The Role of Key Cell Adhesion Receptors in Inhibiting SARS-CoV-2

Cell adhesion and integrin signaling

Focal adhesions are large protein complexes that sense tension and anchor cells to the extracellular matrix. They regulate intracellular reorganizations that result in dynamic changes in the functions and morphologies of cells[1-3]. The hundreds of proteins in a focal adhesion are organized in layers[4] where key proteins mediate crosstalk amongst the layers and the major mediator of cell adhesion, integrin. This cell surface receptor is a heterodimer with an α and β subunit (**Fig. 1**) each harboring an 80-150 kDa extracellular domain, a transmembrane domain, and a short cytoplasmic domain[5]. In mammals, the heterodimeric integrin receptors are composed of 18 distinct α and β chains[6,7] that result in 24 distinct heterodimeric integrins with distinct functions.

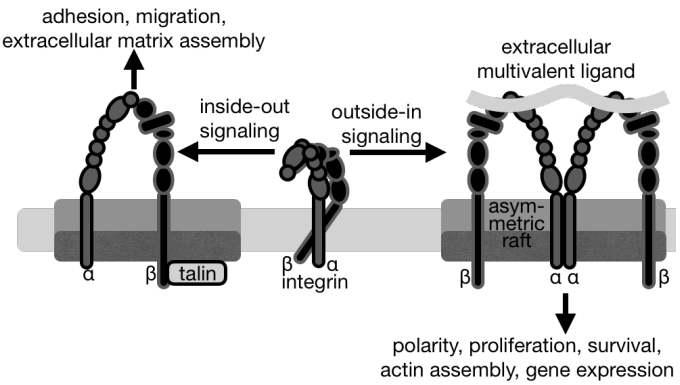

Fig. 1. Integrin signaling in both directions with different outcomes. Integrin adhesion and signaling are linked to the recruitment of integrins to lipid rafts. Several integrins have been found in lipid rafts whereby the activated form of integrin preferably localizes to the cholesterol-enriched membrane lipid rafts. The cholesterol-rich membrane domains cluster integrin at focal adhesions, which regulates integrin activation.

Integrins are bidirectional transmembrane signaling receptors involved in cell-cell adhesion and cell-matrix junctions and play key roles in hematopoiesis, vascular development, immune and inflammatory responses, as well as hemostasis and arterial thrombosis[8,9] (**Fig. 1**). Integrins bind to the extracellular matrix which allows cells to respond to several physical and chemical cues that control a variety of biological processes including hemostasis, differentiation, migration, proliferation, and cell death. By linking focal adhesions to the extracellular matrix, integrins regulate normal cell functions such as signaling, cell migration, cell adhesion, and leukocyte function[10]. These cellular processes are mediated by focal adhesion and controlled by the activation state of integrins. In migrating cell, integrins are active and initiate the formation of focal adhesions and cell attachment. Inactivation of integrin results in the disassembly of focal adhesions and cell detachment.

What is the role of lipid rafts in cell adhesion and integrin activation?

Cell adhesion and integrin biology are profoundly important in cell biology. Yet, the molecular mechanisms of lipid-mediated integrin signaling are understudied. Changes in the thickness and diffusion of the lipid bilayer are known to affect β1 integrin-mediated adhesion as well as nascent focal adhesion formation and cell migration[11]. The composition of the membrane affects clustering or dispersing integrins and the integrin diffusion rate affects integrin binding to ligands[11]. Clearly, the lipid bilayer is not a passive bystander to integrin function and organization and downstream adhesion-related cell behavior. We are currently testing if the thicker raft membrane favors a decreased tilt of the β integrin transmembrane α-helix that correlates with the activated integrin conformation (**Fig. 2**). Integrins depend on lateral diffusion within the membrane and membrane bending is also involved in adhesion[12]. Thus, we are currently exploring how the lipid composition affects several aspects of integrin-mediated adhesion.

Integrins connect the outside and inside of a cell by transmitting signals in either direction across the plasma membrane. The binding of integrins to extracellular ligands is stabilized by

binding to intracellular scaffolding proteins such as vinculin or talin, thereby linking integrins to the actin cytoskeleton and mediating mechano-transduction. During inside-out signaling, the cytoskeletal protein talin plays a key role in regulating integrin affinity. The binding of talin to the membrane proximal region to the cytoplasmic tail of the integrin β subunit[13-18] transitions integrins into an extended conformation with increased affinity for extracellular ligands[6,19].

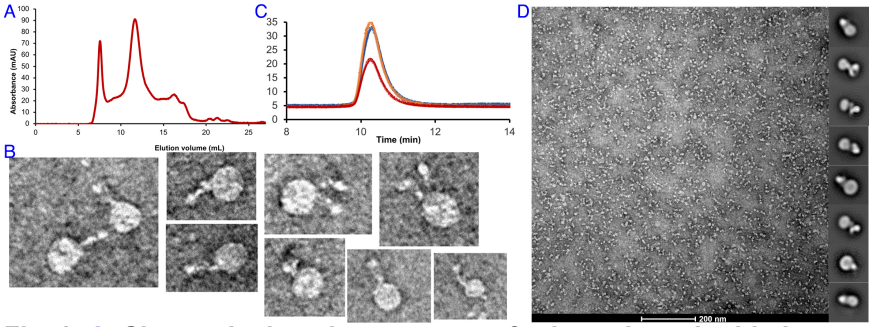

Fig. 2. A, Size exclusion chromatogram for integrin embedded nanodiscs. The integrin embedded nanodiscs, 11.6 ml elution; empty nanodiscs, 16.3 ml elution. **B, Negative stain images of integrin embedded nanodiscs.** The image was obtained on a FEI Tecnai transmission electron microscope and the sample was applied on carbon films on 400 mesh copper grids. **C,** Fluorescence-detection size exclusion chromatograms of nanodisc-embedded integrin which was 3-fold less active in presence of the inhibitor eptifibatide (red) and higher in presence of the activator talin F3 domain (orange) compared with the PAC1 antibody (blue) that binds to the already active integrin. **D, Representative negative stain image of αIIbβ3 integrin reconstituted in nanodiscs of physiological composition.** 2D class averages are shown in the 8 side panels.

During outside-in integrin activation, binding of extracellular matrix ligands results in conformational changes to the cytoplasmic tails and downstream signaling events that impact growth, survival, differentiation, and proliferation of the cell[7,20]. Many integrins recognize a so called RGD motif, which is the single letter for each amino acid (Arg-Gly-Asp) or KGD (Lys-Gly-Asp). These motifs are exposed loops on proteins that bind integrins. RGD recognition is less specific and binds to several integrins including αVβ1, αVβ3, αVβ5, αVβ6, αVβ8, αMβ2, αLβ2, α3β1, and αIIbβ3, while KGD recognition is more specific and restricted to just the underlined integrins[21]. Such RGD motifs have also been identified in the **s**evere **a**cute **r**espiratory **s**yndrome **co**rona**v**irus **2** (SARS-CoV-2) **s**pike (S) protein[22] and the human **a**ngiotensin-**c**onverting **e**nzyme **2** (ACE2)[23], a receptor known to bind to integrins as well *via* its KGD motif (ACE2 residues 353-355) while its RDG motif (ACE2 residues 203-205) is buried.

What is the role of the S protein-integrin interaction in the spread of COVID-19?

The **s**evere **a**cute **r**espiratory **s**yndrome **co**rona**v**irus **2** (SARS-CoV-2) has been shown to be the pathogen of the **co**rona**vi**rus **d**isease 20**19** (COVID-19) that has caused a global pandemic in 2020. SARS-CoV-2 (as well as the earlier identified 2003 SARS-CoV) belongs to the subgenus *sarbecovirus* of *Coronaviridae* common to human as well as animals including bats, camels, cattle, and cats. These beta-coronaviruses originate from bats. The SARS-CoV-2 **s**pike (S) protein is of great importance because it recognizes and binds to the human **a**ngiotensin-**c**onverting **e**nzyme **2** (ACE2) that binds to the receptor binding motifs of the **r**eceptor **b**inding **d**omains (RBD) of SARS-CoV-2 as well as of SARS-CoV.

Many viruses have been shown to attach to specific cell surface receptors often involved in cell adhesion to access the endocytic machinery. Integrins are often the entry for several viruses to the host cell, whereby the integrin-virus interaction leads to membrane permeability, fusion, and endocytosis. We obtained a **s**evere **a**cute **r**espiratory **s**yndrome **co**rona**v**irus **2** (SARS-CoV-2) **s**pike (S) protein construct from our resident virologist, Dr. Michael Farzan. Notably, before joining The Scripps Research Institute, Dr. Farzan published the structure of the SARS coronavirus spike **r**eceptor-**b**inding **d**omain (RBD) in complex with the human **a**ngiotensin-**c**onverting **e**nzyme **2** (ACE2) with his colleague Stephen Harrison at Harvard Medical School in

Science in 2005. We expressed this RBD as a fusion with a **f**ragment **c**rystallizable (Fc) region antibody that we cleaved with thrombin. RBG has an Arg-Gly-Asp motif that binds and activates integrins.

Since we already have NUO_NCCAT-GUP1-TI190926 approved for unbound integrin structure determination, we have already shown that we have become experts in generating stable nanodisc-embedded integrins (**Fig. 2**). The RBD is a known well-folded domain that will provide additional stability to our already stable unbound samples. Excitingly, the size exclusion chromatogram of our integrin-RBD complex runs as a much more single peak (**Fig. 3**) with only a slight shoulder compared to our unbound integrin sample (**Fig. 2A**) that resulted in very encouraging negative stain data.

We propose to determine the cryo-electron microscopy structure of human integrin bound to the SARS-CoV-2 S protein which will provide mechanistic insights into differences between SARS-CoV and SARS-CoV-2. The former binds integrins *via* its KGD motif (S residues 390-392) while SARS-CoV-2 interacts with integrin through its RGD motif (S residues 403-405). S protein binding to integrin occludes the S protein binding site on ACE2. Because RGD binding is permissive to more integrins than KGD and RGD is used by SARS-CoV-2 while KGD is used by SARS-CoV, more types of integrins that bind the S protein in SARS-CoV-2 prevent its S protein binding to ACE2 compared to SARS-CoV. Our structural studies will provide the mechanistic insights into understanding some of the key differences of SARS-CoV-2 *versus* SARS-CoV and provide the mechanism of S protein-integrin interaction to further our understanding on how COVID-19 is spreading. Significantly, integrins have an inhibitory role for viral entry. Our proposed structural studies of the S protein interaction with integrins will provide mechanistic insights into the pathogenesis of SARS-CoV-2 and SARS-CoV and might shed light on their different mortality.

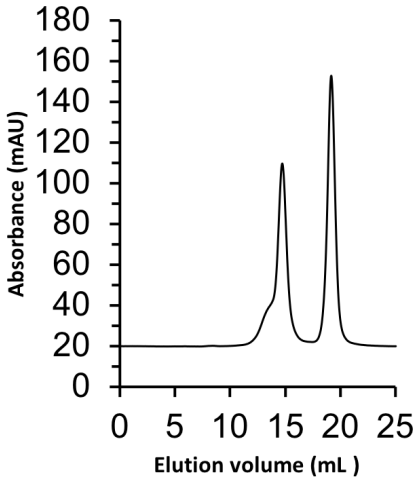

Fig. 3. Size exclusion chromatogram for integrin embedded nanodiscs. The RBD of the spike protein bound to the integrin embedded nanodiscs elutes at 14.8 ml; unbound excess spike protein, 19.2 ml elution.

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