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Review Article

Molecular Aspects of SARS-CoV-2 that Impact Public Health

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Abstract

The novel coronavirus SARS-CoV-2 and its associated disease COVID-19 originated in China and took the world in a storm, having been declared a pandemic on March 11, 2020. Within just three month, a vast amount of research led to the publication of 390 PubMed cited journal articles. While much of the research focuses on the epidemiology and morbidity of the virus, there is also an increasing body of information about the viral molecular biology, genetics and evolution. Among the structural proteins of the virus, the spike protein is important because it mediates the recognition by the host cell through the ACE-2 receptor. Comparative analysis of the spike proteins of multiple coronaviruses led to the identification of bats as the animal of viral origin with multiple intermediate animal hosts being discussed (e.g. turtles). The spike protein has been proposed as a target for the development of vaccines and treatments. Drug targets include the ACE-2 receptor, as well as a serine protease that serves as a primer for the spike protein. Similarity of the spike protein with that of the 2003 SARS-CoV virus may aid the vaccine development.

The replication machinery has been well investigated for SARS-CoV with a special emphasis on the proofreading ability of the RNA polymerase, which is unusual for an RNA virus and lowers the mutation rate. Similar studies on SARS-CoV-2 have by all appearances not been done yet, but first mutations of the virus have been identified in multiple patients, documenting a need to monitor the evolution of the virus. Replication inhibitors in general have been tested for their effectiveness at inhibiting SARS-CoV-2 with promising preliminary results.

Keywords: *SARS-CoV-2; COVID-19; Coronavirus; Pandemic; Spike Protein; Viral Replication*

Abbreviations

SARS: Severe Acute Respiratory Syndrome; MERS: Middle East Respiratory Syndrome; CoV: Coronavirus; COVID 19: Corona Virus Disease 2019; WHO: World Health Organization; ACE-2: Angiotensin Converting Enzyme 2; TMPRSS2: Transmembrane Protease, Serine 2

Introduction

A new virus named SARS-CoV-2 and the associated disease COVID-19 has spread at an alarming speed across the world, with a first detection in China [22,38,39]. On March 11, 2020, this outbreak was declared a worldwide pandemic by the World Health Organization (WHO). SARS-CoV-2 is a member of the coronaviruses of the family Coronaviridae that also includes SARS-CoV (reviewed by [5]) and MERS (reviewed by [4]), which caused outbreaks in 2003 and 2012, respectively. All three of these viruses are RNA viruses with a positive-sense single-stranded RNA genome. Using SARS-CoV as a comparison, this mini review summarizes what is currently known about the molecular biology and genetics of SARS-CoV-2 and how this impacts the unique characteristics of this novel virus. Topics that relate to public health, such as vaccine and drug development are included.

Review

The short history of a novel virus

Including SARS-CoV, MERS, and SARS-CoV-2, the world has by now seen three coronaviruses in two decades [11]. SARS-like coronaviruses are often associated with bats [19] and bats are likely the reservoir for SARS-CoV with the possibility of an intermediate, yet unidentified host [31]. Likewise, the similarity (96% identical) of the SARS-CoV-2 genome sequence to a bat corona virus implies that bats may have served as reservoir for this new virus as well, again with the likelihood of an intermediate mammal that was sold for food on the Wuhan food market [38]. This assessment was confirmed using the non-coding flanks of the SARS-CoV-2 viral genome, though the precise species of bat could not be identified with this method [32]. In this sense, SARS-CoV-2 is a unique case of a respiratory pathogen that got transmitted to humans via a food borne outbreak [16]. Intriguingly, fecal-oral transmission of SARS-CoV-2 has also been discussed [36].

Unique characteristics of SARS-CoV-2

There are to date seven coronaviruses that infect humans. Of these, four cause mild respiratory distress, SARS- CoV MERS caused major respiratory syndromes, and SARS-CoV-2 is the new member of the group (for a comparative review, see [20]). Of these, MERS has by far the highest mortality rate of 35%, accompanied by a small case number of 857 with 334 deaths. In contrast, SARS-CoV infected more than 8,000 people at a mortality rate of approximately 10%. The number of cases for SARS-CoV will not be known for some time, the CDC indicated 3,487 cases with 68 deaths within the United States as of March 16, 2020 (www.cdc.gov).

While the lower mortality rate may already be a good reason for the rapid spread and wide distribution, there appears to be a large variety in the associated symptoms. A study by the Chinese Center for Disease Control and Prevention [33] included 72,314 cases and classified them into three categories; mild (no pneumonia or mild pneumonia), severe (dyspnea, respiratory frequency below 30/min, blood oxygen saturation below 93%), and critical (respiratory failure, septic shock, organ failure, secondary infections). In particular, the possibility of a spread through asymptomatic carriers [12] is of importance, as these people are less likely to get tested and diagnosed. The finding of increased levels of IL-2 and other cytokines in ICU patients implies that immunopathology impacts the severity of the disease [15].

The spike protein: recognition, cell entry, vaccine and treatment

Much of the molecular biology research on coronaviruses is focused on the spike protein, which attaches to the angiotensin converting enzyme 2 (ACE-2) receptor on epithelial cells of multiple organs, including the upper and lower respiratory tracts of the lungs. After cell entry and membrane fusion, the RNA gets released and translated. RNA and protein get packaged into new viruses at the golgi apparatus (for a review on coronavirus replication, see [28]). The genome sequence for SARS-CoV-2 from a patient in the US revealed ~90% amino acid identity between the spike proteins of SARS-CoV-2 and SARS-CoV [13]. As one difference, the 536th amino acid on the SARS-CoV-2 spike protein is an asparagine, while the same position in SARS-CoV is occupied by an aspartate [2]. Among the similarities, the SARS-CoV-2 receptor binding motif that interacts with ACE-2 is similar to that of SARS-CoV and it was concluded that SARS-CoV-2 also uses the ACE-2 receptor [30]. Further insight into the molecular basis of SARS-CoV-2 recognition was obtained from the 2.9 A crystal structure of the spike protein [35]. ACE-2 forms a dimer of heterodimers between ACE-2 and a neutral amino acid transporter B0AT1, while the collectrin-like domain of ACE-2 facilitates the formation of homodimers. The receptor binding domain of the spike protein recognizes the extracellular peptidase domain of ACE-2, primarily via polar amino acids.

The spike protein has been the target of research that is geared towards the development of disease treatments and vaccines. The large degree of identity between the SARS-CoV and SARS-CoV-2 spike proteins permit SARS-CoV antibodies to cross-react with SARS-CoV-2 [13] and sera from recovering SARS patients to cross-neutralize cell entry of SARS-CoV-2 [14]. In an effort to identify vaccine targets, a set of B cell and T cell epitopes were derived from the spike proteins of SARS-CoV and SARS-CoV-2 that mapped identical to both proteins [1].

On the treatment end, a peptide inhibitor of the S2 subunit of the spike protein (designated EK1) was proposed as putative treatment for multiple coronaviruses and is headed towards animal models and clinical trials [34].

After the spike protein binds to the ACE-2 receptor, cell entry by SARS-CoV-2 is dependent on the transmembrane serine protease TMPRSS2 which serves as primer for the spike protein. Camostat mesylate, an inhibitor of TMPRSS2 that is already approved for clinical use in Japan [17] was able to block entry of the SARS- CoV-2 virus [14]. This means the protease inhibitor could potentially be used as a treatment option. Altogether, it seems like virus recognition and host cell entry via the spike protein on the virus, and ACE-2 and TMPRSS2 on the host cell may offer options for the development of both, disease treatments and vaccines (Figure 1). As a word of caution, diabetes patients and people with hypertension who are treated with ACE-2 inhibitors often have increased expression of ACE-2 and might be at an increased risk for COVID-19 [9].

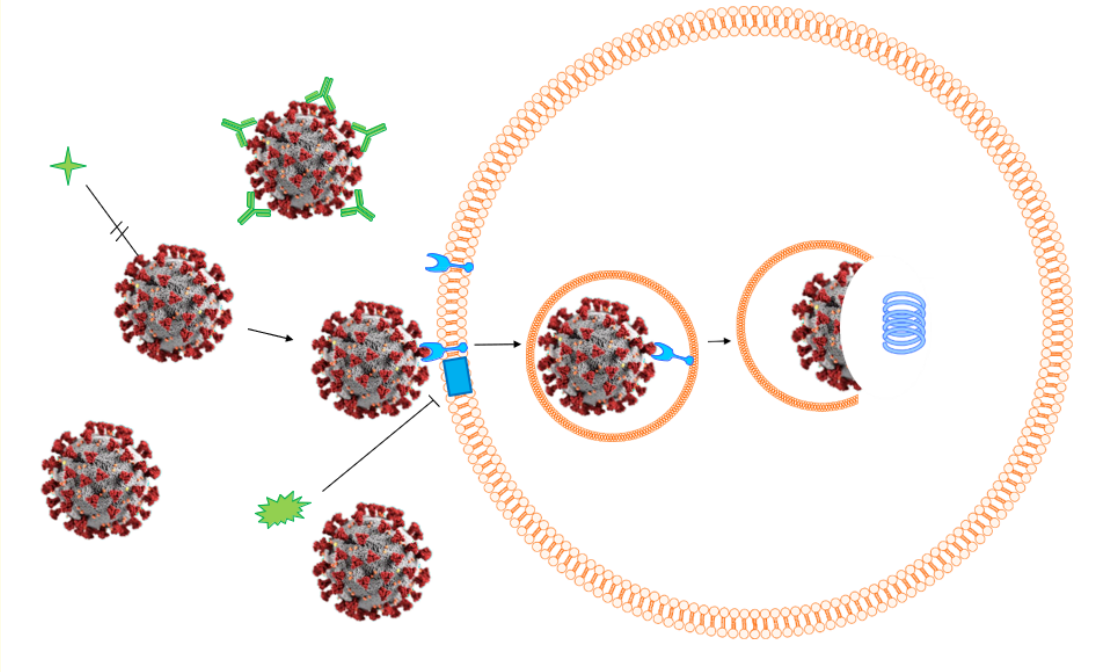

Figure 1: *Schematic of the recognition of SARS-CoV-2 by the ACE-2 receptor and consecutive cell entry and release of the single stranded RNA molecule. Potential actions by SARS-CoV antibodies and camostat mesylate are included. The virus molecule is from the CDC website (www.cdc.gov), all other elements are from Motifolio. The bone like structure in blue is the ACE-2 receptor on the human host cell, accompanied by the TMPRSS2 protease, represented by the blue rectangle. The blue spirale is the viral RNA. In green are antibodies against SARS-CoV spike protein able to recognize SARS-CoV-2 [13], camostat mesylate that inhibits TMPRSS2 [14] and the EK1 peptide that inhibits the S2 subunit of the spike protein [34].*

The spike protein has also been used for comparative analysis between different coronaviruses on the search for an intermediate host. An analysis of the receptor-binding domain on the spike protein and the ACE- 2 host receptor has led to the identification of key amino acids that suggest turtles as a potential intermediate host involved in the transmission of the virus from the original bats to humans [21]. The identification of the intermediate host is important to prevent further spread of the virus and its associated COVID disease.

RNA replication: another drug target

Once the RNA has been released from the endosome, the next step in the coronavirus life cycle is the translation of the replicase gene that makes up two thirds of the viral genome and encodes for two polyproteins, which contain up to 16 non-structural proteins (for a review on coronavirus replication, see [10]. Since the RNA polymerase does not possess any proofreading ability, the mutation rate for most RNA viruses is about one mutation per genome per replication [23]. This puts the virus at risk of accumulating deleterious mutations and poses limitations to the genome of an RNA virus, which is usually around 15 kb [7]. The SARS-CoV genome contains a 3'-5'-exoribonuclease activity (ExoN) on non-structural protein 14 (NSP14) that is involved in the synthesis of multiple RNAs [24] and capable of removing single 3'-mismatched nucleotides during replication [3]. In fact, mutants lacking the ExoN activity were 300 times more sensitive to 5-fluorouracil [27]. This proofreading function that is unusual for an RNA virus may explain the unusually large size of the SARS-CoV RNA genome of 30 kb. ExoN has also been proposed as a pan-CoV drug target [27].

While limited information is available to date on the SARS-CoV-2 replication enzymes, their proof reading ability, and the rate of mutation, first mutations have been identified in patients. One study found mutations in five genes, including the ones encoding the spike and nucleocapsid proteins [37]; of these, more than half were synonymous mutations. A study on the genomic diversity of SARS-CoV-2 in eight patients showed an increased genomic diversity of the virus in these patients [26]. It was concluded that the virus evolves *in vivo* in the patients, which has possible implications on its transmissibility, as well as the severeness of the disease and, therefore, needs to be monitored.

Like the spike protein, the replication machinery of SARS-Co-2 has been proposed as a drug target. In particular, chloroquine is undergoing clinical studies in China with promising preliminary results [8]. The US Food and Drug Administration (www.fda.gov) is looking at widespread clinical trials. The Chinese efforts have been systematically reviewed by a US team of researchers [6], who came to the conclusion that there is sufficient pre-clinical evidence of effectiveness, as well as safety. However, they recommended that clinical use should still follow the normal approval stages of a trial as stated by the WHO. Chloroquine is a drug that has been used to treat malaria for many years with increasing development of resistance [40], numerous side effects [25] and unsuitability for certain categories of patients [18,29].

Conclusion

Altogether, an impressive amount of research has been done over the short period of three month on this new and emerging pathogen and the understanding of the life cycle of coronaviruses in general and SARS-CoV-2 in particular has increased drastically. There is an emergence of aspects of the molecular biology and genetics of the virus, in particular the structural spike protein and several of the non-structural replication proteins that offer targets for the development of vaccines and disease treatments. Some of these are in the experimental stage and others are already headed into trials in individual countries. There is hope that the urgency of the pandemic will intensify research and trial efforts and that we will have a vaccine and possible multiple treatments within the near future.

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Conflict of Interest

The author declares no financial interest of other conflict of interest.

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