SARS-CoV-2 Attachment to Host Cells is Possibly Mediated via RGD-Integrin Interaction in a Calcium-dependent Manner

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Running Title: SARS-CoV-2 Attachment to Human Cells is Calcium-dependent

Abstract

Our study revealed that SARS-CoV-2 harbors a RGD motif in its receptor binding domain (RBD) and the motif is absent in all previously known SARS-CoVs. The RGD motif plays a key role in cell-cell adhesion. Integrins display high affinity for RGD motifs and are known to bind to RGD motifs. Gene expression profiling revealed that expression of integrins is significantly high in lung cells, in particular $\alpha v\beta 6$, $\alpha 5\beta 1$, $\alpha v\beta 8$ and an ECM protein, ICAM1. However, the expression of ACE2, which is a known receptor for SARS-CoV-2, was found to be negligible. The molecular docking experiment showed the RBD of spike protein binds with integrins precisely at RGD motif in a similar manner as a synthetic RGD peptide binds to integrins as found by other researchers. SARS-CoV-2 spike protein has a number of phosphorylation sites for cAMP, PKC, Tyr signaling pathways. These pathways either activate calcium ion channels or get activated by calcium. In fact, integrins have calcium & metal binding sites that were predicted around and in vicinity of RGD-integrin docking site suggesting RGD-integrins interaction occurs in calcium-dependent manner. The higher expression of integrins in lungs along with their previously known high binding affinity ($\sim K_D = 4.0\text{nM}$) for virus RGD motif could serve as a possible explanation for high infectivity of SARS-CoV-2. On the contrary, the lower expression of human ACE2 in lungs and its low binding affinity ($\sim K_D = 15\text{nM}$) for spike RBD suggests that human ACE2 is less likely to be a receptor for SARS-CoV-2, at least in lung cells. A highly relevant evidence never reported earlier which corroborate in favor of RGD-integrins mediated virus-host attachment is an unleashed cytokine storm which causes due to activation of TNF- α and IL-6 activation; and integrins role in their activation is also well established. Altogether, the current study highlighted the possible role of calcium and other divalent ions in RGD-integrins interaction for virus invasion into host cells and suggested that lowering divalent ion in lungs could avert virus-host cells attachment. Since, EDTA is well known chelating agent, we suggest pulmonary EDTA chelation therapy as a mean to phenotypically remodel the Ca^{+2} ion concentration in lungs to prevent RGD-integrin interaction which is dependent upon Ca^{+2} ion for facilitating virus-host cell attachment. The suggested therapy presents a novel, technically simple, quick, safe, affordable and effective solution to prevent SARS-CoV-2 infection and treat COVID 19, especially in the midst of global emergency when a specific treatment is inexistent.

Keywords: Coronavirus, SARS-CoV-2, SARS-CoV, Integrins, COVID-19, Spike Protein

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1. Introduction

Ever since the recent emergence of novel coronavirus (SARS-CoV-2, formally known as SARS-CoV-2) in Wuhan city of China and its subsequent transmission in other countries, scientific community from all over the world are industriously engaged and committed to find a potent therapeutic solution for the treatment of COVID-19. As of 10th April, 15,21,252 confirmed cases and 92,798 deaths were recorded as a cumulative data from different parts of the world (WHO Situation Report no. 81 available at who.int accessed on 12-04-2020). At the onset of the disease, the infected symptomatic patients experience hyperthermia, pharyngeal congestion, cough, and anosmia (in some cases); however, as disease progress more than fifty percent patients develop severe labored breathing, clinically referred to as dyspnoea or tachypnoea (Huang et al., 2020; Qui et al., 2020; Wang et al., 2020). Advance stages are characterized by severe pulmonary inflammation, fibrosis and obstructions of the bronchioles resulting in a pneumonia-like pathophysiological condition (Huang et al., 2020; Qui et al., 2020; Wang et al., 2020). In both symptomatic and asymptomatic patients, SARS-CoV-2 manages to cause significant damages to multiple organs before any patients could quickly overwhelmed because neither any neurological indications nor any signs of heart, kidney and liver failure are seen at the onset of disease (Qui et al., 2020).

As of today, there is no specific antiviral therapy available in any form, either vaccine or drug or others, to combat COVID-19 and its infection. The neutralizing antibodies that were previously tested and found successful against SARS-CoV-1 have displayed inappreciable cross-reactivity against SARS-CoV-2 and for some antibodies (such as CR3022) that could bind to SARS-CoV-2, but their neutralizing efficacy has not be tested and appreciated yet (Tian et al., 2020; Yuan et al., 2020; Wrapp et al., 2020). It has been asserted that the binding sites for these monoclonal antibodies on SARS-CoV-2 are vulnerable and it is only an assumption that antibodies that could bind strongly would possibly neutralize the SARS-CoV-2 also. Moreover, cross-reacting neutralizing antibodies are also doubted for their ability to confer prolonged protection against SARS-CoV-2. So far, we have no other choice than to use previously tested drugs or to solely oblige known tactics as precautionary measures against COVID-19. Recently, Wang and co-authors (2020) suggested the use of combination of remdesivir (a broad-range antiviral drug) and chloroquine for effective control of SARS-CoV-2 infection under *in vitro* condition. Similarly, hydroxychloroquine and azithromycin has also been referred to as a potent therapeutic weapon against the SARS-CoV-2 virus (Colson et al., 2020). Drug repurposing approach, which involved a screening of a thousand of molecules, showed that HIV protease inhibitors, RNA-dependent RNA polymerase (RdRp) inhibitors and some other inhibitors and agonists such as methisazone (an inhibitor of protein synthesis), CGP42112A (an angiotensin AT2 receptor agonist) and ABT450 (an inhibitor of the non-structural protein 3-4A) could become promising treatment options for COVID-19 (Gordon et al., 2020; Li et al.,

2020; Shah et al., 2020). Lu (2020) suggested some treatment options for COVID-19 that include nucleoside analogues, neuraminidase inhibitors, lopinavir or ritonavir, remdesivir, 3TC/TDF/EFV monotherapy or combination therapy (DNA polymerase inhibitors), use of anti-inflammatory or immune-suppressive drugs, just to name a few. Besides this, some traditional Chinese medicine, for instance, ShuFengJieDu and Lianhuaqingwen capsules could also be useful (Lu, 2020). Using virtual screening (Kandeel and Al-Nazawi, 2020), epigenetic dysregulation (Sawalha et al., 2020), protein-protein interaction mapping (Cava et al., 2020), integrated network pharmacological approach (Wang et al., 2020), and similar such approaches, a number of other drugs have also been repurposed for COVID-19 treatment. However, most of the drugs are either in developmental stages or under clinical trials (<https://clinicaltrials.gov/>). In the midst of this pandemic situation, it is obvious that there exists no preventive therapy for highly contagious SARS-CoV-2 and this is strikingly alarming to all of us.

A number of studies demonstrated the key role of human ACE2 in virus attachment to host cells (Wan et al., 2020; Zhao et al., 2020). The three-dimension crystal structure of SARS-CoV-2 spike receptor binding domain complexed with its receptor, human ACE2 (Angiotensin converting enzyme 2), has been already solved (Chen et al., 2020) and all other structural, functional and antigenicity related information are also available (Walls et al., 2020). Based on the biophysical data, it has been demonstrated that the human ACE2 binds to SARS-CoV-2 with greater affinity than SARS-CoV-1 (Wrapp et al., 2020). However, owing to their low copy number (protein expression) (Chen et al., 2020), their high affinity (for SARS-CoV-2 than SARS-CoV-1) alone could not manifest reasonable virus-host cell attachment, at least in lung cells. When this manuscript study was under progress, Sigrist and coauthors (2020) showed that SARS-CoV-2 harbors a RGD motif and integrins (that display high affinity for RGD motifs) may be involved in facilitating virus entry into host cells. However, the study did not explain the full mechanistic state of affairs involved in RGD-integrins interaction and virus entry into the host cells. We also found RGD motif in the spike receptor binding domain of SARS-CoV-2 and studied mechanistic basis of RGD-integrin (and other ECM protein such as ICAM1) mediated virus invasion into host cells. This study is the first study to present striking evidence (substantiated by existing facts in literature) favoring the role of calcium and other divalent ions (magnesium, manganese etc.) in RGD–integrins mediated virus attachment with the host cells for and that lowering the concentration of calcium and other divalent ions in lungs could be a possible mechanism to avert SARS-CoV-2 infection and invasion.

Herein, we did comparative protein sequence analysis, motif scanning, gene expression data analysis and bioinformatics based molecular docking using most trustworthy tools and techniques. The results generated in this study indicated and underscored the key role of calcium and other divalent ions in

mediating virus-host cell attachment for invasion into human lung cells possibly *via* integrins and other cell adhesion proteins present on the host cell surface. We designed and suggested novel pulmonary EDTA chelation therapy as a technical simple (requires nebulizer/inhaler/nasal sprays that are available in all hospitals and clinics), quick (1-3hr a day, 2-3 times a week), safe (EDTA/EGTA are FDA approved for calcium chelation), affordable (estimated cost approx. 50-100 USD) and efficient (based on efficacy data from literature) treatment therapy for COVID 19 patients.

2. Materials and Methods

2.1. Sequence Retrieval

The coronavirus related nucleotide and protein sequences were retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/>), UniProtKB (<https://www.uniprot.org>) and SARS coronavirus 2 data hub of the NCBI (<https://www.nih.gov/coronavirus>).

2.2. Sequence Alignment

The protein sequence of SARS-CoV-1 and SARS-CoV-2 coronaviruses were subjected to pair-wise sequence alignment using Clustal Omega using default setting (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) (Madeira et al., 2019).

2.3. Motif Scanning and Prediction

The Pfam, Prosite and HAMAP profiles of the S protein from the coronavirus were ascertained using MyHits Motif Scan (SIB, Switzerland) (https://myhits.isb-sib.ch/cgi-bin/motif_scan). The putative N-linked glycosylation and other post-translation modification sites were predicted as Frequent Pattern in MyHits outputs (Pagni et al., 2007).

2.4. Gene Expression Profiling

The expression profile of ACE2 receptor protein and integrins in lungs was ascertained using Gene Expression Database at EBI (<https://www.ebi.ac.uk/gxa/home>) and the Human Protein Atlas (<https://www.proteinatlas.org/>) (Uhlén et al., 2015; 2019).

2.5. Calcium and Magnesium Binding Sites in Integrins

In order to understand the role of divalent ions in RGD-integrins mediated virus-host cells attachment. A composite calcium and magnesium binding-site in integrins were predicted using IonCom

(<https://zhanglab.ccmb.med.umich.edu/IonCom/>), which used an integrated approach based on *ab initio* training and template-based transferals (Hu et al., 2016) and predicts calcium binding sites in a given protein by searching four or more oxygen atoms on protein surface arranged in a spherical manner. The input PDB file of integrins such as $\alpha 5\beta 1$ (PDB ID: 3VI3) and $\alpha v\beta 6$ (5FFG) were subjected for ion binding sites' prediction to specifically predict calcium and magnesium binds sites.

2.6. Spike RBD docking with Integrins

The PDB file of the spike receptor binding domain (PDB ID: 6LZG) and integrins such as $\alpha 5\beta 1$ (PDB ID: 3VI3 and 3VI4) and $\alpha v\beta 6$ (PDB ID: 5FFG) were retrieved from Protein Data Bank (<http://www.rcsb.org/structure/>). The protein-protein docking was performed using HDock server which rely on template-based modeling and *ab initio* free docking using default parameters (hdock.phys.hust.edu.cn/). The docked structures were visualized and high resolution photographs were generated in Chimera 1.10.

2.7. Spike RBD Docking with EDTA

The PDB file of the spike receptor binding domain was retrieved from Protein Data Bank (as mentioned above). The PDB file of the EDTA was online obtained and converted in Mol2 file in Chimera 1.10.1 version. The docking was done using Swissdock server of SIB (<http://www.swissdock.ch/docking>). Protein-ligand binding modes were clustered according to their rank based on average FullFitness in output data (Grosdidier et. al., 2007).

Results

3.1. N-terminal of Spike Protein is Variable

Pairwise sequence alignment of SARS-CoV-1 and SARS-CoV-2 spike protein revealed that the N-terminal (especially upto 250 aa) of SARS-CoV-2 is highly divergent than that of the SARS-CoV-1 (Figure 1). The S1 Glycoprotein (from 268-304 aa in SARS-CoV-1 and from 282-317 aa in SARS-CoV-2) domain which plays an important role in recognition of host cell receptor were found divergent at 10 amino acid positions. The spike receptor binding domain is of 253 amino acid residues in SARS-CoV-1 and ranges from 317 to 569 aa; whereas, it is of 254 amino acid residues in SARS-CoV-2 and stretch from 330 to 583 aa. There were 49 mismatches and 1 insertion/deletion that render SARS-CoV-1 and SARS-CoV-2 approximately 80% sequence similarity. The S2 glycoprotein domain is a large 544 amino acid residues long region at the C-terminal of the spike protein which plays crucial role in virus fusion

with host cells. This region has been predicted to be less divergent than S1 glycoprotein and RBD. There were 49 amino acids substitution which amount for approximately 9% sequence divergence between SARS-CoV-1 and SARS-CoV-2. The cysteine rich region at the C-terminal part of the SARS-CoV-2 spike protein has an additional Cys residue (actually Ala>Cys) at 1247 position (corresponds to Ala residue at 1229 position in SARS-CoV-1 spike protein in alignment). This region is also involved in virus-induced membrane fusion (Chang et al., 2000). The presence of extra Cys residue results in the formation of a C_{x6}CC motif, which is usually found in envelop proteins of some viruses, for instance Ebola virus and murine leukemia virus, and is required for virus fusion with host cell (Johnston and Radke, 2000; Lee and Saphire, 2009).

We also found two insertion sequences with the stretch HVSGTNGTKRFD⁶⁹⁻⁸⁰ and RFQTLALHRSYLTPGDSSSGWTAG²³⁶⁻²⁶¹ at the N-terminal region of spike protein and were seen as discontinuous in the amino acid sequence as predicted by the pair-wise sequence alignment. However, considering that these inserts are very short-lengthed and appeared in the hypervariable region of viral spike protein, the most likely assumption for their origin is that they might have arisen naturally. Besides this, the receptor binding domain of the spike protein in SARS-CoV-2 has a stretch of sequence (TEIQAGSTPCNGVEGF⁴⁷⁰⁻⁴⁸⁶) showing mismatch with SARS-CoV-1.

3.2. Increased Number of Phosphorylation Sites in SARS-CoV-2 Led to Increased Viral Infection and Pathogenesis

The number and position of putative N-linked glycosylation sites, post-translation modification sites for cAMP phosphorylation, CK2 phosphorylation, Tyr phosphorylation, PKC phosphorylation and Myristyl sites were predicted to be varying in SARS-CoV-1 and SARS-CoV-2 (Table 1). Although both coronaviruses have equal number of N-linked glycosylation sites but the position of sites differs (Table 1). Furthermore, phosphorylation sites of all types (except PKC phosphorylation) were predicted to be in more number in SARS-CoV-2 spike protein than in SARS-CoV-1. The phosphorylation of viral proteins is actuated by host cells' kinases has a significant impact on viral infection, replication, multiplication and cytotoxicity (Bretaña et al., 2012; Keating and Striker, 2012; Keck et al., 2015), we assume that the increased number of phosphorylation sites in SARS-CoV-2 spike protein could be one of the major reasons for its higher infectivity as observed in case of COVID-19. These pathways either activate calcium ion channels or get activated by calcium. This suggested that virus might be directly involved in regulating these calcium ion channels or might be dependent upon presence of Ca⁺² ion or other divalent ions for attachment with host cells.

3.3. SARS-CoV-2 Spike Receptor Binding Domain Harbors a RGD Motif which is Absent in SARS-CoV-1

The spike protein sequence of SARS-CoV-1 and SARS-CoV-2 were subjected to motif scanning and prediction using MyHits Motif Scan at SIB server. A number of motifs were predicted in the spike protein sequence such as RGD (from 403-405 aa in receptor binding domain of SARS-CoV-2) (Table 2). The RGD motif (K403R substitution) was predicted in spike receptor binding domain of SARS-CoV-2 and the same was not predicted in the SARS-CoV-1 spike protein and other previously known coronaviruses. This motif was originally found in fibronectin, which is an extracellular matrix protein and this motif plays a major role in cell adhesion and attachment. A number of proteins are known to interact with these RGD motifs. One such group of proteins integrins that have strong affinity for RGD motif ($\sim K_D=4.0\text{nM}$) and they employ this motif to efficiently mediate cell adhesion. Eight out of approximately twenty known integrins recognize and exploit RGD motifs for cell adhesion (Ruoslahti, 1996; Teoh et al., 2016). Presence of motif RGD in SARS-CoV-2 spike receptor binding domain is a strikingly alarming as this motif is expected to facilitate even stronger attachment between virus and human target cells, and thus, rendering human cells more susceptible and vulnerable to infection. Motif scan also predicted fusion glycoprotein F0 (from 910-935 aa in S2 glycoprotein of SARS-CoV-1) and FMRFamide (from 437-447 aa in receptor binding domain of SARS-CoV-1). The absence of fusion glycoprotein F0 in SARS-CoV-2 may also have potential deleterious effect because this motif acts as a potent antigen which is targeted by neutralizing antibodies induced by virus infection as a part of humoral immune response and adaptive immunity (Prabakaran et al., 2006) and its absence in SARS-CoV-2 spike protein explains why monoclonal antibodies raised against SARS-CoV-1 could not show cross-reactivity with SARS-CoV-2 spike protein. This also accounts for the reason why an appropriate and adequate immune response could not mount in COVID 19 patients, especially in aged and immunocompromised COVID 19 patients, which eventually led to patients' death. The FMRFamide (Phe-Met-Arg-Phe-amide) like peptides (also known as FLPs) (predicted to be absent in SARS-CoV-2), are one particular group of Arg-Phe-amides (RFamides), are known to play a central role in parasite neuromuscular biology (Peymen et al., 2014); however, their role in pathogenesis of SARS-CoV-2 cannot be ascertained yet and same for other predicted motifs (Table 2).

3.4. ACE2 Expression is High in Digestive Route while Integrins Expression is High in Lung Cells

Presence of RGD motif in the spike receptor binding domain of the SARS-CoV-2 and the fact that integrins display a strong affinity for proteins harboring RGD motifs (Liu, 2009) suggested and indicated that virus possibly make entry into host cells *via* integrins receptor (Ruoslahti, 1988; Van Agthoven et al.,

2014). In order to assess this, we analyzed the gene expression data all eight integrins subunits at Human protein Atlas (<https://www.proteinatlas.org/>) (Uhlén et al., 2015; 2019) and found that expression of three integrins ($\alpha\text{v}\beta 6$, $\alpha 5\beta 1$, $\alpha 8\beta 1$) is significantly high in lung cells (Figure 2A-C). We also found that expression of an ECM protein ICAM1 is significantly higher (even higher than integrins) in lungs (Figure 2D) suggesting that SARS-CoV-2 spike protein might be using these integrins and/or ECM proteins such as ICAM1 (or others) for invading host cells. On the contrary, the ACE2 RNA is detected in very low quantity (almost unidentifiable) in lungs and detectable expression peaks were detected in other organs such as duodenum, small intestine, colon, gallbladder, kidney, testis, and heart muscles (Figure 3). In congruence with this, Xu and coauthors (2020) found high expression of ACE2 in oral mucosa and Zhang and co-authors (2020) demonstrated digestive route as a potential mechanism of ACE2 mediated virus infection based on the single-cell transcriptomic analysis. On the contrary, Chen and coauthors (2020) observed relatively low levels of ACE2 mRNA expression in lungs which further supports my claims.

3.5. SARS-CoV-2 Spike RBD Interacts with Integrins and ICAM1 Precisely at RGD Motif

The RGD motif is the minimal indispensable requirement for integrins to bind with any viral protein which virus can use for attachment with host cells (Hussein et al., 2015). Nagae and coauthors (2012) demonstrated that synthetic RGD tripeptide binds to the integrin $\alpha 5\beta 1$ (PDB ID: 3V14). In order to obtain molecular evidences for integrins mediated SARS-CoV-2 entry into lung cells, we docked the viral spike RBD domain (PDB ID: 6LZG) onto the structure of integrin $\alpha 5\beta 1$ (PDB ID: 3V13 without synthetic RGD tripeptide) (Nagae et al., 2012) and integrin $\alpha\text{v}\beta 6$ (PDB ID: 5FF) (Dong and Springer, 2011) and found that spike RBD binds with integrin precisely *via* the RGD motif as expected (Figure 4A). The predicted docking energy score was -302.33 (ΔG) and the ligand RMSD score was 71.80Å for spike RBD-integrin $\alpha\text{v}\beta 6$ docking; and for the spike RBD-integrin $\alpha 5\beta 1$, the scores were respectively -315.45 and 37.4Å. We found that the spike RGD interacts with loop turns AAG¹⁹⁹⁻²⁰¹, GSYFWQ²²⁵⁻²³⁰ and RQASSIYDDA²⁶⁰⁻²⁷⁰ of the $\alpha 5$ chain of the integrin $\alpha 5\beta 1$ (Nagae et al., 2012) (Figure 4B). The latter (RQASSIYDDA²⁶⁰⁻²⁷⁰) has also been predicted as one of the calcium binding sites as per the UniProt KD database (ITGA5; UniProt ID: P06756) and our IonCon analysis. Presence of Ca⁺² ion binding sites around and in vicinity of RGD-integrins docking site suggest that RGD-integrin interaction is indeed calcium-dependent (Figure 4B). ICAM1 is also known to bind integrins and this mechanism is exploited by some viruses (such as Rhinoviruses) to gain entry into respiratory system for pathogenesis (Bella et al., 1998). Molecular docking results obtained in current study also showed ICAM1 binds with spike receptor binding domain

of SARS-CoV-2 suggesting that virus might also be exploiting ICAM1 along with or in addition to integrins for its entry into respiratory system (Figure 5).

3.6. Molecular Docking Suggests EDTA Chelation Therapy as a Novel Treatment for COVID-19

The RGD motifs are well known in the field of cell biology, cell therapy and tissue engineering because of their remarkable cell-adhesive property. Extracellular matrix proteins, such as fibronectin and laminin, possess RGD motif and are frequently coated onto the surface of biomaterials (petri-dishes, T-flasks) for facilitating human cells adhesion onto the surface of the biomaterials. Bivalent ions such as Ca^{+2} & Mg^{+2} play indispensable role in promoting such cell adhesion and EDTA is used as a chelating agent for disaggregating Ca^{+2} mediated cell bondage.

Two features in SARS-CoV-2 spike protein suggest virus attachment with host cells via integrins in a calcium-dependent manner: 1) presence of RGD motif in receptor binding domain of SARS-CoV-2 and 2) presence of a number of phosphorylation sites for key pathways that regulates calcium ion channels, and secondly, Ca^{+2} signaling. Similarly, integrins possess calcium and magnesium binding sites (as predicted by IonCon) for interaction with RGD motif containing proteins, and the same in current context are spike protein of SARS-CoV-2. Besides this, integrins are also known to regulate cell adhesion by altering intracellular calcium dynamics and have involvement in regulation of ion channels and calcium signaling to exert pathophysiological impact in other diseases (Sjaastad and Nelson, 1997; Arcangeli and Becchitti, 2010). Altogether, it appears that SARS-CoV-2 make entry into human cells *via* interaction of their spike receptor RGD motif with integrins in a Ca^{+2} dependent manner.

Altogether, the current study highlighted the possible role of calcium and other divalent ions in RGD-integrins binding for virus invasion and suggested that lowering divalent ion in lungs could avert virus-host cells binding. This can be accomplished by EDTA or EGTA, both are well known chelating agents. In support of this, we found that EDTA binds to SARS-CoV-2 spike receptor precisely where the RGD motif binds to integrin receptor protein (Figure 6). Besides RGD motif, EDTA also binds to RBD domain of spike protein which interacts with other human receptor protein (such as ACE2) (Figure 7) suggesting that EDTA can play a vital role in blocking the receptor binding sites of viral spike protein in order to discourage virus-host attachment. We strongly believe that phenotypic remodeling and regulation of Ca^{+2} ions (Berridge, 2012) by pulmonary EDTA chelation can discourage the SARS-CoV-2 virus attachment with human lung cells which will lead to attainment of a state of protection against this virus.

Finally, EDTA chelation therapy targeting lungs (as in discussion part) holds immense potential in successful management and treatment of COVID 19 patients.

3.7. RGD-Integrins binding cause activation of downstream integrin-mediated cell signaling (GO:0007229)

Further, it seems that RGD-integrins binding would relay or activate downstream integrins mediated cell signaling pathway (GO:0007229) which comprise of 106 human genes related to different biological processes and cellular pathways (<http://amigo.geneontology.org/>; Data not shown). The experimental evidences collected from UniProt KB databases show that integrins play key role in viral attachment and TGF- β and IL-1 mediated signaling. Other singaling pathways that are known to be regulated by integrins upon interaction with different viruses are FGF1 signaling, FGF2 signaling, NRG1-ERBB signaling, CX3CL1 signaling and CD40-CD40LG signaling (Table 3).

4. Conclusion

In any disease or pathological condition where integrin expression would be high, we can expect patient to be highly vulnerable to COVID-19. Integrin expression has been found high in case of cancer (Bianconi et al., 2016) and diabetes (Roth et al., 1993; Miller et al., 2014) and we assume the susceptibility for 2019-nCoV infection and COVID-19 would be high in patients suffering from any diseases in which integrin expression is high. The immune-diagnosis and profiling of COVID-19 patients showed elevated levels of pro-inflammatory cytokines including IL-6 and IL-1 β in serum (Huang et al., 2020; Shi et al., 2020; Qin et al., 2020; Xu et al., 2020). They also exhibited an increased serum levels of other cytokine and chemokine molecules such as IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1 α (also known as CCL3) and TNF (Huang et al., 2020; Shi et al., 2020; Qin et al., 2020; Xu et al., 2020). Also, C-reactive protein and D-dimer are found to be abnormally high (Cao et al., 2020). High levels of pro-inflammatory cytokines may lead to tissue shedding and damage in lungs, heart, liver and kidney, i.e., multiple organ failure (Cao et al., 2020). The most evident pathophysiological outcome of SARS-CoV-2 infection is activation of immune cells and massive infiltration of neutrophils and macrophages that eventually led to “cytokine storm” resulting in severe lung inflammations (such as pulmonary edema and alveolar damages) and can be characterized by pneumonia-like symptoms and respiratory failure (Mehta et al., 2020). Herein, we underline another strong and convincing evidence in favor of virus-host attachment through RGD-integrins, i.e., the cytokine storm, caused due to activation of

1 pro-inflammatory cytokines such as TNF- α and IL-6 activation; and integrins role in their activation is
2 also well established (Blobe et al., 2000; Annes et al., 2002; Lu et al., 2002; Sheppard, 2008; Hung et al.,
3 2008). Integrin $\alpha 1\beta 1$ regulates Ca^{+2} ion transient responses to IL-1 and TGF- β (Parekh et al., 2014).
4 Integrins also regulate calcium mobilization in associated pathways such as Go-mediated cell signaling
5 (Erb et al., 2001). Besides this, envelop proteins of virus, including previous coronaviruses, are Ca^{+2}
6 channeling viroporins that exploit host cell signaling and involved in virus entry, replication, immune
7 evasion leading to activation of autophagy and inflammasome responses such as that of NLRP3 (Nieto-
8 Torres et al., 2015; Hyser and Estes, 2015). In context of SARS-CoV2 and integrins attachment, what
9 exact role does the dynamics (in terms of mobilization, concentration, frequency, timing) of intracellular
10 and extracellular calcium play is still not clear; however, understanding this would greatly assist us in
11 appropriately and adequately controlling the host cells' response to SARS-CoV-2 so as to counteract
12 virus infection and invasion. However, it is apparently clear that high Ca^{+2} ion concentration in lungs is
13 detrimental in case of SARS-CoV-2 infection.

14 A number of studies demonstrated the key role of human ACE2 in virus attachment to host cells
15 (Wan et al., 2020; Zhao et al., 2020). Previous coronaviruses have also been found to make entry into host
16 cells using clathrin and caveolae-independent pathway (Inoue et al., 2007; Wang et al., 2008). Integrin
17 $\alpha v\beta 6$ has also been implicated in mediating clathrin-dependent endocytosis (Table 3). Based on the
18 biophysical data, it has been demonstrated that the human ACE2 binds to SARS-CoV-2 with greater
19 affinity than SARS-CoV-1 (Wrapp et al., 2020). However, owing to their low copy number (gene
20 expression), their high affinity (for SARS-CoV-2 than SARS-CoV-1) alone could not manifest reasonable
21 virus-host cell attachment, at least in lung cells. On the other hand, the affinity of integrins for RGD motif
22 of the spike RBD is considerably high. The binding affinity of ACE2 for whole spike RBD is $K_D=15.2\text{nM}$
23 (K_D = dissociation constant) (Wrapp et al., 2020) and on the other hand the binding affinity of integrins
24 for RGD is close to $K_D=4.0\text{nM}$ (Bernhagen et al., 2017; 2019; Ma et al., 2017). Since, the binding affinity
25 has inverse relationship with K_D , we can rule out that virus spike protein would prefer binding to integrins
26 rather than ACE2, at least in lung cells. However, ACE2 expression has been found to be considerably
27 high in the digestive tract from duodenum to rectum as well as in gall bladder and kidney. Therefore,
28 ACE2 could be responsible for virus infection in these organs. This study presents striking evidence
29 favoring the role of calcium and other divalent ions (magnesium, manganese etc.) in RGD–integrins
30 mediated virus attachment with the host cells in lung cells for infection and invasion.

31 Chelating agents are long known for treating metal toxicity in humans in context to several
32 disease conditions such as coronary heart disease, neurotoxicity and arthritis etc. (Goyer et al., 1995;
33 Bamonti et al., 2011; Ferrero, 2016; Fulgenzi and Ferrero, 2019). The EDTA is a well-known chelating

1 agent and is a FDA approved drug for treatment of hypercalcemia conditions. In EDTA chelation therapy
2 disodium EDTA (or similar sodium-EDTA derivative) is used as a chelating agent and is administered
3 intravenously for the chelating divalent ions and the resulting chelates then come out in urine after
4 passing through glomerulus filtration (Bamonti et al., 2011; Ferrero, 2016; Fulgenzi and Ferrero, 2019).

5 We propose EDTA chelation therapy as a potent anti-viral therapy against SARS-CoV-2 because
6 EDTA not only acts as a chelating agent but also as an anti-oxidant, anti-inflammatory, immune-
7 suppressive and anti-coagulant. Besides EDTA, EGTA, sodium heparin, and sodium citrate dextrose etc
8 can also be used to prevent coagulation and modulate the concentration of cytokines (Biancotto et al.,
9 2012; Patil et al., 2013). Administration of EDTA into blood using intravenous injections (IV) is one way,
10 and alternatively, pulmonary EDTA chelation therapy can also be used for targeted delivery in lungs.
11 However, whether or not the EDTA would reach to lungs bronchioles and alveoli is not certain in case of
12 pulmonary administration. However, EDTA can reach to lungs alveoli from blood vessels surrounding
13 them in case of IV injection. We also expect that EDTA can effectively ameliorate the proinflammatory
14 cytokines-dependent cell damages in COVID-19 patients if administered via IV injection. There are
15 additional benefits such as the small size of EDTA would also allow it to cross the blood-brain barrier and
16 to protect neuronal cells from getting damaged due to cytokines action (Ferrero, 2016). Similarly
17 cardiovascular complications of diabetes can also be effectively managed and prevented using EDTA
18 based chelation process (Moreno et al., 2019).

19 Pulmonary EDTA chelation therapy has already been executed clinically for asthmatic patients
20 and is also known to increase the efficacy of nebulized bronchodilators (Asmus et al., 2001). Sodium salts
21 of EDTA can be used for chelating Ca^{+2} ions as calcium-EDTA has higher stability than sodium-
22 EDTA (Blaurock-Busch and Busch, 2014) and the resulting Ca-EDTA chelate would be soluble that can
23 easily excrete out of body through glomerular filtration (Figure 8A). We have designed EDTA chelation
24 therapy as a novel therapeutic strategy for safe, fast, cost-effective and efficient treatment of COVID 19
25 based on genomic understanding of SARS-CoV-2 virus spike protein. In EDTA chelation therapy,
26 disodium EDTA will be administered in the body (preferably through nebulizer/inhaler/nasal spray or
27 intravenously) so as to reach to its target organ, i.e., lungs as shown in Figure 8B. In normal disease cells,
28 virus could make entry into host cells *via* binding of RGD motif (in their spike receptor binding domain)
29 to the integrins receptor proteins present of the surface of human lung cells. Ca^{+2} ions (or other bivalent
30 ions) would augment the binding of viral spike protein with host cells and subsequently facilitate viral
31 invasion into cells for infection, replication and pathogenesis. On the contrary, using EDTA chelation
32 therapy, Ca^{+2} or other bivalent ions can be chelated and can be made unavailable to viral spike protein for
33 attaching to host cells' integrins. In parallel, strict control over diet (or medication) for ensuring proper

1 availability of Ca^{+2} in the body may also be required for successful phenotypic remodeling for EDTA
2 chelation therapy. In this way, virus could not enter cells and a state of protection against SARS-CoV-2
3 and COVID 19 can be achieved (Figure 8B).

4 Besides ceasing the RGD-integrin mediated virus attachment to host cells, treatment with EDTA
5 or EGTA is expected to cease SARS-CoV-2 viral infection in a multifaceted way. Firstly, we expect that
6 chelating agents would disintegrate the viral membrane as Ca^{+2} forms major component of retrovirus
7 membrane and are known to stabilize envelop proteins and others (Wunderlich and Sydow, 1982).
8 Second, we also expect chelation of the metal cofactors within the enzyme active site of the SARS-CoV-
9 2, as seen in HIV-1 IN enzyme (Hicks and Gulick, 2009). Third, EDTA is expected to lower metal-ion
10 dependent reactive oxygen species (ROS) production and would also lipid peroxidation (Rousell et al.,
11 2009). EDTA has also been shown to induce protective antioxidant and anti-inflammatory response in
12 case of liver fibrosis (González-Cuevas et al., 2011). Finally, as COVID 19 patients exhibit excessive
13 cytokine secretion (called as cytokine storm) (Mehta et al., 2020), use of EDTA is expected to render an
14 anti-inflammatory and immuno-suppressive effect which would be highly beneficial. As such there are
15 plenty of studies that confirm EDTA safe use in chelation therapy with no potent adversity and fatality
16 (Rousell et al., 2009). The EDTA concentrations greater than 0.6mM may led to reduced energy
17 metabolism in lung cells (Asmus et al., 2001). Besides this, some complications such as hypocalcemia,
18 proteinuria and renal insufficiency are known to associate with EDTA chelation therapy and we suggest
19 that a strict monitoring protocol, comprising routine cytokine profiling, blood examination, urine test and
20 CT scanning etc., must be practiced while treating patients with pulmonary EDTA chelation therapy.

21 In combination with the EDTA/EGTA based pulmonary chelation therapy, modulation of
22 integrins expression using integrins inhibitors or anti-integrin antibodies could also serve as a mechanism
23 to treat COVID 19 patients (Henderson et al., 2013; Hatley et al., 2018; Sigrist et al., 2020). The
24 expression of such integrins can be down regulated by modulation of intracellular redox ions such as
25 superoxide anion ($\text{O}_2^{\cdot-}$) and H_2O_2 (Puchsaka et al., 2016). Besides this, some bioactive
26 compounds/molecules such as Ouabain are also known to affect integrins expression in lung cells
27 (Ninsontia et al., 2014). Application of synthetic RGD tripeptide or its derivatives (linear, cyclic etc.) can
28 also specifically block integrins and could also serve as a possible mechanism to discourage virus-host
29 attachment (Bernhagen et al., 2017; 2019; Ma et al., 2017). Besides this, PGNase treatment is also
30 expected to be equally effective as the mass spectrometric studies revealed that all major glycan classes of
31 human lung glycome can be released effectively by PNGase F (Jia et al., 2020). Since, spike protein of
32 SARS-CoV-2 has a number of sites for cAMP, CK2, PKC and Tyr phosphorylation, we believe
33 synergistic clinical application of other potential drugs such kinase inhibitors can also be beneficial in

COVID 19 treatment. Therapies specifically targeting TNF- α and IL-6 mediated signaling to downregulate cytokines secretion seems to be a promising approach to deal with the situation of cytokine storm. In this regard, tocilizumab (anti-IL6 monoclonal antibody) alone or in combination with infliximab and Emodin are also worth testing for COVID-19 treatment (Cao et al., 2020). Besides this, we also believe that blocking the calcium ion channels (or others) and downregulation of integrin-mediated cell signaling (GO:0007229) pathways such as FGF1 signaling, FGF2 signaling, NRG1-ERBB signaling, CX3CL1 signaling and CD40-CD40LG signaling are also available as a potent immunotherapeutic solutions against COVID-19. Finally, in the era of multi-omics technologies, medicines/therapies based on comparative coronavirus genome analysis and understanding of individual pathophysiology could pave way towards successful development of effective therapeutic solution for combating COVID 19.

Acknowledgements

TCD acknowledges Mohanlal Sukhadia University, Udaipur for the infrastructure facility.

Authors Contribution

TCD conceived the idea and designed the experimental strategy and designed methodology, TCD performed the analyses, wrote the manuscript.

Funding

The work was conducted in absence of any funding source.

References

1. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J. et al., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 395, 497-506.
2. Qiu, H., Wu, J., Hong, L., Luo, Y., Song, Q., Chen, D., 2020. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. *Lancet*. DOI:[https://doi.org/10.1016/S1473-3099\(20\)30198-5](https://doi.org/10.1016/S1473-3099(20)30198-5)

3. Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., Peng, Z., 2020. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 323,1061-1069. doi:10.1001/jama.2020.1585
4. Tian, X., Li, C., Huang, A., Xia, S., Lu, S., Shi, Z., Lu, L., Jiang, S., Yang, Z., Wu, Y., Ying, T., 2020. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. *Emerg. Microbes Infect.* 9, 382–385. doi:10.1080/22221751.2020.1729069
5. Yuan, M., Wu, N. C., Zhu, X., Lee, C-C. D., So, R. T. Y., Lv, H., Mok, C. K. P., Wilson, I. A., 2020. A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV. *Science*. 2020, abb7269. DOI: 10.1126/science.abb7269
6. Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., Graham, B. S., McLellan, J. S., 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*. 2020, abb2507. 10.1126/science.abb2507
7. Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M. et. al., 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res*. 30, 269–271.
8. Colson, P., Rolain, J. M., Lagier, J. C., Brouqui, P., Raoult, D., 2020. Chloroquine and hydroxychloroquine as available weapons to fight COVID-2019. *Int. J. Antimicrob. Agents*. 55, 105932.
9. Gordon, C. J., Tchesnokov, E. P., Woolner, E., K Perry, J. K., Feng, J. Y., Porter, D. P., Gotte, M., 2020. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem.* doi: 10.1074/jbc.RA120.013679
10. Li, H., Zhou, Y., Zhang, M., Wang, H., Zhao, Q., Liu, J., 2020. Updated approaches against SARS-CoV-2. *Antimicrob. Agents Chemother.* doi:10.1128/AAC.00483-20
11. Shah, B., Modi P., Sagar S. R., 2020. In silico studies on therapeutic agents for COVID-19: Drug repurposing approach. *Life Science*, 117652. <https://doi.org/10.1016/j.lfs.2020.117652>.
12. Lu, H., 2020. Drug treatment options for the 2019-new coronavirus (2019-nCoV), *Biosci. Trends* 14, 69-71. doi: 10.5582/bst.2020.01020.
13. Sawalha, A. H.; Zhao, M., Coit, P; Lu, Q, 2020. Epigenetic dysregulation of ACE2 and interferon-regulated genes might suggest increased COVID-19 susceptibility and severity in lupus patients. *Clin Immunol*. 108010. <https://doi.org/10.1016/j.clim.2020.108410>

14. Kandeel, M., Al-Nazawi, M., 2020. Virtual screening and repurposing of FDA approved drugs against COVID-19 main protease. *Life Sci.* 117627. <https://doi.org/10.1016/j.lfs.2020.117627>
15. Cava, C.; Bertoli, ., Castiglioni, I., 2020. In-silico discovery of candidate drugs against Covid-19. *Viruses.* 12, 404. <https://doi.org/10.3390/v12040404>
16. Wang, Y-X., Ma, J-R., Wang, S-Q., Zeng, Y-Q., Zhou, C-Y., Ru, Y-H., Zhang, L., Lu, Z-G., Wu, M-H., Li, H., 2020. Utilizing integrating network pharmacological approaches to investigate the potential mechanism of Ma Xing Shi Gan Decoction in treating COVID-19. *Eur Rev Med Pharmacol Sci.* 24 (6): 3360-3384. DOI: 10.26355/eurev_202003_20704
17. Wan, Y., Shang, J., Graham, R., Baric, R. S., Li, F., 2020. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. *J Virol.* 94, e00127-20. DOI: 10.1128/JVI.00127-20
18. Zhao, Y., Zhao, Z., Wang, Y., Zhou, Y., Ma, Y., Zuo, W., 2020. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. *BioRxiv* doi:<https://doi.org/10.1101/2020.01.26.919985>.
19. Chen, Y., Guo, Y., Pan, Y., Zhuang, J. Z., 2020. Structure analysis of the receptor binding of 2019-nCoV. *Biochem. Biophys. Res. Commun.* 525, 135-140.
20. Walls, A. C., Park, Y. J., Tortorici, M. A., Wall, A., McGuire, A. T., Veersler, D., 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell.* doi:10.1016/j.cell.2020.02.058.
21. Sigrist, C. J. A., Bridge, A., Mercier, P. L., 2020. A potential role for integrins in host cell entry by SARS-CoV-2. *Antiviral Res.* 177, 104759. doi: 10.1016/j.antiviral.2020.104759
22. Zhou, Y., Hou, Y., Shen, J., Huang, Y., Martin, W., Cheng, F., 2020. Network-based drug repurposing for novel coronavirus 2019-nCoV/SARS-Cov-2. *Cell Discov.* 6, 14.
23. Madeira, F., Park, Y. M., Lee, J. Buso N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A. R. N., Potter, S. C., Finn, R. D., Lopez, R., 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* 47, W636-W641. DOI: 10.1093/nar/gkz268.
24. Pagni, M., Ioannidis, V., Cerutti, L., Zahn-Zabal, M., Jongeneel, C. V., Hau, J., Martin, O., Kuznetsov, D., Falquet, L., 2007. MyHits: improvements to an interactive resource for analyzing protein sequences. *Nucleic Acids Res.* 35, W433–W437. doi: 10.1093/nar/gkm352
25. Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigartyo, C. A., Odeberg, J., Djureinovic, D., Takanen, J. O., Hober, S., Alm, T., Edqvist, P. H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J. M., Hamsten, M., von Feilitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von

- 1 Heijne, G., Nielsen, J., Pontén, F., 2015. Tissue-based map of the human proteome. *Science*. 347,
2 1260419. DOI: 10.1126/science.1260419
- 3 26. Uhlen, M., Karlsson, M. J., Zhong, W., Tebani, A., Pou, C., Mikes, J., Lakshmikanth, T.,
4 Forsström, B., Edfors, F., Odeberg, J., Mardinoglu, A., Zhang, C., von Feilitzen, K., Mulder, J.,
5 Sjöstedt, E., Hober, A., Oksvold, P., Zwahlen, M., Ponten, F., Lindskog, C., Sivertsson, Å.,
6 Fagerberg, L., Brodin, P., 2019. A genome-wide transcriptomic analysis of protein-coding genes
7 in human blood cells. *Science*. 366, eaax9198. DOI: 10.1126/science.aax9198
- 8 27. Hu, X., Dong, Q., Yang, J., Zhang, Y., 2016. Recognizing metal and acid radical ion binding sites
9 by integrating ab initio modeling with template-based transferals. *Bioinformatics*. 32, 3260-3269.
- 10 28. Grosdidier, A., Zoete, V., Michielin, O., 2007. EADock: Docking of small molecules into protein
11 active sites with a multiobjective evolutionary optimization. *Proteins*. 67, 1010-1025.
- 12 29. Chang, K. W., Sheng, Y., Gombold, J. L., 2000. Coronavirus-induced membrane fusion requires
13 the cysteine-rich domain in the spike protein. *Virology*. 269, 212-224.
- 14 30. Johnston, E. R., Radke, K., 2000. The SU and TM envelope protein subunits of bovine leukemia
15 virus are linked by disulfide bonds, both in cells and in virions. *J. Virol.* 74, 2930–2935.
- 16 31. Lee J. E., Saphire E. O., 2009. Ebolavirus glycoprotein structure and mechanism of entry. *Future*
17 *Virol.* 4, 621–635.
- 18 32. Bretaña, N. A., Lu, C. T., Chiang, C. Y., Su, M. G., Huang, K. Y., Lee, T. Y., Weng, S. L., 2012.
19 Identifying protein phosphorylation sites with kinase substrate specificity on human viruses.
20 *PLOS ONE*. 7, e40694. <https://doi.org/10.1371/journal.pone.0040694>
- 21 33. Keating, J. A., Striker, R., 2012. Phosphorylation events during viral infections provide potential
22 therapeutic targets. *Rev. Med. Virol.* 22, 166–181. doi: 10.1002/rmv.722
- 23 34. Keck, F., Ataey, P., Amaya, M., Bailey, C., Narayanan, A., 2015. Phosphorylation of single
24 stranded RNA virus proteins and potential for novel therapeutic strategies. *Viruses*. 7, 5257–
25 5273. doi: 10.3390/v7102872
- 26 35. Ruoslahti, E., 1996. RGD and other recognition sequences for integrins. *Annu. Rev. Cell Dev.*
27 *Biol.* 12, 697-715.
- 28 36. Teoh, C. M., Tan, S. S. L., Tran, T., 2016. Integrins as Therapeutic Targets for Respiratory
29 Diseases. *Curr. Mol. Med.* 15, 714–734.
- 30 37. Prabakaran, P., Gan, J., Feng, Y., Zhu, Z., Choudhary, V., Xiao, X., Ji, X., Dimitrov, D. S., 2006.
31 Structure of severe acute respiratory syndrome coronavirus receptor-binding domain complexed
32 with neutralizing antibody. *J. Biol. Chem.* 281, 15829-15836.

38. Peymen, K., Watteyne, J., Frooninckx, L., Schoofs, L., Beets, I., 2014. The FMRFamide-like peptide family in nematodes. *Front. Endocrinol.* 5, 1-21. <https://doi.org/10.3389/fendo.2014.00090>
39. Liu, S., 2009. Radiolabeled cyclic RGD peptides as integrin $\alpha v \beta 3$ -targeted radiotracers: maximizing binding affinity via bivalency. *Bioconjug. Chem.* 20, 2199–2213.
40. Ruoslahti, E., 1988. Fibronectin and its receptors. *Ann. Rev. Biochem.* 57, 375–413.
41. Van, Agthoven, J. F., Xiong, J. P., Alonso, J. L., Rui, X., Adair, B. D., Goodman, S. L., Arnaout, M. A., 2014. Structural basis for pure antagonism of integrin $\alpha V \beta 3$ by a high-affinity form of fibronectin. *Nat. Struct. Mol. Biol.* 21, 383–388.
42. Xu, H., Zhong, L., Deng, J., Peng, J., Hongxia, D., Zeng, X., Li, T., Chen, Q. 2020., High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int. J. Oral Sci.* 12. 10.1038/s41368-020-0074-x.
43. Zhang, H., Kang, Z., Gong, H., Xu, D., Wang, J., Li, Z., Cui, X., Xiao, J., Meng, T., Zhou, W., Liu, J., Xu, H., 2020. The digestive system is a potential route of 2019-nCov infection: a bioinformatics analysis based on single-cell transcriptomes. *bioRxiv.* 2020, 927806; doi: <https://doi.org/10.1101/2020.01.30.927806>
44. Hussein, H. A. M., Walker, L. R., Abdel-Raouf, U. M., Desouky, S. A., Montasser, A. K. M., Akula, S. M., 2015. Beyond RGD: virus interactions with integrins. *Arch. Virol.* 160, 2669–2681. <https://doi.org/10.1007/s00705-015-2579-8>
45. Nagae, M., Re, S., Mihara, E., Nogi, T., Sugita, Y., Takagi, J., 2012. Crystal structure of $\alpha 5 \beta 1$ integrin ectodomain: Atomic details of the fibronectin receptor. *J. Cell Biol.* 197, 131–140. <https://doi.org/10.1083/jcb.201111077>
46. Bella, J., Kolatkar, P. R., Marlor, C. W., Greve, J. M., Rossmann, M. G., 1998. The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. *Proc. Natl. Acad. Sci. U S A.* 95, 4140–4145.
47. Sjaastad, M. D., Nelson, W. J., 1997. Integrin-mediated calcium signaling and regulation of cell adhesion by intracellular calcium. *Bioessays.* 19, 47-55.
48. Arcangeli, A., Becchetti, A., 2010. Integrin structure and functional relation with ion channels. *Adv. Exp. Med. Biol.* 674, 1-7.
49. Berridge, M. J., 2012. Calcium signalling remodelling and disease. *Biochem. Soc. Trans.* 40, 297-309. doi: 10.1042/BST20110766.
50. Bianconi, D., Unseld, M., & Prager, G. W. 2016. Integrins in the Spotlight of Cancer. *International journal of molecular sciences*, 17(12), 2037. <https://doi.org/10.3390/ijms17122037>

51. Roth, T., Podestá, F., Stepp, M. A., Boeri, D., & Lorenzi, M. (1993). Integrin overexpression induced by high glucose and by human diabetes: potential pathway to cell dysfunction in diabetic microangiopathy. *Proceedings of the National Academy of Sciences of the United States of America*, 90(20), 9640–9644. <https://doi.org/10.1073/pnas.90.20.9640>
52. Miller C G, Pozzi A, Zent R, Schwarzbauer J E. 2014. Effects of high glucose on integrin activity and fibronectin matrix assembly by mesangial cells. *Molecular Biology of the Cell*. 25: 2315-2317.
53. Huang, C. et al., 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 395, 497–506.
54. Qin, C. et al., 2020. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin. Infect. Dis.* <https://doi.org/10.1093/cid/ciaa248>.
55. Shi, Y. et al., 2020. Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. Preprint at medRxiv. <https://doi.org/10.1101/2020.03.12.20034736>
56. Xu, Z. et al., 2020. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* 8, 420–422.
57. Cao, X., 2020. COVID-19: immunopathology and its implications for therapy. *Nat Rev Immunol.* <https://doi.org/10.1038/s41577-020-0308-3>
58. Mehta, P., McAuley, D. F., Brown, M., Sanchez, E., Tattersall, R. S., Manson, J. J., 2020. COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet*. 395, P1033-1034. DOI:[https://doi.org/10.1016/S0140-6736\(20\)30628-0](https://doi.org/10.1016/S0140-6736(20)30628-0)
59. Blobel, G. C., Schiemann, W. P., Lodish, H. F. 2000. Role of transforming growth factor- β in human disease. *N. Engl. J. Med.* 342, 1350–1358.
60. Annes, J. P., Rifkin, D. B., Munger, J. S., 2002. The integrin α V β 6 binds and activates latent TGF β 3. *FEBS Lett.* 511, 65–68. pmid:11821050.
61. Lu, M., Munger, J. S., Steadale, M., Busald, C., Tellier, M., Schnapp, L. M., 2002. Integrin α 8 β 1 mediates adhesion to LAP-TGF β 1. *J. Cell Sci.* 115, 4641–4648.
62. Sheppard, D., 2008. The role of integrins in pulmonary fibrosis. *Eur. Respir. Rev.* 17, 157-162. DOI: 10.1183/09059180.00010909
63. Hung, C. F., Wilson, C. L., Chow, Y. H., Schnapp, L. M., 2018. Role of integrin α 8 in murine model of lung fibrosis. *PLOS ONE*. 13, e0197937. <https://doi.org/10.1371/journal.pone.0197937>
64. Parekh, R., Lorenzo, M. K., Shin, S. Y., Pozzi, A., Clark, A. L., 2014. Integrin α 1 β 1 differentially regulates cytokine-mediated responses in chondrocytes. *Osteoarthr. Cartil.* 22, 499-508.
65. Erb, L., Liu, J., Ockerhausen, J., Kong, Q., Garrad, R. C., Griffin, K., Neal, C., Krugh, B., Santiago-Pérez, L. I., González, F. A., Gresham, H. D., Turner, J. T., Weisman, G. A., 2001. An

- RGD sequence in the P2y2 receptor interacts with α V β 3 integrins and is required for Go-mediated signal transduction. *J. Cell Biol.* 53, 491–502. <https://doi.org/10.1083/jcb.153.3.491>
66. Nieto-Torres, J. L., Verdiá-Báguena, C., Jimenez-Guardeño, J. M., Regla-Nava, J. A., Castaño-Rodriguez, C., Fernandez-Delgado, R., Torres, J., Aguilera, V. M., Enjuanes, L., 2015. Severe acute respiratory syndrome coronavirus E protein transports calcium ions and activates the NLRP3 inflammasome. *Virology*, 485, 330–339. <https://doi.org/10.1016/j.virol.2015.08.010>
67. Hyser, J. M., Estes, M. K., 2015. Pathophysiological consequences of calcium-conducting viroporins. *Annu. Rev. Virol.* 2, 473–496. doi: 10.1146/annurev-virology-100114-054846.
68. Inoue, Y., Tanaka, N., Tanaka, Y., Inoue, S., Morita, K., Zhuang, M., Hattori, T., Sugamura, K., 2007. Clathrin-dependent entry of severe acute respiratory syndrome coronavirus into target cells expressing ACE2 with the cytoplasmic tail deleted. *J. Virol.* 81, 8722–8729.
69. Wang, H., Yang, P., Liu, K., Guo, F., Zhang, Y., Zhang, G., Jiang, C., 2008. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res.* 18, 290–301.
70. Bernhagen, D., De Laporte, L., Timmerman, P., 2017. High-affinity RGD-knottin peptide as a new tool for rapid evaluation of the binding strength of unlabeled RGD-peptides to α V β 3, α V β 5, and α 5 β 1 integrin receptors. *Anal. Chem.* 89, 5991–5997.
71. Bernhagen, D., Jungbluth, V., Quilis, N. G., Dostalek, J., White, P. B., Jalink, K., Timmerman, P., 2019. Bicyclic RGD peptides with exquisite selectivity for the integrin α V β 3 receptor using a "Random Design" approach. *ACS Comb. Sci.* 21, 198–206. doi: 10.1021/acscmbosci.8b00144.
72. Ma, Y., Ai, G., Zhang, C., Zhao, M., Dong, X., Han, Z., Wang, Z., Zhang, M., Liu, Y., Gao, W., Li, S., Gu, Y., 2017. Novel linear peptides with high affinity to α V β 3 integrin for precise tumor identification. *Theranostics.* 7, 1511–1523. doi: 10.7150/thno.18401
73. Goyer, R. A., Cherian, M. G., Jones, M. M., Reigart, J. R., 1995. Role of chelating agents for prevention, intervention and treatment of exposures to toxic metals. *Environm. Health Perspect.* 103, 1048–1052.
74. Bamonti, F., Fulgenzi, A., Novembrino, C., Ferrero, M. E., 2011. Metal chelation therapy in rheumatoid arthritis: a case report. Successful management of rheumatoid arthritis by metal chelation therapy. *Biometals.* 24, 1093–1098. doi: 10.1007/s10534-011-9467-9.
75. Ferrero, M. E., 2016. Rationale for the successful management of EDTA chelation therapy in human burden by toxic metals. *BioMed Res. Int.* 2016, 8274504.
76. Fulgenzi, A., Ferrero, M. E., 2019. EDTA chelation therapy for the treatment of neurotoxicity. *Int. J. Mol. Sci.* 20, 1019. <https://doi.org/10.3390/ijms20051019>

77. Biancotto A, Feng X, Langweiler M, Young NS, McCoy JP. Effect of anticoagulants on multiplexed measurement of cytokine/chemokines in healthy subjects. *Cytokine*. 2012 Nov;60(2):438-46. doi: 10.1016/j.cyto.2012.05.019. Epub 2012 Jun 15. PMID: 22705152; PMCID: PMC3449030.
78. Patil R, Shukre S, Paranjape R, Thakar M. 2013. Heparin and EDTA anticoagulants differentially affect the plasma cytokine levels in humans, *Scandinavian Journal of Clinical and Laboratory Investigation*, 73:5, 452-455, DOI: 10.3109/00365513.2013.798869
79. Moreno RC, Navas-Acien A, Escolar E, Nathan DM, Newman J, Schmedtje JF, Diaz D, Lamas GA, Fonseca V. 2019. Potential Role of Metal Chelation to Prevent the Cardiovascular Complications of Diabetes, *The Journal of Clinical Endocrinology & Metabolism*, Volume 104, Issue 7, July 2019, Pages 2931–2941, <https://doi.org/10.1210/jc.2018-01484>
80. Asmus, M. J., Barros, M. D., Liang, J., Chesrown, S. E., Hendeles, L., 2001. Pulmonary function response to EDTA, an additive in nebulized bronchodilators. *J. Allergy Clin. Immunol.* 107, 68-72.
81. Blaurock-Busch, E., Busch, Y. M., 2014. Comparison of chelating agents DMPS, DMSA and EDTA for the diagnosis and treatment of chronic metal exposure. *Br. J. Med. Med. Res.* 4, 1821-1835.
82. Wunderlich, V., Sydow, G., 1982. Disintegration of retroviruses by chelating agents. *Arch. Virol.* 73, 171–183.
83. Hicks, C., and Gulick, R. M., 2009. Raltegravir: the first HIV type 1 integrase inhibitor. *Clin. Infect. Dis.* 48, 931–939. doi: 10.1086/597290
84. Roussel, A. M., Hininger-Favier, I., Waters, R. S., Osman, M., Fernholz, K., Anderson, R. A., 2009. EDTA Chelation therapy, without added vitamin C, decreases oxidative DNA damage and lipid peroxidation. *Altern. Med. Rev.* 14, 56–62.
85. González-Cuevas, J., Navarro-Partida, J., Marquez-Aguirre, A. L., Bueno-Topete, M. R., Beas-Zarate, C., Armendáriz-Borunda, J., 2011. Ethylenediaminetetraacetic acid induces antioxidant and anti-inflammatory activities in experimental liver fibrosis, *Redox Rep.* 16, 62-70. DOI: 10.1179/174329211X13002357050851
86. Henderson, N. C., Arnold, T. D., Katamura, Y., Giacomini, M. M., Rodriguez, J. D., McCarty, J. H., et al., 2013. Targeting of α v integrin identifies a core molecular pathway that regulates fibrosis in several organs. *Nat. Med.* 19, 1617–1624. doi: 10.1038/nm.3282.
87. Hatley, R. J. D., Macdonald, S. J. F., Slack, R. J., Le, J., Ludbrook, S. B., Lukey, P. T., 2018. An α v-RGD integrin inhibitor toolbox: drug discovery insight, challenges and opportunities. *Angew. Chem., Int. Ed. Engl.* 57, 3298–3321. <https://doi.org/10.1002/anie.201707948>.

- 1 88. Puchsaka, P., Chaotham, C., Chanvorachote, P., 2016. α -Lipoic acid sensitizes lung cancer cells
2 to chemotherapeutic agents and anoikis via integrin $\beta 1/\beta 3$ downregulation. *Int. J. Oncol.* 49,
3 1445-1456. DOI:10.3892/ijo.2016.3624.
- 4 89. Ninsontia, C., Chanvorachote, P., 2014. Ouabain mediates integrin switch in human lung cancer
5 cells. *Anticancer Res.* 34, 5495-5502.
- 6 90. Jia, N., Byrd-Leotis, L., Matsumoto, Y. et al., 2020. The Human Lung Glycome Reveals Novel
7 Glycan Ligands for Influenza A Virus. *Sci Rep.* 10, 5320. [https://doi.org/10.1038/s41598-020-](https://doi.org/10.1038/s41598-020-62074-z)
8 62074-z

2019-nCoV Attachment to Host Cells is Possibly Mediated via RGD-Integrin Interaction in a Calcium-dependent Manner

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Legends for Figures & Tables

Legends for Figures

FIGURE 1. Pair-wise sequence alignment of SARS-CoV-1 and SARS-CoV-2 spike protein RBD sequence using Clustal Omega.

FIGURE 2. Gene expression profile of RGD binding integrins $\alpha\text{v}\beta 6$ (A), $\alpha 5\beta 1$ (B), $\alpha 8\beta 1$ (C) and human ICAM1 (D) in lungs as obtained and plotted at the Human Protein Atlas. (<https://www.proteinatlas.org/>).

FIGURE 3. Gene expression profile of ACE2 in lungs as obtained and plotted at Gene Expression Database at EBI (<https://www.ebi.ac.uk/gxa/home>) (A) and the Human Protein Atlas (<https://www.proteinatlas.org/>) (B).

FIGURE 4. Molecular docking of viral spike RBD domain onto the structure of $\alpha 5\beta 1$ (PDB ID: 3VI3) and $\alpha\text{v}\beta 6$ (PDB ID: 5FFG) showing interaction via the RGD motif present in the spike RBD domain (A). The molecular docking of viral spike RBD domain onto the structure of $\alpha 5\beta 1$ (PDB ID: 3VI3) showed that the spike RBD is interacting with the $\alpha 5\beta 1$ through its RGD motif in the same way (B, left panel) as synthetic RGD peptide binds and interact to $\alpha 5\beta 1$ (PDB ID: 3VI4) (B, right panel) as already known in literature (Nagae et al., 2011). The docking was done using HDock server (hdock.phys.hust.edu.cn/).

FIGURE 5. Molecular docking showing binding of SARS-CoV-2 spike receptor binding domain with ICAM1 precisely via RGD domain (A-C) and other regions (D-G).

FIGURE 6. Molecular docking showing EDTA binding to SARS-CoV-2 spike receptor precisely where the RGD motif binds to integrin receptor protein. The docking was done using SwissDock (<http://www.swissdock.ch/docking>).

FIGURE 7. Molecular docking showing EDTA also binds to RBD domain of spike protein in complex with human ACE2 receptor protein (reconstructed from models obtained by Chen et al., 2020). Here, the EDTA is shown to bind to the sites in the viral spike RBD that interacts with the human ACE2.

FIGURE 8. Pulmonary EDTA chelation therapy which can be clinically executed through a nebulizer or inhaler to allow sodium-EDTA to pass into the lungs. The sodium-EDTA will chelates Ca^{+2} ions and other divalent ions making them unavailable for RGD-integrin mediated virus attachment to the host cells (A). A novel strategy for safe, technically simple, quick, cost-effective and efficient treatment of COVID 19 patients (B).

Legends for Tables

TABLE 1. Prediction of putative N-linked glycosylation and other PTM sites using MyHits Motif Scan of SIB. Top scored N-linked Glycosylation sites are represented in bold.

S.No.	Pattern	Position	
		SARS-CoV-1	SARS-CoV-2
1.	ASN_Glycosylation	29, 65 , 73, 109, 118, 119 , 158, 227, 269, 318 , 330, 357, 589, 602 , 691, 699, 783 , 1056, 1080, 1116, 1140 , 1155, 1176	17, 61, 74 , 122, 149, 165, 234, 282 , 331, 343, 603, 616 , 657, 709, 717 , 801, 1074, 1098, 1134, 1158 , 1173, 1194
2.	CAMP_Phospho_Site	343	356, 528
3.	CK2_Phospho_Site	12, 20, 271, 561, 644, 716, 798, 809, 964, 1118, 1129, 1178	50, 108, 151, 221, 250, 284, 734, 816, 827, 982, 1136, 1147, 1160, 1196
4.	Myristyl	86, 104, 225, 246, 298, 368, 418, 531, 587, 634, 653, 682, 739, 862, 871, 890, 953, 1113, 1153, 1228	72, 89, 184, 232, 311, 381, 431, 446, 476, 545, 601, 648, 667, 700, 757, 880, 889, 908, 971, 1093, 1131, 1171, 1246
5.	PKC_Phospho_Site	36, 92, 173, 215, 289, 363, 541, 561, 670, 775, 795, 980, 1019, 1087, 1129	19, 76, 95, 302, 376, 415, 555, 632, 680, 813, 998, 1037, 1105, 1147
6.	Tyr_Phospho_Site	188, 715	417, 733

TABLE 2. Artificial neural network based predicted motifs in the protein sequence of SARS-CoV-1 and SARS-CoV-2.

S.No.	Motifs	Position		Motif's Role/Function
		HCoV-1	HCoV-2	
1	S1 Glycoprotein	268-304	519-592	Viral attachment
2	Potato Inhibitor 1	309-320	Not predicted	NA
3	Spike Receptor Binding domain	317-569	330-583	Attachment with host cells
4	RGD	Not Predicted	403-405	Possibly attachment with host cells
5	FMRP	437-447	Not predicted	NA
6	S2 Glycoprotein	641-1247; 694-1237	684-1265; 712-1255	Fusion of viral and cell membrane as well as fusion of infected cells with adjoining cells.
7	Borrelia Repeat	662-679	Not Predicted	NA
8	DUF16	892-971	Not Predicted	Protein of unknown function

9	Fusion Glycoprotein F0	910-935	Not Predicted	Induces fusion of viral and cellular membranes leading to delivery of the nucleocapsid into the cytoplasm
10	BIG1	Not predicted	1-3	NA
11	Cysteine-rich Domain	1217-1236	1235-1254	Membrane fusion

1 **Table 3.** RGD binding Integrins, their receptors and their role in virus attachment and cell signaling as
2 sourced from UniProt KB database.

S.N.	Integrin	UniProt ID	Receptor	Virus Attachment	Function
1	$\alpha v\beta 3$ ITGAV- ITGB3 CD61	P06756 P05106	CD40LG FGF1/FGF2 CX3CL1 PLA2G2A NRG1 IGF2 IL-1B FBN1 CD40-LG IGF2 Ca⁺² Binding Sites 260-268; 314-322; 379-387; 443-451	Herpes virus 8 HHV-8 Coxsackievirus A9 Hantaan virus Cytomegalovirus/HHV-5 Human metapneumovirus Human parechovirus 1 West Nile virus HIV-1	FGF1 signaling FGF2 signaling NRG1-ERBB signaling CX3CL1 signaling IL-1B signaling CD40-CD40L signaling IGF2 signaling
2	$\alpha v\beta 5$ ITGAV- ITGB5	P06756 P18084	Fironectin	Adenovirus type c Coxsackievirus A9 Coxsackievirus B1	TGF- β 1 singaling
3	$\alpha v\beta 6$ ITGAV- ITGB6	P06756 P18564	Fibronectin Cytotactin TGF- β 1 FBN1	Coxsackievirus A9 Coxsackievirus B1 Herpes simplex virus-1 HHV-1	TGF- β 1 activation Clathrin-mediated endocytosis
4	$\alpha v\beta 8$ ITGAV- ITGB8	P06756 P26012	Fibronectin TGF- β 1	Data not found	TGF- β 1 activation
5	$\alpha 5\beta 1$ ITGA5- ITGB1 CD29	P08648 P05556	Fibrinogen Fibronectin PLA2G2A CD40LG IL1B FBN1 Ca⁺² Binding Sites 280-288; 334-342; 401-409; 465-473	Cytomegalovirus/HHV-5 Epstein-Barr virus/HHV-4 Human parvovirus B19 Mammalian reovirus HIV-1 Human metapneumovirus	CD40-CD40L signaling IL1B signaling
6	$\alpha 8\beta 1$ ITGA8- ITGB1	P53708 P05556	TNC FN1 SPP1 TGFB1/TGFB3 VTN NPNT Ca⁺² Binding Sites 329-337; 395-404; 459-467	Data not found	TGF- β 1 signaling

SARS-CoV-1	AELKCSVKSEIDKGIYQTSNFRVVPSPGDVVRFPNITNLCPFGEVFNATKFPSVYAWERK	343
2019-nCoV	SETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRK	356
	:* **::*** ::***** *: .:*****:*****:*	
SARS-CoV-1	KISNCVADYSVLYNSTFFSTFKCYGVSATKLNDLCFSNVYADSFVVGDDVRQIAPGQTG	403
2019-nCoV	RISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGLEVRQIAPGQTG	416
	:*****: ***** *****:*****:***:*****	
SARS-CoV-1	VIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNKYRYLRHGKLRPFERDISNVFPSPD	463
2019-nCoV	KIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNLYLYRLFRKSNLKPFERDISTEIYQAG	476
	***** **::***:.*:.* ***** ** :*::*:*****. :. .	
SARS-CoV-1	GKPCPTP-PALNCYWPLNDYGFYTTTGIGYQPYRVVVLSELLNAPATVCGPKLSTDLIKN	522
2019-nCoV	STPCNGVEGFNCYFPLQSYGFQPTNGVGYPYRVVVLSELLHAPATVCGPKKSTNLVKN	536
	..** .:***:*.*** *.*:*****:***** **::**	
SARS-CoV-1	QCVNFFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFDTSVDPKTSEILDISPCAFGGVS	582
2019-nCoV	KCVNFFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVS	596
	:***** *.*: *****:.* **::***:*****:***:*****

 Spike Receptor Binding Domain (RBD)
 "RGD" motif

A

1

2

First Helix of the Topological
Extracellular Domain of
human ACE2

Human ACE2 Receptor
complexed with Receptor
Binding domain of Spike
Protein

RGD Motif in Receptor
Binding domain of Spike
Protein

Interacting Region 1

EDTA bound to Interacting
Region 1 & 2 of the RBD of
the Viral Spike Protein

Interacting Region 2

A

B

1

2

3

4

5

6

7

8

9

10