QUALITATIVE ANALYSIS OF THE INVESTIGATIONS OF POTENTIAL DRUG TARGETS AND TREATMENTS FOR NOVEL CORONAVIRUS

By

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Abstract

This project investigates the ongoing effort to determine potential drug targets and treatments for the novel coronavirus (SARS-CoV-2) more commonly known as COVID-19. In the midst of an pandemic, researchers have been able to identify the structure, function, and antigenicity of the virus and its different parts. Of particular interest is the virus's spike glycoprotein, which promotes entry into host cells and is the main target of antibodies. Through various studies, it has been determined that this glycoprotein uses human angiotensin-converting enzyme II (ACE2) in order to enter cells. When the receptor binding domain on the glycoprotein binds ACE2, the enzyme cleaves the spike, exposing the spike fusion peptide, facilitating virus entry. Therefore, the main focus in determining potential drug targets and treatments has been on this mechanism. This paper compiles the major findings of articles that convey different methods in determining these drug targets, and presents possible future treatments.

Acknowledgements

I would like to thank my faculty mentor, Dean Constance Nugent, for her guidance and mentorship throughout this project. Thank you for helping me overcome the problems that arose due to the pandemic, and for inspiring me to use that adversity to pursue this research project. I would also like to express gratitude for my family and friends; Manmeet, Maivy, and Ethan, I could not have completed this project without your constant support and help. I am eternally grateful for you all.

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Introduction

The Novel Coronavirus, SARS-CoV-2, has swept throughout the entire world, with 1.6 million confirmed cases and counting in the United States alone (WHO 2020). The first reports of the virus came from Wuhan, Hubei, China, where a patient reported fever, chest tightness, unproductive cough, pain and weakness for one week on presentation (Wu et. al. 2020). This outbreak was not able to be contained, and due to international travel, the virus spread globally, causing the pandemic. The number of cases has increased significantly, reaching 5.5 million cases, with 350,000 deaths globally as of May27, 2020 (WHO 2020). Facing a pandemic of this magnitude, nations are establishing new methodologies and precautionary measures in order to combat the spread of the virus and acclimate themselves to its dire consequences. This has included methods of social distancing, self-isolation, and quarantine, among other measures, such as closing down thousands of businesses deemed "non-essential". This situational circumstance has affected not only the health and overall wellness of the global population, but also the social norms and practices that fuel the global economy. For example, formalities such as the practice of shaking one another's hand upon introduction are longer considered the norm. Additionally, many people have lost their jobs due to the virus, and as a result the government is having to distribute stimulus checks to compensate people with enough income to survive. Thus, researchers are trying to determine both treatments and vaccines as rapidly and effectively as possible in order to minimize the detrimental socioeconomic and healthcare effects of the novel coronavirus.

Viral structure

SARS-CoV-2 is a novel positive-sense enveloped RNA virus from the family *Coronaviridae* (Lu et. al. 2020). It is structurally similar to other betacoronaviruses, such as SARS-CoV, MERS-CoV, and the bat coronavirus, RaTG13.

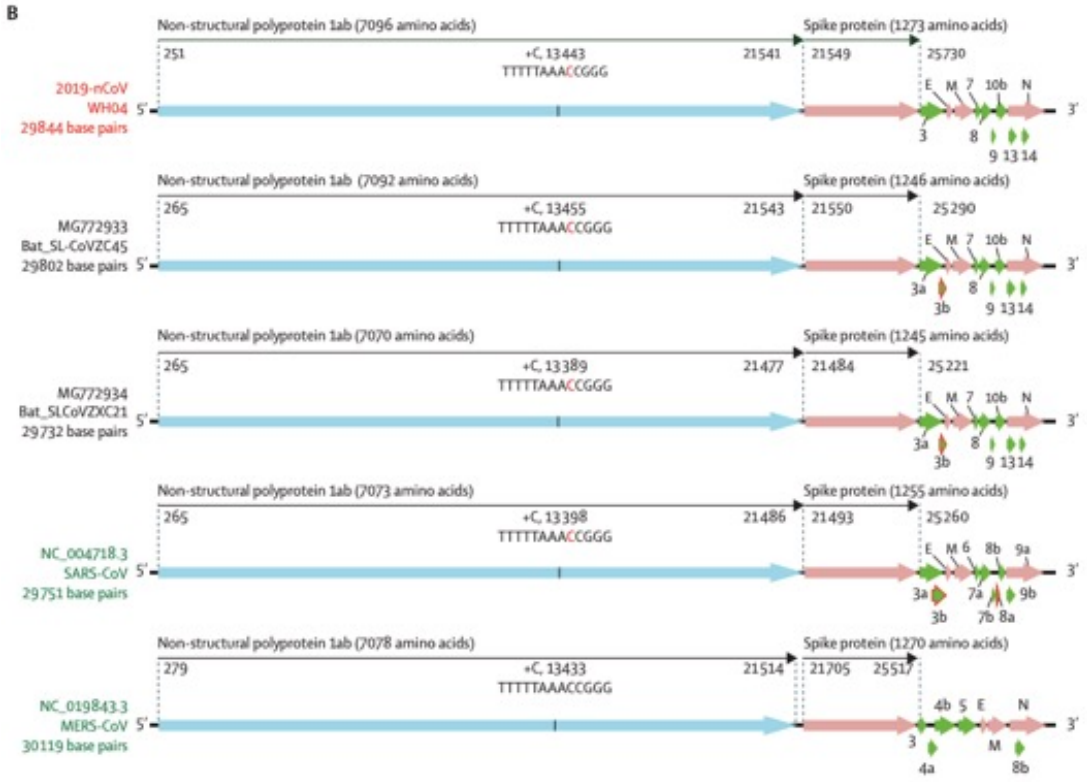

Figure 1: Sequenced Genomes of family *Coronaviridae* (Lu et. al. 2020)

An important structural aspect of a coronavirus is that it is an enveloped virus. This is a virus with an outer wrapping (envelope) that comes from other infected cells, or the host. The process by which this wrapping occurs is known as budding, in which newly formed virions become enveloped in an outer coat that is made from part of the cell's plasma membrane. This structural aspect is significant, as it may play a role in survival of the virus and the spread of infection. In total, SARS-CoV-2 contains four main structural proteins: the nucleocapsid (N), membrane (M), envelope (E), and spike (S) proteins. The N protein forms a complex with viral genomic RNA on

the cytosolic side of the cell and interacts with the M protein during virion assembly. The M protein sorts viral components to be incorporated into virions and is heavily glycosylated with either N-linked or O-linked oligosaccharides. The E protein is a small integral membrane protein that plays roles in virion assembly, budding, envelope formation, and pathogenesis. The S protein is the most important in the mechanism of viral infection, as is plays a role in viral attachment, fusion, and entry into cells (Walls et. al. 2020). As seen in Figure 2 below, the M, E, and S proteins are embedded in the surface of the virus along with the viral envelope glycoprotein, hemagglutinin esterase (HE). This glycoprotein mediates virion attachment by acting as a lectin, a protein that binds specific carbohydrates on the surfaces of cells, or as a receptor-destroying enzyme.

Because the S protein has a significant impact on entry into host cells, it is important to characterize the structure of the protein to understand the infection process. The protein, located on the surface of SARS-CoV-2, forms homotrimers, with each monomer consisting of two subunits, S1 and S2. The S1 subunit is composed of the receptor binding domains (RBDs) that are involved with attachment to the host receptor, while the S2 subunit contains the machinery necessary for the fusion of viral and cellular membranes.

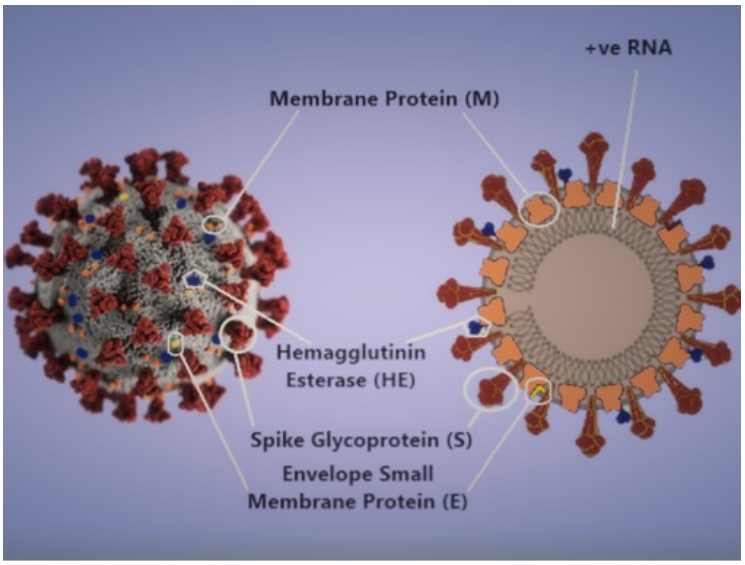

Figure 2: SARS-CoV-2 viral structure (Kakodkar, Kaka, Baig 2020)

For betacoronaviruses, a single region of the spike protein called the receptor-binding domain (RBD) mediates the interaction with the host-cell receptor. (Letko, Marzi, Munster 2020). In comparing SARS-CoV with SARS-CoV-2, the identity of the receptor-binding mtif (RBM), the most variable region of the receptor binding domain, is only 47.8% conserved, as demonstrated in Figure 3 (Yi et. al. 2020). Spike Sequence analysis of SARS-CoV-2 S by a research group at the University of Washington reveals the presence of a four amino acid residue insertion (RRAR) at the boundary between the S1 and S2 subunits in SARS-CoV-2 S compared with SARS-CoV S, resulting in the introduction of a furin cleavage site (Walls et. al.). As it has previously been determined that SARS-CoV undergoes similar proteolytic cleavage via transmembrane serine protease-2 (TMPRSS-2) and/or cathepsin L (Walls et. al. 2020), this novel cleavage site sequence found in SARS-CoV-2 suggests that a furin protease conducts the proteolytic cleavage.

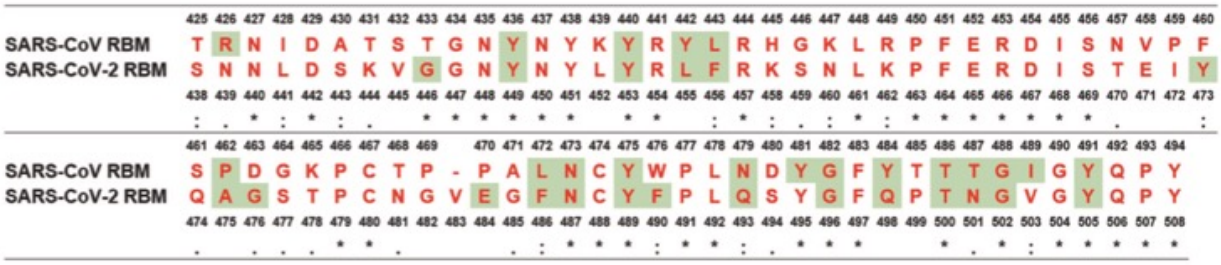

Figure 3: Differences in SARS-CoV RBD and SARS-CoV-2 RBD (Yi et. al. 2020)

Mechanism of Infection

There are two main mechanisms of entry for coronaviruses. The classical route is through receptor-mediated endocytosis. The second, which is found more commonly in highly infectious CoVs, like SARS-CoV-2, is through direct cell-cell fusion. Both methods require binding to a host receptor at the target cell's surface. Previous studies have determined that among human coronaviruses, SARS-CoV binds human angiotensin-converting enzyme-2 (hACE2) (Hoffman et. al. 2004), while MERS-CoV mediates infection by binding to the cellular receptor dipeptidyl peptidase 4 (DPP4) (Mou et. al. 2013). The main host receptor for SARS-CoV-2 found in the human body is also ACE2 (Shang et. al. 2020), possibly explaining why the virus effects the human body in similar ways as SARS-CoV. Important residues of the RBD that specifically interact with hACE2 have been identified, as seen in the residues in Figure 2 that are highlighted green (Yi et. al. 2020). It can be noted that these residue differences from SARS-CoV in the RBD stabilize two virus-binding hotspots at the RBD/hACE2 interface, enhancing the hACE2 binding affinity (Shang et. al.), and thus increasing the likelihood and efficiency of infection. Moreover, the binding affinity of the S protein and ACE2 was found to be a major determinant of CoV replication and disease severity, with increasing affinities corresponding with higher intensity of infection (Sungnak et. al. 2020).

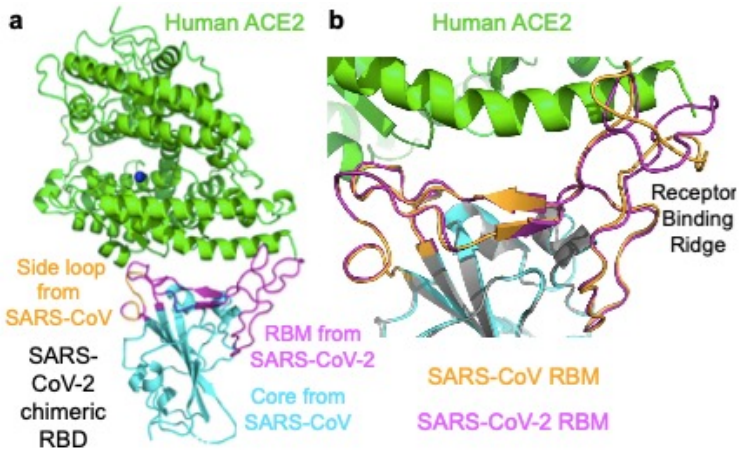

Figure 4: Crystal structure of SARS-CoV-2 RBD bound to human ACE2, depicting differences between SARS-CoV and SARS-CoV-2 (Shang et. al. 2020)

Coronavirus entry into cells through direct cell-cell fusion is mediated by the Spike protein. After the Spike RBD binds to ACE2, the S undergoes protease cleavage, activating the fusion potential of the S protein. It has been determined that this cleavage occurs in two steps: priming cleavage between S1 and S2, and activating the cleavage on the S2' site (Ou et. al. 2020). For many CoVs, the S is cleaved between the two S subunits, which remain non-covalently bound in the pre-fusion conformation (Walls et. al. 2020). CoV S proteins may be cleaved by one or several host proteases, including furin, trypsin, cathepsins, TMPRSS-2, TMPRSS-4, or human airway trypsin-like protease (HAT) (Ou et. al.). For all CoVs, the S is further cleaved at the $S2^0$ site located immediately upstream of the fusion peptide, which is proposed to activate the protein for membrane fusion via extensive irreversible conformation changes (Walls et. al.). After activation of the fusion potential, the heptad repeat 1 (HR1) and 2 (HR2) domains in its S2 subunit interact with each other to form a six-helix bundle (6-HB) fusion core, bringing viral and cellular membranes into close proximity for fusion and infection (Xia et. al. 2020). It is important to distinguish that in the other method of entry into host cells

through receptor-mediated endocytosis, the S protein is not cleaved upon entry, but is cleaved within the cell by host proteases before budding occurs.

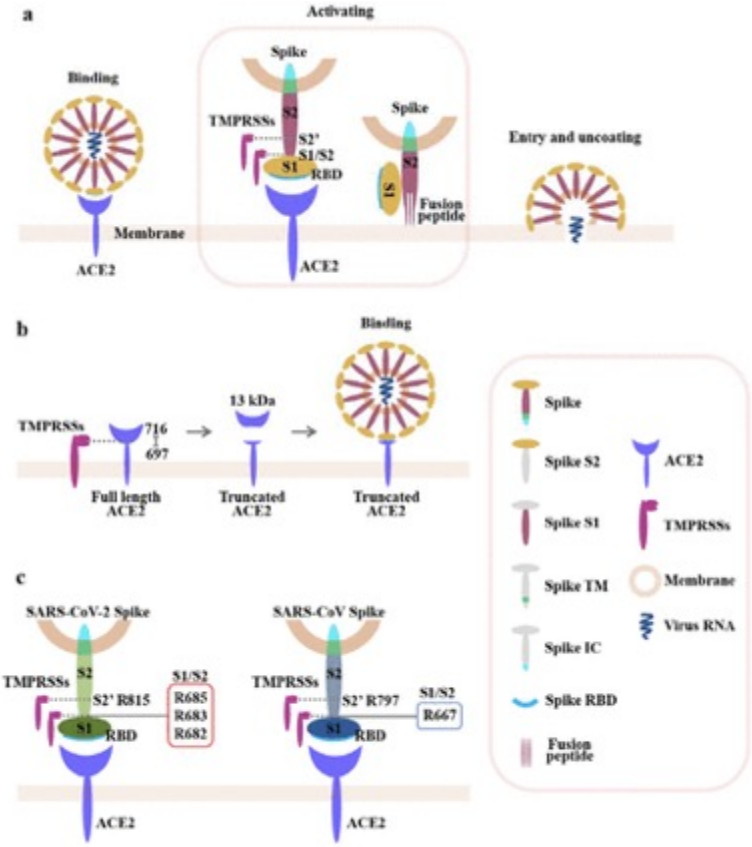

Figure 5: Spike protein cleavage mechanism (Meng et. al. 2020)

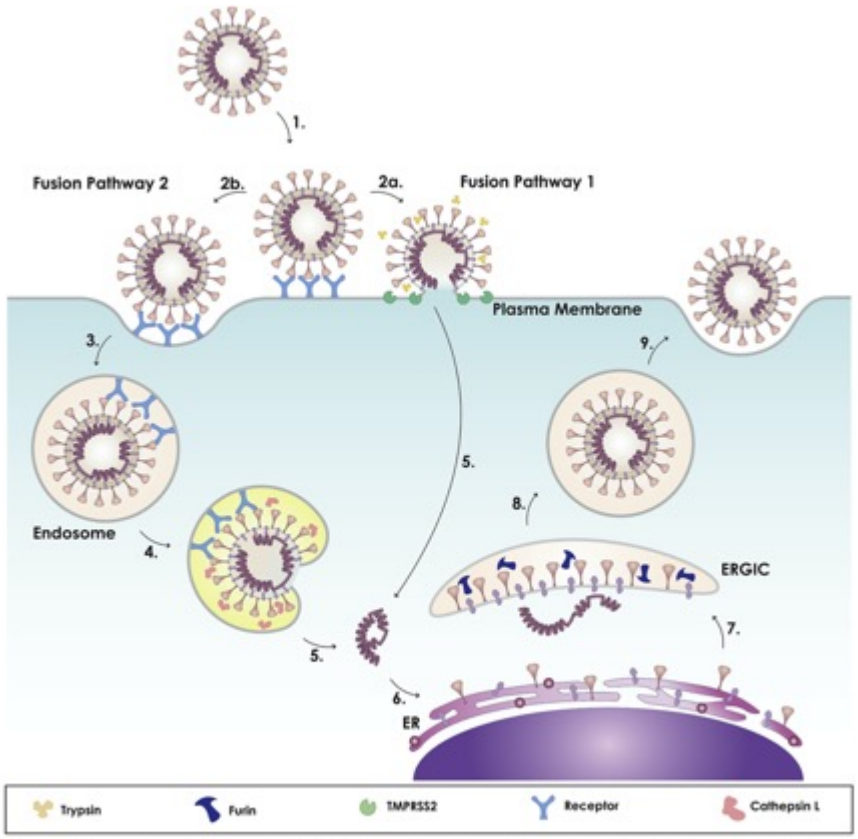

Figure 6: Mechanisms of viral fusion into host target cells (Tang et. al. 2020)

To investigate the importance of the novel furin cleavage site in SARS-CoV-2, researchers at the University of Washington designed a pseudovirus with a mutant furin cleavage site by mutating wildtype SARS-CoV-2 S to SARS-CoV-2 $S_{fur/mut}$. This mutant spike protein lacks the four amino acid residues unique to SARS-CoV-2 S, RRAR, thus mimicking the SARS-CoV S. It is expected to undergo processing upon reaching a target cell, as SARS-CoV S does. This group tested the effects of this mutation in the sequence of the cleavage site in hACE2-expressing BHK cells, and the results revealed that the SARS-CoV-2 $S_{fur/mut}$ remained uncleaved upon budding, suggesting that the furin proteases located within the ER-Golgi intermediate compartment (depicted in Figure 6) are involved in viral protein biosynthesis. Without the novel cleavage sequence, proper protein modification is not able to be completed, thus rendering the viral S protein inactive to mediating infection. These results suggest that the detection of a

polybasic cleavage site (RRAR) in the fusion glycoprotein of SARS-CoV-2 could possibly expand its host response and/or enhance its transmissibility, compared with SARS-CoV and SARS-related CoVs, due to the extensive distribution of furin-like proteases and their reported effects on other viruses (Walls et. al. 2020).

SARS-CoV-2 S protein is capable of triggering protease-independent and receptor-dependent syncytium formation in hACE2-expressing cells. This cell-cell fusion is mediated by the S2 subunit of S protein. Specifically the heptad repeat 1 (HR1) and 2 (HR2) domains in its S2 subunit interact with each other to form a six-helix bundle (6-HB) fusion core, bringing viral and cellular membranes into close proximity for fusion and infection (Xia et. al. 2020). Such a mechanism might enhance virus spreading through cell-cell fusion and this might partially explain the rapid progress of the disease. A clinical feature of COVID-19 is that severe cases are often characterized by formation of such syncytia in the alveolar regions of the lung (Cifuentes et. al. 2018), which explains why the most prominent symptoms present in the respiratory tract.

Human angiotensin-converting enzyme 2 (hACE2) is a negative regulator of the renin-angiotensin system, and functions as the key SARS coronavirus receptor and stabilizer of neutral amino acid transporters. hACE2 catalyzes the conversion of angiotensin II to angiotensin 1-7 (Kuba, Imai, Penninger 2013). HACE2 is abundantly present in the epithelia of the lung and small intestine, which might provide possible routes of entry for the SARS-CoV. However, the enzyme is also present in arterial and venous endothelial cells and arterial smooth muscle cells in oral and nasal mucosa, nasopharynx, lung, stomach, small intestine, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney, and brain (Hamming et. al. 2004). Additionally, entry factors for the virus were found to be highly expressed in nasal epithelial cells that are co-expressed with genes involved with innate immunity (Sungnak et. al. 2020). This evidence

highlights the cells' potential role in the initial viral infection, as well as the role in viral spread and clearance. This also potentially explains the high efficiency of the viral transmission between individuals.

Protein Interactions with virus

In order to replicate and spread, viruses need to hijack normal cellular processes, so understanding interactions of viral proteins with cellular proteins can indicate key processes impacted by the virus. Sequence analysis of SARS-CoV-2 isolates suggests that the 30kb genome encodes as many as 14 open reading frames (Orfs). The Orf1a/Orf1ab encodes a polyprotein, which is auto-proteolytically processed into 16 non-structural proteins (Nsps) that form the replicase/transcriptase complex (RTC) (Gordon et. al. 2020). A study done by a group at the University of California, San Francisco determined a protein interaction map to depict all of these interactions. In doing so, they were able to identify the major cell processes of the interacting proteins. Some of these include DNA replication, epigenetic and gene expression regulators, vesicle trafficking, RNA processing and regulation, nuclear transport machinery, cytoskeleton, mitochondria, and extracellular matrix (Gordon et. al. 2020).

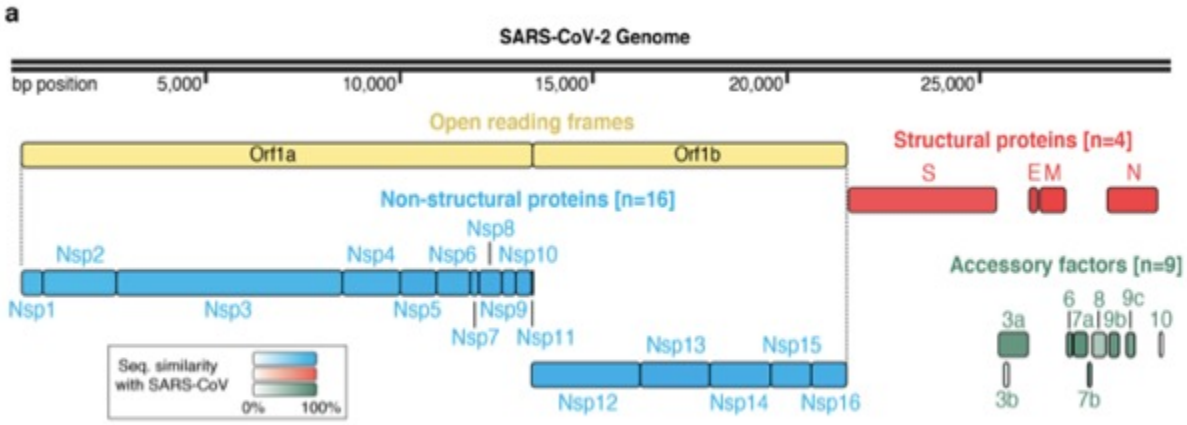

Figure 7: SARS-CoV-2 genes for viral proteins that interact with host cells (Gordon et. al. 2020)

This team discovered that approximately 40% of SARS-CoV-2 interacting proteins were associated with endomembrane compartments or vesicle trafficking pathways. In specific, host interactions of Nsp8 (signal recognition particle), Orf8 (endoplasmic reticulum protein quality control), M (ER morphology), and Nsp13 (centrosome and Golgi organization) may facilitate dramatic reconfiguration of ER/Golgi trafficking during the coronavirus infection, and interactions in peripheral compartments by Nsp2 (WASH), Nsp6 and M (vacuolar ATPase), Nsp7 (Rabs), Nsp10 (AP2), E (AP3), and Orf3a 9 (HOS) may also modify endomembrane compartments to favor coronavirus replication (Gordon et. al. 2020).

Another interaction determined is that of wild-type Nsp5 with the epigenetic regulator histone deacetylase (HDAC). Gordon et. al. (2020) predicted a cleavage site between the HDAC domain and the nuclear localization sequence, suggesting that Nsp5 may inhibit HDAC2 transport into the nucleus, potentially impacting HDAC2's mediation of inflammation and interferon response. Another interaction they identified with Nsp5 is the cleavage of tRNA methyltransferase (TRMT1) by this enzyme, likely resulting in an exclusively mitochondrial localization.

In addition, several innate immune signaling proteins are targeted by SARS-CoV-2 viral proteins. For example, the IFN pathway is targeted by Nsp13 (TBK1 and TBKBP1), Nsp15 (RNF41/Nrdp1) and Orf9b (TOMM70). Also, the NF-κB pathway is targeted by Nsp13 (TLE1, 3, and 5) and Orf9c (NLRX1, F2RL1, NDFIP2). There are also two other E3 ubiquitin ligases that regulate antiviral innate immune signaling, TRIM59 and MIB1, that are bound by Orf3a and Nsp9 respectively (Gordon et. al. 2020). NUP98-RAE1, an interferon-inducible mRNA nuclear export complex that is often taken over or degraded by multiple viruses was identified to have interactions with Orf6. This viral protein in SARS-CoV antagonizes the host interferon signaling by perturbing nuclear transport, and Gordon et. al. suspect that NUP98-RAE1 interaction with Orf6 may perform the same function for SARS-CoV-2.

This study identified many interactional processes between the SARS-CoV-2 viral proteins with the host proteins. This study is of significance, as it allows for the discovery of potential drug targets. This is crucial in the effort to discover any treatment or vaccine to stop the spread of the virus in the growing pandemic.

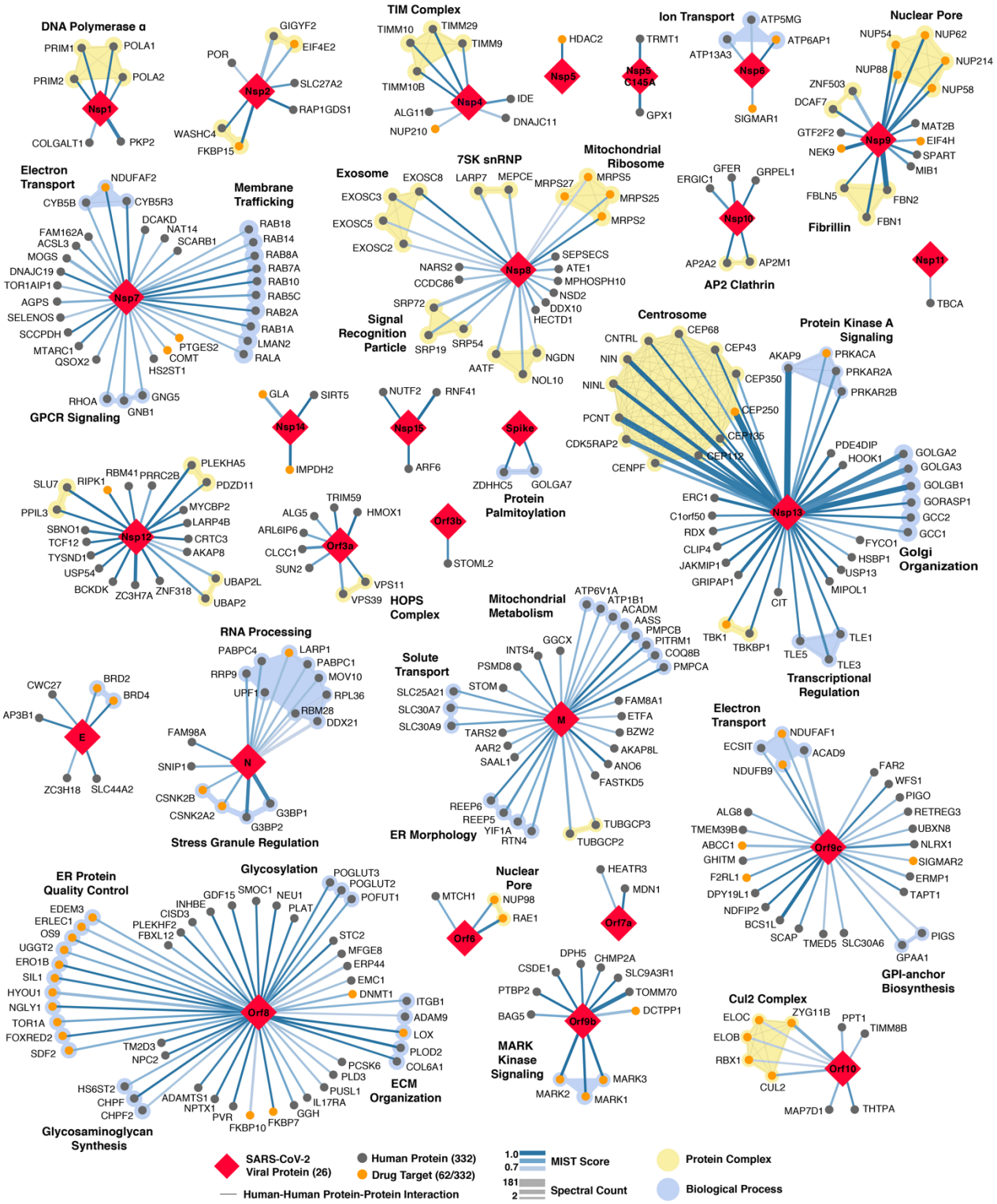

Figure 8: Determined protein Interactions between the viral proteins of SARS-CoV-2 and the host protein in cellular processes (Gordon et. al. 2020)

Potential Drug Targets

With the transmission rate globally increasing during the pandemic, it is vital that researchers work to find both a treatment for the virus, and vaccines to prevent its spread. With limited pre-existing knowledge, work to rapidly determine the viral structure and mechanism of infection, as well as to identify the viral-host interactions has made it possible to determine possible targets for drug therapy and vaccine design. The following analysis comprises the various strategies in determining drug targets, designing and testing both drug treatments and vaccines for COVID-19.

It has been determined that the SARS-CoV-2 S protein plays an important role in the mediating entry into host cells, thus it is an attractive drug target. Walls et. al. (2020) determined cryo-electron microscopy (cryo-EM) structures of the S ectodomain trimer, thus providing blueprints for the design of potential vaccines and inhibitors of entry. The RBD of the SARS-CoV-2 S protein is one of the most likely targets for the development of the virus attachment inhibitors, neutralizing antibodies, and vaccines. As hACE2 is the host receptor for the SARS-CoV-2 S protein RBD, blocking this interaction presents another target for treatment, especially appealing for vaccines. Because the crystal structure of the SARS-CoV-2 RBD/hACE2 complex has been solved (Shang et. al. 2020), other groups are able to use this information to devise a strategy of inhibition. Due to the significance of the receptor function in SARS-CoV-2 infection, Lei et. al. (2020) devised a recombinant protein of the extracellular domain of the hACE2 fused with the Fc region of the human immunoglobulin IgG1 (termed ACE2-Ig) to test whether the ACE2 fusion protein had the potential to neutralize coronaviruses. Results showed that ACE2-Ig shows high-affinity binding to the RBD of both SARS-CoV and SARS-CoV-2, and most importantly, displays potent cross-reactivity against both strains of the virus in vitro (Lei et. al.

2020). Despite the heavy glycosylation of membrane proteins complicating the targeting of the Spike-ACE2 interaction, this study supports further investigations of this recombinant ACE2 receptor for diagnosis, prophylaxis, and treatment for the novel coronavirus.

Another target of interest regarding viral structure is the fusion machinery of SARS-CoV-2. Xia et. al. (2020) demonstrated that SARS-CoV-2 exhibits much higher capacity of membrane fusion than SARS-CoV, suggesting that fusion inhibitors might be an attractive method of treatment. Therefore, they compared the 6-HB fusion core structure of both SARS-CoV-2 and SARS-CoV proteins to investigate the structural basis for membrane fusion mediated by the S proteins. This group used their work from previous studies where they designed a pan-coronavirus fusion inhibitor peptide to test the efficacy of this method of treatment that will be further explained later in this analysis.

It has been determined that protease cleavage is required for activation of the fusion potential of the S protein for coronaviruses. CoV S proteins may be cleaved by one or several host proteases, and the availability of these proteases on target cells largely determines whether CoVs enter cells through the plasma membrane or via endocytosis (Ou et. al. 2020). In studying endosome dynamics, Ou et. al. determined that PIKfyve, the main protease synthesizing PI(3,5)P2 in the early endosome, which regulates the early endosome to late endosome dynamics, might be a potential general drug target for viruses that enter the cell through endocytosis. This group found that inhibition of this enzyme with the drug apilimod significantly reduced entry of SARS-CoV S pseudovirions on human embryonic kidney 293 (HEK293)/hACE2 cells. Thus, this presents data to support further research on testing similar methods with SARS-CoV-2.

A group of researchers in China, Jin et. al. (2020), determined the CoV main protease (M^{pro}) to be another attractive drug target, as it plays an essential role in processing polyproteins that are translated from the viral RNA. It has been determined that inhibiting the activity of this enzyme would block viral replication (Zhang et. al. 2020). Because of the functional importance of M^{pro} in the viral life cycle, in addition to the absence of closely related homologues in humans, the protease as an especially interesting target for antiviral drug design (Jin et. al. 2020).

As a part of the viral life cycle all coronavirus mRNAs rely on cap-dependent translation to produce their proteins. Therefore, Gordon et. al. (2020) determined that the key eIF4F-cap binding complex constituents – the cap binding protein eIF4E, scaffold protein eIF4G, and the DEAD-box helicase eIF4A – are candidates for therapeutic targeting. This group claims that specific targeting of viral translation by interfering with the eIF4F complex formation or the interactions between viral proteins N, Nsp2, Nsp8, and the translation machinery may yield therapeutic benefits. It has also been found that cotranslation entry into the secretory pathway is a potential target for SARS-CoV-2 inhibition. Gordon et. al. predicts that up to ten SARS-CoV-2 proteins undergo ER membrane insertion mediated by the Sec61 translocon, which is known to localize to SARS-CoV replication complexes. Through their protein interaction study, they established high confidence interactions between the viral Nsp8 protein with three SRP components, which suggests viral hijacking of Sec61-mediated protein translocation into the ER. Thus, Sec61 is a potential drug target for the inhibition of protein biogenesis, as it would block SARS-CoV-2 replication and assembly (Gordon et. al.), although discovering drugs that target Sec61 and are selective enough to impede the virus, but not cells generally might prove difficult.

Potential Treatments

Drug Treatments

There are many aspects of SARS-CoV-2 infection that present targets for drug treatment. The first is membrane fusion. As mentioned previously, SARS-CoV-2 exhibits a high capacity for membrane fusion, so this process is especially attractive for drug targeting. Xia et. al. (2020) designed a pan-coronavirus fusion inhibitor peptide, EK1 that targets the HR1 domains of HCoV S proteins, which proved to be effective in inhibiting infection of 5 HCoVs, including SARS-CoV, MERS-CoV, and 3 SARS-related CoVs. To test the inhibitory activity of this peptide on SARS-CoV-2, the team conjugated a cholesterol molecule to the EK1 peptide, and found that one of the lipopeptides created, E1C4, exhibited highly potent inhibitory activity against SARS-CoV-2 S-mediated membrane fusion in 293T/ACE2 cells. Thus, this fusion inhibitor presents positive findings as a possible treatment for COVID-19.

Another aspect to control with treatment is viral infectivity. Gordon et. al.(2020) devised through multiple assays that two classes of molecules emerged as effectively reducing viral infectivity: protein biogenesis inhibitors and the ligands of Sigma1 and Sigma2 receptors. Protein biogenesis inhibitors they tested included the eIF4A inhibitor zotatifin, and the elongation factor-1A (eEF1A0) inhibitor, ternatin-4[50]. Zotatifin, which is currently being used in clinical trials for cancer therapy, demonstrated a strong antiviral effect. They also observed potent antiviral effects of ternatin-4[50], suggesting that the rate of translation elongation is critical for obtaining optimal levels of viral proteins. This information supports further research in testing protein biogenesis inhibitors for treatment of COVID-19. Molecules that target Sigma1 and Sigma2 receptors perturb the virus through different mechanisms than the translation inhibitors, potentially including the cell stress response (Gordon et. al. 2020). In their study, they

tested many ligands for these receptors, including haloperidol, PB28, PD-144418, and hydroxychloroquine, which are undergoing clinical trials in COVID-19 patients. PB28 was found to have substantial selectivity for the Sigma receptors versus side-effect targets, such as the hERG ion channel, whereas hydroxychloroquine showed a lack of selectivity versus hERG and other off-targets. Through assays, Gordon et.al. concluded that PB28, zotatifin, and hydroxychloroquine all decreased the detection of the viral NP protein, indicating the antiviral effect occurs before the viral protein exits the cell.

A third drug target that has been extensively tested is the inhibition of the main CoV protease, M^{pro}. Jin et.al. (2020) designed a Michael acceptor inhibitor, N3, by computer-aided drug design. The team then performed a kinetic analysis to assess the efficacy of N3 for SARS-CoV-2. Through this, they determined that it is a time-dependent irreversible inhibitor of the protease, and they suggested that the Michael acceptor has potent inhibition for the virus. They also conducted an antiviral activity assay using the organoselenium compound, ebselen, which has anti-inflammatory, anti-oxidant and cytoprotective properties. This compound presents an interesting treatment option, as it has extremely low cytotoxicity and its safety in humans has been evaluated by a number of clinical trials (Kil et. al. 2017). In a plaque-reduction assay, ebselen was found to display significant inhibition against SARS-CoV-2, strongly suggesting the clinical potential of ebselen for coronavirus treatment (Jin et. al.).

Vaccines

Many methods of vaccine design have been used in the process of creating a viable COVID-19 treatment. These methods range from using cross-reactivity from other CoV strains, using samples from COVID-19 patients in the recovery stage, to implementing the use of synthetic DNA vaccines. Tai et. al. (2020) found that SARS-CoV RBD-specific polyclonal antibodies

cross-reacted with SARS-CoV-2 RBD protein and inhibited SARS-CoV-2 entry into hACE2-expressing cells. Therefore, since these polyclonal antibodies have the potential to cross-neutralize a SARS-CoV-2 pseudovirus infection, they suggest the potential that a SARS-CoV RBD-based vaccine would have as a candidate for the prevention of infection with COVID-19. Similarly, it has been found that SARS-CoV murine polyclonal antibodies have the potential to inhibit SARS-CoV-2 S mediated entry into cells, indicating that cross-neutralizing antibodies targeting conserved S epitopes can be elicited upon infection (Walls et. al. 2020).

In a study done by Wen et. al. (2020), immune cell profiling of COVID-19 patients in the recovery stage yielded possible candidates for vaccine design. They aimed to characterize the changes in peripheral blood mononuclear cells (PBMCs). Results demonstrated that compared to healthy control cells, monocytes containing high inflammatory gene expression and $IL1\beta^{+}$ subsets predominated, and $CD4^{+}$ T cells decreased remarkably. It was also determined that the antibody IgA was over-represented, demonstrating that the infection had elicited generally mucosal immunity. Although this mucosal immunity will help in the recovery of COVID-19, it presents a cause for concern about whether this method will allow for sustained immunity, as it tends to be less long-lasting. They also found that T and B cell clones were highly expanded during the recovery stage. Because T cells and B cells play an important role in adaptive immunity, the several B-cell receptor and T-cell receptor changes that were found in the recovering patients are believed to be helpful for vaccine and antibody production (Wen et. al.).

The design and pre-clinical testing of a synthetic DNA vaccine candidate was detailed in a study conducted by Smith et. al. (2020). This group created a plasmid designed to encode the SARS-CoV-2 S protein with an IgE attached to it from sequences taken from the published genome of the virus, termed INO-4800. The humoral host responses for this vaccine were then

tested in pre-clinical trials in both mice and guinea pigs. From analyzing the immunogenicity of INO-4800, the results revealed IgG binding against SARS-CoV-2 S protein antigens, with limited cross-reactivity to SARS-CoV S protein antigens. In a neutralization assay, sera from INO-4800 immunized mice neutralized wildtype SARS-CoV-2 virus successfully. Additionally, Smith et. al. used the Hartley guinea pig model to assess the vaccine's immunogenicity. Results showed that, similarly as in mice, INO-4800 was capable of eliciting functional blocking antibody responses to SARS-CoV-2 spike protein. These data show that the vaccine induces cellular and humoral host immune responses that can be observed within days following a single immunization, including cross-reactive responses against SARS-CoV (Smith et. al.). Thus, INO-4800 suggests promising results if it can be continually tested in clinical trials as a potential vaccine candidate for COVID-19.

Limitations

Although investment in biomedical and pharmaceutical research and development has increased significantly over the past two decades, the annual number of new treatments approved by the U.S. Food and Drug Administration (FDA) has remained relatively constant and limited (Zhou et. al. 2020). A recent study estimated that pharmaceutical companies spent $2.6 billion in 2015, up from $802 million in 2003, in the development of an FDA-approved new chemical entity drug (Avorn 2015). Researchers are currently looking for new ways to find treatments in response to the growing global health crisis in the most rapid and cost-effective way possible. Zhou et. al. devised that drug repurposing, represented as an effective drug discovery strategy from existing drugs, could significantly shorten the time and reduce the cost compared to de novo drug discovery and randomized clinical trials. For example, the nucleoside analog RNA-

dependent RNA polymerase inhibitor, Remdesivir, was originally developed as an anti-ebola drug, but is now in clinical trials to treat COVID-19. This drug works to target the viral RNA polymerase to specifically reduce viral infections, and is showing modest results thus far (Beigel et. al. 2020). Additionally, a program of combined structure-assisted drug design, virtual drug screening and high throughput screening to identify new drug leads can lead to the rapid discovery of drug leads with clinical potential in response to new infectious diseases for which no specific drugs or vaccines are available (Jin et. al. 2020).

Conclusion

The COVID-19 pandemic has alarmingly preoccupied the world since its inception and is escalating uncontrollably in many parts of the globe. Using 21st century technology, researchers are working rapidly to discover potential treatments and vaccines for the virus in order to mitigate the virus's impacts and prevent further destruction of the economy and wellness of the world's population. Literature demonstrates various methods of determining viral structure, especially novel characteristics, which have perpetuated the discovery of potential drug targets. Together with the knowledge of previously used drugs and vaccines, researchers have been able to identify many possibly drug treatments and vaccines. Due to the significant effort made by nations to implement practices of social distancing and the recent discovery of potential treatments, the future holds potential to put an end to the global health crisis.

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