Angiotensin Converting Enzyme II

Subjects: Critical Care Medicine

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Angiotensin converting enzyme II (ACE2), a type I transmembrane mono-carboxypeptidase of renin angiotensin system (RAS), in involved in conversion of angiotensin I (Ang I) and angiotensin II (Ang II) to angiotensin (1-9) and angiotensin (1-7), respectively. This enzyme, as the receptor of SARS-CoV-2, plays a crucial role in the virus entrance into the host cells. The docking of the S protein to this receptor, eventually leads to the fusion of the virus membrane with the host cell plasma membrane to release the viral genome into the cell cytoplasm.

ACE2 Ang II angiotensin(1–7) AT1R SARS-CoV-2 ADAM17 soluble ACE2 MAPK PKC RBD ACE2 autoantibody

1. S Protein Structure

S protein of SARS-CoV-2 (of 1273 amino acid residues), a class I fusion and homo-trimeric glycoprotein, is composed of two subunits, S1 and S2, in each of its monomers. The former is in charge of docking of the virion to ACE2 and the latter promotes fusion of viral and host cell lipid outer membranes through a fusogenic process [1]. From N- toward C-terminal ends, S1 comprises a signaling sequence, an N-terminal domain (NTD), a receptor binding domain (RBD) including its receptor binding motif (RBM) and two subdomains (SD1 and SD2) [2][3]. S2, a rather conserved molecule in SARS-CoV and SARS-CoV-2, consists of a fusion peptide (FP), the heptad repeat 1 (HR1), the heptad repeat 2 (HR2), a transmembrane domain (TM) and a cytoplasmic tail (CT) [1][4]. Absent in SARS-CoV, a polybasic amino acid sequence, RRAR (Arg-Arg-Ala-Arg), called the furin-cleavage site is located at the S1/S2 boundary, the deletion of which attenuates SARS-CoV-2 replication in the respiratory cells [5] **(Figure 1).** It has been shown that the presence of the RRAR motif enables SARS-CoV-2 to evade endosomal interferon-induced transmembrane (IFITM) proteins [6].

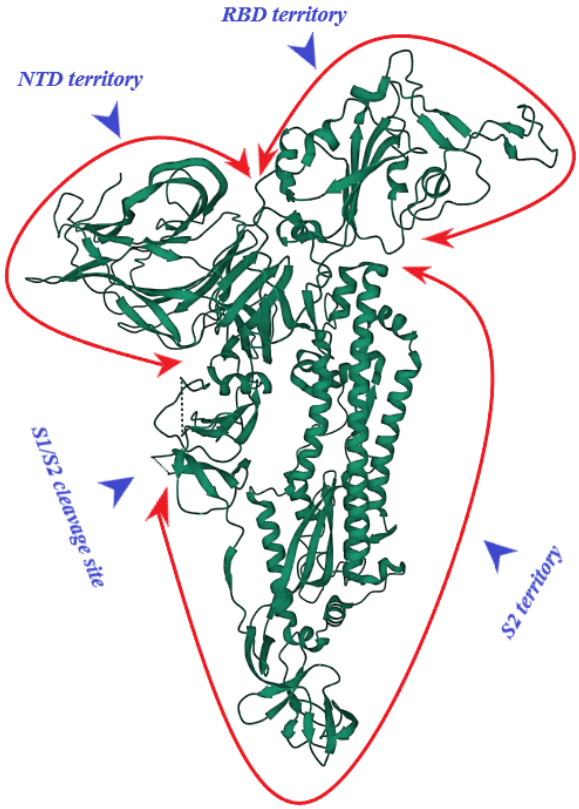

Figure 1. S protein (monomer). PDB (RCSB.org) ID: 7QUS [7]. NTD: N-terminal Domain, RBD: Receptor Binding Domain

2. Binding of ACE2 with RBD Increases the Chance of Further Complexification of ACE2 and SARS-CoV-2

Binding of RBD with ACE2 is the first step of virus cell entry. Glycosylation of ACE2 and RBD residues may block this process [8]. However, RBDs in a trimeric S protein are not always accessible for this complexification and may be packed together [9]. Constitutively, the RBD position within the S1 structure shifts dynamically through an intrinsic hinge-like movement between “open or up (U)” and “closed or down (D)” states [2]. RBD in U status moves outward the apex of S1 domain to be ACE2-accessible while in D position it hides within the protein apex to be ACE2-inaccessible [10] **(Figure 2** and **Figure 3)**.

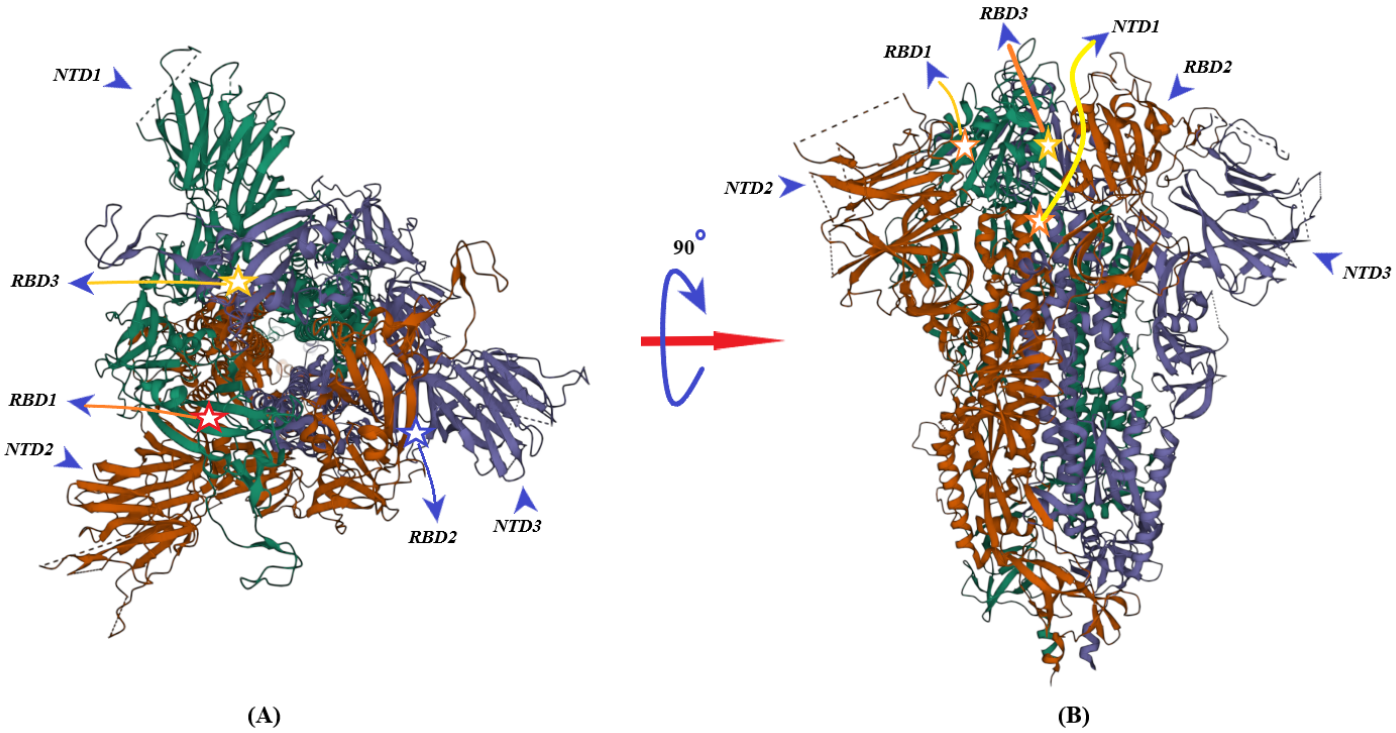

Figure 2. Two views of the trimeric SARS-CoV-2 S protein with the three RBDs in closed (DDD) state. PDB ID (RCSB.org): 7UB5 [11]. (A) Top apex view: the positions of RBDs (stars) show that these domains are hidden within the core of the molecule in the apex in DDD state. (B) Longitudinal lateral view: RBDs hidden position and NTD1 position in behind the molecule are pointed by the stars. All the arrows and the stars show the position of the domain territories. It is observable that the monomers of S protein are intertwined so that S1 in one monomer is in the proximity of S2 of the neighboring monomer (see the text). NTD: N-terminal domain; RBD: receptor binding domain

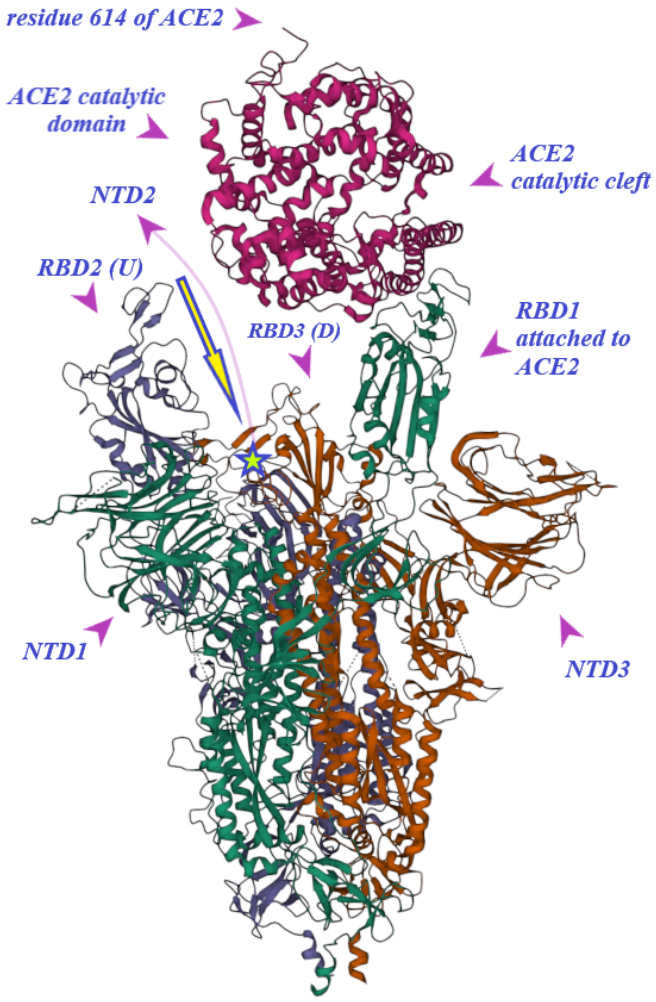

Figure 3. The trimeric form of SARS-CoV-2 S protein. RBD of the first monomer (RBD1) is attached to ACE2 catalytic domain. PDB ID (RCSB.org): 7VXA [12]. The intertwined positions of NTDs and RBDs of each monomer with the other monomers are noticeable. RBD of the second and third monomers (RBD2 and RBD3) are in open (U) and closed (D) states, respectively. NTD2 is identified by the yellow star pointed with the yellow arrow. Residue 614 is the point of attachment of ACE2 catalytic domain with its "Neck" domain (see the text). ACE2: angiotensin converting enzyme 2; NTD: N-terminal domain; RBD: receptor binding domain.

As to the trimeric nature of S protein and considering U or D states of RBD, the apex of the trimeric S1 might harbor symmetric three open (UUU) or three closed (DDD) RBDs as well as intermediate asymmetric conformations of one open/two closed (UDD) or two open/one closed (UUD) ACE2-binding domains **(Figure 2** and **Figure 3)**. The predominant RBD conformation (UUU, DDD or other intermediates) differs in the variants of the SARS-CoV-2 [13]. Recent studies attributed the escalated transmissibility and virulence of the new mutant variants to the abundancy and stability of RBD in the U state [13][14]. Moreover, according to cryo-electron microscopy findings, docking of the single open RBD to ACE2 induces a rotation of the same domain which provokes its center of mass within the UDD complex to move approximately 5.5 Å away from the axis of the whole trimeric S protein

molecule. This rotation is accompanied by moving of the three NTDs in the trimer of about 1.5–3.0 Å [15] which results in a reduction in the contact area among S1 subunits and the neighboring S2 core. This causes successive gaining of U state in the neighboring two D-positioned RBDs within the trimeric S protein [15]. To make it more clear, it should be noticed that in ACE2-unbound form of closed RBD, there are π-π interactions among some residues of S1 in one protomer and Tyr(Y)837 of S2 in the neighboring protomer in addition to a salt bridge between Asp(D)614 of S1 and Lys(K)854 of the S2 which are disordered after ACE2 binding [15]. In D614G (Asp614 to Glycine) mutation, the hydrogen bond between D614 residue of SD2 (of S1 subunit in one protomer) and Threonine(T)859 residue of S2 subunit in the neighboring protomer is deleted which induces S1 to move away from S-trimer axis [2][16]. These conformational changes bringing about successive expression of more U states results in escalating the probability of complexification, not the affinity, of RBD with ACE2 [16]. Clarifying this issue needs to consider that the binding affinity (according to its definition [17]) depends on the quantity and type of the mutations crowded in RBD which determine the density and dynamicity of polar interactions of RBM interfacial and non-interfacial amino acid residues with the counterpart residues of ACE2 [18][19].

3. Membrane-Bound and Soluble ACE2

ACE2 contributes to infectivity and the entry of SARS-CoV-2 into the host cells while physiologically plays a crucial role in protection of the lungs, heart and other tissues against a variety of inflammatory or hypoxia-induced insults [20][21][22][23]. The non-homogenous distribution of ACE2, yet in a limited scale compared to ACE, was shown in a wide variety of tissues with the highest expression in the small intestine, heart, testis, thyroid glands and adipose tissue; intermediate expression in the lungs, colon, liver, bladder and adrenal glands; and the lowest content in the blood, spleen, bone marrow, brain, blood vessels and muscles [24]. Beyond the genetic traits determined by loci near immune related genes such as *IFNAR2* and *CXCR6* [25], the distinct genetic variants of highly polymorphic *Ace2* (gene located on chromosome Xp22.2 encoding for ACE2 protein [26]) in different populations have also been shown to affect the susceptibility to and severity of COVID-19 in some cohorts of patients [27][28][29][30].

ACE2, a type I transmembrane protein of 805 residues, comprises an extracellular segment (residues 19–740) including a signal peptide of 18 amino acids, an N-terminal claw-like protease domain (PD_{ACE2}, residues 19–615) and a collectrin-like domain (residues 616–740) including ferredoxin-like fold “Neck” domain (residues 616–726) attached to a long transmembrane domain (residues 741–763) ending with a cytosolic C-terminal tail [31][32][33][34][35] (**Figure 4** and **Figure 5**). The cytosolic tail containing a conserved endocytosis short linear motif had been demonstrated to play a role in SARS-CoV-S protein-induced shedding of ACE2, TNF-α production and SARS-CoV infection [36][37] although a combined in silico and in vitro study strengthened by confocal imaging denied any role for C-terminal tail of ACE2 in SARS-CoV and SARS-CoV-2 cell entry [38]. The collectrin-like domain contributes to dimerization of two ACE2 molecules (assuming ACE2-A and ACE2-B) through interacting with Arg652, Glu653, Ser709, Arg710 and Asp713 in ACE2-A with Tyr641, Tyr633, Asn638, Glu639, Asn636 and Arg716 in ACE2-B [31].

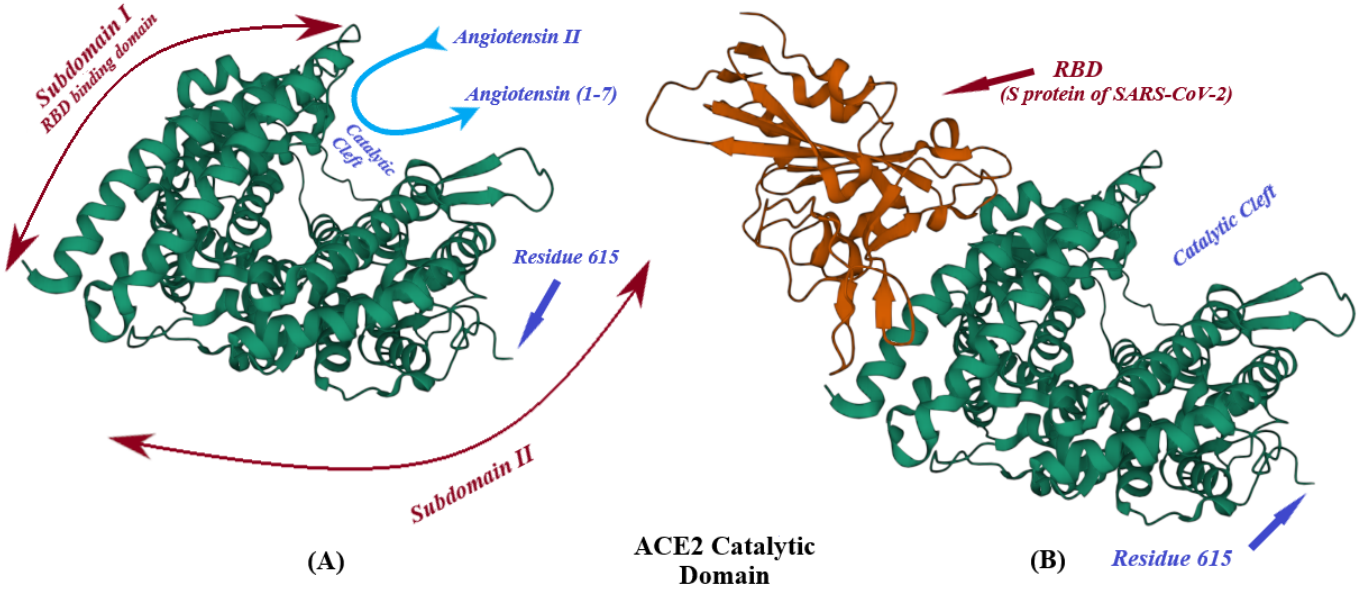

Figure 4. Catalytic domain of ACE2 alone **(A)** and in binding with RBD of SARS-CoV-2 S protein **(B)**; PDB ID (RCSB.org): 6M0J [3]. ACE2: Angiotensin Converting Enzyme 2; RBD: receptor binding domain of SARS-CoV-2 S protein.

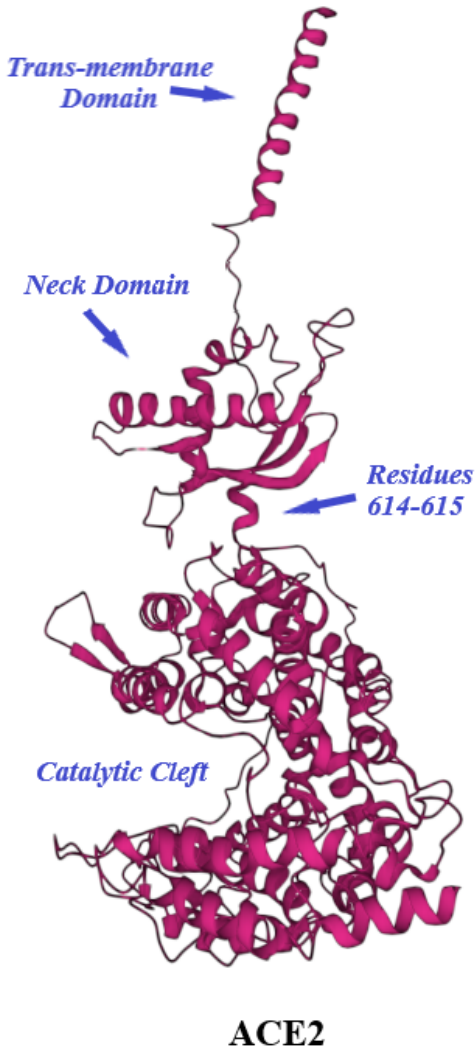

Figure 5. Full length of ACE2 molecule with the catalytic, the collectrin-like (Neck) and the trans-membrane domains; PDB ID (RCSB.org): 6M1D [39]. Residues 614-615 are the C-terminal amino acids of the catalytic domain of ACE2 which bind with the N-terminus of Neck domain of ACE2. ACE2: Angiotensin Converting Enzyme 2.

Physiologically, PD_{ACE2} with its catalytic site removes one amino acid from the C-terminal end of angiotensin I (Ang I) and angiotensin II (Ang II) to turn them into angiotensin (1–9) and angiotensin (1–7), respectively [40]. An X-ray study uncovered the presence of a wide and deep cleft within the metallopeptidase catalytic domain of ACE2 with two subdomains I and II comprising its wall [41] **(Figure 4)**. The catalytic efficiency of ACE2 for hydrolysis of Ang II is 400 times more than that of Ang I (as the precursor of Ang II) [40]. This means that any deficiency of ACE2 affects hydrolysis of Ang II 400 times more than that of its precursor, Ang I. Physiologically, this enzymatic hydrolysis leads to an increase in angiotensin(1–7)/Ang II concentration ratio resulting in attenuation of proinflammatory and organ-damaging effects of Ang II/AT1R pathway [42]. Conversely, deficiency of ACE2, which leads to an increase in tissue or plasma Ang II, may deprive the cell of one of its anti-inflammatory and protective tools [20]. An animal study on Ace2 (ACE2 gene) knockout mice proved that cardiac contractility is impaired, hypoxia-inducible genes such as

BINP3 (encoding pro-apoptotic Bcl2 interacting protein) are upregulated and Ang II increases in the kidneys, heart and plasma [43][44]. Reciprocally, Ang II, while upregulates angiotensin converting enzyme (ACE), was shown, both in vitro and in vivo, to downregulate ACE2 expression at both mRNA and protein levels rather through a cellular inflammatory reaction induced by AT1R mediated ERK/p38MAPK pathway [45][46]. Furthermore, as a positive feedback effect, Ang II through stimulation of AT1R promotes shedding of the membrane bound ACE2 (mACE2) ectodomain off the cell surface into the plasma as soluble ACE2 (sACE2; residues 18-708) [47] by inducing a sheddase protein, ADAM17 [48][49]. Both of these two effects, which results in a decrease in mACE2 and an increase in circulating sACE2, can be blocked by Ang II type I blockers (ARBs), e.g., losartan [45][46][49]. Consequently, the concentration of sACE2 increases in pathologies associated with higher AT1R stimulation such as myocardial infarction, heart failure, metabolic syndrome and diabetes mellitus [50][51][52].

4. ADAM17, the Sheddase of ACE2

Regulated selective cleavage and release of the extracellular segment of many of transmembrane proteins into the extracellular space called “ectodomain shedding” modifies a diverse array of trans- and cis-signaling pathways [53]. ADAM17, the first identified shedding protease, is a Zn^{2+}-dependent metalloproteinase and a member of the family of membrane-anchored ADAMs, which plays a role in both innate and humoral adaptive immunity, among the others [54]. This sheddase promotes proinflammatory effects through dissociating a variety of ligand proteins as its substrates including ACE2, TNF-α and transmembrane CX3CL as well as transmembrane receptors including IL6Ra and TNF receptors I and II [48][55][56][57][58]. Additionally, an animal study has revealed that ADAM17-induced degradation of interferon-γ (IFN-γ) inhibits the latter’s anti-tumorigenic and anti-osteoclastogenic properties [59].

This metalloprotease exists in two forms: the full length pro-ADAM17 of 100KDa and the mature form of 80KDa lacking the inhibitory prodomain: the latter comprises almost two thirds of its total cell content which resides predominantly in perinuclear space along with TNF-α [60][61]. ADAM17 as a type I transmembrane multidomain proteinase comprises an N-terminal signal sequence, an inhibitory prodomain, a metalloproteinase catalytic domain, a disintegrin-like domain, a cysteine-rich and membrane proximal domains (MPD, involved in substrate recognition) attached to a single transmembrane domain (TMD) ending into a cytoplasmic tail [58][62]. The TMD, but not the cytoplasmic domain, contributes to the rapid activation of this metalloprotease by different signaling pathways [63]. However, phosphorylation of the cytoplasmic domain of ADAM17 by mitogen activating protein kinase (MAPK) network including p38MAPK and extracellular signal-regulated kinase (ERK) as well as Polo-like kinase2 (PLK2) keep this metalloprotease proteolytically functional through prevention of its dimerization and dampening of its binding to tissue inhibitor of metalloproteinase3 (TIMP3) at the cell surface [64][65]; TIMP3 plays an inhibitory role for the dimerized ADAM17 on the cell surface [66].

A proteolytically inactive rhomboid protein (iRhom2) encoded by *Rhbdf2* gene involved in innate immunity against viral infections [67], regulates trafficking of pro-ADAM17 from endoplasmic reticulum to Golgi apparatus where furin detaches ADAM17 inhibitory prodomain to make it mature, substrate specific and be prepared for expression on the cell surface in complex with iRhom2 [68][69]. Regulation of the function and substrate-selectivity of iRhome2/ADAM17 complex seem to be critically dependent on the interactions between TMDs and juxtamembrane

domains (JMDs) of ADAM17 and iRhom2 [70]. Infection with DNA and RNA viruses (influenza A, RSV) upregulates *Rhbdf2* and *ADAM17* genes [69]. However, an in vivo study revealed that ADAM17-dependent ectodomain shedding is a saturable phenomenon: it does not increase significantly when ADAM17 expression rises above a certain level [71]. It must be mentioned that this metalloprotease is rapidly and reversibly switched “on” and “off” through conformational changes making its catalytic domain accessible and inaccessible, respectively, yet independent of removal of the inhibitory pro-domain or dissociation of TIMP3 [72].

Given that ADAM17 small interfering RNA (siRNA) is capable of attenuating Ang II-mediated inflammation in vascular smooth muscle cells [73] it is implied that along with other downstream mediators, ADAM17 is also involved in Ang II-induced inflammatory responses. Additionally, Ang II-mediated stimulation of AT1R, a G-protein coupled receptor (GPCR), provokes oxidative stress, induces phospholipase C (PLC)-mediated protein kinase C (PKC) activation and increases calcium influx [74][75][76][77]. Oxidative stress in tumor cells and platelets could previously be found to activate ADAM17 with pro-inflammatory effects (see previous paragraph) [78][79]. Reciprocally, ADAM17 in a mouse model could also increase NADPH oxidase 4 (Nox4) activity resulting in oxidative stress [80] which by inducing pro-inflammatory genes is intertwined with inflammation [81][82]. It is, however, noticeable that ADAM17 positively regulates Thioredoxin-1 (Trx-1) activity as the key effector of intracellular reducing system and downregulates ADAM17 as a negative feedback effect [83][84].

Furthermore, the PKC pathway, depending on the nature of its activator, affects ADAM17-dependent ectodomain shedding. Short-term (minutes) activation of protein kinase C (PKC) by phorbol ester (the strongest non-physiologic stimulator of PKC) increases ADAM17 content on the cell plasma membrane and the sheddase activity while prolonged (hours) exposure to phorbol ester downregulates mature form of ADAM17 on the cell surface abolishing the ectodomain shedding without reducing the total cell content of ADAM17 (mature plus pro-ADAM17) [85]. Conversely, ligand-activated GPCRs, such as thrombin-mediated protease activated receptor1 (PAR1), with the potential to activating of PKC signaling [86][87], induces sheddase function without any change in the content of ADAM17 on the plasma membrane [85]. Moreover, Ang II, via AT1R, was found to upregulate PAR1 and PAR2 in rat aorta which lead to pro-inflammatory responses accompanied by raising IL-6 and monocyte chemoattractant protein-1 (MCP-1), the substrates of ADAM17 [61][88][89]. Along with the natural homo- and hetero-merization of GPCRs [75], an in vitro study revealed synergistic interaction between AT1R and PAR1 [90], implicating a positive influence of these receptors in activating ADAM17. Moreover, AT1R stimulation alone is associated with a rise in IL-1β which by itself promotes ectodomain shedding by ADAM17 [91][92]. Considering that Ang II via AT1R could induce PKCδ/p38MAPK [93] which also activates ADAM17 [94][95], it is logically implied that AT1R also potentiates ADAM17-induced ectodomain shedding through p38MAPK pathway. Ca^{2+} influx and calmodulin inhibition stimulate ADAM10 [96]. Given that ADAM17 is involved in processing of pro-α2δ-1 and α2δ-3 subunits of voltage gated calcium channels which enhances calcium influx [97] it is implied that ADAM17 also indirectly contributes to activating ADAM10 in ectodomain shedding.

5. Shedding of ACE2 Ectodomain and sACE2 in SARS-CoV-2

As was mentioned previously, ADAM10 and ADAM17, the predominant sheddases, contribute to cleaving mACE2 off the cell surface which lead to a vast array of physiological and pathological cis- and trans-signaling [55]. Cleavage of the ectodomain of mACE2 occurs through a basal low level constitutive or a metalloproteinase-dependent fashion [98]. A cell culture study showed that inhibition of both ADAM17 and ADAM10, widely found on pneumocyte type I and II, results in ACE2 increment on the surface of these cells [99]. Consistently, an animal model of diabetes proved that ADAM17 gene knockdown and overexpression could reduce and raise mACE2 shedding off the cardiomyocytes, respectively [100].

Oxidative stress, MAPK network, PKC and Ca^{2+} signaling activation in various viral infections including SARS-CoV and SARS-CoV-2 has already been described [101][102][103][104][105][106][107][108]. Ang II-mediated AT1R stimulation activates these signaling pathways, as well [109][110]. All these signaling pathways directly promote ADAM17-dependent ACE2 shedding which by itself decreases angiotensin(1–7)/Ang II concentration ratio and promotes Ang II/AT1R pathway which also induces ADAM17 sheddase function (see previous section). Additionally, COVID-19 has been shown to promote ADAM17 expression both at the protein and transcriptional level [111].

In this context, neither should it be forgotten that the downregulation of mACE2 in COVID19 can be due to internalization of the virus-ACE2 complex [112][113][114][115] nor should the cleaving effects of TMPRSS2 along with ADAM17 be ignored. These two proteases compete in shedding, yet at different sites, of ACE2: ADAM17 and TMPRSS2 cleave Arginine and Lysine amino acids in residues 652–659 and 697–716, respectively [116]. However, TMPRSS2-mediated dissociation of mACE2 does not result in the release of sACE2 [117].

It was also shown that docking of the SARS-CoV S protein with ACE2 could induce detachment of the latter off the cell membrane by ADAM17, which can be blocked by the TNF protease inhibitor 2 (TAPI-2) [36]. Concordantly, SARS-CoV infection was shown to downregulate mACE2 in Vero E6 and the cells of the lungs in mice [118][119]. Anti-ADAM17 compounds after SARS-CoV infection could, in vitro and in vivo, inhibit ACE2 shedding, reduce sACE2 and suppress infectivity of the virus [120]. Consistently, a cell culture study confirmed that SARS-CoV-2 S protein induces metalloprotease-dependent ACE2 shedding [121]. Kornilov et al., in the early months after the COVID-19 pandemic, considered the elevated plasma level of sACE2 as a biomarker or possible cause of more severe SARS-CoV-2 infection seen in pathologies associated with higher levels of sACE2, e.g., diabetes, heart failure, metabolic syndrome and myocardial infarction as well as in the old, men and postmenopausal women [122]. Concordantly, in a study on 42 hospitalized patients with moderate to severe SARS-CoV-2 infection, sACE2 level was higher in all patients compared to healthy control group on the day of admission (DOA) which even rose in a meaningful manner within the next 7 days of hospitalization. During this period, the upward slope of sACE2 level was even significantly steeper in severe vs moderate cases and the escalating trend continued in male sharper than that of female patients and in all hypertensive as well as diabetic COVID-19 cases under study. The strong positive correlation of the raised sACE2 with D-dimer levels in this study implies that sACE2 may contribute to hypercoagulability state seen in SARS-CoV-2 infection albeit it needs to be studied more [123].

Whatever the mechanism is, cleavage of mACE2 reduces its presence on the cell membrane and raises its soluble form (sACE2, containing PD_{ACE2} including RBD docking domain) in extracellular space including airway surface

liquid covering the lung epithelial cells [124][125]. Furthermore, a recent prospective study on 119 SARS-CoV-2 infected patients compared to 23 adult non-COVID controls revealed that autoantibodies against ACE2 (AA-ACE2; with antagonistic effect against mACE2) and AT1R (AA-AT1R; with agonistic effect on the receptor) developed in COVID patients. Intriguingly a more rise in AA-ACE2 and AA-AT1R levels was observed in the moderate and severe groups than in the mild group. The presence of AA-ACE2 was postulated to have originated from immunologic reaction against SARS-CoV-2-sACE2 complex; the autoantibodies are likely anti-idiotypic antibodies against antigen binding sites of the antibodies developed against RBD of SARS-CoV-2 [126][127]. In addition, the activity of plasma sACE2 decreased in the presence of AA-ACE2 [127].

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