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Pathogenic Mechanism of Coronavirus infection

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Abstract

Human coronavirus (HCoV) infection causes respiratory diseases with mild to severe outcomes. In the recent years, two zoonotic, highly pathogenic HCoVs have been identified : severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus(MERS-CoV). An outbreak of mystery pneumonia in Wuhan since December 2019 has been drawing tremendous attention around the world. A novel coronavirus (nCoV) was suggested as the causative agent of the pneumonia infection. It was confirmed by deep sequencing and etiological investigations by more than five laboratories in China. Later, on 12 January 2020, the World Health Organization temporarily named the new virus as 2019 novel coronavirus or Covid 19. CoVs have now become a severe global threat for humans because of its sporadic emergence and outbreaks. Coronaviruses are enveloped viruses of round shape with pleiomorphic virions of approximately 80 to 120 nm in diameter. Each group of coronaviruses encodes a group of various types of proteins which are involved in the pathogenic mechanism of infections by Coronaviruses. In this review, we summarize the updated knowledge of pathogenic proteins, host factors and signaling pathways that are activated during HCoV infection, with an emphasis on the pathogenesis of HCoV-infection.

Keywords: Human coronavirus (HCoV), pleiomorphic virions, signaling pathways.

Introduction

Coronaviruses (CoVs) are the pathogens causing human infections usually affecting respiratory, gastrointestinal and central nervous system of humans, birds, bat, mouse, and many other wild animals. [1]

The name “coronavirus” was coined in 1968, that refers to its “corona”-like or crown-like morphology as by suggested by electron microscopic studies [2]. In 1975, the International Committee on the Taxonomy of Viruses established term Coronaviridae family. Later in June 2005, in the 10th International Nidovirus Symposium in Colorado, the Coronaviridae family was divided into two subfamilies, the coronaviruses and the toroviruses. Toroviruses have been found to cause enteric diseases in cattle and rarely in humans. [3]

CoVs belong to the subfamily Coronavirinae in the family of Coronaviridae, and this subfamily of Coronavirinae includes four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. [4,5]. The viral genome of CoVs constitutes a single stranded positive sense RNA (+ssRNA) of 30kb length with 5′ cap structure and 3′ polyA tail. The RNAof the genome is used as template to directly translate polyprotein 1a/1ab (pp1a/pp1ab), encoding nonstructural proteins (nsps) to form the replicationtranscription complex (RTC) in a double membrane vesicles (DMVs). [6] Subsequently, another set of subgenomic RNAs (sgRNAs) are manufactured by RTC. These genomic messenger RNAs (mRNAs) are all composed of common 5′ and 3 terminal sequences. Transcription termination and subsequent acquisition of aof the 5 terminal of RNA occurs at transcription regulatory sequences that is situated between open reading frames (ORFs). [7]

The outbreaks of severe acute respiratory syndrome (SARS) in 2002/2003 and the Middle East respiratory syndrome (MERS) in 2012 have revealed the possibility of transmission of animal to human and human to human CoVs. [8,9] An outbreak of mystery pneumonia in Wuhan since December 2019 has been drawing tremendous attention around the world. A novel coronavirus (nCoV) was suggested as the causative agent of the pneumonia infection. It was confirmed by deep sequencing and etiological investigations by more than five laboratories in China. Later on 12 January 2020, the World Health Organization temporarily named the new virus as 2019 novel coronavirus or Covid 19. CoVs have now become a severe global threat for humans because of its sporadic emergence and outbreaks. [1]

Coronaviruses are subclassified into three groups (I to III). Group I coronaviruses include animal pathogens, such as porcine epidemic diarrhea virus (PEDV), and feline infectious peritonitis virus (FIPV), as well as the human coronaviruses HCoV229E and HKU1, that are responsible respiratory infections. GroupII also include pathogens of animal origin, such as BCoV, porcine hemagglutinating encephalomyelitis virus, and equine coronavirus, also human coronaviruses viruses OC43 and NL63, which, like HCoV-229E, also cause respiratory infections. It also includes viruses infecting both mice and rats. Group III includes only avian coronaviruses, such as IBV, turkey coronavirus, and pheasant coronavirus [10]. Recently, using reverse transcription-PCR (RT-PCR), coronavirus sequences were detected in a few species like graylag goose (Anser anser), feral pigeon (Columbia livia), and mallard (Anas platyrhynchos). [11] Sequences suggested that these viruses belong to group III only. Coronaviruses affect many species of animals,

including humans causing acute and chronic respiratory, enteric, and central nervous system (CNS) disorders.

The recently identified CoV, which was shown to cause a severe acute respiratory syndrome was the first example of serious illness in humans. [12] This review pertains to the pathogenic mechanism of Coronavirus. Since the identification of SARS-CoV, two new human coronaviruses have been reported to be associated with respiratory disease. HKUI is a group II coronavirus which was isolated from an elderly patient with pneumonia. [13] HCoV-NL63 is a group I coronavirus isolated from a 7-month-old child in Netherlands affected with bronchiolitis and conjunctivitis. [14,15] Later it was reported in other parts of the world like including Canada, Japan, Australia and Belgium. [16] HCoV-NL63 is an infection associated with serious respiratory symptoms including bronchiolitis and pneumonia.

Viral Structure

Coronaviruses are enveloped viruses of round shape with pleiomorphic virions of approximately 80 to 120 nm in diameter. These contain positive-strand RNA, with the largest RNA genome of approximately 30 kb. The genome RNA is complexed with the basic nucleocapsid (N) protein forming a helical capsid found within the viral membrane. The membranes of all coronaviruses possess three viral proteins. These are spike (S) which is type I glycoprotein that forms the peplomers on the virion surface that gives the virus its corona- or crown-like morphology when seen under electron microscope; second is the membrane (M) protein, a protein that increases the strength of the membrane three times. [17] The third type is E protein which has a short N-terminal ectodomain and a cytoplasmic tail; it is a small membrane protein (E), a highly hydrophobic protein. E protein of IBV has a short ectodomain, a transmembrane domain, and a cytoplasmic tail. [7]

Some group II coronaviruses have an additional membrane protein, hemagglutinin esterase (HE). [18] While the function of HE is not known, it is not an essential protein, but has been suggested to play a role in viral entry and/or pathogenesis. Another structural viral protein is group II virion protein called Internal (I) protein, as it is encoded within the nucleocapsid open reading frame (ORF). This is also a nonessential protein of unknown function. [19]

The genomes of all coronaviruses have a similar structure. The replicase gene products in the genome are encoded within two very large open reading frames, ORFs 1a and 1b, which are translated into two large polypeptides, pp1a and pp1ab, via a frame shifting mechanism involving a pseudoknot structure formed by the genomic RNA. [20,21,22] The structural proteins are encoded within the 3 one-third of the genome, for all coronaviruses. In addition, each group of coronaviruses encodes a group of unique small proteins; while these protein are nonessential and have been speculated to serve as accessory proteins.

Pathogenesis

Coronaviruses attach to the receptors present on the host cell via their spike protein. This triggers a change in spike configuration which then mediates fusion between the viral and cell membranes resulting in release of nucleocapsid into the cell. Once it get entry into the cell, the 5 end of the genome RNA, ORFs 1a and 1b, are translated into pp1a and pp1ab. After receptor interaction and fusion of viral and plasma membranes, synthesis of virus-specific RNA and proteins take place, probably entirely with in the cytoplasm. Expression of coronaviruses reveals after

translation of two polyproteins, pp1a and pp1ab, that undergo proteolytic processing by cotranslation into the proteins that form the replicase complex. This complex is then used to transcribe a3 -coterminal set of nested subgenomic mRNAs, as well as genomic RNA, that have a common 5“leader” sequence derived from the 5end of the genome. There is translation of proteins from the 5 end of each mRNA. New virions enter the host intracellular membranes and released through vesicles by the cell secretory mechanisms.

ORF 1a encodes papain-like proteases (PLpro or PLP) and a picornavirus 3C-like protease (3CLpro), that process pp1a and pp1ab into the mature replicase proteins. Also, ADP-ribose 1-phosphatase activity is encoded in the X domain of ORF 1a is a (putative) [23,24] and an RNA-dependent RNApolymerase(RdRp) and a helicase [21], as well as other enzymatic activities, including (putative)3-to-5 exonuclease (ExoN), poly(U)-specific endoribonuclease (XendoU), and (putative)S-adenosylmethionine-dependentribose2-O-methyltranferase are encoded in ORF 1b. [25] Also, an additional activity, cyclic phosphodiesterase, is encoded downstream in ORF 2a. These collective enzymatic activities are observed to play roles in metabolism and pathogenic mechanism of coronavirus RNA. [24]

For coronaviruses to cause infection, replication of genome and transcription of mRNAs must occur. Replication of the genome involves the synthesis of a negative-strand RNA of full length that serves as template for full-length genomic RNA.

The mechanism by which the group of positive- and negative-strand RNAs are synthesized involves a transcription mechanism that is yet to be completely understood. However, various proteins present in the viral structure play a definite role pathogenesis of Coronavirus.

Role of Coronavirus proteins in Pathogenesis

Spike proteins

The protein of the coronavirus spike is a type I glycoprotein which forms the peplomers on coronavirus framework. Some coronaviruses spikes are divided into two subunits by a furin-like enzymatic activity in the Golgi apparatus during processing. The prototype MHV (Murine coronavirus strain) spike is 180 kDa; which is cleaved into two noncovalently associated subunits of about 90 kDa. [26] The amino-terminal S1 subunit, which is believed to form the globular head of the mature protein, forms the receptor binding domain (RBD) within the first 330 amino acids. [27] The carboxyterminal S2 subunit, forms a stalk-like structure anchored in the membrane, contains two (or perhaps three) heptad repeat (HR) domains. [28,29,30] A cysteine-rich domain is also there that bridges the putative junction of the anchor and the cytoplasmic tail necessary for fusion, as is the transmembrane domain. Coronaviruses attach to their specific receptors in the cell with the help of spike protein. Viral attachment triggers a conformational change in the spike protein that facilitates the fusion of viral and cellular membranes. [31,32] The coronavirus spike protein plays vital roles in the entry of virus, cell-to-cell spread, and determining its tropism. CoV spike is cleaved by the proteases produced by inflammatory cells in the lungs of patients and thus enter cells by plasma membrane route [33] The ability of a coronavirus to replicate in a particular cell type depends entirely on the ability to interact with its receptors [34]. Several coronavirus receptors have been identified on the cell. The group I coronaviruses human HCoV-229E, feline FIPV, and porcine TGEV all use aminopeptidase N

(APN), a zinc-binding protease, of their respective host species as their receptors. [35]

CoV is believed to have jumped to humans from civets. Changes within the RBD is responsible for the adaptation of CoV to humans. There are six amino acid differences within the RBD of the spike. The spike protein of civet CoV has low affinity for the human ACE2 CoV receptor. But substitution of the two residues within the civet spike with the human amino acids confers the ability to infect cells expressing the human receptor. Thus, it is likely that amino acids 479 and 487 are important for receptor interaction that allowed the adaptation of CoV to humans. [36,37] CoV spike protein also plays a role in pathogenesis by inducing interleukin-8 (IL-8) in the lungs by activating MAPK and AP-1. Such an activity was mapped to amino acids 324 to 688 of the CoV spike. This activity was detected in epithelial cells and fibroblasts by using virus expressed CoV spike. [38]

Hemagglutinin-Esterase Protein

A second type of spike is the coronavirus HE glycoprotein that forms, smaller (5 to 7nm) spike protein peplomers, on the envelopes of coronaviruses. [39,40] HE is synthesized as 42-kDa apoprotein, glycosylated to 65 kDa, and disulfide linked to form a homodimer; when expressed, the CoV HE displays hemagglutinating and esterase activities. The MHV HE displays 30% sequence homology to the HA1 subunit of the hemagglutinin-esterase fusion protein of influenza C virus. [41] HE is thought to have been obtained via homologous RNA recombination involving a group II coronavirus before the split of CoV, which does not encode an HE protein. [23] HE proteins of some coronaviruses are sialic acid-specific lectins, as demonstrated by hemagglutination and/or hemadsorption assays, thus supporting in receptor binding. [42,43,44] In tissue culture also, it has been observed that viruses expressing HE have a relative growth disadvantage with respect to viruses that do not express HE. [45] It has long been presumed that HE play a role in acute and/or chronic disease induced by MHV, especially in cellular tropism,[46,47,48] or may facilitate spread of virus by enhancing attachment and/or exit from the cell. [39]

Membrane Protein

The M protein is the most abundant virion membrane protein in the viral structure. It plays an important role in host interactions. It may be subclassified into O glycosylated (groups I and III) or N glycosylated (group II). The glycosylation state of M protein functions in virus-host interaction, however this state is not essential for viral assembly or infectivity. Also, it has been shown to have interferogenic activity, and any mutation in the M protein ectodomain that impair N glycosylation decrease this activity. It also may affect the ability to induce IFN in vitro and also to replicate in the liver in vivo. [49]

Nucleocapsid protein

N protein is a structural protein which plays a role in transcription and also in pathogenesis of infection. Expression of N protein is essential for sufficient recovery of virus from infectious cDNA clones. Recently it is believed to enhance the replication capacity of HCoV-229 E genome RNA. The ability to upregulate transcription of this gene maps to the nucleocapsid gene and correlates with the development of fulminant hepatitis [50] the nucleocapisd proteins of coronaviruses representing groups I, II, and III were shown to localize to the nucleolus as well as to the cytoplasm. This report suggests that N protein induces a cell cycle delay or arrest, most likely in the G2/M phase, possibly by inhibition of cytokines. [51]

Small envelop protein

The coronavirus E protein is an integral membrane protein. [52] E protein along with the M protein, plays an important role in viral assembly. [53] Virus-like particles are formed when E protein is expressed alone or when expressed together with M forms. Surprisingly, it was possible to select a recombinant MHV with a deletion of the E gene. This recombinant MHV has low infectivity and poor replication. This suggests that while it is nonessential for MHV, E plays an important role in production of infectious virus. Apart from having aprominent role in viral assembly causing infection, It has recently been demonstrated that the E protein of CoV has cation selective ion channel activity. [54] E protein ion channel could function at budding site to enhance viral morphogenesis and assembly. E protein also play a role in host virus interaction, leading to induction of apoptosis, usually by a caspase-dependent mechanism. [55]

Internal protein

The genomes of several group II coronaviruses, including MHV, contain an internal ORF within the nucleocapsid gene. [19,56] This ORF, translated in the reading frame with respect to the N protein, encodes a mostly hydrophobic 23-kDa polypeptide. This gene product is expressed in MHV-infected cells and found within the virions as well. However, the exact role of this gene is still not known.

Replicase proteins

The replicase proteins generally affect viral tropism and pathogenesis of infection by determining the rate of viral replication, probably by interactions with noncoding 5 and/or 3UTR sequences in the viral genome, with cell type-specific factors, or with elements of the immune response. The several enzymatic activities that are encoded in ORFs 1a and 1b, are supposed to be involved in host cell metabolism. [24] In the presence of infectious cDNA clones, the replicase gene is now available for genetic analysis, and gaining further information regarding the role of replicase proteins.

Group specific proteins

The coronavirus genes encoding these proteins are sometimes referred to as “small ORFs” or “group-specific” genes. The MHV genome contains ORFs 2a, 4, and 5a; the proteins encoded in these three ORFs are usually nonessential for replication. In some strains of MHV, like A59, ORF 4 is cleaved and becomes ORFs 4a and 4b [57], and also it has been seen that there are reports of an MHV (JHM strain) isolate with a deletion of ORF2a [58] as well as an MHV strain isolate with deletions of ORFs 4 and 5a. [59] Recent data suggests that these small ORFs may br different for different CoV isolates. For example, there are reports that in the genomes of CoV isolates from humans there are deletions within a single ORF 8, resulting in two ORFs, 8a and 8b (60, 23). Variations in these ORFs participate in variety of adaptation patterns to the human host and/or subversion of the host innate immune response.

Conclusion

The knowledge that multiple viral genes contribute to pathogenesis and the type of immune response indicates that small changes in sequence can have greater effects on pathogenic phenotype. The observations that coronavirus tropism variants may be readily selected during replication in tissue culture and/or animals and also that variants with changes and increased host range are also readily selected in tissue culture are all helpful in the understanding of the emergence of CoV into the human population. The identification and characterization of the proteases and the replicase as well as the identification of several

putative enzymatic activities encoded within ORFs 1a and 1b of other coronaviruses have provided possible targets for which to evaluate potential drug therapies. The experience with development of coronavirus vaccines will aid the developments of vaccines for CoV as well. Future directions for CoV research include further understanding of the mechanisms of replication; elucidation of the molecular determinants of virulence and tropism and the immune response, with attention to the possible roles of group-specific proteins; development of vaccine strategies and antiviral therapies for animal and human viruses; and very likely the isolation and characterization of new pathogenic human coronaviruses.

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