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How tetraspanin-mediated cell entry of SARS-CoV-2 can dysregulate the shedding of the ACE2 receptor by ADAM17

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1. Introduction

The novel Severe Acute Respiratory Syndrome CoronaVirus (SARS-CoV2) is the causative agent of COVID-19, and like the original SARS-CoV invades the cell through attachment of a spike (S) protein to the angiotensin converting enzyme 2 (ACE2) receptor, leading to internalization of the virion [1]. ACE2 is a type 1 transmembrane protein that serves as a key component of the renin-angiotensin-aldosterone system (RAAS) that mediates numerous effects in the cardiovascular system. In addition to being membrane-bound ACE2 can also be cleaved by ADAM17 (A Disintegrin And Metalloprotease), releasing a catalytically active soluble form of ACE2 that has been shown to block the association of the SARS-CoV spike protein with the ACE2 receptor [2]. By way of contrast the serine protease TMPRSS2 (TransMembraneProtease2), releasing a catalytically active soluble form of ACE2 that has been shown to block the association of the SARS-CoV spike protein with the ACE2 receptor [3]. Since cleavage of ACE2 by ADAM17 was found to be dispensable for cell entry by the virion it becomes important to understand how TMPRSS2 pre-empts ADAM17 for cleavage of ACE2 following infection by the virus.

The ectodomain of proteolytically-active ADAM17 consists of a catalytic, a disintegrin and a membrane proximal domain (MPD), as well as a CANDIS (Conserved Adam seventeen Dynamic Interaction Sequence) adjacent to the juxtamembrane region, Fig. 1A. This follows conformational displacement and cleavage of an auto-inhibitory prodomain used to maintain an inactive state during cellular routing [4]. In the quiescent state the protease is assumed to orient freely at the cell surface [5], but translocation of the negatively charged phospholipid phosphatidylserine (PS) to the outer leaflet of the lipid bilayer reorients the catalytic domain proximate to both its substrate and the cell membrane, Fig. 1B [6]. The PS-binding motif is a triplet cluster of cationic residues, RK_K in ADAM17 [7] and R_KK in closely related ADAM10 [8], that is located in the MPD and mediates the interaction of the protease with the membrane. This scenario accounts for why ADAM17 substrates are cleaved at positions very close to the membrane of the same cell [9], and why increased shedding in vivo does not follow from over-expression of the protease [10]. Disruption of these spatio-temporal events would be expected to influence the severity of COVID-19 by dysregulating the physiological shedding of ACE2 by ADAM17. We have previously shown how the heat shock response in COVID-19 can disrupt this repositioning of the ADAM17 catalytic domain [11], and have separately demonstrated how the M. tuberculosis heat shock protein Acr can dysregulate the shedding of the inflammatory cytokine CXCL16 by ADAM10 [12].

Tetraspanins (Tspans) are a family of small transmembrane proteins that interact with membrane-associated proteins, including cell receptors, transmembrane preoteases and other Tspans, to provide platforms critical for viral entry [13,14]. Tspan enriched microdomains (TEMs) are associated with the entry sites...
of several viruses including HPV [15], HCV [16], HIV [17], CoV [18,19] and Influenza [19]. Tspans consist of four conserved transmembrane helical segments, a small extracellular loop (SEL) and a more variable larger extracellular loop (LEL). In addition to cell adhesion Tspans are also implicated in the formation and function of extracellular vesicles (EV) used for intercellular transport of proteins, lipids and RNA [20]. Mechanistically these protrusive activities of the cell membrane are facilitated by the ability of Tspan clusters to generate local membrane curvature and induce cell membrane reorganization [21].

ADAM10 and ADAM17 are atypical members of the ADAM protease family due to the presence of the MPD that facilitates sheddase regulation by PS translocation. ADAM10 activity is also regulated by Tspans, specifically the subfamily consisting of Tspans 5,10,14,15,17 and 33 [22] that together allow ADAM10 to adopt different “scissor” complexes primed for cleavage of specific substrates [23]. By contrast ADAM17 activity is regulated by the seven membrane-spanning family of rhomboid proteins [24], with the Tspan CD9 having an inhibitory effect on ADAM17 activity through direct association [25] that limits both movement of the protease and accessibility by the substrate [26]. CD9 has also been shown to facilitate MERS-CoV cell entry by scaffolding cell-associated receptors and proteases, leading to the hypothesis that a TEM containing CD9 might function to assist in both MERS-CoV cell entry and inhibition of the shedding of the ACE2 receptor by ADAM17. By first modeling the open CD9 conformer responsible for interacting with membrane-bound partners we use protein-protein docking to generate a CD9-mediated ACE2/ADAM17 complex that demonstrates how the Tspan can induce cell-membrane curvature and in the process reorient the protease catalytic domain away from the substrate cleavage site, Fig. 1C.

2. Methods

Crystal structures for the complete ACE2 receptor, available as pdb id 6M1D [27], the catalytic domain of ADAM17, available as pdb id 1BKC [28], and the open conformation of the tetraspanin CD53, 6WVG [29], are available from the RCSB (www.rcsb.org) After insertion and refinement of missing residues using the MODELLER loop refinement algorithm [30], hydrogen were added and the structures subjected to a short energy minimization using the CHARMM force field [31] as implemented in the Discovery Studio program suite. To generate the open structure of CD9 the query sequence of hCD9 (Uniprot ID P21926) was aligned to the CD53 template, and the alignment used to create a set of 20 homology models. The best model, selected as the structure with the most negative value of the normalized discrete optimized protein energy (DOPE) [32], was minimized using the CHARMM force field, and superimposed on the CD9 closed conformation, available as 64 KJ [33]. We have previously described the use of homology modeling, using vascular apoptosis-inducing protein-1 (VAP1) [34] as a template, to generate a structure of the ADAM17 ectodomain [11].

The ClusPro docking server [35] was employed to generate an ACE2/ADAM17 complex using the complete structure of ACE2 and the catalytic domain of ADAM17. A CD9/ADAM17 complex was generated using the homology-modeled ectodomain of ADAM17 and the partial structure of the open conformation of CD9, CD9EC2, previously characterized as interacting with the nanobody 4E8 [36] and available as pdb id 6Z1V. The ClusPro rigid-body protocol involves Fast Fourier Transform (FFT)-based global sampling of the rotational/translational space, followed by clustering of the one thousand lowest-energy structures, finishing with a CHARMM minimization to remove steric clashes. Ranking is based on cluster population, using the cluster center as the model complex. The structures shown in Figs. 2 and 3B represent the center of the most populated cluster when scored using either electrostatic-favored, hydrophobic-favored, Van der Waals-electrostatic-favored, or balanced weight coefficients For the ACE2/ADAM17 complex the ACE2 attractor set was comprised of residues Arg652, Lys657, Arg659, Arg708, Ser709 and Arg710. The attractor set for the ligand was chosen as the His405, Glu406, His409, Gly412 and His415 residues that incorporate the zinc-binding consensus motif for ADAM17. For the CD9/ADAM17 complex the attractor set for CD9 included Val159, Glu160, Gin161, Ser164, Lys165, Leu173, Thr175, Phe176 and Val178, while the ADAM17 ligand was left unbiased.
The orientation of the ACE2/CD9/ADAM17 complex relative to the cell membrane was determined by first docking the ACE2 TM to the membrane spanning domain of the open conformer of CD9 generated by homology modeling, and then embedding the complex in an explicit membrane configuration containing a 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayer. The optimal orientation was defined as the tilt angle of the TM relative to the membrane normal that corresponds to the minimum solvation energy [37]. Docking of this complex to the TM of ADAM17 and embedding in POPC reveals a new membrane orientation now angled relative to that of the original ACE2/CD9 transmembrane complex. Superimposing both solutions and removal of overlapping POPC components allows for an interpolated model of the localized curvature predicted for ACE2/CD9/ADAM17 transmembrane complexation, Fig. 3B. The ternary complex in Fig. 4 was generated by first grafting the CD9EC2/ADAM17 complex onto the open CD9 conformer in the ACE2/CD9/ADAM17 transmembrane complex embedded in the explicit membrane. The C-terminus of ADAM17 was extended by adding the α-helical CANDIS domain [38] as generated by a-Fold [39], and connected to the TM domain with a juxtamembrane segment generated using the MODELLER loop refinement algorithm. The orientation of the ACE2 receptor was determined by extending the juxtamembrane as dictated by the ACE2 TM in the transmembrane complex, optimizing the geometry to relieve any resulting steric clashes. The complete complex was subjected to energy minimization using CHARMm.

3. Results and discussion

The ACE2/ADAM17 complex in Fig. 2 is formed through an array of hydrogen bonding and salt bridge interactions as well as several tightly-coupled hydrophobic interactions. The interface contacts are summarized in Table S1, and can be characterized as representing three distinct regions centering on the ACE2 residues Lys657, Arg652 and Arg710 respectively, residues previously identified as critical for protease recognition [3,40]. As can be seen in Fig. 3A the open conformer of CD9 involves a reorientation of the LEL relative to the SEL as seen in the 4E8 nanobody complex and predicted as the interface for complexation with ADAM17; B: local membrane curvature induced by complexation of the CD9 transmembrane domain (cyan) with the TM domains of ACE2 (green) and ADAM17 (blue).
static, indicating that activation of CD9 can be regarded as essentially a rigid-body movement of the large extracellular loop that in turn angles the C and D loops, highlighted in Fig. 3A, in a manner that permits interaction with the membrane-bound target. For ADAM17 this interface is predicted to occur at the disintegrin domain, a domain that also functions as an alternative ligand for the integrin family of cellular receptors abrogating both the adhesive capacity of the integrin as well as the protease activity of ADAM17 [41]. The interface contacts for the CD9/ADAM17 complex are summarized in Table S2.

The architecture of CD9 is such that helices 1 and 2 are spanned by the SEL while the larger loop spans helices 3 and 4. The CD9/ACE2 membrane complex in Fig. 3B positions the ACE2 TM more proximal to helices 1 and 3, in contrast to that observed in the previously reported for the CRYO-EM images of the complex of CD9 with the immunoglobulin (Ig) EWI-2 [33], where the interaction was primarily with helices 3 and 4. For the CD9/EWI-2 complex mutational analysis confirmed a complementary arrangement of small residues between the glycine zipper motif of the Ig and the Cd9 helical bundle but no such motif is evident in the ACE2 TM. Instead complexation with ACE2 is due to hydrophobic interactions, primarily between valine residues on one side of the ACE2 TM helix and alternating isoleucine and valine residues on helix 4 of CD9. Interaction with helix 1 of CD9 is through longer contacts involving isoleucine residues on the distal side of the ACE2 TM helix. Subsequent complexation with the TM of ADAM17 primarily involves a nest of hydrophobic contacts among residues Ile583, Phe683, Trp685 and Pro686 of ADAM17, Pro209 of helix 4 on CD9 and Phe762 on the TM of ACE2.

Embedding the ternary ACE2/CD9/ADAM17 transmembrane complex in a linear membrane bilayer would either expose portions of the CD9 and ADAM17 TM domains to the cytosol or alternatively embed a portion of the ACE2 cytosolic domain in the POPC bilayer. The resulting prediction that the complex induces localized membrane curvature is reflected in the x-ray structure reported for CD9 and characterized using the lipid cubic phase method (LCP) [42]. The wavy layer observed in the crystalline lattice obtained by LCP demonstrates how the asymmetric shape of CD9 produces a cone-like, curved arrangement of the quasisolid lipidic layer. The conical structure of a CD9/CD81 Tspan network is also predicted to induce membrane curvature, amplified by a cholesterol-induced reorientation of the upper leaf of the bilayer from conical to cylindrical [43]. Connecting the ectodomain and transmembrane structures yields a ternary complex, Fig. 4, that orients the protease active site significantly distal to the predicted ACE2/ADAM17 interface. A ternary complex has been previously confirmed for the interaction of the integrin α5β1 with both CD9 and ADAM17 [41], and it has been shown separately that ADAM17 complexation with both the integrin and CD9 inhibits ADAM17 activity [44]. The strength of the CD9/ADAM17 interface responsible for complexation can be evaluated by comparing the buried surface area (BSA) of the CD9-ADAM17 interface with that of the previously characterized CD9EC2 nanobody complex (pdb 6Z1V).

The combined buried surface area (BSA) for the CD9EC2 complex with the 4E8 nanobody is 1274 Å². For CD9 complexation with the disintegrin domain of ADAM17 the BSA is 1506 Å² reflecting a tighter fit at the interface. Comparison of BSA by residue, Table S2, predicts an ADAM17 interaction nearly identical, but marginally more efficient, to that characterized for the nanobody with the notable exception of the Phe176 residue on CD9. In the complex with 4E8 Phe176 is sandwiched between nanobody residues Trp98, Trp102 and Arg105. The superior fit of Phe176 between the contiguous residues Thr541 and Cys542, located on the turn of the disintegrin loop of ADAM17, is reflected in a 50% increase in the BSA of phenylalanine. This loop has been identified as the interface for attachment of ADAM17 to multiple integrins [45], and apart from Thr541 and Cys542 the loop remains exposed in the predicted CD9/ADAM17 complex, and so capable of further complexation to yield a ternary α5β1/CD9/ADAM17 complex, Fig. 4. By anchoring the disintegrin loop CD9 this model predicts that CD9 provides an exposed but rigid platform for attachment of the integrin to ADAM17, a model that explains results from ligation assays and immunoprecipitation experiments which indicate that CD9 inhibits α5β1-mediated cell adhesion by reinforcing cis, or same cell, interaction between α5β1 and ADAM17 [41].

Over 80 cell-bound substrates of ADAM17 have so far been identified, and at least nine are either cytokines or cytokine receptors that have been reported as triggering inflammation when dysregulated [46]. Likewise CD9 influences many cellular activities in leukocytes and endothelial cells including the regulation of adhesion. As such it is expected that in addition to facilitating cell entry by SARS-CoV-2 the dysregulation induced by formation of the ternary complex in Fig. 4 would also impact COVID-19 symptomology, especially those associated with inflammation and cytokine production, as well as other aspects of the immune response. Indeed a recent search to identify articles published since 2005 and relevant to ADAM17 and SARS-CoV or SARS-CoV2 results in a clinical picture consistent with dysregulation of many of the molecular pathways in which ADAM17 participates, leading the authors to ask: “is ADAM17 the Missing Link?” [47].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Appendix A. Supplementary data

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