



The role of lipids in the pathophysiology of coronavirus infections

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ABSTRACT

Coronaviruses, which have been known to cause diseases in animals since the 1930s, utilize cellular components during their replication cycle. Lipids play important roles in viral infection, as coronaviruses target cellular lipids and lipid metabolism to modify their host cells to become an optimal environment for viral replication. Therefore, lipids can be considered as potential targets for the development of antiviral agents. This review provides an overview of the roles of cellular lipids in different stages of the life cycle of coronaviruses.

Keywords: Human coronavirus; Lipids; Metabolism; SARS-CoV-2

Introduction

Coronaviruses are a group of viruses that belong to the *Coronaviridae* family. This viral family is subdivided into 4 genera, including alpha-coronaviruses, beta-coronaviruses, gamma-coronaviruses, and delta-coronaviruses [1]. Human coronaviruses (HCoVs) belong to the alpha-coronavirus or beta-coronavirus genera. Although HCoVs generally cause mild to severe respiratory diseases [2], some coronaviruses have evolved to cross the species barrier [3], giving rise to diseases such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), which caused viral outbreaks in 2003 and 2012, respectively [4,5].

In late December 2019, a new member of *Coronaviridae* family, named SARS-CoV-2, was discovered in China [6]. SARS-CoV-2, like SARS-CoV and MERS-CoV, is a zoonotic virus that has crossed the species barrier. Thus, SARS-CoV-2 is an emerging virus, and it causes coronavirus disease 2019 (COVID-19) [7,8]. As of September 14, 2021, the World Health Organization has

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reported more than 200 million confirmed cases of COVID-19, including more than 4 million deaths [9].

Coronaviruses have a positive-strand RNA enclosed in a protein shell that is surrounded by a host cell-derived membrane [10]. Coronaviruses cross the host cell membrane at least twice in the cell entry and exit stages of viral particles during the replication cycle. Replication of coronaviruses depends on their interactions with cellular lipids, which are essential for the successful life cycle of coronaviruses [7,11].

Cellular lipids play key roles in viral infections, particularly during the entry process and virion maturation. Lipid signaling and its synthesis machinery in host cells can be remodeled via proteins encoded by viruses [12]. Moreover, viruses alter cellular metabolism to promote proficient viral replication [13]. In this paper, we review the interactions of coronaviruses with the cellular lipids required for an effective viral life cycle.

Lipids and Viral Infections

Viruses have complex interactions with cells. According to recent studies, cellular lipids play important roles in the viral life cycle, such as viral-to-host cell membrane fusion, viral replication, and endocytosis and exocytosis [12]. Viral entry involves specific lipids, which vary among viruses [14]. The combination of lipids and proteins in the host cell membrane and the viral envelope plays a key role in viral infections [10]. In fact, viruses can alter the metabolism and signaling of lipids in host cells in order to facilitate their replication, and such viral interactions with cellular lipids have shown to be different among viruses [15]. The pathways of cellular lipid biogenesis are among the most important cellular pathways hijacked by viruses. Lipids play a crucial role in the formation of viral replication organelles, as well as energy production for viral replication. Lipids are also important for regulating the proper cellular arrangement of viral proteins and the assembly, trafficking, and release of viral particles [16–18].

Lipid rafts are specialized microdomains (10 to 200 nm) of the cell membrane that are found on the membranes of endosomes and exosomes in the endoplasmic reticulum (ER) and the Golgi complex [19]. These microdomains contain sphingolipids, cholesterol, various receptors, and other proteins [20]. Lipid rafts play important roles in viral infection, for instance in endocytosis or during different stages of pathogenesis [21]. A vital component of lipid rafts is cholesterol, which plays a major role in viral entry and release for enveloped viruses such as coronaviruses and influenza virus [22]. The major surface glycoproteins of

influenza virus, hemagglutinin (HA) and neuraminidase, are associated with lipid rafts, and depletion of cholesterol by methyl- β -cyclodextrin (M β CD) can reduce the transport of HA from the trans-Golgi network to the cell membrane [23]. The requirement of cholesterol for viral pathogenesis may differ in respiratory RNA viruses, and the depletion of cholesterol by M β CD is involved in the increased budding of influenza A virus (IAV) from the host cells during pathogenesis [24]. In fact, cholesterol is essential as a vital component for sustaining IAV and respiratory syncytial virus (RSV) infectivity [25].

In a previous study, pretreatment of influenza virions with M β CD efficiently depleted cholesterol in the envelope and considerably reduced the infectivity of the virus in a dose-dependent manner [15]. In addition, the depletion of cholesterol by M β CD decreased RSV infection and interrupted lipid raft microdomains, implying that cholesterol in lipid rafts is essential for the interactions of viral proteins during infection [25]. According to prior research, human rhinovirus serotype 2 can enter into the cell through clathrin-mediated endocytosis, and the depletion of cholesterol by M β CD can prevent clathrin-mediated endocytosis and decrease viral entry [26]. Some studies showed that ceramide-rich platforms play key roles in rhinovirus infections. This suggests a novel target to treat rhinovirus infections [27]. For hepatitis C virus (HCV), it is clear that the utilization of very low-density lipoprotein secretion machinery during HCV infection supports the exit of the virus from its cellular host [28].

The Genome of Coronaviruses

Coronaviruses are enveloped positive-stranded RNA viruses with a genome size of about 27 to 30 kb, 5'-cap structure, and 3'-poly-A tail [29]. The genome of coronaviruses contains several segments—including untranslated regions (UTRs), the spike (S) protein, the envelope (E) protein, the membrane (M) protein, and the nucleocapsid (N) protein—organized as follows: 5'-leader-UTR-replicase-S-E-M-N-3'UTR-poly(A) tail [30,31]. There are 2 overlapping open reading frames (ORFs), referred to as ORF1a and ORF1b, in the replicase gene. These ORFs encode 2 polypeptides (pp1a and pp1ab), which are processed into 16 nonstructural proteins by viral-encoded enzymes including 3-chymotrypsin-like protease or main protease and 1 or 2 papain-like proteases (Figure 1) [31,32].

Structural Proteins of Coronaviruses

The surface spike proteins of coronaviruses are formed by

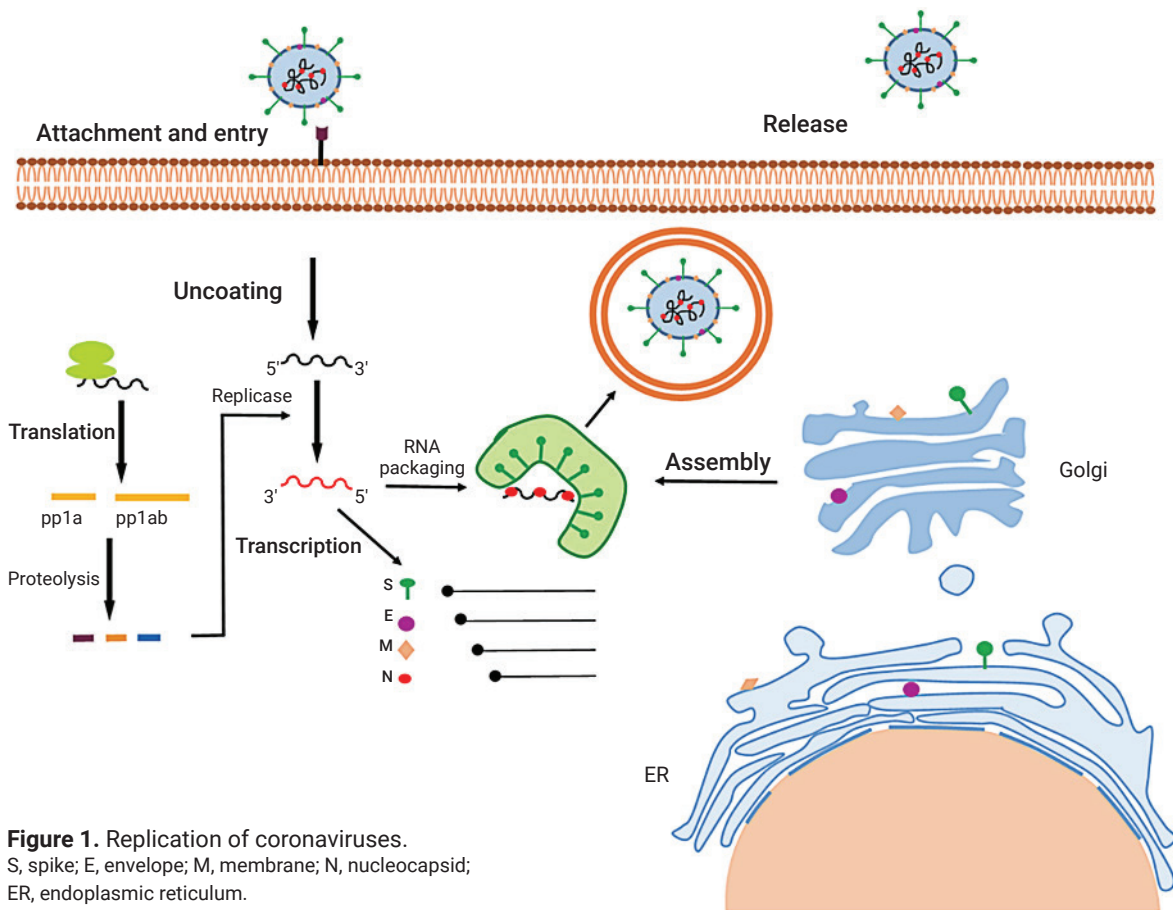


Figure 1. Replication of coronaviruses. S, spike; E, envelope; M, membrane; N, nucleocapsid; ER, endoplasmic reticulum.

trimers of S molecules. The S protein is a class I viral fusion protein that plays a major role in viral binding to cellular receptors in order to enter the host cell [33]. The S protein undergoes modification by N-linked glycosylation in the ER [34]. The S glycoprotein contains 2 subunits (S1 and S2). The S1 subunit is variable, whereas the S2 subunit is conserved among diverse isolates of a single coronavirus. The S1 subunit is responsible for the binding of the virus to the cell receptor, whereas S2 mediates the fusion of the viral envelope and cellular membranes [35]. The M protein, which is considered to be the most abundant structural protein in coronaviruses, is N-glycosylated [36], while some beta-coronaviruses are modified by O-linked glycosylation [37]. The glycosylation of the M protein influences the interferon-inducing ability of some coronaviruses and also shapes the virion envelope [36]. The E protein is the smallest protein and is found in virions in limited amounts. Furthermore, the E protein is essential for viral infectivity and also plays a key role in virion assembly [38]. Some studies have shown that the E protein plays a role in viral pathogenesis [39]. The N protein, which is the only protein

located in the ribonucleoprotein core, is made up of 3 domains, including the N-terminal domain and C-terminal domain, which are separated by an intrinsically disordered central region. The N protein is a phosphoprotein that binds to the RNA genome and is involved in the formation of the nucleocapsid [40]. In addition, HA esterase as a structural protein is encoded by beta-coronaviruses of lineage A, such as OC43-CoV. During viral infection, viruses can alter host cell metabolism and reprogram it to use cellular factors [13].

Lipids and Coronavirus Entry

To initiate infection, coronaviruses need to enter into the cell through interactions between the viral spike proteins and the cellular receptors located on the surface of the host cell [33]. The cellular plasma membrane contains subdomains of lipid rafts composed of cholesterol and glycosphingolipids [41]. SARS-CoV-2 and SARS-CoV use the angiotensin-converting enzyme-2 (ACE2) receptor for cellular entry [42]. Interactions between the ACE2 receptor and the spike protein are facilitated by cholesterol-rich

microdomains. Studies have shown that the depletion of cholesterol by M β CD in cells expressing ACE2 leads to a decrease in binding viral S glycoproteins in 50% of SARS-CoV infections. Therefore, M β CD affects cholesterol levels and ACE2 receptor expression. The depletion of cholesterol by M β CD also prevents the attachment of coronaviruses to the cell membrane [43]. Human coronavirus 229E (HCoV-229E) binds to the cellular receptor (aminopeptidase N or cluster of differentiation 13 [CD13]) for cellular infection, CD13 localized in lipid rafts, so the depletion of cholesterol by M β CD decreases the likelihood of HCoV-229E infection and prevents viral entry into host cells [44]. According to *in vitro* experiments, cholesterol supplementation enhances the propensity of the virus to fuse with the cell membrane. Clathrins, caveolins, and dynamin in lipid rafts play an important role in viral entry [43]. Some coronaviruses utilize lipid rafts for the cellular entry process. It has been reported that lipid rafts are required in the attachment process during infectious bronchitis virus infection [45], and another study also showed that lipid rafts are required

for the entry of SARS-CoV into Vero E6 cells [46].

Cholesterol in the plasma membrane of target cells is also important for SARS-CoV infection [47]. It has been reported that drugs causing cholesterol depletion can inhibit the entry of murine hepatitis virus and HCoV-229E into host cells [48]. Since coronaviruses are enveloped particles, fusion with the host cell membrane is necessary before the internalization of viral particles into cells [49], SARS-CoV uses various endocytic routes including clathrin-mediated dependent, lipid raft-dependent, and clathrin- and caveolae-independent endocytosis [50]. However, HCoVs such as human coronavirus OC43 (HCoV-OC43) use caveolae-dependent endocytosis as the entry pathway [51] and feline infectious peritonitis virus uses clathrin-independent and caveolin-independent endocytosis to enter the host cell (Table 1) [33,44–46,48–60]. Therefore, it is necessary to investigate the entry pathways of coronaviruses and the mechanisms of those pathways in order to design selective inhibitors for the entry stage of coronaviruses.

Table 1. Lipid interactions in the coronavirus life cycle

Virus	Receptor	Steps of the coronavirus life cycle	Lipid interactions	Endocytic pathway	References
CCoV serotype 2	APN	Entry	Plasma membrane cholesterol	Unknown	[54,55]
MHV	CEACAM1	Entry	Lipid rafts	Clathrin-mediated endocytosis	[33,48,49]
FCoV serotype 2	APN	Entry	Cholesterol	Clathrin- and caveolae-independent pathway that depends strongly on dynamin	[33,54]
PEDV	APN	Entry	Cholesterol, lipid rafts	Clathrin- and caveolae-mediated endocytosis pathways	[33,56]
TGEV	APN	Entry	Cholesterol	The route of entry is not yet known in terms of which endocytosis pathway occurs (clathrin- or non-clathrin-dependent mechanism or both)	[57,58]
IBV	Not recognized	Entry	Lipid rafts	Macropinocytosis	[45]
HCoV-229E	APN	1. Entry 2. Replication	1. Cholesterol 2. LA and AA	Caveolae-dependent endocytic	[44,49,53,59]
HCoV-OC43	HLA class I, sialic acids, Neu5,9	Entry	Cholesterol	Caveolae-dependent endocytosis	[33,51]
MERS-CoV	DPP4 or CD26	Replication	LA and AA	Clathrin-mediated endocytosis	[51,53,60]
SARS-CoV	ACE2	Entry	Lipid rafts	Clathrin- and caveolae-independent mechanism; may involve a clathrin-mediated or clathrin-dependent mechanism	[46,50]

CCoV, canine coronavirus; APN, aminopeptidase N; MHV, murine hepatitis virus; CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; FCoV, feline coronavirus; PEDV, porcine epidemic diarrhea virus; TGEV, transmissible gastroenteritis virus; IBV, infectious bronchitis virus; HCoV-229E, human coronavirus 229E; LA, linoleic acid; AA, arachidonic acid; HCoV-OC43, human coronavirus OC43; HLA, human leukocyte antigen; MERS-CoV, Middle East respiratory syndrome coronavirus; DPP4, dipeptidyl peptidase 4; SARS-CoV, severe acute respiratory syndrome coronavirus; ACE2, angiotensin-converting enzyme-2.

Lipids and Proliferation of Coronaviruses

The replication of most positive-strand RNA viruses occurs in the cytoplasm of the host cell. These viruses induce the formation of subcellular membranes known as virus factories or viroplasm, where they can efficiently replicate, recruit host components, and escape from the defense mechanisms of host cells [61,62].

Depending on the viral family and genus, these remodeled intracellular membranes may originate from various organelles, including the ER, late endosomes/lysosomes, or the mitochondrial outer membrane. Positive-sense RNA viruses induce the formation of 2 types of vesicles: (1) spherules, which are generated by viruses in the *Togaviridae*, *Bromoviridae*, and *Nodaviridae* families; and (2) double-membrane vesicles (DMVs), generated by the *Coronaviridae*, *Arteriviridae*, and *Picornaviridae* families [63,64].

Although the role of DMVs has not been fully clarified, they may act as autophagosomes in autophagy. The virus uses DMVs to protect against host antiviral responses or in viral replication [65]. DMV formation requires both viral and host factors. In coronaviruses, the nonstructural proteins nsp3, nsp4, and nsp6, which contain predicted transmembrane domains, play an important role in DMV biogenesis [66].

The replication process of coronaviruses that occurs in the cytoplasm of the infected host cells is similar to that of other positive-strand RNA viruses and is associated with intracellular lipid membranes derived from various organelles. Moreover, coronaviruses can also utilize cellular lipids for their replication, and thus the replication of viruses induces cellular membrane remodeling [67]. HCoV-229E infection can rearrange the cellular lipid profile, and lysophosphatidylcholine, lysophosphatidylethanolamine, and fatty acids are upregulated after infection with HCoV-229E. However, HCoV-229E replication is suppressed by exogenous supplementation of linoleic acid (LA) or arachidonic acid (AA) in infected cells. LA and AA have potent modulatory effects on MERS-CoV infection and replication of HCoV-229E [53].

The nonstructural proteins nsp3, nsp4, and nsp6 facilitate the formation of replication/transcription complexes by inducing the formation of DMVs [68]. Some cellular enzymes such as cytosolic phospholipase A2 α are involved in the formation of DMVs, which take part in the replication of coronaviruses [69]. As described above, the development of new inhibitors could play a strategic role in preventing virus transmission from infected individuals to the healthy population.

Lipid Pathways as Potential Therapeutic Targets in HCoV Infections

Since lipids play vital roles in the viral life cycle, using drugs that can target lipid metabolism may therefore interfere with infections of SARS-CoV-2 and other coronaviruses [70]. Phytosterols can affect viral infection by decreasing the levels of cholesterol in cell membrane [71]. In this regard, umifenovir and chloroquine are antiviral drugs that inhibit the process of endocytosis [72]. The mechanism of actions of these antiviral drugs suggests the significance of the viral membrane for developing potent drugs. Statins can reduce cholesterol levels and disrupt lipid rafts, thereby inhibiting coronavirus infection [73].

Conclusion

Overall, the replication process of coronaviruses relies on cellular lipids, and these viruses alter the cellular lipid profile. Coronaviruses can stimulate membrane lipid remodeling in host cells and utilize cellular lipids to form viral particles or viral replication complexes, which are involved in the replication and infection process of coronaviruses. In conclusion, the study of cellular lipids and remodeling of lipid metabolism in coronavirus infections provides a good background for the development of antiviral drugs and vaccines.

Notes

Ethics Approval

Not applicable.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Availability of Data

All data generated or analyzed during this study are included in this published article. For other data, these may be requested through the corresponding author.

Authors' contributions

Conceptualization: MZ; Data curation: PH, SS, AR; Investigation: PH, SS; Supervision: MZ; Writing—original draft: SN, MM, FB; Writing—review & editing: MZ, PH.

Additional Contributions

The images that constitute Figure 1 were provided by Milad Zandi (Tehran University of Medical Sciences, Tehran, Iran).

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