Human Coronavirus-229E, -OC43, -NL63, and -HKU1

Ding X Liu, Jia Q Liang, and To S Fung, South China Agricultural University, Guangzhou, Guangdong, China

Nomenclature

ACE2   Angiotensin converting enzyme 2
APN   Aminopeptidase N
BAX   Bcl-2-associated X protein
BST2   Bone marrow stromal antigen 2
CNS   Central nervous system
CsA   CyclosporinA
CypA   Cyclophilin A
DMV   Double-membrane vesicle
DUB   Deubiquitinating
EIA   Enzyme-linked immunoassays
eIF2α   Eukaryotic initiation factor 2
ER   Endoplasmic reticulum
ERGIC   ER/Golgi intermediate compartment
ERK   Extracellular signal-regulated kinase
ExoN   Exoribonuclease
GRP   Glucose regulated protein
HCoV   Human coronavirus
HR   Heptad repeat
IBV   Avian infectious bronchitis coronavirus
IC   Ion channel
ICTV   International Committee on Taxonomy of Viruses
IFITM   Interferon inducible transmembrane protein
IFN   Interferon
IRE1   Inositol requiring enzyme 1
JNK   c-Jun N-terminal kinase
MAP   Mitogen-activated protein
MDA5   Melanoma differentiation-associated 5
MERS-CoV   Middle East respiratory syndrome coronavirus
MHV   Mouse hepatitis virus
Mpro   Main protease
NendoU   Endoribonuclease
NF-κB   Nuclear factor-κB
nsp   Non-structural protein
ORF   Open reading frames
PERK   PKR-like ER kinase
PLpro   Papain-like proteases
RBD   Receptor-binding domain
RdRP   RNA-dependent RNA polymerases
RTC   Replication-transcription complex
RT-LAMP   Reverse transcription loop-mediated isothermal amplification
SARS-CoV   Severe acute respiratory syndrome coronavirus
sgRNA   Subgenomic RNA
TMPRSS2   Transmembrane serine protease 2
TRS   Transcription-regulated sequences
UPR   Unfolded protein response
UTR   Untranslated region
XBP1   X-box protein 1

Glossary

Apoptosis   A form of programmed cell death that occurs in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death.
Autophagy   The natural, regulated mechanism of the cell that removes unnecessary or dysfunctional components. It allows the orderly degradation and recycling of cellular components.
ER stress   A cellular process that is triggered by a variety of conditions that disturb the folding of proteins in the endoplasmic reticulum.
Innate immunity   Refers to nonspecific defense mechanisms that come into play immediately or within hours upon pathogen recognition in the body. These mechanisms include physical barriers such as skin, chemicals in the blood, cytokine production and immune cells that attack foreign cells in the body.

Mitogen-activated protein kinase   Serine-threonine protein kinases, important molecules in mediating the signal transduction from cell surface to nucleus, regulating cellular activities such as gene expression, mitosis, differentiation, and cell survival/apoptosis.
Type I interferon   A large subgroup of interferon proteins that help regulate the activity of the immune system, and a pleiotropic cytokine with antiviral, antitumor and immunoregulatory functions.
Unfolded protein response   A cellular stress response related to the endoplasmic reticulum stress. It has been found to be conserved between all mammalian species, as well as yeast and worm organisms.
Viroporin   A group of small hydrophobic viral proteins that tend to oligomerize to form hydrophilic pores or ion channels in cellular membrane.

Background

The first human coronavirus (HCoV), strain B814, was isolated in 1965 from the nasal discharge of a patient with a common cold. Since then, more than 30 additional strains were identified. Among them, the prototypic stain HCoV-229E (named after a student specimen coded 229E) was isolated using standard tissue culture. HCoV-OC43 (Organ Culture 43) was later recovered using tracheal organ culture and found to be serologically distinct from HCoV-229E. These two viruses were the focus of HCoV research
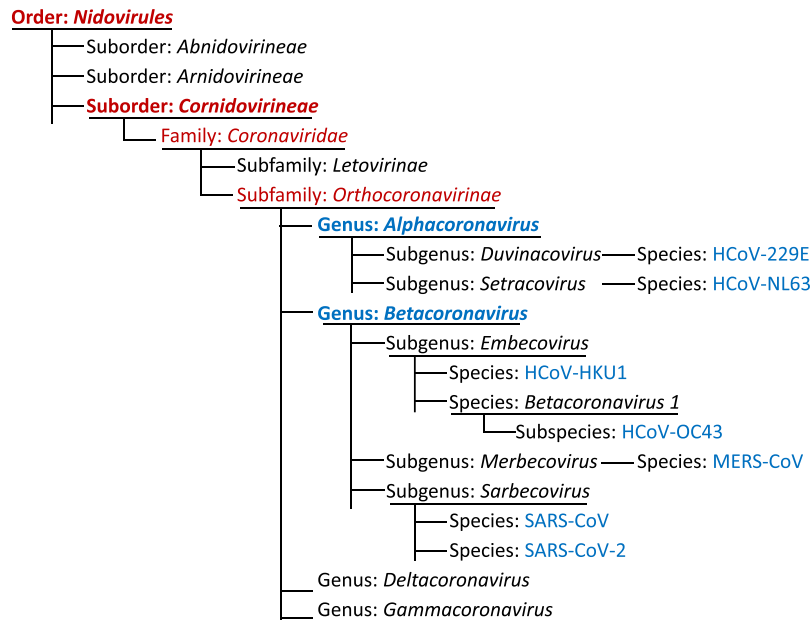
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Order: Nidovirules
├──Suborder: Abnidovirineae
├──Suborder: Arnidovirineae
├──Suborder: Cornidovirineae
   └──Family: Coronaviridae
      ├──Subfamily: Letovirinae
      └──Subfamily: Orthocoronavirinae
         ├──Genus: Alphacoronavirus
         │  ├──Subgenus: Duvinacovirus──Species: HCoV-229E
         │  └──Subgenus: Setracovirus    ──Species: HCoV-NL63
         ├──Genus: Betacoronavirus
         │  ├──Subgenus: Embecovirus
         │  │  ├──Species: HCoV-HKU1
         │  │  └──Species: Betacoronavirus 1
         │  │     └──Subspecies: HCoV-OC43
         │  ├──Subgenus: Merbecovirus ── Species: MERS-CoV
         │  └──Subgenus: Sarbecovirus
         │     ├──Species: SARS-CoV
         │     └──Species: SARS-CoV-2
         ├──Genus: Deltacoronavirus
         └──Genus: Gammacoronavirus
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Fig. 1   The updated classification scheme of HCoVs according to the ICTV.

in the following years, until the emergence of the highly pathogenic severe acute respiratory syndrome coronavirus (SARS-CoV) in 2002–2003. In the post SARS era, two more HCoVs were identified. HCoV-NL63 (NetherLand 63) was isolated from the aspirate of a 7-month-old infant with bronchiolitis in 2004, whereas HCoV-HKU1 (Hong Kong University 1) was isolated from a Hong Kong patient with pneumonia in 2005. Since then, two more zoonotic HCoVs have emerged, namely as the Middle East respiratory syndrome coronavirus (MERS-CoV) and the 2019 novel coronavirus (2019-nCoV, a.k.a. SARS-CoV-2). Unlike SARS-CoV, MERS-CoV and SARS-CoV-2 that are associated with severe respiratory disease, the four common HCoVs (229E, OC43, NL63, and HKU1) generally cause mild to moderate upper-respiratory tract illness, presumably contributing to 15%–30% of cases of common colds in human.

Classification

On the basis of the 10th International Committee on Taxonomy of Viruses (ICTV) report, coronaviruses are classified under the order *Nidovirales*, suborder *Cornidovirineae*, family *Coronaviridae*, subfamily *Orthocoronavirinae* (**Fig. 1**). According to serology studies and genomic analysis, *Orthocoronavirinae* is further divided into four genera: *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. Under the genus *Alphacoronavirus*, HCoV-229E and HCoV-NL63 belong to the subgenus *Duvinacovirus* and *Setracovirus*, respectively; under the genus *Betacoronavirus*, both HCoV-OC43 and HCoV-HKU1 belong to the subgenus *Embecovirus*.

Virion Structure

Under the electron microscope, coronavirus virions are spherical or pleomorphic. Coronavirus particles are enveloped, about 80–120 nm in diameter, with club-like projections of the spike (S) protein decorating the surface. In some *betacoronaviruses*, including HCoV-OC43 and HCoV-HKU1, shorter projections of the hemagglutinin-esterase (HE) protein are also observed. The viral envelope is supported by the membrane (M) protein and contains a small amount of the envelope (E) protein. Inside the viral envelope, the genome is bound by the nucleocapsid (N) protein to form a helical symmetric nucleocapsid. The common structural and functional features of HCoV structural proteins are briefly summarized as follows.

The S protein is a type I transmembrane protein, with a molecular weight of 128–160 kDa before glycosylation and 150–200 kDa after N-linked glycosylation. As a class I viral fusion protein, the S protein forms homotrimer and is cleaved by host proteases into a S1 subunit for receptor binding and a S2 subunit for membrane fusion. The ectodomain of the S protein is also modified by disulfide bonds, whereas the very short cytosolic tail is modified by palmitoylation. The S protein is the major determinant of host and tissue tropism, and may also contribute to viral pathogenesis by activating the endoplasmic reticulum (ER) stress response.

The HE protein is also a type I transmembrane protein, about 48 kDa before glycosylation and 67 kDa after N-linked glycosylation. It forms homodimer via disulfide bonds. With its sialic acid-binding hemagglutinin activity, the HE protein may serve as a cofactor of S protein and facilitate virion attachment. Additionally, as it possesses esterase activity that removes acetyl groups from O-acetylated sialic acids, it has been postulated to have a role as a receptor-destroying enzyme that facilitates the

release of progeny virions from nonpermissive host cells, thereby enhancing virion spreading in the extracellular milieu. In fact, the HE protein of HCoV-HKU1 mediated receptor-destroying enzyme activity specific to the *O*-acetylated sialic acids recognized by its own S protein.

The M protein (25–30 kDa) is the most abundant structural protein and possesses three transmembrane domains. The short N-terminal ectodomain of the M protein is modified by *O*-linked glycosylation in HCoV-OC43 and some animal coronaviruses including mouse hepatitis virus (MHV) and bovine coronavirus (BCoV). However, in HCoV-229E, HCoV-NL63, and most other coronaviruses, the ectodomain of M protein is modified by N-linked glycosylation. The M protein forms homodimer and interacts with other viral structural proteins to orchestrate the assembly of the coronavirus particle. This protein may also contribute to viral pathogenesis. For example, retinoic acid-inducible gene 1 (RIG-I)-dependent induction of type I interferon (IFN) is observed in cells overexpressing the M protein of SARS-CoV but not HCoV-HKU1.

The E protein is a small (8–12 kDa) integral membrane protein found in low amounts in the virion. Current evidence strongly suggests that the E protein adopts an N-ecto/C-endo topology with one transmembrane domain. The SARS-CoV E protein is modified by N-linked glycosylation and three cysteine residues in its endodomain are modified by palmitoylation. Additionally, the E protein of SARS-CoV and avian infectious bronchitis coronavirus (IBV) has been shown to form homopentamers with ion channel (IC) activity. The IC activity may modulate the process of virion release and contribute to viral pathogenesis. Although the deletion of the E gene is not lethal for SARS-CoV, the mutant virus is severely defective in virion morphogenesis and attenuated in vivo compared with the wild type control.

Underneath the viral envelope, the N protein (43–50 kDa) forms dimer and binds to the genomic RNA in a beads-on-a-string fashion, forming a helically symmetric nucleocapsid. In SARS-CoV and other coronaviruses, the N protein is phosphorylated by cellular kinases such as glycogen synthase kinase 3 (GSK3) and ataxia-telangiectasia mutated and Rad3-related. Other modifications such as SUMOylation, ADP-ribosylation, and proteolytic cleavage by caspases has also been demonstrated in the N protein of some coronaviruses. The N protein facilitates RNA packing and is involved in many other processes, including viral genome replication and evasion of the immune response.

Genome

With a single-stranded, positive-sense RNA genome containing approximately 27 to 32 kilobases (kb), coronavirus has the largest viral RNA genome described so far. The genome sizes are approximately 27.5 kb for HCoV-229E and HCoV-NL63, and more than 30 kb for HCoV-OC43 and HCoV-HKU1 (Fig. 2). Because the genomic RNA harbors a 5′-cap structure and a 3′-polyadenylate tail, it can act directly as a messenger RNA (mRNA) encoding the viral replicase. Additionally, the genome also serves as a template for RNA replication and the genome is packed into progeny virions. There are two untranslated regions (UTRs) flanking the coding region. The 5′-UTR is 292, 210, 286, and 205 nucleotides long in HCoV-229E, -OC43, -NL63, and -HKU1, respectively, and contains a leader sequence (∼70 nucleotides long) at its 5′-terminus. At the other end of the genome, the 3′-UTR is 462, 288, 287, and 281 nucleotides long in HCoV-229E, -OC43, -NL63, and -HKU1 respectively, and it contains a highly conserved octameric sequence ∼70 nucleotides upstream from the poly(A) tail.

The HCoVs replicase gene comprises the 5′- two thirds of the genome and is made up of two overlapping open reading frames (ORFs)—ORF1a and ORF1b. ORF1a is directly translated from the RNA genome, producing the polyprotein pp1a; whereas the translation of ORF1b requires a programmed ribosomal frameshift near the 3′ end of ORF1a, leading to the production of polyprotein pp1ab. The autoproteolytic cleavage of pp1a and pp1ab then gives rise to 16 non-structural proteins (nsp1–16).
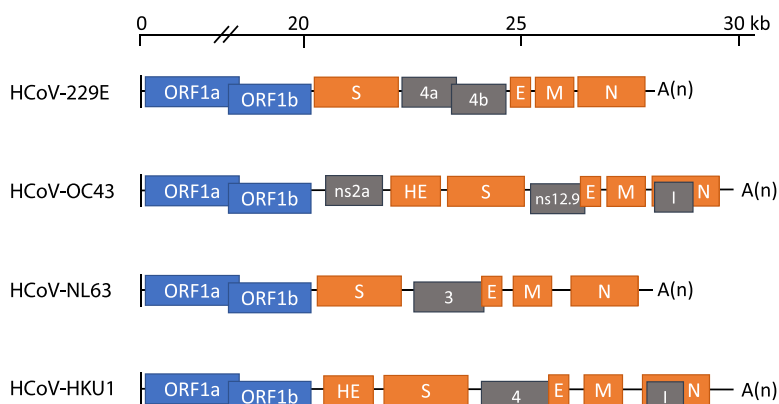

Fig. 2 Genome structure of human coronaviruses (HCoVs) – HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1. The open reading frame 1a (ORF1a) and ORF1b are represented as shortened navy blue-boxes. The genes encoding structural proteins spike (S), envelope (E), membrane (M), nucleocapsid (N), and hemagglutinin-esterase (HE) are shown as orange boxes. The genes encoding accessory proteins are shown as dark gray boxes.

The remaining one third of the HCoV genome contains ORFs for viral structural proteins in the order of 5′-(HE)-S-E-M-N-3′, as well as several accessory proteins that are distinct among HCoVs of different species and genus. Specifically, HCoV-229E encodes two accessory proteins 4a and 4b; HCoV-OC43 encodes three accessory proteins ns2a, ns12.9 (a.k.a. ns5a), and protein I (a.k.a. N2 or N internal ORF protein); HCoV-NL63 encodes a single accessory protein 3; and HCoV-HKU1 encodes two accessory proteins: protein 4 and protein I. Although these accessory proteins are dispensable for viral replication in cell culture, they may be involved in viral pathogenesis and contribute to virulence in vivo. Unlike the viral replicase, structural proteins and accessory proteins are translated from a 3′-nested set of subgenomic RNA (sgRNA) species. The coding sequences of some accessory proteins overlap with those of the structural proteins, but are translated in distinct reading frames. In fact, the accessory gene encoding protein I of HCoV-OC43 and HCoV-HKU1 are encoded internally of the N genes.

Non-Structural Proteins

The HCoV polyproteins pp1a and pp1ab are autocatalytically processed by viral proteases into 16 non-structural proteins. Nsp1, the most N-terminal cleavage product of the polyproteins, has been shown to suppress host protein synthesis and IFN response.

Nsp3 encodes one or two papain-like proteases (PLpro). Nsp3 of the four common HCoVs contains two PLpro domains (PLP1 and PLP2), whereas the nsp3 of SARS-CoV and MERS-CoV contains only one PLpro domain. In general, PLP1 processes at cleavage site 1 to release nsp1, whereas PLP2 is responsible for the processing at both cleavage sites 2 and 3 to release nsp2 and nsp3. The deletion of PLP2 is lethal, whereas the proteolytic activity of PLP1 is dispensable for HCoV-229E replication. Notably, a recent study reported that ectopic expression of HCoV-NL63 PLP2 induces proteasomal degradation of p53, thereby inhibiting p53-dependent production of type I IFN and the innate immune response.

Following the translation of ORF1a and ORF1ab, nsp5 is properly folded within the context of the replicase polyprotein and orchestrates its own autoproteolytic processing. Nsp5 cleaves pp1a/pp1ab at as many as 11 sites to produce a total of 13 mature proteins, and is therefore indispensable for virus replication. Nsp5 is also referred to as the coronavirus main protease (Mpro).

Nsp6 of some coronaviruses, such as IBV, MHV, or SARS-CoV, activates the formation of autophagosomes from the ER via an omegasome intermediate. Nsp3, nsp4, and nsp6 are also responsible for remodeling cellular membranes to form double-membrane vesicles (DMVs) or ER spherules, onto which the coronavirus replication-transcription complex (RTC) is assembled and anchored.

The complex of HCoV-229E nsp7 and nsp8 is capable of synthesizing short RNA strands of ∼6 nucleotides. Coronavirus nsp8 protein also has template-dependent RNA polymerase activities resembling those of RNA primases or even canonical RNA-dependent RNA polymerases (RdRP). Current evidence suggests an essential cofactor function of nsp7 and nsp8 for the RNA-dependent RNA polymerase activity of nsp12. In a recent study, it is found that nsp8 of HCoV-229E has a metal ion-dependent RNA 3′-terminal adenylyl transferase (TATase) activity, when a partially double-stranded RNA with a short 5′ oligo-U sequence is provided as the template strand. This supports the notion that nsp8 may catalyze the 3′-polyadenylation of HCoV genomes.

Nsp9, a single-stranded DNA/RNA-binding protein which exists as a dimer in physiological situations, has been found to be indispensable for virus replication based on reverse-genetics experiments. Nsp10 is a double-stranded RNA-binding zinc-finger protein. Nsp7, nsp8, nsp9, and nsp10 are all closely associated with the replication complex built around the RNA-dependent RNA polymerase (nsp12).

Nsp13 separates the double-stranded replicative intermediates to provide single-stranded templates for RNA synthesis. HCoV-229E nsp13 contains an N-terminal zinc-binding domain and a C-terminal superfamily 1 helicase domain. It exhibits a variety of enzymatic activities including NTPase, dNTPase, and RNA/DNA helicase activity. Using the NTPase active site, this protein also has an RNA 5′-triphosphatase activity, which may be involved in the capping of viral RNAs.

Nsp14 demonstrates exoribonuclease (ExoN) activities. RNA viruses generally have high mutation rates that allow for rapid viral adaptation in response to selective pressure. Nsp14-ExoN is the first proofreading enzyme identified for an RNA virus, and it functions together with other CoV replicase proteins to perform the crucial role of maintaining CoV replication fidelity.

Nsp15 is an endoribonuclease (NendoU) and a type I interferon antagonist. HCoV nsp15-NendoU can excise both single- and double-stranded RNA and specifically recognize uridylates to produce 2-3′-cyclic phosphodiester products, thereby preventing the activation of the host innate immune response.

Nsp16 has a 2′-O-methyltransferase activity. The 2′-O-methylation capping protects viral RNA from recognition by melanoma differentiation-associated protein 5 (MDA5) and thus prevents MDA5-dependent production of type I interferon in virus-infected cells.

Accessory Proteins

Although not essential for viral replication in cell culture, coronavirus accessory proteins may play in vivo functions that have not yet been fully elucidated. Most HCoV accessory proteins are genus-specific and show low homology to known proteins. But an accessory gene between the S and E gene is encoded by three HCoVs (3a of SARS-CoV, 4a of HCoV-229E, and ns12.9 of HCoV-OC43), suggesting a conserved role during HCoV infection. Indeed, all three proteins have been shown to serve as viroporins that regulate viral replication. Viroporins are oligomeric hydrophobic viral proteins that form and insert ion channels into the host cell membrane. The HCoV-OC43 ns12.9 protein is a recently identified viroporin that facilitates virion
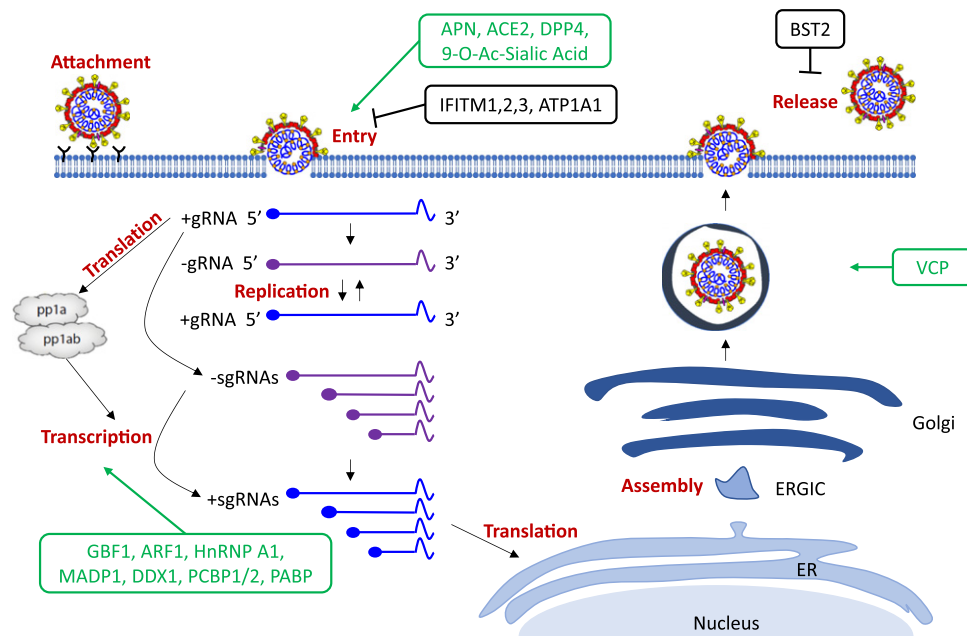
Fig. 3 HCoV replication cycle. HCoV infection is initiated by binding of virions to cellular receptors, which drives the conformational change in the S2 subunit in S, promoting the fusion of the viral and cell plasma membrane. The gRNA serves as the template for translation of polyproteins pp1a and pp1ab, which are cleaved to form nsps. Nsps induce the rearrangement of cellular membrane to form DMVs, where the viral RTCs are anchored. Full-length gRNA is replicated via a negative-sense intermediate, and a nested set of sgRNA species are synthesized by discontinuous transcription. These sgRNAs encode viral structural and accessory proteins. The viral products produced will be assembled in the ERGIC, and bud out as a smooth-wall vesicle to the plasma membrane to egress via exocytosis. Host factors that promote infection and inhibit infection are highlighted in green and black, respectively.

morphogenesis and pathogenesis. The expression of important mediators of innate immune response was downregulated in cells expressing of HCoV-OC43 ns12.9, presumably due to inhibited promoter activity of ISRE, IFN-β, and NF-κB.

Interestingly, because of a scenario in which HCoV-229E acquired an out-of-frame insertion or deletion, instead of an ORF4a and ORF4b, an intact ORF4 was present in some HCoV-229E clinical isolates. ORF4a of HCoV-229E was expressed in infected cells and localized at the ER/Golgi intermediate compartment (ERGIC). The ORF4a protein formed homo-oligomers through disulfide bridges and possessed ion channel activity in both Xenopus oocytes and yeast.

The NS2 protein of HCoV-OC43 has cyclic phosphodiesterase activity, which may modulate cAMP-mediated signaling and important physiological processes such as lipid metabolism and apoptosis. Finally, although the accessory protein encoded by HCoV-NL63 ORF3 appears to be nonessential in cell culture, there were differences in RNA synthesis, protein expression, plaque morphology, and virus growth in cells infected with the ORF3-deleted mutant compared with the control.

Life Cycle

The replication cycle of HCoV can be arbitrarily divided into five steps: attachment to host cells, viral entry and uncoating, expression of the viral replicase and formation of the replication-transcription complex, viral RNA synthesis, and virion assembly and release. Each step will be briefly introduced as follows (Fig. 3).

Attachment

Coronavirus infection is initiated by binding of virions to cellular receptors. The S protein includes two functional domains: S1 (bulb) is the part binding to the receptor(s) and S2 (stalk) is responsible for fusion between virion and cell membranes. The receptor-binding domain (RBD) of S1 varies among different coronaviruses. RBDs of HCoV-229E, HCoV-NL63 and HCoV-HKU1 are located in the C-terminal region but not the N-terminal domains of the respective S1 subunits.

Receptor binding is critical to initiate viral infection. HCoV has been shown to use either cellular proteins or carbohydrates displayed on the plasma membrane as receptors. Interestingly, all known protein receptors for HCoVs are cell surface peptidase, such as aminopeptidase N (APN) for HCoV-229E, dipeptidyl peptidase 4 (DPP4) for MERS-CoV, and angiotensin converting enzyme 2 (ACE2) for HCoV-NL63, SARS-CoV and SARS-CoV-2. On the other hand, HCoV-OC43 and HCoV-HKU1 employ glycan-based receptors carrying 9-O-acetylated sialic acid.

In addition to the receptor binding by the S protein, other HCoV structural proteins may also facilitate the early stage of attachment. For example, the M protein of HCoV-NL63 binds to target cells using heparan sulfate proteoglycans as the initial attachment factors. This is followed by the engagement of the S protein with the ACE2 receptor protein.

Viral Entry and Uncoating

Specific binding between S1 and the cognate receptor leads to a conformational change in the S2 subunit and large-scale rearrangements of the S protein, resulting in the fusion between virus and cell membranes and the release of viral nucleocapsid into the cytoplasm. Many host factors are involved in the entry and uncoating of HCoVs.

The cleavage of S protein into S1 and S2 subunits is mediated by one or more cellular proteases. HCoV-229E can enter host cells via two distinct pathways: one is mediated by surface proteases like type II transmembrane protease serine 2 (TMPRSS2), and another is mediated by endosomal cathepsin L. However, to avoid triggering an innate immune response, it is more likely that HCoV-229E enters cells via the TMPRSS2 pathway, as the endosome is a main site for recognition by toll-like receptors. Similarly, the entry of SARS-CoV depends on the endosomal cysteine protease cathepsin L and another trypsin-like serine protease to activate the S protein.

Coated vesicles and the cytoskeleton are utilized by some HCoV for entry. For example, HCoV-OC43 employs caveolin-1-dependent pathway of endocytosis, and the scission of virus-containing vesicles from the cell surface is dynamin-dependent. Internalization of these vesicles also requires rearrangements of the actin cytoskeleton. The interaction between HCoV-NL63 S protein and the ACE2 receptor molecule triggers the recruitment of clathrin. Subsequent vesicle scission by dynamin results in virus internalization, and the newly formed vesicle passes the actin cortex, which requires active cytoskeleton rearrangement.

Finally, acidification of the endosomal microenvironment is required for successful fusion and release of the viral genome into the cytoplasm. Additionally, virion release of HCoV-229E and IBV from early endosomes is shown to be dependent on the host factors valosin-containing protein (VCP), an AAA ATPase family protein that normally facilitates the export of misfolded proteins from the ER to the cytoplasm.

On the other hand, some host factors could prevent the entry and uncoating of HCoVs. Interferon inducible transmembrane proteins (IFITMs) exhibit broad-spectrum antiviral functions against various RNA viruses. IFITMs restricted the entry of HCoV-229E and HCoV-NL63, as well as SARS-CoV and MERS-CoV. Conversely, IFITM2 or IFITM3 serve as an entry factor to promote the infection of HCoV-OC43.

Formation of the Replication-Transcription Complex

Among RNA viruses, the transcription of coronavirus RNA is unique. First, in order to maintain genetic stability, the large genome size requires unusual enzymatic activities, such as an exoribonuclease and an endoribonuclease activity. Also, the synthesis of a nested set of sgRNAs by discontinuous transcription demands a huge and complicated RTC.

Following the release of viral nucleocapsid into the cytoplasm, the genomic RNA serves as a transcript encoding the viral replicase. The replicase gene includes two ORFs, ORF1a and ORF1b. ORF1a is translated into the polyprotein pp1a (440–500 kDa). Owing to a slippery sequence and an RNA pseudoknot near the end of ORF1a, a programmed -1 ribosomal frameshifting event can occur with a frequency of 25%–30%. This allows a continuous translation into ORF1b, producing a larger polyprotein pp1ab (740–810 kDa). Similar to likely all coronaviruses, the pp1a and pp1ab are autoproteolytically processed into 16 nsps, collectively forming the RTC for viral RNA synthesis. Notably, nsp3, 4, and 6 appear to be responsible for remodeling of cellular membranes to form DMVs or spherules, onto which the HCoV RTC is assembled and anchored. Host factors of the early secretory pathway, such as Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (GBF1) and its effector ADP ribosylation factor 1, may also contribute to DMV formation and RTC assembly.

Viral RNA Synthesis

The assembly of RTC sets a foundation for viral RNA synthesis. By using the genomic RNA as a template, new copies of full-length viral genome are synthesized, using the full-length negative sense genomic RNA as the intermediate template. Meanwhile, the polymerase can switch templates at short motifs called transcription-regulated sequences (TRS) during negative sense RNA synthesis, producing a 5′-nested set of negative sense sgRNAs. These are in turn used as templates to synthesize a 3′-nested set of positive sense sgRNAs, which serve to encode the structural and accessory proteins. The core sequence of HCoV TRS is a conserved hexamer CUAAAC in HCoV-229E and HCoV-NL63, and a conserved heptamer UCUAAAC in HCoV-OC43 and HCoV-HKU1.

Different numbers of sgRNAs are produced by the four common HCoVs. For instance, seven major viral RNA species are produced during HCoV-229E infection. The full-length genome (mRNA1) encodes the viral replicase, whereas mRNAs 2, 4, 5, 6, and 7 encode the S protein, accessory protein 4, E protein, M protein, and N protein, respectively. Notably, mRNA 3 is considered defective, because it contains a truncated version of the S gene that is not translated.

Although the replication and transcription of viral genome is mainly carried out by the replicase, the involvement of other factors including viral structural protein and host proteins has been implicated. For instance, by serving as an RNA chaperone, the coronavirus N protein can facilitate template switching during sgRNA synthesis. Host proteins such as

heterogeneous nuclear ribonucleoprotein A1, polypyrimidine tract-binding protein, mitochondrial aconitase, and polyadenylate-binding protein have been suggested to participate in coronavirus RNA synthesis, presumably mediated by their RNA binding activity.

Assembly and Release of Virion

Coronavirus structural and accessory proteins that are membrane-associated (such as S, HE, M, and E) are translated by ribosomes in the ER, whereas other viral proteins (such as N) are translated by free ribosomes. Most coronavirus structural proteins are also subjected to posttranslational modifications that modulate their functions. The building parts converge at the assembly site of ERGIC. Assembly is orchestrated by the M protein: homotypic interaction of M protein provides the scaffold for virion morphogenesis, whereas heterotypic interactions of M protein with other structural proteins, such as M-S and M-E, facilitate their recruitment and incorporation. Virion assembly is completed by the condensation of the nucleocapsid with the envelope components, a process mediated by M-N interactions. A small amount of E protein may provide the driving force for envelope morphogenesis by inducing membrane curvature.

After assembly, progeny virions are transported in smooth-wall vesicles, trafficked to the plasma membrane via the secretory pathway, and released by exocytosis. The cytoskeletal system also participates in HCoV assembly and release. For instance, the interaction between tubulin and the cytosolic domain of S protein are required for the assembly and release of infectious virions during HCoV-229E and HCoV-NL63 infection. In addition, bone marrow stromal antigen 2 (BST2, a.k.a. tetherin), an interferon-inducible antiviral protein, blocks the release of various envelope viruses by interfering with the budding step at the plasma membrane. Although budding of HCoV-229E occurs at the ERGIC, it is recently shown that BST2 can trapp HCoV-229E virions in the intracellular vesicles, thereby suppressing the release of progeny viruses. Interestingly, the S protein of SARS-CoV downregulates BST2 at the protein level by promoting its lysosomal degradation, thus antagonizing the BST2 tethering of SARS-CoV, HCoV-229E, and HIV-1 virus-like particles.

HCoV-Host Interactions

As intracellular obligate parasites, HCoVs exploit the host cell machinery for their own replication and spread. Since virus–host interactions also form the basis of viral pathogenesis, knowledge about their interplay is of great research interest (Fig. 4).
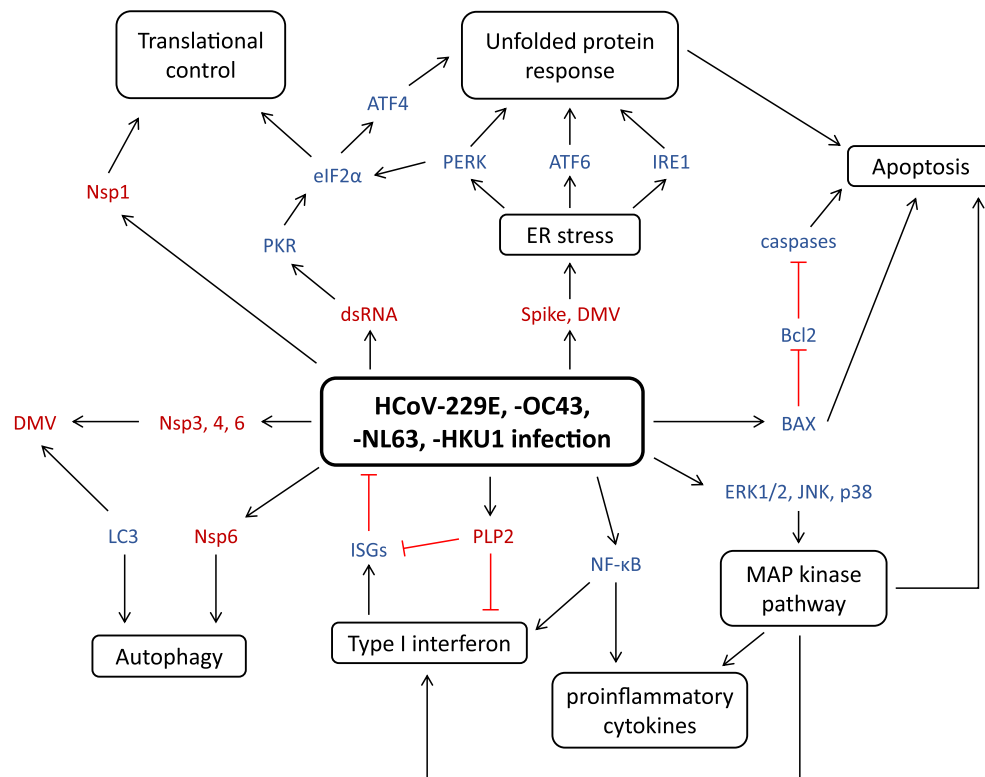

Fig. 4 HCoV-host interaction. Schematic diagram showing the host signaling pathways activated during HCoV infection. Black pointed arrows indicate activation, and red blunt-ended lines indicate inhibition. Viral components modulating the pathway are in red, and host proteins are in blue. See text for detail.

Translational Control

Viruses must utilize the host translation machinery to ensure efficient viral protein translation. In response to acute viral infection, host cell would shut down the protein translation system to cope with the infection stress, which is regarded as an integrated stress response. Integrated stress response is marked by the phosphorylation of the α-subunit of eukaryotic initiation factor 2 (eIF2α), downregulation of the general cap-dependent protein synthesis, and up-regulation of the expression of certain transcription factors, such as activating transcription factor 4 (ATF4). Due to the translation competition between cellular and viral mRNAs for limiting number of ribosomes and associated factors, coronavirus must hijack the host translational machinery to produce its own proteins.

Some HCoVs, such as SARS-CoV and MERS-CoV, have been shown to induce host translation shutoff in susceptible cells. Specifically, SARS-CoV infection leads to sustained phosphorylation of eIF2α in 293T/ACE2 cells. Coronavirus nsp1 also suppresses host protein synthesis and IFN response. For example, SARS-CoV nsp1 suppresses the expression of host genes including type I IFN, thereby counteracts the host innate immune response and contributes to virulence. MERS-CoV nsp1 also negatively regulates host gene expression by inhibiting the translation and inducing the degradation of host mRNAs. Nsp1 of HCoV-229E and HCoV-NL63 inhibits the expression of reporter genes, probably by binding to ribosomal protein S6 and blocking the mRNA binding to the 40S ribosomal subunit.

ER Stress Response

The ER is a cellular organelle important for protein synthesis, folding, and post-translational modifications. In normal circumstances, the ER can be loaded with a very high concentration of proteins without perturbing its unique luminal environment. However, when the protein load exceeds the ER folding and processing capacity, misfolded or unfolded proteins will accumulate within the ER, resulting in ER stress. ER stress activates the signaling pathways collectively known as unfolded protein response (UPR). These are initiated by three ER transmembrane sensors: protein kinase R (PKR)-like ER kinase (PERK), inositol requiring enzyme 1 (IRE1) and activating transcriptional factor 6 (ATF6). The activation of UPR restores the ER homeostasis by enhancing protein folding, attenuating protein translation and ER-associated degradation (ERAD).

HCoV infection triggers ER stress and activates UPR in virus-infected cells. Overexpression of the S protein of HCoV-HKU1 and SARS-CoV can activate PERK and the promoters of GRP78 and GRP94. Infection with HCoV-OC43 activates IRE1 and induces X-box protein 1 (XBP1) mRNA splicing, thereby upregulating downstream UPR effector genes. Introduction of two point mutations (H183R and Y241H) into the S protein of HCoV-OC43 induces a higher degree of XBP1 mRNA splicing and results in a more pronounced apoptotic cell death. It is also reported that when the E gene is deleted from SARS-CoV, the mutant virus also induces a higher level of XBP1 mRNA splicing and apoptosis, compared with the wild type control. This suggests that activation of the IRE1-XBP1 pathway may be generally pro-apoptotic during HCoV infection.

MAP Kinase Pathway

The family members of the mitogen-activated protein (MAP) kinases mediate a wide variety of cellular processes in response to extracellular stimuli. Four distinct subgroups within the MAP kinase family have been described: extracellular signal-regulated kinase 1/2 (ERK1/2), ERK5, c-Jun N-terminal kinase (JNK), and the p38 group of protein kinases. Activation of the ERK pathway has been observed in cells infected with a number of HCoVs, including SARS-CoV, MERS-CoV, and HCoV-229E. Phosphorylation of the 90-kDa ribosomal protein S6 kinase (p90RSK), a key substrate of ERK, was also observed in SARS-CoV-infected Vero E6 cells. Activation of p38 and its upstream kinases has been detected in cells infected with HCoV-229E, SARS-CoV, and MERS-CoV. In fact, the replication of HCoV-229E is suppressed by treatment with p38 inhibitor SB203580 or chloroquine, which attenuates p38 activation. As for JNK, phosphorylation of JNK and its upstream kinases was observed in cells infected with SARS-CoV or in cells overexpressing the SARS-CoV S protein. Notably, treatment with JNK inhibitor abolished persistent infection of SARS-CoV. Apart from their involvement in cell survival and apoptosis, MAP kinases also contribute significantly to the induction of pro-inflammatory cytokines during HCoV infection.

Autophagy

Autophagy is a conserved cellular process involving self-eating. Specifically, cells under stress conditions, such as starvation, growth factor deprivation, or infection by pathogens, initiate autophagy in nucleation sites at the ER, where part of the cytoplasm and/or organelles are sequestered in DMVs (autophagosomes) and degraded is response to a fusion with lysosomes. Autophagy is regulated by highly conserved autophagy-related genes.

Coronavirus infection activates the formation of autophagosomes, but inhibition of autophagy does not affect viral replication. The non-structural protein 6 (nsp6) is a transmembrane protein implicated in the formation of DMVs during SARS-CoV infection. Overexpression of nsp6 of IBV, MHV, or SARS-CoV induced the formation of autophagosomes from the ER via an omegasome intermediate. However, autophagosomes induced by IBV infection or overexpression of coronavirus nsp6 had smaller diameters compared with those induced by starvation, indicating that nsp6 may also restrict the expansion of autophagosomes. Notably, recent studies have shown that coronavirus employs the host machinery for COPII-independent vesicular ER export to derive

cellular membranes for DMV formation. Although this process requires an autophagy-related gene called LC3, it is independent of host autophagy.

Apoptosis

Apoptosis is a form of programmed cell death that is tightly regulated. When cells undergo apoptosis, they demonstrate specific hallmarks such as cell shrinkage, extensive plasma membrane blebbing, nuclear condensation, and DNA fragmentation. During viral infections, apoptosis is induced as one of the host antiviral responses to limit virus replication and production. Two main mechanisms of apoptosis have been established – the extrinsic and intrinsic pathways. The extrinsic pathway is initiated by the binding of extracellular death ligands to death receptors from the tumor necrosis factor super-family. The intrinsic pathway occurs internally in the cell and involves changes in the mitochondrial outer membrane permeability based on the ratio of pro-apoptotic and anti-apoptotic B-cell lymphoma 2 (Bcl2) family proteins.

As HCoVs are known to infect tissue cultures, they have been associated with apoptosis induction in a wide spectrum of cell types during infection, including intestinal mucosal cells, kidney tubular cells and neuronal cells. Apoptosis in neuronal cells infected with HCoV-OC43 involved mitochondrial translocation of Bcl-2-associated X protein (BAX) but this phenomenon was independent of caspase activation. HCoV-229E infection resulted in massive cytopathic effect (CPE) and cell death in dendritic cells, albeit independent of apoptosis induction. Since dendritic cells are prevalent throughout the human body, it is possible that they are used as a vehicle to facilitate viral spread. Induction of apoptosis during HCoV infection is also regulated by cellular stress response pathways such as the UPR and MAP kinase pathways described above.

Pathogenesis

HCoV-229E, -OC43, -NL63, and -HKU1 are considered as pathogens causing upper respiratory tract disease and responsible for up to 15%–30% of common colds in adults. Unlike SARS-CoV that spreads from the upper airway to cause a severe lower respiratory tract infection, HCoV-229E and HCoV-OC43 replicate principally in the upper respiratory tract epithelial cells, where they produce virus and cause local respiratory symptoms.

There are striking differences in the extent of genetic variability when isolates of HCoV-OC43 and HCoV-229E are compared. HCoV-229E isolated at geographically distinct locations shows little genetic variability, whereas for HCoV-OC43 the opposite is true. The ability of HCoV-OC43 to tolerate mutations probably accounts for its ability to grow in mouse cells and to infect the mouse brain as well. As for HCoV-NL63, it shares homology with HCoV-229E and phylogenetic analyses suggest that HCoV-NL63 and HCoV-229E diverged approximately 1000 years ago.

HCoVs attach to cellular receptors by the S protein on the surface of the virion. Internalization into host cells occurs by direct fusion with the plasma membrane or by endocytosis. Viral receptors, components that actively promote host cell entry, differ greatly from one virus to another, and each with its own distinct physiological functions. Both APN, the receptor for HCoV-229E, and ACE2, the receptor for SARS-CoV and HCoV-NL63, exist as prominent zinc-dependent peptidases on the plasma membrane. However, unlike SARS-CoV, HCoV-NL63 does not require cathepsin L or endosomal acidification to infect ACE2-expressing cells. In addition to the receptor ACE2, the entry of HCoV-NL63 also requires heparan sulfate proteoglycans on the cell surface, which act as attachment factors that increase the virus density and possibly facilitate receptor binding. Proteolytic cleavage of the HCoV S protein is also an important regulatory mechanism. In recent studies, the proteolytic activation of the HCoV-229E S protein is analyzed using trypsin-like serine proteases. It is found that fusion activation is not dependent on the cleavage of the S1/S2 site, but is highly dependent on the cleavage in the S2′ region. This is very similar to the fusion activation of the IBV S protein, which requires furin-dependent cleavage at the S2′ site.

Compared with SARS-CoV and MERS-CoV, the ability of other HCoVs causing mild infections to suppress the host antiviral response is less well studied. HCoV-229E replication is shown to attenuate the inducible activity of the transcription factor nuclear factor kappa-B (NF-κB) and to restrict the nuclear accumulation of NF-κB subunits. Overexpression of structural or accessory proteins of HCoV-OC43 also leads to downregulation of over 30 genes related to innate immune response, including genes encoding MAP kinases, toll-like receptors, interferons, interleukins, and signal transduction proteins. Similar to the PLPro of SARS-CoV and MERS-CoV, the PLP2 of HCoV-NL63 possesses deubiquitinating (DUB) activity. This DUB activity may remove ubiquitins from critical intermediates in the innate immune signaling pathway, thereby suppressing host antiviral response. PLPro of SARS-CoV and MERS-CoV also recognizes and catalyzes the removal of another ubiquitin-like modifier called interferon-stimulated gene 15 (ISG15). Such deISGylating activity has not been fully characterized for other HCoVs.

HCoV infection is not always confined to the upper respiratory tract and can invade the central nervous system (CNS) under circumstances that are presently uncharacterized. Although evidence for a significant correlation between the presence of HCoV-229E and HCoV-OC43 RNA and multiple sclerosis has not been demonstrated, accumulating evidence from cell culture and animal models highlights their neurotropic and neuroinvasive potential. HCoV-229E RNA is detected in about 44% (40 of 90) of human brains tested, with similar frequencies in brains from multiple sclerosis patients and patients who died from other neurologic diseases or normal control subjects. The detection of HCoV RNA in human brain samples clearly demonstrates that these respiratory pathogens are naturally neuroinvasive in humans and suggests that they may establish a persistent infection in

the human CNS. Therefore, the close structural and biological relatedness of HCoV to the neurotropic animal coronaviruses has led to speculation of the possible involvement of HCoV in neurological diseases. Research proved that classical apoptosis associated with the BAX protein does not play a significant role in HCoV-OC43-induced neuronal cell death and that RIP1 and MLKL, two cellular proteins that are usually associated with necroptosis, another form of regulated cell death when apoptosis is not adequately induced. It is likely that in HCoV-OC43-infected neuronal cells, RIP1 and MLKL are activated to induce necroptotic cell death in an attempt to limit viral replication. However, this regulated cell death also leads to neuronal loss in the mouse CNS and accelerates the neuroinflammation process, reflecting the severity of neuropathogenesis.

Epidemiology and Clinical features

HCoV-229E, -OC43, -NL63, and -HKU1 are distributed globally. By spreading via coughing and sneezing, they cause mild upper respiratory tract diseases in adults. However, in infants, young children, elderly, and immunocompromised individuals, they may sometimes cause life-threatening bronchiolitis and pneumonia. Apart from respiratory illnesses, they may also result in enteric and neurological diseases. Natural infection is probably acquired in a fashion similar to that of many other respiratory viruses, with primary infection of ciliated epithelial cells in the nasopharynx. Although HCoVs were found to cause epidemics every 2–3 years with a high probability of reinfections, there is a paucity of evidence on the epidemiology and clinical manifestations of these four HCoVs worldwide. Reinfection of HCoVs demonstrates that infection does not induce long-lasting protective immunity.

HCoV-229E

First isolated in 1966, HCoV-229E is proposed to originate from African hipposiderid bats and adopt camelids as intermediate hosts. The evolutionary history of HCoV-229E likely shares important characteristics with that of the recently emerged highly pathogenic MERS-CoV. HCoV-229E infection is associated with common cold symptoms in healthy adults. But younger children and the elderly are vulnerable to lower respiratory tract infections. In particular, immunocompromised patients have been reported to suffer severe and life-threatening lower respiratory tract infections attributed to HCoV-229E. Moreover, the serological test suggested the possible involvement of HCoV-229E in the development of Kawasaki syndrome.

The incubation period of HCoV-229E is approximately 2–5 days, followed by illness lasting 2–18 days. Symptoms of HCoV-229E infection include headache, nasal discharge, sneezing, sore throat and general malaise. A few patients exhibit fever and cough. The clinical features can be distinguished from respiratory tract infections caused by other pathogens such as influenza A virus. HCoV-229E tends to be epidemic during winter in temperate-climate countries, and tests of a HCoV-229E laboratory strain suggested it is relatively stable in the environment.

HCoV-OC43

First isolated in 1967, HCoV-OC43 has no serological cross-reactivity with HCoV-229E. However, patients infected with HCoV-OC43 or HCoV-229E cannot be distinguished based on clinical symptoms alone; although coryza (inflammation of the mucous membrane of the nose) occurs more often during HCoV-229E infections, while sore throat manifestations occur more often during HCoV-OC43 infections.

HCoV-OC43 is generally associated with mild upper respiratory tract infections, although it has also been shown to have neuroinvasive properties. In vivo studies in mice have shown that HCoV-OC43 can infect neurons and cause encephalitis. The virus has also been shown to cause persistent infections in human neural cell lines. Since the first isolation of HCoV-OC43 in the 1960s, seven genotypes (A–G) have been identified by phylogenetic analysis. HCoV-OC43 is also transmitted primarily during the winter in temperate climates.

HCoV-NL63

HCoV-NL63 was isolated from a 7-month-old girl with coryza, conjunctivitis, fever, and bronchiolitis in the Netherlands in 2004. HCoV-NL63 and HCoV-OC43 cause most of the respiratory infections leading to hospitalization. Although HCoV-NL63 infections often exists in mixed viral infections, the mixed infection does not usually increase the severity of the disease. In about 71% of the cases, patients are co-infected with other respiratory viruses, such as human rhinovirus, enterovirus and parainfluenza viruses. In most patients, HCoV-NL63 infection is associated with relatively mild symptoms like fever, cough, sore throat and rhinitis. Furthermore, HCoV-NL63 is one of the main causes of croup in children.

HCoV-NL63 infects people in all ages, with the highest infection rate occurring before 5 years of age. It is estimated that 1%–10% of the population suffers annually from cold-like symptoms related to infection with HCoV-NL63. HCoV-NL63 epidemic shows a peak during the spring and summer in Hong Kong, indicating that the seasonality of HCoV-NL63 infection may not be restricted to the winter in tropical and subtropical regions. Surprisingly, studies show that HCoV-NL63 virions are exquisitely stable in liquid media and can be stored also without preservatives at ambient temperature for up to 14 days.

HCoV-HKU1

HCoV-HKU1 was isolated from an adult who had a chronic pulmonary disease in Hong Kong. The clinical symptoms of HCoV-HKU1 infection included rhinorrhea, cough, nasal congestion, fever, sputum, sore throat, chills, postnasal discharge, and tonsillar hypertrophy. About 50% of patients infected with HCoV-HKU1 experience febrile seizures, but the symptoms of HCoV-HKU1 infections in the respiratory tract are not easily separable from those caused by other respiratory viruses. HCoV-HKU1 co-circulates with respiratory syncytial virus and an epidemic usually appears prior to influenza season. In addition, HCoVs have been associated with wheezing and exacerbations of asthma.

Similar to other three mild disease causing HCoVs, HCoV-HKU1 is distributed worldwide. HCoV-HKU1 infection is relatively frequent in adults. One recent study has examined the circulation of HCoV in Israel during 2015–2016. In the winter influenza survey, 10.36% of patients were infected with the other three common HCoVs; among them, 43.43% were infected with HCoV-OC43, 44.95% with HCoV-NL63, and 11.62% with HCoV-229E. Although it is absent from the winter survey, 22.6% of the hospitalized patients were positive for HCoV-HKU1, mainly during the spring-summer period.

Diagnosis

In most cases, HCoV infections, other than SARS-CoV or MERS-CoV, are not well identified by clinical diagnosis because they cause mild, upper respiratory tract disease, and no specific therapy is available. At the same time, HCoV detection is carried out in a limited number of virological laboratories, because HCoV infections cannot be easily distinguished clinically from other causes of upper respiratory tract infections. The presence of multiple genotypes and the co-infection with other respiratory viruses, such as human rhinoviruses, enteroviruses and parainfluenza viruses, render the detection of HCoV even more challanging. HCoVs are also sometimes detected in asymptomatic patients/individuals; so that the presence of these viruses may not be etiologically related to the illness.

Electron microscopic examination of the clinical materials, although laborious, has contributed to the identification and characterization of many HCoVs. Electron microscopy has been extensively used in most of the initial studies, also because isolation of HCoVs from infected individuals and their propagation in tissue culture cells have been technically challanging. HCoV-229E has sometimes been isolated in human diploid cell lines. However, HCoV-OC43 initially required cell organ culture systems for isolation, although this virus can now be grown in tissue culture cells. HCoV-NL63 can infect monkey kidney LLC-MK2 cells or Vero cells, whereas HCoV-HKU1 has been grown only in primary human airway epithelial cells. Sometimes, these cell or organ culture techniques are labor intensive, time consuming, and relatively insensitive.

HCoVs can be detected by RT-PCR with greater sensitivity than standard culture techniques. Nasopharyngeal aspirates are the most common respiratory samples for diagnosis. PCR primers can be designed to be broadly reactive or strain specific, based on primer binding sites (usually in the viral replicase and/or the N gene). A multiplex real-time RT-PCR assay capable of detecting all four common HCoVs (HCoV-229E, OC43, NL63 and HKU1) has become the diagnostic method of choice. A simple and sensitive assay for rapid detection of human coronavirus NL63 (HCoV-NL63) has been developed by colorimetric reverse transcription loop-mediated isothermal amplification (RT-LAMP). This method employs six specially designed primers that recognize eight distinct regions of the HCoV-NL63 nucleocapsid protein gene for amplification of target sequences under isothermal conditions at $63°C$ for 1 h. This RT-LAMP assay can achieve a high sensitivity of 1600 RNA copies per reaction with high specificity, and is suitable for clinical settings without conventional PCR equipment.

Various serologic assays can also be used to detect HCoV infections, including complement fixation assays, neutralization assays, immunofluorescence assays (IFA), enzyme-linked immunoassays (EIA), and hemagglutination inhibition (HI) for viruses with an HE protein. Monoclonal antibodies have also been generated for direct detection of HCoV antigens in nasopharyngeal aspirates samples. Complement-fixing and enzyme-linked immunosorbent assays (ELISA) for HCoV-229E and HCoV-OC43 have been published, but are not yet available in clinical laboratories.

Serologic tests for specific antibody responses against HCoVs are mainly reserved for epidemiologic studies. Initially, virus lysates or inactivated whole viruses were used as antigens for serologic assays. More recently, cloned expressed proteins, synthesized peptides, and pseudoviruses are also used. For example, recombinant N protein of HCoV-HKU1 expressed by E. coli and baculovirus has been used for IgG and IgM detection of sera from patients and normal individuals, using Western blot and EIA.

Treatment

At present, there are no specific antiviral drugs available for treating HCoV infections, and therapy is therefore primarily supportive. Studies have shown that, if diagnosed early, HCoV-NL63 replication can be suppressed by treatment with PEG-IFN. The effect of various chemicals to inhibit HCoV replication has been studied in cell culture. Potential targets for anti-HCoV drugs include inhibiting virus entry, viral RdRP, and viral protease such as Mpro.

Similar to SARS-CoV and MERS-CoV, peptide-based membrane fusion inhibitors targeting the heptad repeat 1 (HR1) and HR2 of HCoV-229E S protein have been developed. They effectively inhibit S-mediated membrane fusion and the replication of HCoV-229E in cell culture. Moreover, when administered intranasally to a mouse model, these inhibitors could widely distribute in the

upper and lower respiratory tracts and maintain fusion-inhibitory activity. Some polymers, such as a cationically modified chitosan known as N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride and its derivatives, have been shown to block HCoV-NL63 entry by inhibiting the receptor binding of S protein. Viral entry can also be inhibited using serine proteases (such as camostat) and cysteine protease inhibitor (such as K11777). These compounds inhibit the critical proteolytic activation of HCoV S protein, thereby blocking the entry step of HCoV infection. Chloroquine, a compound that inhibits acidification of endosomes, can also strongly inhibit the replication of HCoV-OC43 in vitro.

The nucleoside analog ribavirin has been used to treat RSV infection, hepatitis C, and some viral hemorrhagic fevers. However, ribavirin only shows inhibition of HCoV-OC43 replication in cell culture at high concentrations ($IC_{50} \sim 10$ μM), which may not be applicable to humans in clinical treatment. Remdesivir (GS-5734) is a novel adenosine analog prodrug. By targeting the viral RdRP, remdesivir exhibits potent antiviral activity against HCoV-OC43 and HCoV-229E with submicromolar IC_{50} values in cell culture.

Targeting host factors required for replication may be another viable therapeutic approach against HCoV infection. A systemic investigation of HCoV-host interactome has identified cyclophilins as targets for pan-coronavirus inhibitors. In fact, knockdown of cyclophilin A (CypA) in Caco-2 cells inhibits the replication of HCoV-NL63, suggesting that CypA is required for virus replication. Notably, when cells are treated with cyclosporinA (CsA) or FK506, potent immunosuppressive drugs that bind to CypA or related immunophilins, the replication of HCoV-NL63, HCoV-229E, and SARS-CoV is inhibited. Importantly, recent studies also show that CsD Alisporivir, NIM811, and other novel non-immunosuppressive derivatives of CsA and FK506 can also strongly inhibit the growth of HCoV-NL63 in cell culture at low micromolar, non-cytotoxic concentrations. Therefore, non-immunosuppressive CypA inhibitors may be promising candidates as antivirals against HCoV infection. Interestingly, the replication of HCoV-229E and MERS-CoV is also suppressed when exogenous arachidonic acid or linoleic acid is added to the culture medium, suggesting that the regulation of host lipid metabolism could also be a common and druggable target for coronavirus infections.

Prevention

Due to the mild diseases associated with the infection by the four common HCoVs, no vaccines are currently available or being developed. Risk of infection can be reduced by good hand hygiene and coughing etiquette and avoiding close contact with infected individuals.

Acknowledgment

Conflict of Interest

The authors declare no conflict of interest.

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