity to the ACE2 receptor and hence, causing lower pathogenicity. Changes in these mutations may reduce their binding affinity or pathogenesis, which can be a therapeutic approach for a better vaccine alternative. However, there could be a risk of higher binding affinity and pathogenesis in subsequent mutations and, therefore, must be adequately investigated.

Furthermore, the S-protein of MERS-CoV (Kleine-Weber et al. 2018), and SARS-CoV-2 (Coutard et al. 2020), harbors a particular S1/S2 furin cleavage site, which is distinct from SARS-CoV, possessing peculiar infectious properties (Coutard et al. 2020; Xia et al. 2020). In SARS-CoV-2 insertion of four amino acids (residues of proline, two arginines, and alanine, underlined, SPRRAR↓S, this indicating the motifs (**K/R**)–$(X)_n$–(**K/R**)↓, where $n = 0$, 2, 4 or 6 and X is any amino acid) was observed at the S1/S2 site making it potential cleavage site for the furin protease and considered as a "polybasic" or "multibasic" site (site cleaved by a wide variety of proteases to activate precursor proteins) (Coutard et al. 2020; Jaimes et al. 2020; Seidah and Chretien 1999).

The binding of MERS-CoV to DPP4, and SARS-CoV and SARS-CoV-2 to ACE2 triggered each of the three units of heptad repeat 1 (HR1) and HR2 domains of S2 subunit to interact with each other through hydrophobic forces, thus forming a six-helix bundle (6-HB) fusion core (Yuan et al. 2017). The fusion core brings the virus and cell plasma membranes close to each other for fusion and infection (Bosch et al. 2004). The X-ray crystallographic studies show that the 6-HB fusion core established by the assembly of HR1 and HR2 of SARS-CoV-2 shares similarities to MERS-CoV 6-HB and SARS-CoV 6-HB with RMSD 0.36 Å and 0.66 Å respectively for all the Cα atoms proved that they have highly conserved regions (Zhou et al. 2020a; Xia et al.

Table 1 Three-dimensional (3D) protein structural details of coronaviruses (SARS-CoV, SARS-CoV-2 and MERS-CoV) (Source: https://www.rcsb.org/)

PDB entry	Method	Resolution (Å)	Chains	Sequence position	Sequence length
SARS-CoV					
S-Protein					
5X58	EM	3.20	A/B/C	14–1193	1179
5XLR	EM	3.80	A/B/C	1–1196	1195
6ACC	EM	3.60	A/B/C	1–1196	1195
6CRV	EM	3.20	A/B/C	14–1190	1176
M-Protein					
3I6G	X-ray	2.20	C/F	88–96	8
E-Protein					
2MM4	NMR		A	8–65	57
5X29	NMR		A/B/C/D/E	8–65	57
N-Protein					
2OFZ	X-ray	1.17	A	49–174	125
2OG3	X-ray	1.85	A	49–174	125
SARS-CoV-2					
S-Protein					
6VSB	EM	3.46	A/B/C	1–1208	1207
6VXX	EM	2.80	A/B/C	14–1211	1197
6VYB	EM	3.20	A/B/C	14–1211	1197
N-Protein					
6VYO	X-ray	1.70	A/B/C/D	47–173	126
MERS-CoV					
S-Protein					
4L72	X-ray	3.00	B	382–585	203
4NJL	X-ray	2.30	A	984–1063	79

SARS-CoV severe acute respiratory syndrome coronavirus, *MERS-CoV* Middle East Respiratory Syndrome coronavirus, *S* spike, *M* membrane, *E* envelope, *N* nucleocapsid, *PDB* Protein Data Bank, *EM* electron microscopy, *NMR* nuclear magnetic resonance

2020). The shape of coronaviruses' 6-HB structure is rod-like, attained a length of 115 Å and a diameter of 25 Å (Xia et al. 2020). S2 subunit's 6-HB structure plays a vital role in the membrane fusion process and can be targeted to develop inhibitors against viral fusion and infection (Lan et al. 2020; Xia et al. 2020).

The similarity in the HR2 domain of SARS-CoV and SARS-CoV-2 could also serve as a common target for designing monoclonal antibodies and drugs by destabilizing the 6HB core structure to prevent virus cell entry and pathogenesis. A study indicated that the 47D11 mAb (monoclonal antibody) targeting S-protein trimer inhibits SARS-CoV-2, including SARS-CoV (Wang et al. 2020a).

Besides, the SARS-CoV and SARS-CoV-2 also have infectious proteases involved in transcription and replication, which are aided by the nsps, RNA dependent RNA polymerase (RdRp) (Masters 2006; Astuti 2020), and other subunits of the replicase-transcriptase complex (RTC) (Liu et al. 2020a; Ou et al. 2020), produced via cleavage of bulk replicase polyprotein 1a (pp1a) and pp1ab by 3 C like protease ($3CL^{pro}$). The $3CL^{pro}$ (M^{pro}) (PDB_ID: 6LU7), the main protease in SARS-CoV-2, has a similar structure and sequence concerning SARS-CoV involved in viral replication and transcription (Jin et al. 2020a). The M^{pro} Cys145 residue can serve as an attractive drug target in playing a vital role in inhibiting viral pathogenesis (Liu et al. 2020a; Jin et al. 2020a). The in silico computational studies identified compounds such as Itacitinib, Oberadiol, Telcagepant, Vidupiprant, Pilaralisib, Poziotinib, Fostamatinib, CL-275838, Ziprasidone, Leucal/Folinic Acid, and ITX506 (Liu et al. 2020a), inhibit both SARS-CoV and SARS-CoV-2 by targeting M^{pro} at Cys145. Hepatitis C virus protease inhibitors, Boceprevir and Telaprevir (Ma et al. 2020a), calpain inhibitors II and XII, GC-376 (Ma et al. 2020a), and α-ketoamide inhibitors (Zhang et al. 2020a) effectively block the activity of M^{pro} to inhibit SARS-CoV-2. In contrast, some M^{pro} inhibitors were shown to be ineffective for SARS-CoV and SARS-CoV-2. However, Boceprevir displayed a broad-spectrum anti-viral activity by inhibiting SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-229E,

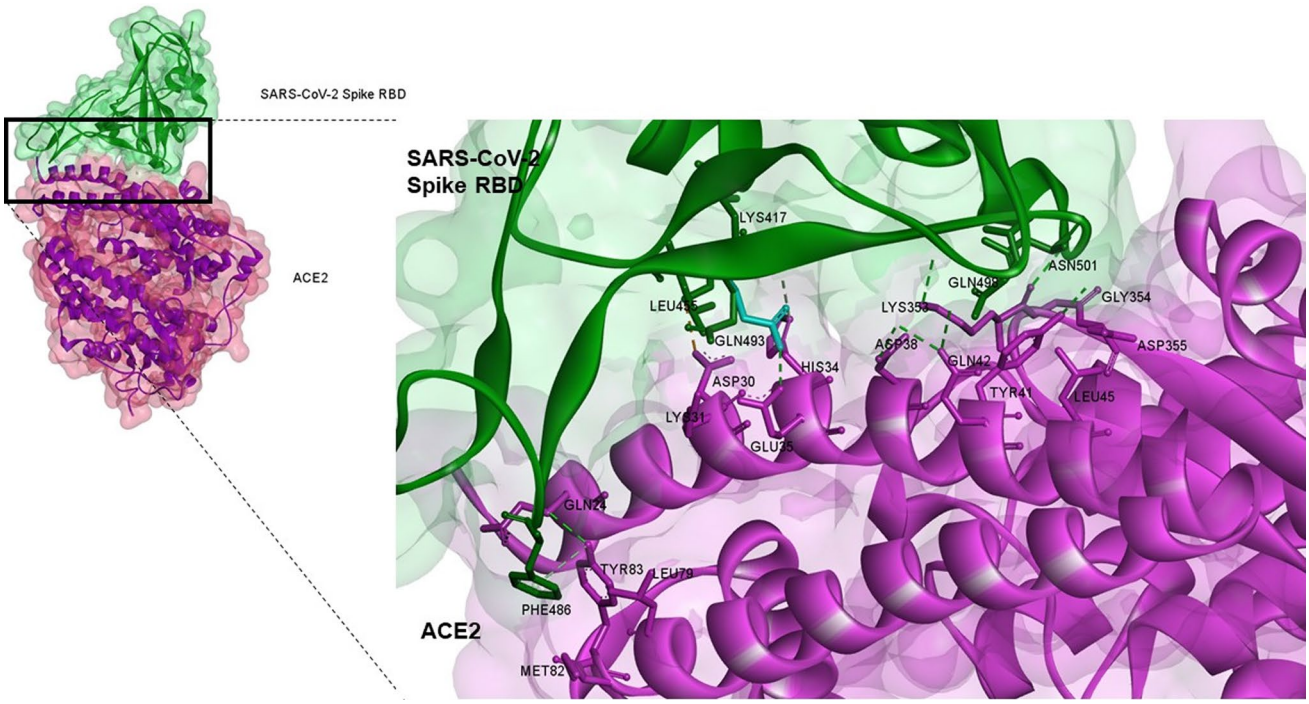


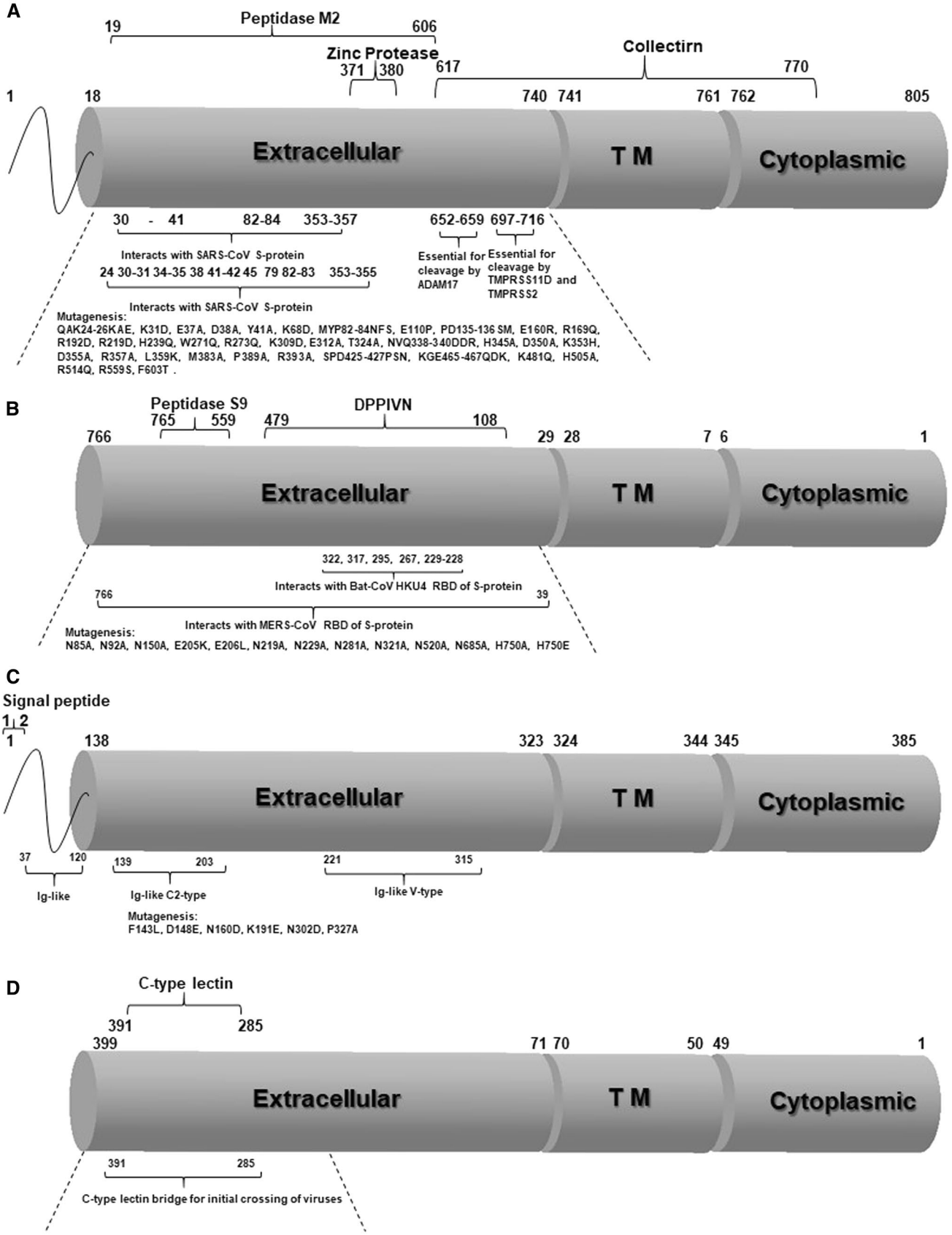
Fig. 2 3D complex structure of SARS-CoV-2 Spike RBD with human ACE2 and their interacting residues (PDB_ID: 6M0J)

HCoV-OC43, and HCoV-NL63 (Hu et al. 2020). The human rhinovirus protease inhibitor Rupintrivir (Hilgenfeld 2014) is effective against HCoV-229E, but it could not inhibit the activity of M^{pro} in SARS-CoV and SARS-CoV-2 (Ma et al. 2020a; Kim et al. 2012). The in silico studies indicated that HIV-1 protease inhibitors Lopinavir and Ritonavir (Nutho et al. 2020) is a novel M^{pro} inhibitors. However, in vitro and in vivo studies showed that these inhibitors could not reach the effective pharmacokinetic concentration for inhibiting M^{pro} (Zhang et al. 2020b).

An in-depth structural analysis of M^{pro} would be likely to identify or design an anti-viral compound. There are three nsps of SARS-related viruses, namely nsp1, nsp2, and nsp3, processed by papain-like protease (PL^{pro}) found in the SARS virus that cleaves the N terminal of these nsps (Hilgenfeld 2014). A lead compound GRL0617 and its modified form suppress the activity of PL^{pro} (Hilgenfeld 2014). It could also be used to find the overall function of PL^{pro}, which further needs to be investigated. Hence, understanding the viral infection identifies the potential targets involved that could be inhibited or neutralized accordingly.

Mode of infection

SARS-CoV-2 infection is triggered through the binding of its S1 subunit of S-protein with ACE2 (Walls et al. 2020). Afterward, SARS-CoV-2 entry also relays upon priming by the serine proteases, transmembrane protease serine subfamily 2 (TMPRSS2) (Li et al. 2003; Matsuyama et al. 2010). Also, the S2 subunits of SARS-CoV-2, responsible for membrane fusion, share 89.8% sequence identity to SARS-CoV (Zhou et al. 2020a). As the SARS-CoV-2 comes in close proximity to the host cell, the RBD of the S1 subunit of S-protein binds to the ACE2 of the target cells (Xia et al. 2020). Subsequently, the 6-HB fusion core is formed, in which the three units of both HR1 core and HR2 domain intertwine antiparallelly with each other in a coiled-coil manner, thus facilitating the fusion of virus and cell plasma membranes and causing infection (Bosch et al. 2004). However, clathrin and non-clathrin mediated endocytosis are an alternate route for internalization and coronaviruses' entry in the absence of membrane-bound or exogenous proteases (Inoue et al. 2007; Wang et al. 2008). After the viral entry into the cells, SARS-CoV-2 and SARS-CoV RNA replication and transcription are aided by the nsps like RdRp and helicase produced through cleavage of pp1a and pp1ab by their respective proteases M^{pro} (Jin et al. 2020a) ($3CL^{pro}$ (Zumla et al. 2016)), while the N terminal of the nsps is cleaved by the PL^{pro} (Hilgenfeld 2014). The RTC formation by the nsps occurs in the double-membrane vesicles, the RdRp and helicase containing subunits. Subsequently, the transcription of an endogenous genome template mediated by the RTC occurs in the sub-genomic RNA's negative-sense genes and the progeny genome, which then transcribes the positive-sense mRNAs. The transcribed sub-genomic RNA further translates into structural and accessory proteins:


A
Peptidase M2
19
606
Zinc Protease
371
380
Collectirn
617
770
1
18
740
741
761
762
805
Extracellular
T M
Cytoplasmic
30 - 41
82-84
353-357
652-659
697-716
Interacts with SARS-CoV S-protein
24 30-31 34-35 38 41-42 45 79 82-83 353-355
Interacts with SARS-CoV S-protein
Essential for cleavage by ADAM17
Essential for cleavage by TMPRSS11D and TMPRSS2
Mutagenesis:
QAK24-26KAE, K31D, E37A, D38A, Y41A, K68D, MYP82-84NFS, E110P, PD135-136 SM, E160R, R169Q, R192D, R219D, H239Q, W271Q, R273Q, K309D, E312A, T324A, NVQ338-340DDR, H345A, D350A, K353H, D355A, R357A, L359K, M383A, P389A, R393A, SPD425-427PSN, KGE465-467QDK, K481Q, H505A, R514Q, R559S, F603T .
B
Peptidase S9
765
559
DPPIVN
479
108
766
29
28
7
6
1
Extracellular
T M
Cytoplasmic
322, 317, 295, 267, 229-228
Interacts with Bat-CoV HKU4 RBD of S-protein
766
39
Interacts with MERS-CoV RBD of S-protein
Mutagenesis:
N85A, N92A, N150A, E205K, E206L, N219A, N229A, N281A, N321A, N520A, N685A, H750A, H750E
C
Signal peptide
1
2
1
138
323
324
344
345
385
Extracellular
T M
Cytoplasmic
37
120
139
203
221
315
Ig-like
Ig-like C2-type
Ig-like V-type
Mutagenesis:
F143L, D148E, N160D, K191E, N302D, P327A
D
C-type lectin
391
285
399
71
70
50
49
1
Extracellular
T M
Cytoplasmic
391
285
C-type lectin bridge for initial crossing of viruses

◂**Fig. 3** The line Diagram of prominent receptors for coronavirus infection. These receptors represent the main topological domains: extracellular, transmembrane (TM), and cytoplasmic along with mutagenesis and interacting regions with viruses: **a** ACE2 (Uniprot ID: Q9BYF1) (Lan et al. 2020), **b** DPP4 (Uniprot ID: P24487) (Wang et al. 2013, 2014). **c** CD147 (Uniprot ID: P35613), **d** CD209L (Uniprot ID: Q9H2X3). The protein mutagenesis data has also been mentioned along with major protein domains

S, M, and E and are packed into the endoplasmic reticulum and then moved to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). The N protein is then transcribed and joins the prior replicated genome program forming nucleocapsid before moving into ERGIC. Within the ERGIC, assembly of nucleocapsid with other structural proteins results in small virion coated vesicles released out of the cell through exocytosis (Astuti 2020). Moreover, MERS-CoV interacts with receptor DPP4 for their entry into the host's cells (Wang et al. 2014; Tai et al. 2020; Raj et al. 2013). However, the complete pathway of infection further needs to be investigated to understand its potential domain that causes infection.

Human cellular receptors as targets of coronaviruses

Prominent binding receptors like DPP4 for MERS-CoV, ACE2 for SARS-CoV and SARS-CoV-2 (Xu et al. 2020; Kim et al. 2020; Zhou et al. 2020a), other receptors such as CD147 and CD209L are also identified to be involved in SARS-CoV-2 infection (Wang et al. 2020; Amraie et al. 2020). Furthermore, human proteins and enzymes involved during the viral entry could serve to be potent targets for coronavirus.

DPP4 or CD26: a binding receptor for MERS-CoV

DPP4 or CD26 is a type-II transmembrane protein that helps in regulating different physiological processes (Gorrell et al. 2001). The complex structure analysis indicates that bat coronavirus, HKU4 RBD is associated with human CD26 (hCD26) for entry into the cell (Wang et al. 2014; Lau et al. 2013). The complex structure of HKU4 RBD and hCD26 (PDB_ID: 4QZV) revealed that RBD of MERS-CoV and HKU4 have a higher identity (54.4%) and also share a similar binding mode to hCD26 (Wang et al. 2014; Tai et al. 2020; Raj et al. 2013). The receptor-binding subdomain in the RBD of MERS-CoV has key residues critical for binding with DPP4 (Wang et al. 2013). Although a recent study on HKU4 suggests that MERS-CoV would have originated from bats (Wang et al. 2014). The expression of DPP4 is high in alveolar type 1 (AT1) and alveolar type 2 (AT2) cells of lung alveoli, endothelial cells, non-ciliated bronchial epithelial cells of the liver, intestine, kidneys, thymus, and hemopoietic cells (HSCs) of bone marrow (Memish et al. 2020). These cells with high DPP4 expression would correspond to MERS-CoV spreading extensively across the body.

CD147 an alternative target of SARS-CoV-2

CD147 receptor is also known as Basigin or Extracellular Matrix Metalloproteinase Inducer (EMMPRIN), a highly glycosylated transmembrane protein of immunoglobulin superfamily that acts as a leading upstream simulator of matrix metalloproteases (MMPs). The MMPs are highly expressed in asthma, diabetes, influenza, and cancer. The cancer cells have a high expression of CD147 (Mattos et al. 2002; Moheimani et al. 2018; Bao et al. 2010), which is associated with inflammatory responses. In malaria patients, CD147 serves as a receptor for cell entry of *Plasmodium falciparum* (Crosnier et al. 2011). Studies reported that CD147 also serves as a new route for SARS-CoV-2 infection (Wang et al. 2020). The virus invaded the host cell by S-protein binding to CD147 (Wang et al. 2020). Abundant CD147 expression is observed in macrophages and AT2 cells in pulmonary fibrosis (Guillot et al. 2006), leading to acute effects of COVID-19. This de novo route of entry is plausibly because of adaptation, and both the pathogens (*Plasmodium* and SARS-CoV-2) likely share a common domain for their entry into the cells via CD147, which needs to be further studied. Also, blocking CD147 would be able to reduce pulmonary fibrosis due to SARS-CoV-2 infection.

CD209L a SARS-CoV and SARS-CoV-2 target

CD209L or dendritic cell-specific intercellular adhesion molecule (ICAM) 3-grabbing nonintegrin (DC-SIGN) is an HIV-1 receptor. CD209L is expressed on dendritic cells commonly, but its expression level is higher in lymphatic endothelial cells than that of ACE2 (Li et al. 2007). Despite of having lower affinity, it serves as a substitute receptor in SARS-CoV infection (Magrone et al. 2020), and facilitates entry passage for other RNA viruses like Sindbis and Ebola. While Hepatitis C virus (HCV) and Human Immunodeficiency Virus (HIV) may bind to CD209L, it does not necessarily guide their entry into the host cells. Immunohistochemical (IHC) studies show that along with ACE2, CD209L is also expressed in AT2 and endothelial cells of the human respiratory system. Studies suggest that other than ACE2, the S-protein of SARS-CoV can also use CD209L for viral infectivity and pathogenesis (Jeffers et al. 2004). The C-type lectin present in CD209L (UniProt ID: Q9H2X3) (Fig. 3) acts as an entry portal for various pathogens. Mutagenesis studies occur in this region will help in preventing the entry of these pathogens. Like SARS-CoV, a recent study also revealed that CD209L could also serve as

receptors for SARS-CoV-2 infection (Amraie et al. 2020). The line diagram of receptors ACE2, DPP4, CD147, and CD209L, along with interacting regions is represented in Fig. 3.

ACE2 a target of SARS-CoV and SARS-CoV-2

ACE homologous zinc metalloprotease carboxypeptidase enzyme, ACE2, is composed of 805 amino acids (Fig. 3). The full-length form of ACE2 is a type 1 transmembrane glycoprotein that consists of a single extracellular catalytic domain (N terminal), a small transmembrane segment, and a short intracellular cytoplasmic tail (C terminal) (Patel et al. 2014).

The membrane-anchored protein enzyme, A Disintegrin and Metalloproteases 17 (ADAM17) cleaves ACE2 and converts it into a shorter and soluble form, with the ability of circulating in the blood at low levels. Ang II type 1 receptor (AT_1R) upregulates the expression of ADAM17 and increases soluble ACE2 (sACE2) levels (Serfozo et al. 2020; Arendse et al. 2019; Xu et al. 2017). Accumulation of Ang II through AT_1R produces pro-inflammatory effects that may lead to acute lung injury or myocarditis (Zhonghua et al. 2020). Nonetheless, membrane-bound ACE2 inhibits Ang II activity in the Renin-Angiotensin System (RAS) by degrading Ang II into Ang 1–7, which elicits anti-inflammatory effects through Angiotensin type 2 receptor (AT_2R) and Mas receptor pathway (Fig. 4). Also, ACE2 cleaves Ang I to release Ang 1–9 during the suppression of ACE2, although ACE2 has 400 folds higher affinity and higher catalytic efficiency for Ang II than Ang I (Arendse et al. 2019; Cheng et al. 2020; Vickers et al. 2002). The structural information of binding receptors is listed in Table 2.

Table 2 3D structural details of human cell receptors that bind with coronaviruses (SARS-CoV, SARS-CoV-2, and MERS-CoV) (Source: https://www.rcsb.org/)

PDB entry	Method	Resolution (Å)	Chains	Sequence position	Sequence length
ACE2					
1R42	X-ray	2.20	A	1–615	614
1R4L	X-ray	3.00	A	1–615	614
6M0J	X-ray	2.45	A	19–615	596
6M17	EM	2.90	B/D	18–805	787
DPP4					
1J2E	X-ray	2.60	A/B	33–766	733
1PFQ	X-ray	1.90	A/B	36–766	730
4A5S	X-ray	1.62	A/B	39–766	727
4N8D	X-ray	1.65	A/B	39–766	727
CD147					
3I84	X-ray	2.00	A/B	13–219	206
4U0Q	X-ray	3.10	B/D	1–385	384
CD209L					
1SL6	X-ray	2.25	A/B/C/D/E/F	216–399	183
1XAR	X-ray	2.25	A/B	216–399	183
1XPH	X-ray	1.41	A	250–399	149

ACE2 angiotensin-converting enzyme 2, *DPP4* dipeptidyl peptidase 4, *PDB* Protein Data Bank, *EM* electron microscopy

The overall function of ACE2 in the entry of SARS-CoV and SARS-CoV-2 is to act as a counter-regulatory force for

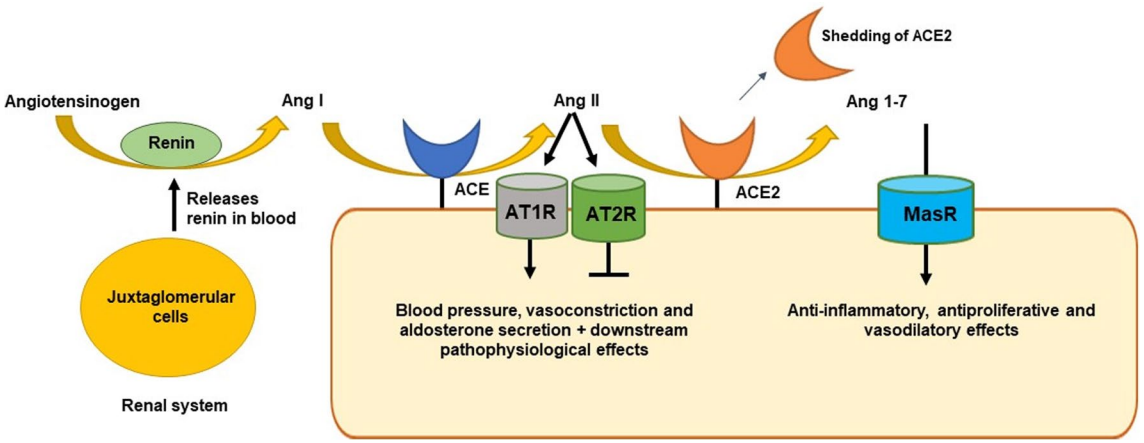


Fig. 4 The physiological role of ACE and ACE2 in the RAS pathway. During hypotension, the juxtaglomerular cells of the renal system releases renin into the blood that circulates into the body. Renin subsequently breaks down Angiotensinogen to Ang I. The Ang I, with the help of ACE is further converted to Ang II, which leads to elevated blood pressure, vasoconstriction, aldosterone secretion and downstream pathophysiological effects through AT1R pathway. Alternatively, these effects would be inhibited if Ang II mediates its effects through AT2R pathway (resulting in vasodilatory effect). The Ang II is further broken down to Ang 1-7 by ACE2 that elicits antagonistic effect to Ang II as Ang 1-7 that binds with Mas receptor (MasR) resulting in anti-inflammatory, antiproliferative, and vasodilatory effects. ADAM17 is known to be responsible for shedding membrane anchored proteins, thus releasing soluble ACE2

RAS. It balances multiple ACE functions by targeting Ang II, protects the organs especially the cardiovascular system, and plays a crucial role in binding and absorption of amino acids in the renal system and GI tract by regulating the expression of Sodium-dependent neutral amino acid transporter (B^0AT1) (Sun et al. 2020; Clotet-Freixas et al. 2018; Ortiz-Melo and Gurley 2016; Vuille-Dit-Bille et al. 2015).

Conclusively, the mutagenesis studies revealed the favorable mutations in DPP4, ACE2, and CD147 that would likely have active interacting or dissociating sites for MERS-CoV, SARS-CoV, and SARS-CoV-2 (Fig. 3). The COVID-19 patients with comorbid conditions like cancer have an additional possibility of the mutations in the ACE2 receptor, which would make the patient susceptible or resistant to the SARS-CoV-2 infections. Such cases must be further investigated to understand the probable reasons of the virus binding to the mutated receptor. Similarly, in the course of evolution, mutations in receptors including DPP4 and CD147 could also either promote or prevent the binding affinity with the coronaviruses. Alternatively, there may also be a few mutations that would not affect the binding affinity. Hence, an understanding of these receptors and viral domains' mutation rate is required to delineate the mutations that would prevent viral entry and pathogenesis.

Miscellaneous targets

In the coronavirus pathophysiology, the enzymes such as serine cystine proteases, trypsin, furin, thermolysin, and elastase are required for viral entry (Xia et al. 2020; Belouzard et al. 2010; Tang et al. 2020). Meanwhile for endocytosis pathway, enzymes such as cathepsins, phosphatidylinositol 3-phosphate 5-kinase and two-pore segment channel are known to play key role during the coronavirus infection (Ou et al. 2020; Hoffmann et al. 2020).

Transmembrane serine proteases are the target for SARS-CoV, SARS-CoV-2 and MERS-CoV

The membrane-bound proteases family member, type-II transmembrane serine proteases (TTSPs) are involved in the influenza virus' infectivity. The influenza virus' activation and transmissibility are mainly through transmembrane protease serine subfamily 2 (TMPRSS2) and subfamily 4 (TMPRSS4) (Tang et al. 2020; Choi et al. 2009; Bertram et al. 2010; Böttcher et al. 2006; Chaipan et al. 2009). TMPRSS2 is a membrane-bound serine protease that takes part in proteolytic cascades to maintain the homeostasis of the prostate (Lucas et al. 2014).

Studies have shown that membrane-bound TMPRSS2 activates MERS-CoV, SARS-CoV, and their pseudo-particles (pp) like SARSpp and MERSpp for infection (SARSpp includes lentiviral particles incorporated with S-protein of SARS-CoV (Simmons et al. 2004), replication defective Vesicular stomatitis virus (VSV) particles having S-protein of SARS-CoV-2 (Hoffmann et al. 2020), and MERSpp includes MERS-CoV S-protein expressing murine leukemia virus (MLV) vector (Zmora et al. 2018)). TMPRSS2 cleaves MERS-CoV, SARS-CoV, and SARS-CoV-2 at the S2 site to activate plasma membrane fusion (Tang et al. 2020; Hoffmann et al. 2020; Gierer et al. 2013; Qian et al. 2013; Shirato et al. 2013; Matsuyama et al. 2020), and facilitates cell entry of the SARS-CoV-2 after the binding of S-protein to the ACE2 (Danser et al. 2020). Likewise, TMPRSS4 activates cell-mediated fusion of MERS-CoV and SARS-CoV, but it does not activate infection. Other TTSPs, TMPRSS11a, and TMPRSS11e, activate S-protein fusion of MERS-CoV, while TMPRSS11a activates SARS-CoV fusion S-protein, which causes infection (Tang et al. 2020; Kam et al. 2009). Human airway trypsin-like protease (HAT) known as TMPRSS11d, can activate MERS-CoV (Tang et al. 2020; Kam et al. 2009; Bertram et al. 2011). Although, transient expression of TMPRSS2, TMPRSS4, TMPRSS11a, TMPRSS11d, and TMPRSS11e enhances SARS-CoV-2 S-protein-mediated cell–cell fusion, these proteases activating the S-protein for infection are yet to be investigated (Ou et al. 2020; Tang et al. 2020).

Furin-like proteases that cleave S1/S2 sites of MERS-CoV and SARS-CoV-2

Furin is known to activate the fusion machinery of viral glycoproteins (Jaimes et al. 2020). Furin cleavage sites are available for the influenza virus, making it highly pathogenic (Kawaoka and Webster 1988). Similarly, SARS-CoV-2 and MERS-CoV also contain S1/S2 site cleaved by the furin or furin-like proteases present on the trans-Golgi network that attack the newly synthesized S-protein cleavage sites of these coronaviruses (Xia et al. 2020; Tang et al. 2020). The TMPRSS2 cleaves S2's position to establish plasma membrane fusion. However, other than the S1/S2 sites of SARS-CoV-2 and MERS-CoV, the furin activity was not observed in the S2 site of SARS-CoV and SARS-CoV-2 that needs to be analyzed further (Walls et al. 2020; Kleine-Weber et al. 2018; Coutard et al. 2020; Mille and Whittaker 2014).

Trypsin plays a role in activating membrane fusion during infection

Trypsin is an exogenous protease acting as an activator for the fusion of the plasma membrane (Belouzard et al. 2009). This serine endopeptidase expression is commonly present in respiratory and digestive cells, especially in the small intestine where the enzyme is highly active (Jaimes et al. 2020). It consists of various cleavage conditions, unlike TMPRSS2 that functions even in the absence of the S1/S2 cleavage site.

Trypsin shows components of a two-step activation process in the SARS virus, such as cleaving arginine in the S1/S2 site and providing the S2 site for fusion (Belouzard et al. 2009). In a study, it has been indicated that trypsin induces protein-mediated cell–cell fusion in Vero E6 cells infected with SARS-CoV. Similarly, trypsin also induced MERS-CoV and SARS-CoV-2 S-protein-mediated cell–cell fusion in Vero E6 and 293 T cells, respectively. Also, the increased undergo treatment with trypsin have been observed after the binding of retroviral pseudo-particles like SARSpp and MERSpp to ACE2 and DPP4, respectively. This resulted in enhancement in their effectiveness of infection at the plasma membrane surface indicating that trypsin is involved in the coronavirus infection (Tang et al. 2020). Alternatively, it has been hypothesized that trypsin pre-treatment prior to receptor binding would cause irreversible conformational change in S-protein and thus prevent infection. However, this phenomenon is yet to be investigated for SARS-CoV-2 (Tang et al. 2020).

Elastase cleaves the S2 site of the S-protein in SARS-CoV

The S2 cleavage of SARS-CoV is mediated by elastase near the trypsin cleavage site, implying that more than one cleavage site is present on the S-protein on the SARS virus (Belouzard et al. 2010). The S-protein induces flexibility in cleavage location, and mutagenic studies prove that elastase cleave at the trypsin cleavage site and enhance the virus's fusion ability. However, similarity and uniqueness of S1/S2 and S2 cleavage sites in both SARS-CoV-2 and MERS-CoV are yet to be identified (Tang et al. 2020).

Role of thermolysin in entry and replication of SARS-CoV and MERS-CoV

Thermolysin acts as an exogenous enzyme known to activate unidentified sites of SARS-CoV and MERS-CoV (Tang et al. 2020; Shirato et al. 2013; Matsuyama et al. 2005). The treatment with a high concentration of thermolysin and trypsin enhances the MERS-CoV entry and replication in VeroE6 cells, indicating that thermolysin may also play a role in infection (Matsuyama et al. 2005).

Role of cathepsins in the endosomal pathway of SARS-CoV, SARS-CoV-2, and MERS-CoV

Cathepsins are a group of cysteine proteases commonly found in lysosomes and endosomes (Regan et al. 2008) which take part in several degradative and antigen-presenting processes (Jaimes et al. 2020). They are activated in acidic pH, wherein cathepsin B and L are the Coronaviridae family activators in Early and Late endosomes, respectively (Regan et al. 2008; Qiu et al. 2006). A cathepsin B/L inhibitor, MDL28170, inhibits the entry of MERS-CoV and SARSpp into MRC-5 cells (Gierer et al. 2013; Simmons et al. 2005; Huang et al. 2006). These inhibitors were also effective against SARS-CoV-2 (Ou et al. 2020; Hoffmann et al. 2020). However, more recent studies indicated that cathepsin L specific inhibitor, SID 26681509, prevented the SARS-CoV-2 entry into human embryonic kidney 293/hACE2 cells. However, cathepsin B specific inhibitor, CA-074, did not affect the viral entry into the cells (Ou et al. 2020; Pišlar et al. 2020). This indicated that cathepsin L has a more important role in the SARS-CoV-2 expression (Pišlar et al. 2020; Gomes et al. 2020). Moreover, the expression of cathepsin L is associated with the extracellular matrix's degradation and was found significantly higher during chronic inflammation, a key attribute of SARS-CoV-2 infection (Gomes et al. 2020). The X-ray crystallographic studies indicated that the calpain inhibitors II and XII, GC-376 analogs: UAWJ246, UAWJ247, and UAWJ248, showed dual inhibition properties by inhibiting both M^{pro} and cathepsin L (Sacco et al. 2020). The early and late cathepsin inhibitors confirmed that these viruses get activated for fusion in late endosome (Ou et al. 2020; Simmons et al. 2005). However, the indirect dependencies of the SARS-CoV, SARS-CoV-2, and MERS-CoV on low-acidic pH activates the cathepsin L in the endosomal pathway, which acts on the S-protein followed by virus undergoing the next steps of fusion (Tang et al. 2020). Conversely, HCoV-NL63 is unable to utilize cathepsin L protease in the infection of ACE2 expressing host cells (Huang et al. 2006). Currently, the in silico and in vivo analysis of TMPRSS2 and cathepsin inhibitors have been studied, the in vivo anti-viral efficacy is yet to be confirmed.

Phosphatidylinositol 3-phosphate 5-kinase and two-pore segment channel are essential for SARS-COV-2 entry

Since endocytosis is an alternative mode of infection of the virus into the cells, phosphatidylinositol 3-phosphate 5-kinase (PIKFYVE) is required during SARS-CoV-2 infection through the endocytic pathway (Ou et al. 2020). This initiates phosphoinositides synthesis to regulate the formation of early endosome. Besides, a calcium channel, a two-pore segment channel (TPC2) activated by phosphoinositides in membranes of the lysosome is essentially required for endocytosis (Ou et al. 2020).

Epithelium sodium channel C (ENaC)-α mimicking S1/S2 subunit

Other than furin present on human cells for cleavage of S1/S2 sites, a similar peptide for the furin cleavage site on the ENaC-α subunit is present (Anand et al. 2020). ENaC

Table 3 Human receptors and enzymes involved in SARS-CoV-2, SARS-CoV, and MERS-CoV infection

Type of Coronavirus (Genebank accession number)	Disease	Genome sequence identity to SARS-CoV-2 (NC_045512.2) (%)	Receptor of interaction	Other proteases involved for fusion and activation of S-protein	Mortality rate (%)	PubMed IDs
SARS-CoV-2 (NC_045512.2)	COVID-19	100	ACE2; CD147	TMPRSS2; TMPRSS4; TMPRSS11a; TMPRSS11d; TMPRSS11e; Furin; trypsin; cathepsin, PIKFVE, TPC2	2.3	32170806, 32275855, 32307653, 32231345, 32221306, 32272173, 24098509, 32057769, 32234451
SARS-CoV (AY278488.2)	SARS	80.04	ACE2; CD209L	TMPRSS2; TMPRSS4; TMPRSS11a; TMPRSS11d; TMPRSS11e; Trypsin; Cathepsin, Elastase; Thermolysin	9.5	32015507, 30102747, 15496474, 32272173, 24227843, 32142651, 23468491, 32165541, 19924243, 19321428, 16339146, 20507992, 16116101, 32234451
MERS-CoV (KT225476.2)	MERS	54.86	DPP4 (CD26)	TMPRSS2; TMPRSS4; TMPRSS11a; TMPRSS11d; TMPRSS11e;Furin; Trypsin; Cathepsin, Elastase; Thermolysin	34.4	23486063, 32272173, 29976755, 24027332, 25288733, 30413791, 32234451, 32149036

ACE2 angiotensin-converting enzyme 2, *DPP4* dipeptidyl peptidase 4, *IHC* immunohistochemistry, *RNA-Seq* RNA sequencing, *TMPRSS* transmembrane proteases serine subfamily, *SARS* severe acute respiratory syndrome, *MERS* Middle east respiratory syndrome, *CoV* coronavirus, *COVID* coronavirus disease

absorbs sodium ions in the airway surface liquid (ASL) along with transmembrane member 16 A (TMEM16A) and cystic fibrosis transmembrane conductance regulator (CFTR) that secretes chloride ions, thereby maintaining the hydration of the mucus layers (Gaillard et al. 2010). Hence among its subunits α, β and γ (Gaillard et al. 2010), ENaC-α plays a vital role in the homeostasis of ASL (Rossier and Stutts 2009) that generates inward sodium ion current (Canessa et al. 1994; Tarjus et al. 2017). The specific genetic mutations of ENaC-α lead to a regulation imbalance of aldosterone in patients, impeding that the furin site is crucial for activating ENaC and S1/S2 cleavage site SARS-CoV-2 has targeted mimicry of ENaC-α (Rossier and Stutts 2009). The single-cell RNA sequencing (scRNA-Seq) data of 65 samples shows a prominent overlapping expression of ACE2 and ENaC-α in cell types linked to the cardiovascular-renal-pulmonary physiopathology of SARS-CoV-2 infection (Anand et al. 2020). The potent receptors, enzymes, and active proteins involved during SARS-CoV-2, SARS-CoV, and MERS-CoV infection are summarized in Table 3.

Certain inhibitors block the virus entry by suppressing the interacting enzymes like TTSPs, trypsin, elastase, and thermolysin. The serine protease inhibitors like Camostat were partially able to block SARS-CoV infection and HCoV-NL63 in HeLa cell lines. However, its synergistic effect with cathepsin inhibitor trans-epoxysuccinyl-L-leucylamindo3-methylbutane ethyl ester (EST) in human Calu-3 airway epithelial cells efficiently prevents SARS-CoV entry inside host (Parks and Smith 2020; Memish et al. 2020; Kawase et al. 2012). The use of Camostat also prevented MERS-CoV entry in human Calu-3 bronchial submucosal gland cells by 10×, and the viral growth reduced by 270×. In contrast, co-treatment with EST, Leupeptin, or other serine, cysteine, and threonine peptidase inhibitors was not effective in preventing MERS-CoV infection (Shirato et al. 2013). Hence, these inhibitors could be used as a possible treatment in preventing the entry of SARS-CoV-2 growth and infection. Interfering the endosomal route of viral entry could also be accomplished by blocking the activities of cathepsins, PIKFYVE and TPC2, essential for the SARS-CoV-2 entry, to prevent further infection to organs. Further understanding of ENaC-α for its resemblance to that of the S1/S2 site could be a significant breakthrough in developing a recombinant protein that could design a suitable vaccine candidate.

Role of ACE2 in the RAS pathway

The RAS is an essential system required for regulating homeostasis in the body by playing a pivotal role in monitoring and controlling extracellular fluid volume and atrial vasoconstriction (Arendse et al. 2019; Cheng et al. 2020). During hypotension (reduced renal blood circulation and

blood sodium levels), renin (a protease enzyme that cleaves Angiotensinogen) from the renal system's juxtaglomerular cells is released into the blood circulation (Arendse et al. 2019). It hydrolyzes serum globulin and converts Angiotensinogen to Ang I, Ang I into a vasoactive peptide Ang II in the presence of a zinc metalloprotease ACE. Ang II, facilitated by AT_1R highly expressed in the cardiovascular system, increases blood pressure through vasoconstriction, aldosterone secretion, and sympathetic nervous system tension, ultimately eliciting downstream pathophysiological effects in classical RAS (Fig. 4) (Hanff et al. 2020; Arendse et al. 2019; Li et al. 2017; Tikellis et al. 2011). It also promotes the inflammatory response of cells, fibrosis, and myocardial hypertrophy. In the counter-regulatory RAS pathway, ACE2 plays an antagonistic role by cleaving Ang II and releasing Ang 1–7 that binds with G-protein Coupled Receptor (GPCR) and Mas receptor. This interaction causes anti-inflammatory, antiproliferative, and vasodilatory effects. Ang II also binds to AT_2R to mediate vasodilatory effects. The expression of AT_2R is low in the cardiovascular system of healthy adults (Arendse et al. 2019; Cheng et al. 2020). In the case of hypotension, the classical RAS axis plays an important role. The ACE2 antagonizes these effects through the negative regulatory RAS axis, thus preventing many diseases like hypertension, diabetes, and CVD (Cheng et al. 2020; Tikellis et al. 2011). The Ang II elicits downstream pathophysiological effects, it could be the primary cause for the symptoms of the COVID-19 and SARS disease. However, a partial neutralization of Ang II could be an effective way to treat the disease and not entirely prohibiting its function as a vasoconstrictor. ACE2 knock out (KO) mice developed mild liver fibrosis and promote migration of inflammatory cells, when wild type (WT) and ACE2-KO mice were subjected to different acute and chronic type lung injury, this study implies that ACE2 inhibits the liver fibrosis. Subsequently, ACE2-KO mice were treated with recombinant ACE2, which could terminate the fibrosis during the cholestatic liver injury. Hence, ACE2 acts as a RAS antagonist, to inhibit liver fibrosis by degrading Ang II and forming Ang 1–7. The absence of ACE2 activity augments liver fibrosis in chronic liver injury models, and recombinant ACE2 could have a therapeutic effect in this regard (Österreicher et al. 2009). Figure 4 represents the physiological role of ACE and ACE2 in the RAS pathway.

ACE2 expression in human tissues

ACE2 is highly expressed in the heart, lungs, kidney, testis, and GI tract (Turner et al. 2004; Chen et al. 2020). ACE2 is abundantly present and has high level of activity in human epithelial AT2 cells, small intestinal epithelial cells, endothelial cells of arteries and veins, and smooth muscle cells of arteries. It was expressed in the Stratum Basale of the non-keratinized squamous epithelium of nasal, oral mucosa, and nasopharynx and basal cell layer of skin. In contrast, a lower expression level is reported in glomerular tubules, while expression is absent in glomerular endothelial and glomerular mesangial cells. ACE2 expression has not been indicated in B cell, T cells, macrophages, Kupffer cells, and liver cells, and tissues such as bone marrow, spleen, thymus, and lymph (Hamming et al. 2004; Santos et al. 2018). Higher expression of ACE2 is observed in tissue than in plasma and varies between different ages and sex. Aging factor also plays an essential role in ACE2 expression, in which chances of COVID-19 rise with increasing age. Also, it has been reported that females have higher ACE2 expression compared to males (Haber et al. 2014; Xudong et al. 2006). The ACE2 at the gene level is expressed in almost all the organs and tissues (Table 4). The IHC studies and microarray-based gene expression data demonstrate the ACE2 expression in glandular cells of the adrenal gland, colon, gall bladder, and small intestine. The higher expression of ACE2 is observed in testis, small intestine, heart, kidney, colon, and adipose tissues, whereas tubular cells in the kidney and seminiferous ducts of testis have the highest expression of ACE2.

Moreover, the ACE2 protein expression is also found in the heart and kidney. The ACE2 gene expresses in the colon and adipose tissue, but corresponding protein expression is not observed. A positive ACE2 gene expression was observed in organs such as tongue, salivary gland, and lungs that serve a significant route of viral entry (Table 4), thus posing a higher risk of infection. Fluidic secretions from the body like pancreatic juice and urine have higher protein expression of ACE2 (probably soluble form), signifying a higher viral load. The fetal gut and heart have significant ACE2 protein expression, indicates possibility to have a higher risk of viral infection. Ovaries have the highest expression levels followed by kidney, heart, gall bladder, and testis. Urine and fecal discharge from the infected patients would cause virus spread and must thoroughly investigate. The clinical data analysis indicates that COVID-19 patients tested positive due to the presence of SARS-CoV-2 in feces and urine (Wang et al. 2020), indicating the possibility that the GI tract might have abundant ACE2 protein expression as observed in gall bladder and pancreatic juice along with kidney and testis (Table 4).

Furthermore, the ACE2 protein expression is high in cancers of stomach, liver, colon, renal system, and urothelium (Table 4). Comparatively, diseased conditions like cancer, heart disorders, diabetes, and smoking associated ailments have higher ACE2 gene and protein expression on tissues than seen in healthy individuals (Table 4) (Chen

Table 4 ACE2 gene and protein expression levels in various organs, cells, and tissues in human body. (Source: IHC and microarray data of normal cells and cancer cell expression data obtained from: https://www.proteinatlas.org/about/download; Gene Card RNA, microarray and protein data obtained from: (GCID: GC0XM015562))

Tissues/organs	Cells	Gene expression			Protein expression	
		Human Protein Atlas (based on IHC and microarray)	Gene card RNA-Seq expression	Gene card Microarray data	Gene card	Human Protein Atlas Cancer cell expression
Adipose tissue	Adipocytes	–	++	NA	–	NA
Adrenal gland	Glandular cells	+	+	NA	–	NA
	Adrenal cortex	NA	NA	+		
Amniocyte	–	NA	NA	NA	–	NA
Amygdala	–	NA	NA	+	NA	NA
Appendix	Glandular cells	–	NA	+	NA	NA
	Lymphoid tissue				NA	
Artery	–	NA	+	NA	NA	NA
Blood	Serum	NA	+	NA	–	NA
	Plasma					
	Neutrophil					
	Platelets					
Bone	Skeletal muscle (myosites)	NA	+	NA	–	NA
	Synovial fluid	NA	NA			
	Bone marrow hematopoietic cells	–				
	Bone marrow mesenchymal stem cells	NA				
	Bone marrow stromal cells					
Brain	–	NA	+	NA	–	NA
Breast	Adipocytes	–	+	NA	–	–
	Glandular cells					
	Myoepithelial cells					
	Milk	NA	NA			
Cardia	–	NA	NA	NA	–	NA
Caudate	Glial cells	–	NA	NA	NA	NA
	Neuronal cells					
Cerebellum	Cells in granular layer	–	+	NA	NA	NA
	Cells in molecular layer					
	Purkinje cells					
Cerebellar peduncles	–	NA	NA	+	NA	NA
Cerebral cortex	Endothelial cells	–	+	NA	–	NA
	Glial cells					
	Neuronal cells					
	Neuropil					
Cerebrospinal fluid	–	NA	NA	NA	–	NA

Table 4 (continued)

Tissues/organs	Cells	Gene expression			Protein expression	
		Human Protein Atlas (based on IHC and microarray)	Gene card RNA-Seq expression	Gene card Microarray data	Gene card	Human Protein Atlas Cancer cell expression
Cervix (uterine)	Glandular cells	–	+	NA	–	–
	Squamous epithelial cells	–				
	Cervix	NA				
	Endometrial stromal cells	–				–
	Endometrial glandular cells	–				
	Uterus corpus	NA		+	NA	NA
Ciliary ganglion (eyes)	–	NA	NA	+	NA	NA
Cingulate cortex	–	NA	NA	+	NA	NA
Colon	Glandular cells	+	++	NA	–	+
	Endothelial cells	–				
	Peripheral nerve/ganglion	–				
	Colon muscle	NA				
Caudate nucleus	–	NA	NA	+	NA	NA
Dorsal root ganglion	–	NA	NA	+	NA	NA
Endothelial	–	NA	NA	+	NA	NA
Epididymis	Glandular cells	–	NA	NA	NA	NA
Esophagus	Squamous epithelial cells	–	+	N A	–	NA
Fetal brain	–	NA	NA	+	–	NA
Fetal gut	–	NA	NA	NA	++	NA
Fetal heart	–	NA	NA	NA	+	NA
Fetal liver	–	NA	NA	+	–	NA
Fetal lung	–	NA	NA	+	–	NA
Fetal ovary	–	NA	NA	NA	–	NA
Fetal testis	–	NA	NA	NA	–	NA
Fetal thyroid	–	NA	NA	+	NA	NA
Fallopian tube	Glandular cells	–	NA	NA	NA	NA
Gallbladder	Glandular cells	+++	+	NA	+	NA
Globus pallidus	–	NA	NA	+	NA	NA
Hair follicle	–	NA	NA	NA	–	NA
Head an neck	–	NA	NA	NA	NA	–
Heart	Cardiac myocytes	–	++	+	++	NA
	Atrioventricular node cells	NA		+		
	Heart muscle	–		NA		
Hippocampus	Glial cells	–	NA	NA	NA	NA
	Neuronal cells		NA	NA		
Hypothalamus	–	NA	NA	+	NA	NA

Table 4 (continued)

Tissues/organs	Cells	Gene expression			Protein expression	
		Human Protein Atlas (based on IHC and microarray)	Gene card RNA-Seq expression	Gene card Microarray data	Gene card	Human Protein Atlas Cancer cell expression
Immune cells	Myeloid	NA	NA	+	NA	NA
	Dendritic cells					
	Monocytes				−	
	NK Cells					
	T CD4+ cells					
	T CD8+ cells					
	B-cells					
Kidney	Cells in tubules	+++	++	NA	++	+++
	Cells in glomeruli	−				
Liver	Bile duct cells	−	+	NA	−	+
	Hepatocytes					
	Liver secratome	NA				
Lung	Bronchial epithelium	NA	+	+	−	−
	Respiratory epithelial cells	−		NA		
	Macrophages					
	Pneumocytes					
Lung alveolar lavage	–	NA	NA	NA	−	NA
Lymph	Germinal center cells	−	+	NA	−	−
	Lymph node					
	Non-germinal center cells					
Medulla oblongata	–	NA	NA	+	NA	NA
Nasopharynx	Respiratory epithelial cells	−	NA	NA	−	NA
Occipital lobe (brain)	–	NA	NA	+	NA	NA
Olfactory bulb	–	NA	NA	+	NA	NA
Oral mucosa	Squamous epithelial cells	−	NA	NA	NA	NA
	Oral epithelium	NA	NA		−	NA
Ovary	Follicle cells	−	+	NA	+++	−
	Ovarian stroma cells					
Pancreas	Exocrine Glandular Cells	−	+	NA	NA	−
	Islets of langerhans			+	−	
	Pancreatic juice	NA		NA	+++	
Parathyroid gland	Glandular cells	−	NA		NA	NA
Parietal lobe (brain)	–	NA	NA	+	NA	NA
Pineal gland	–	NA	NA	+	NA	NA
Pituitary	–	NA	+	+	NA	NA
Placenta	Decidual cells	−	NA	NA	NA	NA
	Trophoblastic cells					
Pons	–	NA	NA	+	NA	NA
Prefrontal cortex	–	NA	NA	+	NA	NA
Prostate	Glandular cells	−	+	NA	−	−
Rectum	Glandular cells	+	NA	NA	−	+

Table 4 (continued)

Tissues/organs	Cells	Gene expression			Protein expression	
		Human Protein Atlas (based on IHC and microarray)	Gene card RNA-Seq expression	Gene card Microarray data	Gene card	Human Protein Atlas Cancer cell expression
Retina (eyes)	–	NA	NA	NA	−	NA
Salivary gland	Glandular cells	−	+	NA	−	NA
	Saliva	NA	NA			
Seminal vesicle	Glandular cells	+	NA	NA	NA	NA
Skin	Fibroblasts	−	NA	NA	−	−
	Keratinocytes					
	Langerhans					
	Melanocytes					
	Epidermal cells					
Small intestine	Glandular cells	+++	+++	NA	NA	NA
	Duodenal glandular cells					
Smooth muscle	Smooth muscle cells	−	NA	NA	NA	NA
Soft tissue	Chondrocytes	−	NA	NA	NA	NA
	Fibroblasts					
	Peripheral nerve					
	Fibroblasts					
	Peripheral nerve					
Spinal chord	–	NA	+	NA	−	NA
Spleen	Cells in red pulp	−	+	NA	−	NA
	Cells in white pulp					
Stomach	Glandular cells	−	+	NA	−	+
	Glandular cells					
Subthalamic nucleus	–	NA	NA	+	NA	NA
Sup cervical ganglion	–	NA	NA	+	NA	NA
Temporal lobe (brain)	–	NA	NA	+	NA	NA
Testis	Cells in seminiferous ducts	+++	+++	+	+	−
	Leydig cells					
	Interstitial cells	NA		+++		
	Germ cells			+		
Thalamus	–	NA	NA	+	NA	NA
Thyroid gland	Glandular cells	−	+	NA	−	−
Tibial nerve	–	NA	+	NA	NA	NA
Tongue	–	NA	NA	+	−	NA
Tonsil	Germinal center cells	−	NA	+	−	NA
	Non-germinal center cells					
	Squamous epithelial cells					
Trachea	–	NA	NA	+	NA	NA
Trigeminal ganglion	–	NA	NA	+	NA	NA
Urinary bladder	Urothelial cells	−	NA	NA	−	+
urine	–	NA	NA	NA	+++	NA
Vagina	Squamous epithelial cells	−	NA	NA	NA	NA
Vitrious humor	–	NA	NA	NA	−	NA

IHC immunohistochemistry, *RNA-Seq* RNA sequencing, *NA* not available, +++: highest expression, ++: moderate expression, +: lower expression; −: no/negative expression

et al. 2020; Uhlen et al. 2017; Brake et al. 2020; Nicin et al. 2020). Therefore, the COVID-19 patients suffering from these comorbidities have higher risks of infection, it may lead to severe conditions. The transcriptome analysis of 700 comorbid COVID-19 patients' lung samples indicate that the higher ACE2 expression in these patients than control. This data suggests that individuals indicating such comorbid SARS-CoV-2 infections may have chances of developing severe COVID-19 conditions (Pinto et al. 2020). A higher ACE2 protein expression facilitates higher viral load, therefore cells lacking ACE2 can find a possible treatment option against COVID-19. Recently, convalescent plasma from recovered patients was used to treat four critically ill COVID-19 patients, including a pregnant woman, and all four recovered eventually, though its efficacy has to be further investigated (Zhang et al. 2020c). It is also suggested that immune cells like monocytes, NK cells, B-cells, and T cells have no ACE2 protein expression (Table 4), would be used instead of plasma therapy from recovered patients.

Effects of COVID-19 on human tissues

The viral infection reduces the activity of ACE2, which triggers an imbalance of the Ang II/ACE2 regulation system and led to the accumulation of Ang II (Cheng et al. 2020).

SARS-CoV-2 infection in liver

Both liver and bile duct cells have ACE2 expression, but the expression is higher in bile duct cells than in liver cells. The bile duct epithelial cells play a crucial role in immune responses and liver generation. However, COVID-19 patients suffered from a liver injury more commonly in case of infection that damages the bile duct's cells rather than liver (Xu et al. 2020; Chai et al. 2020; Banales et al. 2019; Liu et al. 2020c).

SARS-CoV-2 infection in lungs

ARDS is the most severe condition of lung injury and has a high mortality rate (Ware and Matthay 2000). Diseases like SARS-CoV infection, HIV, and Bird flu may cause sepsis, aspiration, and pneumonia (Cheng et al. 2020). These diseases directed to elevate pulmonary vascular permeability and pulmonary oedema, ultimately causing ARDS (Gonzales et al. 2015). ACE2 is highly expressed in the lung and has a protective effect in acute lung injury (Imai et al. 2005). The lungs' alveoli consist of a majority of AT1 cells (95%) (mainly constitutes alveolar surface for gas exchange and play a key role in maintaining permeability barrier function of alveolar membrane (Gurka and Balk 2008)), and very few AT2 cells (5%). AT2 cells play a crucial role in maintaining lung elasticity by producing pulmonary surfactant that monitors cellular functions through specific protein–protein, lipid–protein, and lipid–lipid interactions. Further, these alveolar stem cells act as progenitor for AT1 and perform function in gas exchange (Aoshiba et al. 2003; Beers and Moodley 2017; Mason and Dobbs 1980; Pérez-Gil 2008). Since ACE2 expresses in mucosal and AT2 cells in the lungs and alveoli, therefore, SARS-CoV-2 targets AT2 cells and also damages the regenerative capacity of lung AT1 cells. Reduced levels of AT2 cells led to lung damage as there would be surfactant deficiency, causing fibrotic obliteration and only partial repair of alveolar epithelial injury (Rivellese and Prediletto 2020; Barkauskas et al. 2013). The virus may also downregulate ACE2, leading to toxic overaccumulation of Ang II, thus inducing ARDS (Hanff et al. 2020).

SARS-CoV-2 infection in intestine

The SARS-CoV-2 infection in the gastrointestinal (GI) tract causes GI disorders (showing prominent symptoms of nausea, vomiting and/or diarrhea), and are common in severe COVID-19 patients compared to mild cases (Jin et al. 2020b). Clinical data of 651 COVID-19 patients in China showed that about 11% of patients had GI disorders, most commonly diarrhea in 5–8% patients which persisted for an average of 4 days before the onset of respiratory symptoms. Another study conducted in China and Hong Kong reported that of the total COVID-19 patients considered in the study, with a sample size of 1099, 138, 58, 204, 59, and 254, the section of patients showing in GI disorders are 8.7%, 13.7%, 11%, 18.6%, 25.4% and 26% respectively. While in United States and Europe, 61% and 35% patients in the former, and 55% patients in the latter among a total of 318, 278, and 40 COVID-19 patients respectively, showed GI disorder signs and symptoms (Guan et al. 2020; Wang et al. 2020; Lin et al. 2020; Pan et al. 2020; Cheung et al. 2020; Zhou et al. 2020b; Redd et al. 2020; Nobel et al. 2020; Effenberger et al. 2020; Trottein and Sokol 2020). Moreover, the presence of the SARS-CoV-2 virus in the stool samples indicated that the virus could also infect the GI tract (Guan et al. 2020; Holshue et al. 2020). A study identified the presence of SARS-CoV-2 RNA in 29% of specimens that indicates positive results for COVID-19 in stool specimen (Wang et al. 2020). In addition, SARS-CoV-2 infectivity is higher in GI tract compared to lungs (Ma et al. 2020b). The viral infection in the intestinal cell may be due to the high

expression of ACE2 in the small intestine colon (Table 4) (Hamming et al. 2004).

There could be various factors attributed to GI tract disorders in COVID-19 patients. During the infection, the release of inflammatory cytokines may weaken the epithelial barrier. High viral load and replication in the GI tract may damage the gut epithelium. The GI symptoms may also be due to dysregulation of the ACE2-RAS mechanism (Trottein and Sokol 2020). However, the actual cause of GI disorders in COVID-19 patients is yet to be studied.

SARS-CoV-2 infection in the heart

Downregulation of ACE2 and toxic Ang II overaccumulation in COVID-19 patient causes fulminant myocarditis (Zhonghua et al. 2020). Nonetheless, Cardiovascular disease (CVD) and pharmacologic RAS inhibition both increase the levels of ACE2 expression. Increased ACE2 expression provides easier viral entry, which may increase the virulence of SARS-CoV-2 in the lungs and heart (Imai et al. 2005; Crackower et al. 2002; Kuba et al. 2005). The virus has potential susceptibility to infect the heart as ACE2 expression is observed in the cardiac muscle cells, higher than in the lungs, but lower than that in the intestine and kidney (Table 4). Patients with primary heart failure may have higher chances of having a heart attack and severe repercussions in case of the viral infection as they exhibit higher ACE2 expression at both mRNA and protein levels (Table 4). ACE2 is also highly expressed in pericytes of adult human hearts. Pericytes play a crucial role in maintaining endothelial cell function in capillary vessels (Chen et al. 2020). Over-accumulation of Ang II can cause an inflammatory response of cells, promoting oxidative stress and migration of vascular smooth muscle and endothelial cells and causing atherosclerosis (Chen et al. 1998).

Moreover, ACE2 is mainly involved in the RAS pathway and acts as an essential factor in heart function. However, in hypertensive rats, ACE2 mRNA and protein expression was significantly declined, signifying that it is a crucial gene for this quantitative trait locus (QTL). The disruption in ACE2 expression may lead to cardiac contractility disorder and increased Ang II expression, with upregulation of genes inducing hypoxia in the heart muscles (Crackower et al. 2002).

SARS-CoV-2 infection in the reproductive system

A recent clinical study for the presence of SARS-CoV-2 in semen samples has shown that 6 out of 38 patients were COVID-19 positive (Li et al. 2020). This could be due to high ACE2 gene expression and presence of the transcribed protein in low amounts in the Testis (Table 4). However, in oocytes of two female COVID-19 patients, the presence of SARS-CoV-2 RNA was undetectable, and the further clinical investigation is yet to be performed (Barragan et al. 2020). It has been reported that male SARS-CoV-2 infected patients had reduced sperm count and sperm motility, and asymptomatic female patients developed respiratory symptoms postpartum (Segars et al. 2020). Transcriptome sequencing data indicated ACE2 expression in the germ cells, Leydig and Sertoli cells of the testis, and infection of SARS-CoV-2 virus may cause impairment of male fertility (Vishvkarma and Rajender 2020). ACE2 transcripts were also observed in human spermatogonia, spermatocyte, and spermatids in 500 individual cells through laser-dissection, micro-capture followed by RNA sequencing (Jan et al. 2017). Apparently, to high expression of ACE2, as observed in testis, could increase the risk of SARS-CoV-2 infection (Table 4). This is in contradiction to the low expression of ACE2 and TMPRSS2 observed in the female reproductive organs like ovaries, fallopian tube, uterus, and myometrium that indicates to have a lower susceptibility for SARS-CoV-2 infection (Goad et al. 2020).

Target based current approaches for COVID-19 treatment

Presently, various approaches have come up to combat COVID-19 pandemic. However, effective treatment against SARS-CoV-2 are still under investigation. Treatments targeting host cellular receptors such as ACE2 based therapies, recombinant RBD, soluble ACE2 therapy, and inhibitors targeting other human cellular receptors like CD147, TMPRSS2, trypsin and cathepsin could serve as effective ways to overcome SARS-CoV-2 infection.

ACE2 based therapies

As ACE2 serves to be receptor for entry of SARS-CoV and SARS-CoV-2, targeting this receptor would help preventing viral entry along with mitigating the adverse effects of infection. Therefore, use of ACE blockers and MSCs based therapy could be a practical approach for the treatment of COVID-19 patients.

Use of ACE blocker

RAS inhibitors like ACEi and ARBs have been used to diminish the unfavorable effects of Ang II. These inhibitors are used for the treatment of CVD, hypertension, and diabetes (Arendse et al. 2019; Sparks et al. 2020). According

to the National Health and Nutrition Examination Survey's current analysis, these are the widely used and prevalent classes of antihypertensive agents (Derington et al. 2020; Vuille-Dit-Bille et al. 2015). ACEi inhibits the enzyme ACE, thereby decreasing the production of Ang II (Miller and Arnold 2019). They also increase levels of Ang 1–7 and Ang 1–9 for reducing hypertension and protect the cardiovascular system as ACE helps in the degradation of Ang 1–7. Since ACE and ACE2 are different enzymes, thus, ACEi does not inhibit ACE2. These inhibitors increase renin secretion as well as the flux through RAS by suppressing counter-regulatory RAS. This causes declination of pharmacologic efficacy of ACEi in the long-term treatment, wherein Ang II is not entirely inhibited, and abundant Ang I is present due to renin's high activity in the blood plasma. Hence, a new steady state is established where there is no Ang II suppression, while higher Ang 1–7 levels is present (Arendse et al. 2019; Lindholm et al. 2002; McMurray et al. 2003; Dahlöf et al. 2002; Pitt et al. 2000). Animal studies have shown that ACEi and ARBs upregulate the expression of ACE2, which would also aggravate SARS-CoV-2 infection (Danser et al. 2020; Talreja et al. 2020), but the studies vary due to various ARBs in different tissues. Clinically, it has been investigated that ACEi treatment in healthy human increases the ACE2 duodenal mRNA expression level compared with control (Vuille-Dit-Bille et al. 2015). ARBs (Azilsartan, Losartan, Telmisartan, and Olmesartan) have been known to increase ACE2 mRNA and protein expression in other animal models with heart and chronic kidney disorders (Iwanami et al. 2014; Kai and Kai 2020; Sukumaran et al. 2011, 2012a, b, 2017; Ishiyama et al. 2004; Lakshmanan et al. 2012). Conversely, Ramipril and Valsartan, independently or in co-treatment, did not affect cardiac ACE2 expression in case of myocardial infarction (Burchill et al. 2012). The ARB treatment to patients shows no significant change in the expression level of ACE2 (Vuille-Dit-Bille et al. 2015). Few reports demonstrated that ACEis/ARBs were able to reduce the harsh effects of COVID-19. The impact of ACEi and ARBs in higher doses is analyzed in the animal models, while in clinical trials the dosage given is comparatively low (Kai and Kai 2020). This results in certain conflicting opinions among physicians and patients about the treatment of COVID-19 with ACEis/ARBs (Sparks et al. 2020).

On the contrary, significant ACE2 mRNA upregulation was observed in the heart with ACEis and ARB's treatment, suggesting that these inhibitors may cause detrimental effects on COVID-19 (Ferrario et al. 2005). The COVID-19 patients with comorbidities are being treated with drugs that perhaps stimulate ACE2 expression, which in turn increases the risk of developing severe and fatal conditions of COVID-19 infection and are likely to be investigated further. Likewise, ACEi and ARB increase ACE2 expression, hence increasing the viral load (Peiris et al. 2003), but also need to be investigated clinically (Chen et al. 2020). There are two issues associated with heart diseases (Guo et al. 2020). First, SARS-CoV-2 infection is aggravated in pulmonary tissues due to the upregulated expression of ACE2. Second, downregulation of ACE2 occurs after the infection and symptoms of ARDS arise, which is in contradiction to the first issue. This suggests that administering patients with ACEis/ARBs would increase the viral infection risk factor, and once infected, ACE2 downregulation would be a distinctive feature of COVID-19 progression. Hitherto, no substantial evidence has been reported in supporting the link between RAS inhibition and increased ACE2 expression that led to enhanced infection and virulence of SARS-CoV-2 (Sparks et al. 2020).

Therefore, COVID-19 could be affected by RAS inhibition, but the pathway of impact is ambiguous; it may decrease the pro-inflammatory activity of Ang II. Subsequently, the risk of ARDS, myocarditis, or mortality in COVID-19 or RAS inhibition, may increase ACE2 expression, leading to the enhanced virulence of SARS-CoV-2 in the lungs and heart, eventually causing ARDS, myocarditis, and death (Hanff et al. 2020). Therefore, the current evidence does not link COVID-19 and hypertension to ACEi and ARB medication and is subject to vary. Hence, it is dubious to conclude that these drugs enhance ACE2 expression and its activity in tissues to cause the viral infection to be critical (Talreja et al. 2020).

SARS-CoV-2's S-protein binds to ACE2 that leads to internalization and shedding of ACE2 into a soluble form. It causes increased Ang II, thereby declining Ang (1–7), which increases net inflammation and fibrosis. On the contrary, when treated with ACEis and ARBs, they can induce alterations in RAS. ACEi can inhibit Ang I, and converted into Ang II, while ARB inhibit the activity of AT_1R. Subsequently, the residual Ang II bind to AT_2R, and Ang (1–7), which further bind to the MasR pathway by diminishing the effects of Ang II and increase the Ang (1–7) levels leading to attenuated inflammation and fibrosis (Sparks et al. 2020).

MSCs based therapy

MSCs are adult tissue-derived multipotent cells having the property of self-renewal and differentiation into various types of cells (Hayes et al. 2012). MSCs perform immunomodulatory effects by releasing various cytokines through paracrine secretions and interacting with immune cells (Galipeau and Sensébé 2018; Bernardo and Fibbe 2013). These properties of MSCs make it a suitable candidate in cell-based therapy and have been used for treatment to reverse the cytokine storm (Leng et al. 2020).

A study was conducted on seven COVID-19 patients infused with MSCs for two days. This shows a significant improvement in lung functions after 14 days. It was also

observed that three patients in which one was critically infected were able to recover within ten days of the MSCs treatment. There was an increase in the level of peripheral blood lymphocytes, like $CXCR3^{+}CD4^{+}$ and $CD8^{+}$ T cells, while CXCR3 NK cell level was diminished in 3–6 days of treatment. The levels of TNF-α were reduced while IL-10 levels stayed elevated. Concluding that, MSCs could be used for effective treatment in SARS-CoV-2 infected patients (Leng et al. 2020).

The in vitro and in vivo studies suggest that bone marrow MSCs could differentiate and exhibit specific pulmonary epithelial cells (Krause et al. 2001; Li et al. 2012). Preclinical investigation on mice revealed that bone marrow-derived MSCs reduces the pulmonary vascular permeability, normalized lung endothelial nitric oxide synthase (eNOS), and enhanced the integrity of the endothelial barrier compared to control (He et al. 2015). The study demonstrated that ACE2 transduced MSCs in lipopolysaccharide (LPS) induced-acute lung injury (ALI) treated mice were able to increase the expression and activity of ACE2, thereby reducing the level of Ang II and elevated Ang 1–7 and diminishing the adverse effects of Ang II with an overall improvement of pulmonary endothelial function (He et al. 2015). Similarly, the mice were pre-treated with bleomycin followed by ACE2 gene expressing umbilical cord derived MSCs (uMSCs) treatment mitigated the lung damage having better efficacy than ACE2 or uMSCs alone. Nonetheless, noted a reduced expression of malondialdehyde (MDA), TNF- α, oxidized glutathione (GSSG), interferon (IFN)-γ, transforming growth factor (TGF)-β, IL-1, IL-2, IL-6, MMPs, hydroxyproline, tissue inhibitors of metalloproteinases (TIMPs), and collagen type 1 mRNA. Subsequently, this increases the level of superoxide dismutase (SOD), Glutathione (GSH), ACE2, and IL-10. Since uMSCs are accessible and have the least ethical issues than bone marrow-derived MSCs, they are convenient for therapeutic purposes for ARDS/ ALI (Min et al. 2015).

Currently, enough clinical data is not present regarding the use of MSCs in viral-induced ARDS, making it challenging for its use. Furthermore, the production and use of current Good Manufacturing Practice (cGMP) grade MSCs has major limitations, first, lack of cost effectiveness makes this therapy more expensive. Second, in this regard, limited number of clinical has been reported (Golchin 2020; Khoury et al. 2020). However, technological improvements, adequate clinical reports, and the nations' economic sustainability can overcome such limitations.

Recombinant RBD binding to ACE2

ACE2 is a common receptor for SARS-CoV and SARS-CoV-2 RBD binding (Zhou et al. 2020a), it can act as the primary target for inhibitors, antibodies, and vaccines. The recombinant RBD of SARS-CoV-2 S-protein has a strong binding affinity with human ACE2 (hACE2) and bat ACE2 (bACE2 (Tai et al. 2020)), and it's transfection prevents the viral entry into hACE2 expressing cells. The studies also demonstrated that polyclonal antibodies specific to RBD of SARS-CoV cross-reacted with the RBD of SARS-CoV-2, indicating that these antibodies could also be effective against the SARS-CoV-2 (Tai et al. 2020). The recombinant RBD could serve as an attractive vaccine target, and there could be a chance of low immunogenicity. This could be optimized by incorporating appropriate adjuvants or selecting potent target sequences that would elicit good immune response (Wang et al. 2020; Dai et al. 2020).

Soluble ACE2 and Cyclodextrin complex therapy targets viral domains

The soluble ACE2 (sACE2) retain the enzymatic activity of the membrane-bound ACE2 and interact with SARS-CoV S-protein (Hong et al. 2009). The sACE2 suppresses the effect of SARS-CoV, whereas SARS-CoV and SARS-CoV-2 follow the same infectivity mechanism, thus it is hypothesized that sACE2 would likely suppress the SARS-CoV-2 infection. Also, sACE2 used to treat ALI and reduces the mortality as prolonged coronavirus infection leading to the downregulation of ACE2, causing ARDS and pulmonary oedema. It can also promote the expression of ACE2 in the heart, kidney, and testis (Sun et al. 2020; Imai et al. 2005). A complex water-soluble drug designed by combining a macrocyclic molecule, Cyclodextrin with sACE2, which enhances the water solubility of the sACE2 for drug atomization inhalation. Cyclodextrin is linked by a pyran ring monosaccharide with the α-1,4-glycoside bond and contains hydrophobic cavities (host), which encompasses hydrophobic molecules (guest molecules), resulting in a host–guest complex. After the administration into the body, the conjugate can release sACE2 that will combine with S-proteins of SARS-CoV-2, blocking the viral infection (Sun et al. 2020; Hong et al. 2009).

Inhibitors targeting CD147

CD147 has been observed to be the alternate route of entry of SARS-CoV-2. Azithromycin drug reduces the viral load in some patients. This might be a possibility that Azithromycin would interrupt the interactions of CD147 with ligand and suppress the expression of some metalloproteases that are downstream to CD147 (Ulrich and Pillat 2020). Anti-CD147 Humanized Meplazumab (NCT04275245) uses monoclonal antibodies to block CD147 against SARS-CoV-2 infection and is also undergoing clinical trials in China. A tetracycline analog, Doxycycline decreases the

expression of CD147 in gingival crevicular fluid in chronic periodontitis patients (Emingil et al. 2008), and gall-bladder carcinoma cell lines (He et al. 2017). Hence, these could also be effective in the treatment of SARS-CoV-2 infection in addition to other therapies.

Serine and cysteine protease inhibitors targeting TMPRSS2, trypsin and cathepsin

Cell line studies indicate the partial prevention of SARS-CoV, HCoV-NL63 by serine protease inhibitor, Camostat in HeLa cell. Camostat also prevents the entry of MERS-CoV in human Calu-3 bronchial submucosal gland cells (Shirato et al. 2013; Kawase et al. 2012). Similarly, SARS-CoV-2 entry is prevented by Camostat mesylate that acts as a TMPRSS2 inhibitor (Hoffmann et al. 2020). Also, the Cathepsin inhibitor, MDL28170, prevents SARS-CoV (Simmons et al. 2005) and MERS-CoV (Gierer et al. 2013; Simmons et al. 2005; Huang et al. 2006) infection. Cysteine protease inhibitor like vinyl sulfones and K11777 ((2S)-N-[(1E,3S)-1-(benzenesulfonyl)-5-phenylpent-1-en-3-yl]-2-{[(E)-4-methylpiperazine-1-carbonyl]amino}-3-phenylpropanamide) targets closely related vinyl sulfones to act as broad-spectrum antivirals by targeting cathepsin-mediated cell entry of Ebola and SARS-CoV (Zhou et al. 2015). These inhibitors could act on the human targets, preventing viral entry and pathogenesis.

Conclusion

The contagion SARS-CoV-2 had a significant impact on a global scale. However, it has a lower fatality rate with a higher transmissibility rate than SARS-CoV and MERS-CoV. SARS-CoV-2 structure and genetic make-up are almost similar to all other coronaviruses, with few genetic alterations making it highly infectious (Petrosillo et al. 2020; Zhonghua et al. 2020; Kim et al. 2020; Lu et al. 2020). Likewise, SARS-CoV utilizes the ACE2 receptor for its entry and pathogenesis. Alternatively, it also uses CD147 and CD209L for infection, while MERS-CoV enters through DPP4 (Xu et al. 2020; Kim et al. 2020; Zhou et al. 2020a; Wang et al. 2020; Amraie et al. 2020). The infection through these receptors downregulates ACE2 expression upon virus binding and causes severe abnormalities in various tissues leading to critical conditions like ARDS and sepsis, later septic shock, and death (Peiris et al. 2003; Chen et al. 1998). ACE2 plays a vital role in the RAS system regulating Ang II levels, regulating heart function, and preventing fibrosis in the liver (Österreicher et al. 2009). The membrane-bound ACE2 is highly expressed in the heart, kidney, lungs, and GI tract (Turner et al. 2004; Chen et al. 2020). The ACE2 expression levels can be used as a molecular signature to determine the risk of infection in the body. Currently, the use of ACEi and ARBs against coronavirus infection is still under observation due to other side effects on comorbid patients. It could hence, be used for the treatment of COVID-19 patients with mild or asymptomatic conditions. Since MSCs based therapy has a promising therapeutic effect, but the feasibility is low. Therefore, plasma therapy could also be used for initial treatments until proper vaccines and drugs are available. An alternative to plasma therapy uses of immune cells like monocytes, NK cells, B and T lymphocytes could be used for treatment. The enhancement of immune response by producing immune cells could prevent SARS-CoV-2 infection, thereby reducing COVID-19. The coronaviruses' entry is also mediated by other endogenous and membrane-bound proteases like TTSPs, furin, trypsin, elastase, thermolysin, PIKFYVE, and TPC2, depending upon the pathway of their entry and identifying inhibitors and blockers for these proteins would aid in the entry of the viruses. The viral domains involved during infection include the RBDs of S1 and S2 subunits, the S1/S2 cleavage sites of MERS-CoV and SARS-CoV-2 (Xia et al. 2020; Tang et al. 2020), 6HB core structure, proteases like $3CL^{pro}/M^{pro}$ and PL^{pro} (Bosch et al. 2004). These domains will design potent antivirals, drugs, inhibitors, and vaccines against this contagion and control its infectivity and spread.

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Declarations

Conflicts of interest The authors declare no potential conflict of interest.

Research involving human participants and/or animals No human or animal studies have been used in this study.

Informed consent All figures are original, and no patient studies were involved; thus, no consent is required.

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