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Case Report

Lighting Up Dark Areas of COVID-19

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Summary

Binding of SARS-CoV-2 S protein to angiotensin converting enzyme 2 on host cells is the main mechanism of viral entry into human cells but SARS-CoV-2 may infect cells using multiple mechanisms. We propose and present evidence to support an alternative infection mechanism involving interaction between the SARS-CoV-2 N protein and the transmembrane glycoprotein CD147 that is mediated by Cyclophilin A (CypA). If SARS-CoV-2 does use a SARS-CoV-2 N-CypA-CD147 infection mechanism, a CypA inhibitor could prove useful against SARS-CoV-2. Cyclosporin A (CsA) is an immunosuppressant drug used in cancer therapy, with a mechanism based on binding/inhibition of the CypA protein in the cytoplasm. Inhibition of CypA by CsA, in addition to preventing SARS-CoV-2 infection, could also stop the life cycle of the intracellular virus. Therefore, SARS-CoV-2 N-CypA-CD147 may play an important role in viral infection, and CsA could be a very useful therapy against SARS-CoV-2 infection.

Keywords: Covid-19; Mechanism of infection; Pandemic; SARS-CoV-2; SARS-CoV

Introduction

In December 2019, an epidemic due to a new coronavirus, which preferentially causes lung disease, originated in Wuhan, China. This new coronavirus was provisionally named 2019-nCov [1]. However, it was phylogenetically characterized as having 79.6% of its genome similar to that of SARS-CoV [2], and was therefore called SARS-CoV-2 [1]. Currently, COVID-19 refers to disease caused by SARS-CoV-2 [1], and today it is a pandemic [3].

Both SARS-CoV and SARS-CoV-2 have four structural proteins: Spike (S), Membrane (M), Envelope (E), and Nucleocapsid (N) [4]. Proteins S, M, and E are envelope proteins, and protein N wraps viral RNA inside the virion [5]. The S protein of known coronaviruses is responsible for both binding to receptors in host cells and fusion of the virus and cell membranes [4]. However, protein N may also play a role in SARS-CoV invasion of host cells. In 2005, Chen et, al. found that protein N can bind to Cyclophilin A (CypA), a host cell protein that was localized on the surface of SARS-CoV in infected cells, as observed by electron microscopy [5]. This seems to be contradictory to the finding that protein N is completely located inside the virus. However, previously Saphire et al. reported detection of CypA linked with nucleocapsid viral proteins on the surface of viruses [6]. Thus, there are N proteins outside the virion as well. We have called internal N proteins Nin, whereas those linked to CypA and appearing on the surface we have called Nout, emulating this relationship between CypA and N protein described by Chen, et al. [5] (Figure1).

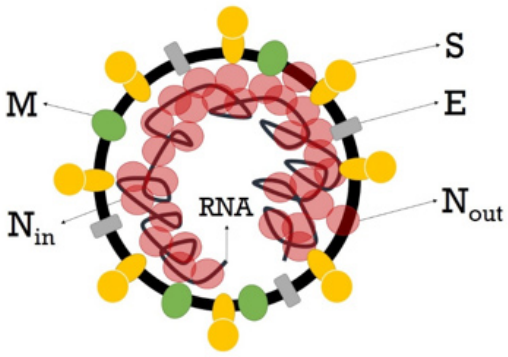

Figure 1: Structure of coronavirus, with the structural proteins shown. Protein N is called Nout when it is presented on viral surface, and Nin, the same protein, inside the virion.

Given the presence of N protein both inside and outside the virion, we have investigated whether this protein might be a potential target for the treatment of patients with COVID-19.

Results and Discussion

SARS-CoV and SARS-CoV-2: Homology and Host Cell Receptors

Binding of SARS-CoV-2 S to Angiotensin Converting Enzyme 2 (ACE2) on host cells is the main mechanism of viral entry into human cells [7], and SARS-CoV-2 S is thus being targeted by researchers. SARS-CoV also uses this receptor to infect human cells [8]. Another, recently described, receptor target for SARS-CoV-2 involves the transmembrane glycoprotein CD147, also known as EMMPRIN, Basigin and Hab18G, which interacts with the extracellular matrix [9]. Interaction between the SARS-CoV-2 S protein and CD147 was shown to enhance viral invasion of host cells [8] (Figure 2A). This was the first time that SARS-CoV-2 had been linked to the CD147 receptor. However, we are not surprised because an association between CD147 and SARS-CoV has previously been described, but with a difference in that viral recognition of CD147 in the host cell could have been mediated by CypA, which binds to the N protein of the coronavirus (SARS-CoV N) [5]. CypA has important roles in protein folding and inflammatory responses that are mediated by CD147 [10]. We know that the interaction of CypA with SARS-CoV and CD147 possibly enables SARS-CoV to infect the host cell using SARS-CoV N [5], and SARS-CoV N has been shown to bind to CypA with high affinity [11].

Potential New Mechanism of SARS-CoV-2 Cellular Infection

Lu, et al. states that SARS-CoV and SARS-CoV-2 have a similar genomic organization and that where they differ most is in protein S [12]. This genomic homology between SARS-CoV and SARS-CoV-2, may suggest that SARS-CoV-2 may use a SARS-CoV-2 N-CypA-CD147 infection mechanism (Figure 2B).

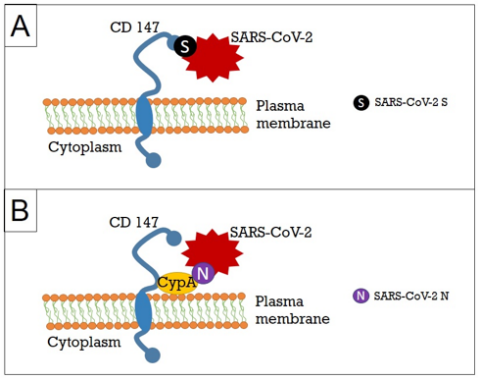

Figure 2: Newly identified mechanisms of cellular entry by SARS-CoV-2. (A) Recently published recognition mechanism involving SARS-CoV-2 S protein. (B) Proposed recognition mechanism involving SARS-CoV-2 N protein.

Genomic homology between coronaviruses cannot be the only proposal to conclude that SARS-CoV-2 also uses the same CypA-mediated mechanism of SARS-CoV (a SARS-CoV-2 N-CypA-CD147 interaction). This hypothesis can be raised only if the SARS-CoV N and SARS-CoV-2 N proteins have sufficient structural homology. In this respect, we found in a review of the literature that SARS-CoV-2 N has an amino acid sequence comparable to SARS-CoV N, with 90% coincidence [13]. Based on our 3D structure and sequence homology model, we demonstrated that, for SARS-CoV N protein and SARS-CoV-2 N protein, there is a 95.76% sequence identity between the SarS-CoV-2 N Asn140-Ala419 segments with the SARS-CoV N oligomerization domain. In addition, we found almost complete overlap of the 3D structures analysed in both proteins.

CypA is also known to bind to the N-terminal domain of the HIV-1 capsid protein [14]. In fact, there is a crystallographic model of the CypA complex with this domain of HIV-1 protein for use as a template [11]. Knowing this, Luo, et al. performed a sequence alignment between the N-terminal domains of the HIV-1 viral capsid protein with the SARS-CoV N protein [11]. Sequence alignment indicated that the SARS-CoV N segment Val235-Pro369 is homologous to the N-terminal domain of the HIV-1 capsid protein with a sequence identity of 25.1%, consequently the binding site of SARS-CoV N to CypA was deduced as being the same segment [11]. Further analysis of SARS-CoV N show that the Val235-Pro369 segment is a special domain of the sterol carrier protein family [11]. The 3D-modeled structure of the SARS-CoV N Val235-Pro369 segment was manually coupled into the CypA active site. A loop Trp302-Pro310 of N in SARS-CoV (homologous with Pro85-Pro93 loop in HIV-1) fell on the CypA active site [11]. Computational studies revealed a SARS-CoV N (Trp302-Pro310) segment determinant in CypA-SARS-CoV N binding [11].

In further comparison of the SARS-CoV and SARS-CoV-2 N proteins using the 3D structure and sequence homology model, we confirmed the presence, although displaced, of a Trp302-Pro310 segment in SARS-CoV-2 N. The SARS-CoV-2 segment involved in SARS-CoV-2-CypA lies in a downstream residue, Trp301-Pro309.

Our sequence analysis to determine homology of the potential binding segment of the SARS-CoV N and SARS CoV-2 N proteins comprised the Asn140-Ala419 segment, which is longer than the segment proposed by Luo, et al. [11]. The result was a 95.76% sequential homology between our SARS-CoV-2 N segment and SARS-CoV N, specifically with the SARS-CoV N oligomerization domains (PDB 2CJR) (Figure 3A). Using 3D structures to compare the Asn140-Ala419 segment of SARS-CoV-2 N with the oligomerization domain of SARS-CoV N revealed an almost completed overlap (Figure 3B).

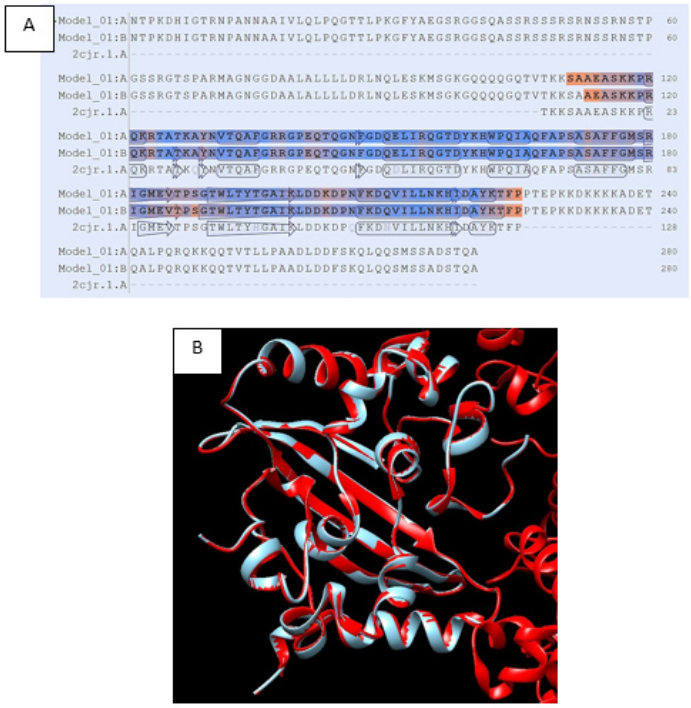

Figure 3: Sequence analysis and 3D modelling to compare SARS-CoV2 and SARS-CoV. A) Sequence homology by SWISS-MODEL of Asn140- Ala419 (SARS-CoV-2) (model 01:A/B), on the oligomerization domain (SARS-CoV) (model 2cjr.1.A). B) Overlapping 3D structures of Asn140-Ala419 segment of SARS-CoV-2 N (blue) on the oligomerization domain of SARS-CoV N (red). As it should be noted, there is a practically total overlap. (Software: "Chimera 1.14")

We also found that the Trp302-Pro310 segment of SARS-CoV N (PDB 2CJR) and Trp301-Pro309 segment of SARS-CoV-2 N are alpha helixes, and not loops (Figure 4), as reported by Luo, et al. [11], which implies that the site of interaction with CypA is not the one reported by Luo, et al. [11]. Therefore, based on experimental results of high affinity of SARS-CoV N-CypA, and sequences of 3D structures of both N proteins (SARS-CoV N and SARS-CoV-2 N), we conclude that a binding CypA site in the Asn140-Ala419 SARS-CoV-2 N segment can exist.

This new proposed mechanism suggests that a known CypA inhibitor would be useful against SARS-CoV-2. Cyclosporin A (CsA) is an immunosuppressant drug used in cancer therapy, with a mechanism based on binding/inhibition of the CypA protein in the cytoplasm, that recently decreased CypA expression in *Leishmania donovani* [15].

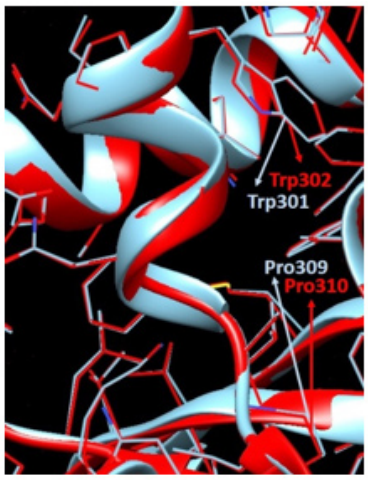

Figure 4: Overlapping segments of Trp302-Pro310 (SARS-CoV N) (red) and Trp301-Pro309 (SARS-CoV-2 N) (blue), where they are shown to be alpha helixes. (Software: "Chimera 1.14")

Inhibition of CypA by CsA, in addition to preventing SARS-CoV-2 infection, could also stop the life cycle of the intracellular virus. SARS-CoV N protein not only acts in the infection mechanism but is necessary for intracellular viral multiplication and for viral evasion of the innate immune response [16]. In the event that the virus uses another mechanism of infection (for example: SARS-CoV-2 S-CD147 or SARS-CoV-2 S-ACE2) and manages to penetrate the host cell, viral multiplication may possibly be stopped, or the effects of immune evasion reduced, by blocking the action of intracellular CypA.

CsA is a medication that has been used in humans for years. Therefore, the hypothesis we propose could be tested in a clinical essay using patients affected by COVID-19. Specifically, a study using SARS-CoV-2 infected cell culture can be developed to measure viral multiplication capacity after treatment with CsA. We are developing a computational model that allows the identification of specific amino acid residues in the SARS-CoV-2 N-CypA interaction and the site where CsA would act to interfere with such binding. Similarly, it would be necessary to obtain by means of various experimental methods (X-Ray diffraction, solution nuclear magnetic resonance, or others) the structure of the SARS-CoV-2 N-CypA complex and then perform a docking of protein-ligand using CsA.

Conclusions

Recent publications on SARS-CoV-2 may suggest that S protein is the only antiviral therapeutic target. Although it is a

protein directly involved in infection, it is not the only one. We have proposed a new route of infection for SARS-CoV-2 that involves the CD147 receptor interacting with protein N through CypA. It is known that SARS-CoV uses this alternative mechanism of infection. Following recent findings and the homology between N proteins of both coronaviruses, we suggest this mechanism (SARS-CoV N-CypA-CD147) is also involved in SARS-CoV-2 infection. Furthermore, we demonstrated that the CypA binding site of the Trp302-Pro310 segment of SARS-CoV N (PDB 2CJR) and Trp301-Pro309 segment of SARS-CoV-2 N are alpha helixes and not loops. Finally, we conclude that, based on sequence homology and the complete overlap of SARS-CoV N and SARS-CoV-2 N 3D structures, a binding CypA site in the Asn140-Ala419 SARS-CoV-2 N segment can exist.

Therefore, SARS-CoV-2 N protein bound to CypA may play an important role in viral infection. Since CypA can be inhibited through the use of CsA, a CypA inhibitor known and widely used by the medical community, it could be a very useful therapy against SARS-CoV-2 infection.

Materials and Methods

Development of the SARS-CoV-2 N, 3D Homology Model

To develop a SARS-CoV-2 N, 3D homology model, we first copied the primary structure of SARS-CoV-2 N (available in FASTA format in https://www.ncbi.nlm.nih.gov/protein/1798174255 with NCBI code: YP_009724397.2) and selected the segment Asn140-Ala419 (280 amino acids). Luo, et al. explained that the SARS-CoV N segment involved in the binding of CypA must be Val235-Pro369 (135 amino acids) [11]. However, to expand on this and better frame the portion of protein for study, we studied the longer Asn140-Ala419 segment (280 amino acids).

Next, using SWISS-MODEL (https://swissmodel.expasy.org), homologous polypeptides to Asn140-Ala419 segment were obtained. Of all these homologous polypeptides, we selected the most homologous model so far: 2cjr.1 (CRYSTAL STRUCTURE OF OLIGOMERIZATION DOMAIN OF SARS CORONAVIRUS NUCLEOCAPSID PROTEIN), resolution 2.50 Å and with sequence identity of 95.76%. From this, we built the corresponding model using the 2cjr.1 template. The PDB files of the target and temple 3D structures were downloaded and analysed using the free software "Chimera 1.14". With the Tools/Structure Comparison/MatchMaker function, the selected 3D structures of SARS-CoV N and SARS-CoV-2 N were overlapped.

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Authors' Contributions

Both authors participated equally in the generation of this paper (conception of idea, data collection, analysis, interpretation, writing and literature search).

Conflicts of Interest

Dr. Cervera-Grau is employed as a Clinical Oncologist at Lilly Spain, but declares that the intellectual conception of this idea is independent. Dr. Bermejo-Valdés has nothing to disclose.

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