The biology of SARS-CoV-2 and the ensuing COVID-19

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Abstract

Recently there has been a worldwide concern about the novel coronavirus SARS-CoV-2 which originated in China but rapidly spread internationally posing a global health emergency. As a Betacoronavirus (βCoV), SARS-CoV-2 is a positive single-stranded RNA virus and infects the respiratory tract. The ensuing disease has been named COVID-19. Genome structural analyses of SARS-CoV-2 have revealed genomic similarities but low evolutionary relationship to existing SARS. The S glycoprotein is vital for cell adhesion and virus entrance to host cells. Cell entry depends on ACE2, as already described for βCoVs, but recent studies proposed a newly discovered receptor, CD147. Genome RNA translation encodes structural and unstructural proteins starting with ORF1a and ORF1ab which produce non-structural proteins (nsps) with different functions. Although nsps are conserved among βCoVs, mutations in nsp2 and nsp3 may play an important role in viral transmission and cell and tissue tropism. Currently, no vaccine or specific antiviral treatments are available for COVID-19. As a result, preventive measures are the main strategy to limit the spread of the virus.

Key words: *COVID-19; SARS-CoV-2; genome structure; viral entry; ACE2, therapy*

INTRODUCTION

The 2019 novel coronavirus (2019-nCoV) was recently named SARS-CoV-2 by the World Health Organization (WHO). The disease caused by SARS-CoV-2 has been named COVID-19 [1]. The origin of the novel coronavirus is not known with certainty. There are those who claim that SARS-CoV-2 originated through laboratory manipulations. However, the study of coronaviruses implicates the use of reverse genetic systems using BACs, in vitro ligation or vaccinia virus vectors to study virus RNA replication [2]. Genetic and structure analyses among sequences derived from different existing CoVs indicate that SARS-CoV-2 is a novel coronavirus which originated due to natural selection either in an animal host before zoonotic transfer or following zoonotic transfer [3]. Mostly, it is believed that bats and palm civets are the natural reservoirs for SARS-CoV, and it was reported that SARS-Cov-2 infected humans in an animal market [4].

Coronaviruses cause respiratory and intestinal infections in humans. Human-to-human transmission of SARS-CoV-2 has been confirmed. Its sequence (MN908947) that was released by GenBank, is 96% identical to a bat coronavirus [5-7]. RNA viruses have an average evolutionary range of 10^{-4} nucleotide substitutions per site per year. This means that alterations to genome sequence arise during each replication site [8]. Coronaviruses are enveloped positive single-stranded RNA viruses ((+)RNA) with a genome of 27-32

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kb. They are classified into four genera: Alphacoronavirus (αCoVs), Betacoronavirus (βCoVs), Gammacoronavirus (γCoVs) and Deltacoronavirus (δCoVs), that share common ancestors and genomic structures. Evolutionary analyses have shown that bats and rodents are the gene sources of most αCoVs and βCoVs whereas avian species are the gene sources of most δCoVs and γCoVs [4]. SARS-CoV-2 belongs to the same family of viruses as the well-known severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus MERS-CoV and more precisely to Betacoronavirus, lineage b [6, 9, 10]. Virions have average diametres of 80-160 nm. Structural analysis has revealed genomic similarity between the three virus genomes. Genome phylogenetic analyses have revealed a greater similarity of 2019-nCoV to SARS-CoV than MERS-CoV [11]. Nevertheless, because SARS-CoV and MERS-CoV showed approximate similarities of 79% and 50% with SARS-CoV-2, respectively, that are considered low evolutionary relationships, SARS-CoV-2 is considered a novel human Betacoronavirus [8]. Currently, diagnosis is based on robust molecular techniques such as Real-Time PCR by detecting amplicons for the most preserved viral genes [12]. Although, to date, there is no available treatment for COVID-19 or a vaccine against the virus SARS-CoV-2, anti-viral and anti-inflammatory therapies used in other diseases are being tried. Drugs that have been designed for other viruses, such as Ebola or HIV-1, and treatment regimens for other diseases such as malaria, are being tested in COVID-19 patients with promising results [13-16].

GENOME STRUCTURE

SARS-CoV-2 genome consists of a single positive-strand RNA of almost 29,900 nucleotides and 38% G+C content, encoding 9,860 amino acids. Genome characterization showed two flanking untranslated regions, the 5'UTR -265 nucleotides- and the 3' UTR -358 nucleotides-long [4]. The genomic RNA has a 5' cap and a poly-A 3' tail and numerous open reading frames (ORFs). The virus replicase is encoded by two large ORFs, ORF1a and ORF1b. This RNA codes for both structural and unstructural proteins with different functions. Structural proteins are encoded by the 3'-terminus and include envelope glycoprotein spike (S), envelope (E), membrane (M) and nucleocapsid (N) protein [17, 18]. The 5'-terminal of the genome consists of accessory genes that are species-specific and encode polyproteins pp1a and pp1b. Polyprotein pp1a is further divided into nonstructural proteins that participate in genome transcription and replication [4, 6, 19]. Genome structure analysis revealed a main difference between lineage A β-CoVs and SARS-CoV-2 because the latter lacks the hemagglutinin-esterase gene in the 3'-terminus [4]. Structural proteins M, N, and E of SARS-CoV-2 showed a similarity over 90% to the known coronaviruses. However, the reference sequence-based analysis confirmed a reduced genetic similarity of the S protein [8]. Although great genome similarities are observed throughout the Betacoronaviruses, it is known that the gene that encodes for the spike glycoprotein is the least conserved, with genome identity reaching 74-83%. Full-length genome analysis showed that the S gene of SARS-CoV-2 is longer than in other SARSr-CoVs. There are three short insertions in the N-terminal domain and alterations in four of five of the residues in the receptor-binding motif. The role of the spike glycoprotein is to form spikes on the surface of coronaviruses and is responsible for the entrance of the viruses into the host cells [6, 18].

CORONAVIRUS SARS-COV-2 STRUCTURE AND MAIN STRUCTURAL PROTEINS

Coronaviruses are spherical with spikes on the surface. Recently it has been argued that not all coronaviruses encode for the same structural proteins for a complete virion [20]. SARS-CoV-2 in comparison to other viruses of the same family lacks hemagglutinin-esterase (HE) protein. The protein that has been studied most is the S-spike glycoprotein. It is a type I transmembrane protein and consists of a large ectodomain, a single-pass transmembrane anchor and a short C-terminal intracellular tail. The role of that protein is well defined. S-spike glycoprotein is crucial for cell adherence and entry to the host cell [1, 8, 21]. However, the entry requires priming of the S protein. SARS-CoV-2 needs serine protease TMPRSS2 to cleave S into S1/S2 and S'; this was deduced because an inhibitor for TMPRSS2 could block viral entry [22]. The envelope (E) is a small transmembrane protein involved in the life cycle of the virus. It consists of three domains and functions as an ion-channeling viroporin. E contains a binding motif which acts as a protein-protein interaction module and is involved in host cell processes and SARS-CoV pathogenesis [20]. The M protein is a membrane glycoprotein that supports the viral envelope and is the most abundant structural protein. M consists of three transmembrane domains, can adopt two conformations and plays a key role in virion shape and size. M plays a key role in organizing

coronavirus assembly and interacts with some of the major structural proteins [20, 23]. Finally, the N protein binds to the RNA genome forming the helically symmetric nucleocapsid. Phosphorylation of protein residues by glycogen synthase kinase 3 (GSK3) activates the N protein. Inhibition of GSK3 in cells infected with SARS-CoV, resulted in a restriction of viral replication [17, 21].

ATTACHMENT AND ENTRY TO THE HOST CELL

Infection starts when the virus attaches to its cellular receptor on the surface of the host cell and is endocytosed. The trimeric S transmembrane protein is responsible for the virus attachment and entrance and consists of two subunits, S1 and S2, which have distinct roles. S1-ectodomain binds to the receptor and initiates a structural change to the S2 subunit, which is essential for membrane fusion between the virus envelope and the host membrane. The activation of the S protein requires sequential cleavage by endosomal proteases. For example, cysteine protease cathepsin L activates the S protein in SARS-CoV [9, 21]. The S1/S2 cleavage of coronavirus S protein is mediated by host proteases at the time of infection. The SARS-CoV S1-protein contains a receptor binding domain (RBD) and implicates 14 amino acids, 9 of which recognize the angiotensin-converting enzyme 2 (ACE2) [24, 25]. The S2-protein consists of fused peptides, internal fusion peptides and a second proteolytic site S2' which is furin-like, that are totally preserved in SARS-CoV-2 compared to SARS-CoV. It is possible that since furin is highly expressed in lungs, an enveloped virus such as SARS-CoV-2, that infects the respiratory tract, could take advantage of this convertase to activate the S protein and enter the host cell [9], although previous experimental data suggest that insertion of a furin cleavage site at the S1/S2 domain enhances cell to cell fusion without affecting viral entry [26]. To note, ACE2 in not exclusively expressed by lung cells; it is therefore possible that SARS-CoV-2 could also spread to other cells and organs, as is the case with SARS-CoV [27].

ROLE OF HOST ACE2 IN VIRAL ENTRY - OTHER HOST RECEPTORS

The recognition of the host receptor is vital for viral entry. As previously mentioned, the S1 protein of the SARS-CoV recognizes the angiotensin-converting enzyme 2 ACE2. Many studies include structural analyses that predict that SARS-CoV-2 also identifies ACE2 as a host receptor [4, 25, 28]. The spike glycoprotein structure analysis of SARS-CoV has revealed an RBD with a core structure and a receptor-binding motif (RBM) that binds to ACE2. When analyzing the RBM of both SARS-CoV and SARS-CoV-2, neither deletions nor insertions were found. The only alteration predicted was a one-residue insertion away from the binding domain; other coronaviruses that do not use ACE2 lack these residues [22, 25, 29]. In vitro experiments have also determined ACE2 as a functional receptor for SARS-CoV-2 and additionally showed that the novel virus does not use aminopeptidase N (APN) or dipeptidyl peptidase 4 (DPP4) to enter the cell, as is the case for MERS-CoV [12, 18, 23]. In addition to ACE2, it is likely that other receptors exist for S binding on the host cell. CD209L, which is expressed in human lung alveolar and endothelial cells, was previously reported as a putative cell receptor for SARS-CoV [30], and could be tested for SARS-CoV-2. A recent study reported that the S protein can bind to the CD147 receptor on the host cell, based on in vitro experiments that demonstrated that an anti-CD147 humanized antibody significantly inhibited SARS-CoV-2 entry into the cells [31]. The identification of all the receptors and the exact mechanism SARS-CoV-2 uses for cell entry will aid the quest for antiviral targets.

ssRNA REPLICATION AND VIRION SECRETION

Once the virus manages to enter the host cell, the viral nucleocapsid is released to the cytoplasm. Due to the nature of their genome, viruses have to exploit the host's replication machinery. Single stranded RNA positive sense is the same sense as mRNA. Sense strand contains the exact nucleotide sequence to mRNA, which encodes for a functional protein. The genomic RNA serves as a template and polyproteins pp1a and pp1ab are at first encoded and cleaved by virally produced chymotrypsin-like protease (3CLpro) or main protease (Mpro) and one or two papain-like proteases to form 16 nsps which participate in minus-strand RNA synthesis, genome replication and subgenomic RNA [21, 32, 33]. A number of these nsps, the N protein, host proteins and the endoplasmic reticulum (ER), compose coronavirus replicative structures where viral RNA synthesis takes place [34]. The role of each nsp is well defined. For example, the RNA-dependent RNA polymerase is encoded in nsp12 [35], nsp3 participates in polyprotein processing as a protease, nsp8 and nsp9 bind to cis-acting elements of the viral RNA [36, 37]. Although genome replication and transcription are catalyzed by the viral replicase, in some cases the host machinery is implicated. Full-length gRNA is replicated using a complementary

negative sense RNA molecule as an intermediate, while smaller RNA molecules (subgenomic RNA) produce structural and accessory proteins. New virions form in the ER and mature virions are secreted [21]. Obviously, the nsps play an important role in genome replication and virus formation. The ORF1ab of SARS-CoV-2 was recently analyzed to investigate possible alterations in genome structure caused by selective pressure on the virus. The analysis predicted alterations in position 321 of the nsp2 protein and positions 192 and 543 of the nsp3 protein. These alterations may be related to the differences between SARS-CoV-2 from SARS-CoV, and to SARS-CoV-2 contagion [38].

COVID-19 THERAPEUTIC STRATEGIES

No specific treatment is available for COVID-19, and investigations are under way to test whether existing treatment regimens for other viruses are also effective for COVID-19. The most notable examples include remdesivir (GS-5734), a nucleotide analog prodrug currently in clinical trials for treating Ebola, that inhibited the replication of SARS-CoV and MERS-CoV in tissue cultures and displayed efficacy in non-human animal models, and a combination of HIV-1 protease inhibitors lopinavir/ritonavir and IFN-β (LPV/RTV-INFb) that were shown to be effective in animal models and patients infected with SARS-CoV [13]. Additionally, chloroquine phosphate, an approved malaria drug, is being used in certain patients with COVID-19. Previous studies showed its therapeutic activity against viruses, including in vivo and in vitro experiments with existing human coronavirus OC43 and SARS-CoV [39]. Results from 100 Chinese patients demonstrated that treatment with chloroquine inhibited the exacerbation of pneumonia and shortened the disease course; it was proposed that chloroquine exerts anti-viral and anti-inflammatory activities by interfering with the glycosylation of cellular receptors for SARS-CoV-2 [40]. In addition to antiviral therapies, immunological therapies are also under consideration for COVID-19 treatment, mainly to lower the elevated cytokine levels observed in COVID-19 patients. Tocilizumab, a humanized antibody against the IL-6 receptor was recently used with encouraging results [15]. Baricitinib, fedratinib and ruxolitinib, which are selective JAK inhibitors approved for rheumatoid arthritis and myelofibrosis treatmen, are also likely to be effective against the consequences of the elevated levels of proinflammatory cytokines in COVID-19 patients [16]. The antibiotic teicoplanin, used to treat Gram-positive bacterial infections, was previously shown to inhibit cellular entry of SARS-CoV and MERS-CoV by inhibiting the activity of cathepsin-L; a recent study suggested that teicoplanin also inhibits the entry of SARS-CoV-2 into the host cells [41]. Another proposed method to treat COVID-19 is to transfuse patients with plasma from patients that have recovered from it; this method was used in the past with Ebola and H1N1 outbreaks. Plasma from recovered COVID-19 patients should include specific antibodies against SARS-CoV-2 capable of viremia suppression [14].

INFECTION, PATHOPHYSIOLOGY AND EPIDEMIOLOGY

COVID-19 is acquired by inhalation through droplets by symptomatic patients as well as from asymptomatic people and even by touching contaminated surfaces. Some of the most common symptoms include fever, cough, fatigue, pneumonia which can develop to acute respiratory distress syndrome, metabolic acidosis, liver, kidney and heart failure [42]. All ages are susceptible and certain groups of patients are at a higher risk [43]. Available epidemiological data show that most patients are 30 to 79 years old and the case-fatality rate increases in patients aged 70 years and older [44].

Hypertension has been studied and approved as a host risk factor associated with severe COVID-19 [45]. Other studies suggest several underlying comorbidities such as diabetes mellitus, cardiovascular diseases and respiratory system diseases [46].

Particular attention is being paid to patients with underlying cardiovascular diseases and other inflammatory disorders. ACE2, the functional receptor for SARS-CoV-2, exists as a membrane-bound enzyme (98%) and in a soluble state in blood and other body fluids (2%). In pathological cases, the concentration of soluble ACE2 is increased. Although there are conflicting reports about the role of ACE inhibitors in the treatment of cardiovascular diseases, to date there are no supporting evidence that ACE inhibitors or angiotensin II type 1 receptor blockers enhance coronavirus entry by increasing ACE2 expression [43, 47].

Factors that affect the disease severity are not fully investigated. Previous studies have revealed the cytokine profiles of SARS-CoV infected patients. Lymphopenia and CD4 and CD8 T-cell lymphocyte depletion, often encountered in viral infections, are probably associated with the disease. Recently, routine complete blood counts of COVID-19 patients confirmed the low levels

of lymphocytes [42]. Specific cytokines (IL-1β, IL-6, IL-10) showed an increase in COVID-19 patients; however, the plasma concentrations of Th1 cytokine IL-2 and Th2 cytokine IL-4 did not show a significant increase [48].

CONCLUDING REMARKS

There is no vaccine or effective treatment for COVID-19 at present. The epidemiological characteristics of the novel coronavirus SARS-CoV-2 differ dramatically from those of the previous coronavirus outbreaks, SARS-CoV in 2002-2003 and MERS-CoV in 2012. SARS-CoV-2 is more transmissible from human to human and the mortality rate is higher. Anti-retroviral therapies such as LPV/RTV-INFb and remdesivir have shown some hope. Another proposed method to treat COVID-19 is to transfuse patients with plasma from patients that have recovered from it; this method was used in the past with Ebola and H1N1 outbreaks [14]. The use of chloroquine, used for malaria treatment, as well as other anti-inflammatory drugs and especially Baricitinib may also help [16]. However, the need for direct and effective treatment is emerging and great efforts are made to develop a vaccine against SARS-CoV-2.

Since the COVID-19 epidemic outbreak, several genetic structure analyses have been conducted that revealed similarities among genomes extracted from existing coronaviruses. Notably, the feature of the genome that differs most in the novel coronavirus, is the S gene that encodes for the S protein that is responsible for viral attachment and entry to the host cell. An obvious target of a protein-based vaccine is therefore the S protein, which binds to the ACE2 receptor on the host cell, and especially the S1-subunit which plays a pivotal role in the adherence of the virus to the cell [12, 18, 22, 27, 29]. Another approach is to mask ACE2 by delivering an excessive soluble form of ACE2 as a competitor for the virus [49, 50]; a similar strategy may be tried for the CD147 receptor on the host cells [31]. In addition to targeting the receptor or its ligand, another approach could be the inhibition of the proteases that participate in S cleavage. TMPRSS2, a transmembrane protease serine may do this, thus inhibiting the priming of S protein and subsequent viral entry to the host cells [22, 50].

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