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REVIEW ARTICLE

Properties of Coronavirus and SARS-CoV-2

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Abstract

Zoonotic coronaviruses were discovered in the 1960s. Since then pathogenic human coronaviruses were identified beginning with the discovery of SARS-CoV in 2002. With the recent detection of SARS-CoV-2, there are now seven human coronaviruses. Those that cause mild diseases are the 229E, OC43, NL63 and HKU1, and the pathogenic species are SARS-CoV, MERS-CoV and SARS-CoV-2

Coronaviruses (order *Nidovirales*, family *Coronaviridae*, and subfamily *Orthocoronavirinae*) are spherical (125nm diameter), and enveloped with club-shaped spikes on the surface giving the appearance of a solar corona. Within the helically symmetrical nucleocapsid is the large positive sense, single stranded RNA. Of the four coronavirus genera (α,β,γ,δ), human coronaviruses (HCoVs) are classified under α-CoV (HCoV-229E and NL63) and β-CoV (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1). SARS-CoV-2 is a β-CoV and shows fairly close relatedness with two bat-derived CoV-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21. Even so, its genome is similar to that of the typical CoVs.

SARS-CoV and MERS-CoV originated in bats, and it appears to be so for SARS-CoV-2 as well. The possibility of an intermediate host facilitating the emergence of the virus in humans has already been shown with civet cats acting as intermediate hosts for SARS-CoVs, and dromedary camels for MERS-CoV. Human-to-human transmission is primarily achieved through close contact of respiratory droplets, direct contact with the infected individuals, or by contact with contaminated objects and surfaces.

The coronaviral genome contains four major structural proteins: the spike (S), membrane (M), envelope (E) and the nucleocapsid (N) protein, all of which are encoded within the 3' end of the genome. The S protein mediates attachment of the virus to the host cell surface receptors resulting in fusion and subsequent viral entry. The M protein is the most abundant protein and defines the shape of the viral envelope. The E protein is the smallest of the major structural proteins and participates in viral assembly and budding. The N protein is the only one that binds to the RNA genome and is also involved in viral assembly and budding.

Replication of coronaviruses begin with attachment and entry. Attachment of the virus to the host cell is initiated by interactions between the **S** protein and its specific receptor. Following receptor binding, the virus enters host cell cytosol via cleavage of S protein by a protease enzyme, followed by fusion of the viral and cellular membranes. The next step is the translation of the replicase gene from the virion genomic RNA and then translation and assembly of the viral replicase complexes. Following replication and subgenomic RNA synthesis, encapsidation occurs resulting in the formation of the mature virus. Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis.

Keywords: Coronavirus, SARS-CoV-2, classification, genetic characteristics, replication

INTRODUCTION

Before the SARS-CoV outbreak in 2002, two human coronaviruses, namely HCoV-229E and HCoV-OC43, were often seen as just one of the causes of the common cold. With the subsequent emergence of MERS-CoV in 2012 and the current COVID-19, understanding of the properties of coronavirus is needed to help determine the characteristics of SARS-Cov-2.

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It began on 31 December 2019, when the World Health Organization (WHO) was informed of cases of pneumonia of unknown aetiology in Wuhan City, Hubei Province, China.[1] A novel coronavirus was officially announced as the causative agent on 7 January 2020 and the viral genome sequence was subsequently released three days later for immediate public health support through the community online resource virological.org,[2] followed by four other genomes deposited on 12 January in the viral sequence database curated by the Global Initiative on Sharing All Influenza Data (GISAID). The genome sequences suggest presence of a virus closely related to SARS- CoV.

On 11 February 2020, the WHO named the novel coronavirus-induced pneumonia as Coronavirus Disease 2019 (COVID-19). At around the same time, the International Virus Classification Commission announced that the provisionally known 2019-nCov was named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).[3] SARS-CoV-2 is not a descendent of SARS-CoV. The name was chosen based on the established practice for naming viruses in this species and the relatively distant relationship of SARS-CoV-2 to the prototype SARS-CoV in a species tree and the distance space.

EPIDEMIOLOGY

As of 5 April 2020, 1,133,758 laboratory-confirmed human cases of COVID-19 have been notified to WHO with 62,784 deaths (fatality rate of 5.5%). Meanwhile, Malaysia reported 3483 confirmed cases and 57 (fatality rate of 1.6%) deaths.[4]

The available yet limited epidemiological and clinical data for SARS-CoV-2 suggest that the disease spectrum and transmission efficiency of this virus differ from those reported for SARS-CoV.[5-7]

Transmission of COVID-19

Symptomatic transmission refers to transmission from an individual with symptoms. This is the primary mode of transmission and it is via close contact through respiratory droplets, direct contact with the infected individuals, or by contact with contaminated objects and surfaces.[8-10] Evidence has shown that viral shedding is highest in the upper respiratory tract within the first 3 days from onset of symptoms.[11-12]

Pre-symptomatic transmission refers to transmission just before the symptoms appear. A few cases have been reported where individuals who were tested positive, actually transmitted the disease 1-3 days before they became symptomatic.[13] This indicates that the viral load may be high enough to enable transmission just before the symptoms appear.

Asymptomatic transmission refers to transmission during the incubation period which averages 5-6 days, and can extend up to 14 days. There are few reports of laboratory-confirmed cases who are truly asymptomatic, and to date, there has been no documented asymptomatic transmission.

Human-to-human Transmission of SAR-CoV

Transmission of SARS-CoV was fortunately relatively inefficient. Even though it also spread through direct contact, via droplets with infected individuals after the onset of illness, the outbreak was largely contained within households and healthcare settings.[14] There was of course, the exception of the few cases of super spreader events where one individual was able to infect multiple contacts due to the high viral load.

Animal to Human Transmission

Coronaviruses primarily infect birds and mammals, causing a variety of lethal diseases that particularly impact the farming industry.[15,16] Some CoVs were originally found as enzootic infections, limited only to their natural animal hosts, but have crossed the animal-human species barrier and progressed to establish zoonotic diseases in humans.

It has been widely accepted that SARS-CoV originated in bats since a large number of Chinese horseshoe bats contain SARS-related CoV sequences and showed serologic evidence of prior exposure to a related CoV.[17,18] Two novel bat SARS-related CoVs were actually found to be more similar to SARS-CoV.[19] These viruses were also found to use the same receptor as the human virus, angiotensin converting enzyme 2 (ACE2), providing further evidence that SARS-CoV originated in bats.

Next-generation sequencing work on SARS-CoV-2 shows 88% homology to two bat-derived CoV-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21.[20] Epidemiological data also suggests the possibility of an intermediate host facilitating the emergence of the virus in humans. This was already shown with civet cats acting as intermediate hosts for SARS-CoVs and

dromedary camels for MERS-CoV. Structural analysis also suggests that SARS-CoV-2 has a similar receptor-binding domain structure to that of SARS-CoV, which is the ACE2 in humans.

CORONAVIRUS CLASSIFICATION AND STRUCTURE

Classification of Coronaviruses

Coronaviruses (CoVs) are spherical and approximately 125 nm in diameter,[21,22] with club-shape spikes projecting from the surface of the virus giving the appearance of a solar corona, prompting the name, coronaviruses. Within the envelope is the helically symmetrical nucleocapsids, which is actually uncommon among positive-sense RNA viruses.

CoVs are classified under the order *Nidovirales*, family *Coronaviridae*, and subfamily *Orthocoronavirinae* (Fig. 1). With genome sizes ranging from 26 to 32 kilobases (kb) in length, CoVs have the largest genome for RNA viruses. Based on genetic and antigenic criteria, CoVs have been organised into four groups: alphacoronavirus (α-CoV), betacoronavirus (β-CoV, gammacoronavirus (γ-CoV) and deltacoronavirus (δ-CoV).[23,24]

For SARS-Cov-2, next-generation sequencing also shows 79% homology to SARS-CoV and 50% to MERS-CoV. Phylogenetic analysis has placed SARS-CoV-2 under the subgenus Sarbecovirus of the genus Betacoronavirus.[20]

Genomic Structure and Function of Coronaviruses

The organization of the coronavirus genome is 5′-leader-UTR- replicase-S (Spike)–E (Envelope)-M (Membrane)-N (Nucleocapsid)-3′UTR-poly (A) tail with accessory genes interspersed within the structural genes at the 3′ end of the genome (Fig. 2).

The four structural proteins are required by most CoVs to produce a structurally complete viral particle,[25,26] suggesting that some CoVs may encode additional proteins with overlapping compensatory functions.[27,28,29] While each of the major protein plays a primary role in the structure of the virus particle, they are also involved in other aspects of the replication cycle.

The S protein (~150 kDa) mediates attachment of the virus to the host cell surface receptors resulting in fusion and subsequent viral entry.[30,31] In some CoVs, the S protein also mediate cell-cell fusion between infected and adjacent, uninfected cells resulting in formation of multinucleated giant cells, a strategy that allows direct viral

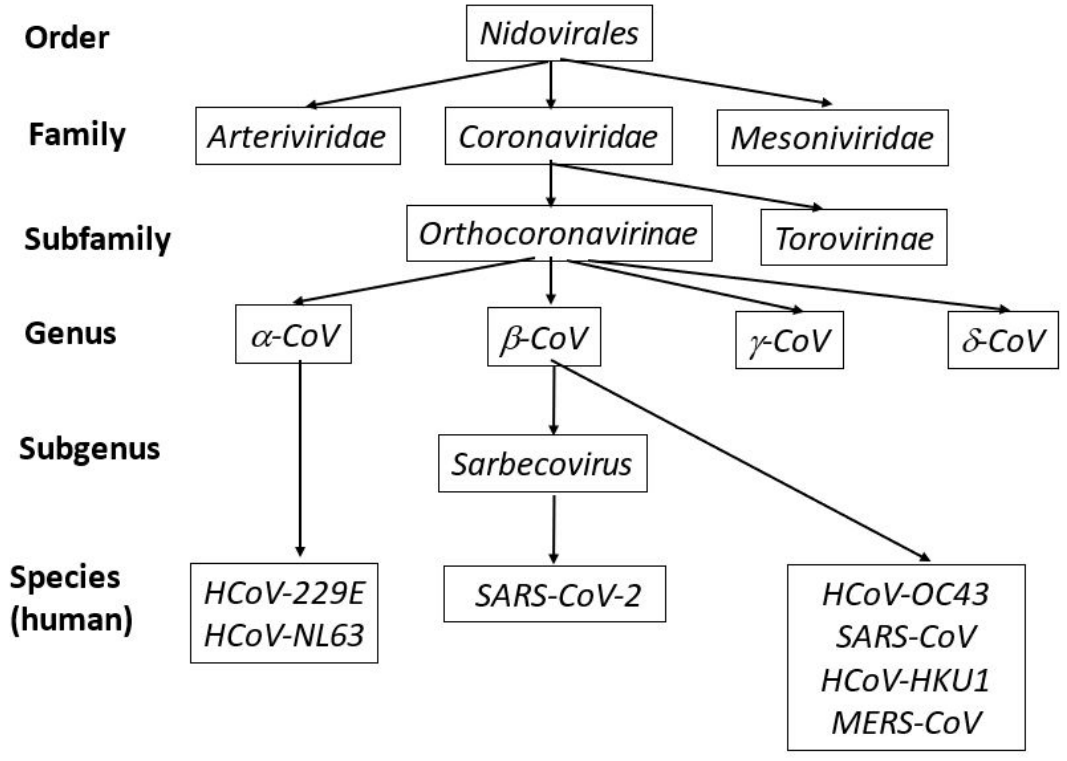

FIG. 1: Classification of Human Coronaviruses

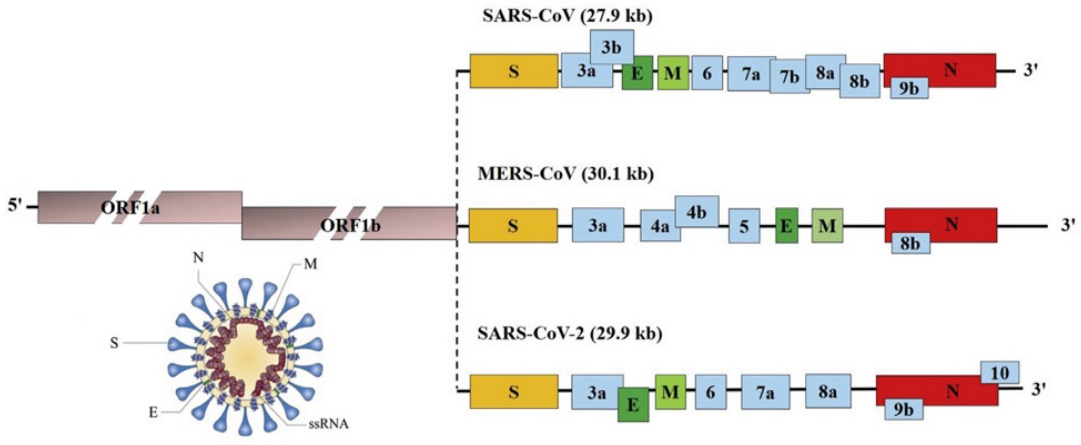

FIG. 2: Genomes of SARS-CoV, MERS-CoV and SARS-CoV-2 (Li *et al*. 2020)[71]
Available from https://doi.org/10.1016/j.jpha.2020.03.001

spread between cells while avoiding virus-neutralising antibodies.[32,33]

The S protein utilizes an N-terminal signal sequence to gain access into the endoplasmic reticulum (ER), and is heavily N-linked glycosylated. Homotrimers of the virus-encoded S protein make up the distinctive spike-like structure.[34,35] This trimeric S glycoprotein is a class I fusion protein[36] that mediates attachment to the host receptor.[37] In most coronaviruses, S is cleaved by a host cell furin-like protease into two separate polypeptides, namely S1 and S2.[38,39] S1 makes up the large receptor-binding domain of the S protein and S2 forms the stalk of the spike.[40]

The M protein (~25–30 kDa) with three transmembrane domains[41] is the most abundant structural protein and defines the shape of the viral envelope.[42] It has a small N-terminal glycosylated ectodomain and a much larger C-terminal endodomain that extends 6–8 nm into the viral particle.[43] Studies have shown that the M protein exists as a dimer, and may adopt two different conformations allowing it to promote membrane curvature as well as bind to the nucleocapsid.[42] Interaction of S with M protein is necessary for retention of S in the ER-Golgi intermediate compartment (ERGIC)/ Golgi complex and its incorporation into new virions, but is not required for the assembly process.[44] Binding of M to N protein stabilises the nucleocapsid (N protein-RNA complex), as well as the internal core of virions, and, ultimately, helps complete the viral assembly.[45] Together, M and E proteins make up the viral envelope and their interaction is sufficient for the production and release of virus-like particles (VLPs).[46]

The E protein (~8–12 kDa) is the smallest of the major structural proteins. This transmembrane protein has a N-terminal ectodomain and a C-terminal endodomain with ion channel activity. During the replication cycle, E is abundantly expressed inside the infected cell, but only a small portion is incorporated into the virus envelope.[47] The majority of the protein participates in viral assembly and budding.[48] Recombinant CoVs without E have been shown to exhibit significantly reduced viral titres, crippled viral maturation, or yield incompetent progeny, thereby demonstrating the importance of E protein in virus production and maturation.[49,50]

The N protein is the only one that binds to the RNA genome.[51] The protein is composed of two separate domains, an N-terminal domain (NTD) and a C-terminal domain (CTD). It has been suggested that optimal RNA binding requires contribution from both these domains.[52] It is also involved in viral assembly and budding,[53] resulting in complete virion formation.

Genomic structure of SARS-CoV-2

The SARS-CoV-2 genome is similar to that of typical CoVs and contains at least ten open reading frames (ORFs). The 5'-terminal two-thirds of the genome ORF1a/b encodes two large polyproteins, which form the viral replicase transcriptase complex. The other ORFs of SARS-CoV-2 on the one-third of the genome encode the same four main structural proteins: spike (S), envelope (E), nucleocapsid (N) and membrane (M) proteins, as well as several accessory proteins with unknown functions which do not

participate in viral replication (Fig. 2).

REPLICATION CYCLE OF THE CORONAVIRUS

Attachment and Entry

Attachment of the virus to the host cell is initiated by interactions between the **S** protein and its receptor. The site of receptor binding domains (RBD) within the S1 region of a coronavirus S protein varies for each coronavirus. MHV have the RBD at the N- terminus, whereas SARS-CoV have the RBD at the C-terminus.[54,55] The S-protein/receptor interaction is the primary determinant to infect a host species and also controls viral tissue tropism. Many coronaviruses utilize peptidases as their cellular receptor however, SARS-CoV and HCoV-NL63 use angiotensin- converting enzyme 2 (ACE2) as their receptor.

Following receptor binding, the virus enters host cell cytosol via acid-dependent proteolytic cleavage of S protein by a cathepsin, TMPRRS2 or another protease, followed by fusion of the viral and cellular membranes. S protein cleavage occurs at two sites within the S2 portion of the protein, with the first cleavage for separating the RBD and fusion domains of the S protein.[56] and the second to expose the fusion peptide (cleavage at S2′). Cleavage at S2′ exposes a fusion peptide that inserts into the membrane, followed by the joining of two heptad repeats in S2 forming an antiparallel six-helix bundle.[36] The formation of this bundle results in fusion and ultimate release of the viral genome into the cytoplasm.

Replicase Protein Expression

The next step in the coronavirus lifecycle is the translation of the replicase gene from the virion genomic RNA. Coronaviruses encode either two or three proteases that cleave the replicase polyproteins. Next, many of the non-structural proteins (nsps) assemble into the replicase-transcriptase complex (RTC) to create an environment suitable for RNA synthesis, and ultimately are responsible for RNA replication and transcription of the sub-genomic RNAs. The nsps also contain other enzyme domains and functions as listed in Table 1.

Replication and Transcription

Viral RNA synthesis follows the translation and assembly of the viral replicase complexes. Viral RNA synthesis produces both genomic and sub-genomic RNAs. Sub-genomic RNAs serve as mRNAs for the structural and accessory genes which reside downstream of the replicase polyproteins. All positive-sense sub-genomic RNAs are 3′ co-terminal with the full-length viral genome and thus form a set of nested RNAs, a distinctive property of the order *Nidovirales*. Both genomic and sub-genomic RNAs are produced through negative-strand intermediates. These negative-strand intermediates are only about 1% as abundant as their positive-sense counterparts and contain both poly-uridylate and anti-leader sequences.[57]

Coronaviruses are also known for their ability to recombine using both homologous and non-homologous recombination.[58,59] The ability of these viruses to recombine is tied to the strand switching ability of the RNA-dependent RNA polymerase (RdRp). It is likely that recombination plays a significant role in viral evolution and is the basis for targeted RNA recombination, a reverse genetics tool used to engineer viral recombinants at the 3′ end of the genome.

Assembly and Release

Following replication and subgenomic RNA synthesis, the S, E, and M proteins are translated and inserted into the endoplasmic reticulum (ER). These proteins move along the secretory pathway into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC).[60,61] In the compartment, the viral genomes that are encapsidated by the N protein, will bud into the membrane resulting in formation of the mature virus.[62]

The M protein directs most of the protein-protein interactions required for coronaviruses assembly. However, virus-like particles (VLPs) can only be formed when M protein is expressed along with E protein, suggesting the need for these two proteins to produce coronavirus envelope.[63] Additional roles of the E protein include inducing membrane curvature[64-66] and preventing M protein aggregation.[67] Following assembly, virions are transported to the cell surface in vesicles and released by exocytosis.

In light of their ability to recombine, mutate, and infect a number of animals, novel strains of coronaviruses will continue to evolve, emerge and cause new outbreaks.

CONCLUSION

Coronaviruses have a number of non-structural and accessory proteins that are yet to be characterised with no known function. If their mechanisms of action are identified and their

TABLE 1: Functions of coronavirus non-structural proteins (nsps)

Protein	Functions
nsps1	Promotes cellular mRNA degradation and blocks host cell translation, results in blocking innate immune response
nsps2	No known function, binds to prohibitin proteins
nsps3	Large, multi-domain transmembrane protein, activities include: Ubl1 and Ac domains, interact with N protein ADRP activity, promotes cytokine expression PLPro/Deubiquitinase domain, cleaves viral polyprotein & blocks host innate immune response Ubl2, NAB, G2M, SUD, Y domains, unknown functions
nsps4	Potential transmembrane scaffold protein, important for proper structure of DMVs
nsps5	Mpro, cleaves viral polyprotein
nsps6	Potential transmembrane scaffold protein
nsps7	Forms hexadecameric complex with nsp8, may act as processivity clamp for RNA polymerase
nsps8	Forms hexadecameric complex with nsp7, may act as processivity clamp for RNA polymerase; may act as primase
nsps9	RNA binding protein
nsps10	Cofactor for nsp16 and nsp14, forms heterodimer with both and stimulates ExoN and 2-O-MT activity
nsps12	RdRp
nsps13	RNA helicase, 5′ triphosphatase
nsps14	N7 MTase) and 3′-5′ exoribonuclease, ExoN; N7 MTase adds 5′ cap to viral RNAs, ExoN activity is important for proofreading of viral genome
nsps15	Viral endoribonuclease, NendoU
nsps16	2′-O-MT; shields viral RNA from MDA5 recognition

Note: Ubl, ubiquitin-like; Ac, acidic; ADRP, ADP-ribose-1'-phosphate; PLPro, papain-like protease; NAB, nucleic acid binding; SUD, SARS-unique domain; DMVs, double-membrane vesicles; Mpro, main protease; RdRp, RNA-dependent RNA polymerase; MTase, methyltransferase; Viral exoribonuclease, ExoN; Viral endoribonuclease, NendoU; 2'-O-MT, 2'-O-Methyltransferase; MDA5, Melanoma differentiation associated protein 5.

roles are defined in viral replication, this will result in an increase in the number of suitable therapeutic targets. A number of suitable anti-viral targets, such as viral proteases, polymerases, and entry proteins have been identified to inhibit viral replication.

A few strategies have been developed in an attempt to produce a candidate vaccine that can reduce recombination by making large deletions in the E proteins,[68] rearranging the 3′ end of the genome[69] or using mutant viruses with abnormally high mutation rates that significantly attenuate the virus.[70]

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