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Autophagy in severe acute respiratory syndrome coronavirus 2 infection

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) orchestrates host factors to remodel endomembrane compartments for various steps of the infection cycle. SARS-CoV-2 also intimately intersects with the catabolic autophagy pathway during infection. In response to virus infection, autophagy acts as an innate defensive system by delivering viral components/particles to lysosomes for degradation. Autophagy also elicits antiviral immune responses. SARS-CoV-2, like other positive-stranded RNA viruses, has evolved various mechanisms to escape autophagic destruction and to hijack the autophagic machinery for its own benefit. In this review, we will focus on how the interplay between SARS-CoV-2 viral proteins and autophagy promotes viral replication and transmission. We will also discuss the pathogenic effects of SARS-CoV-2-elicited autophagy dysregulation and pharmacological interventions targeting autophagy for COVID-19 treatment.

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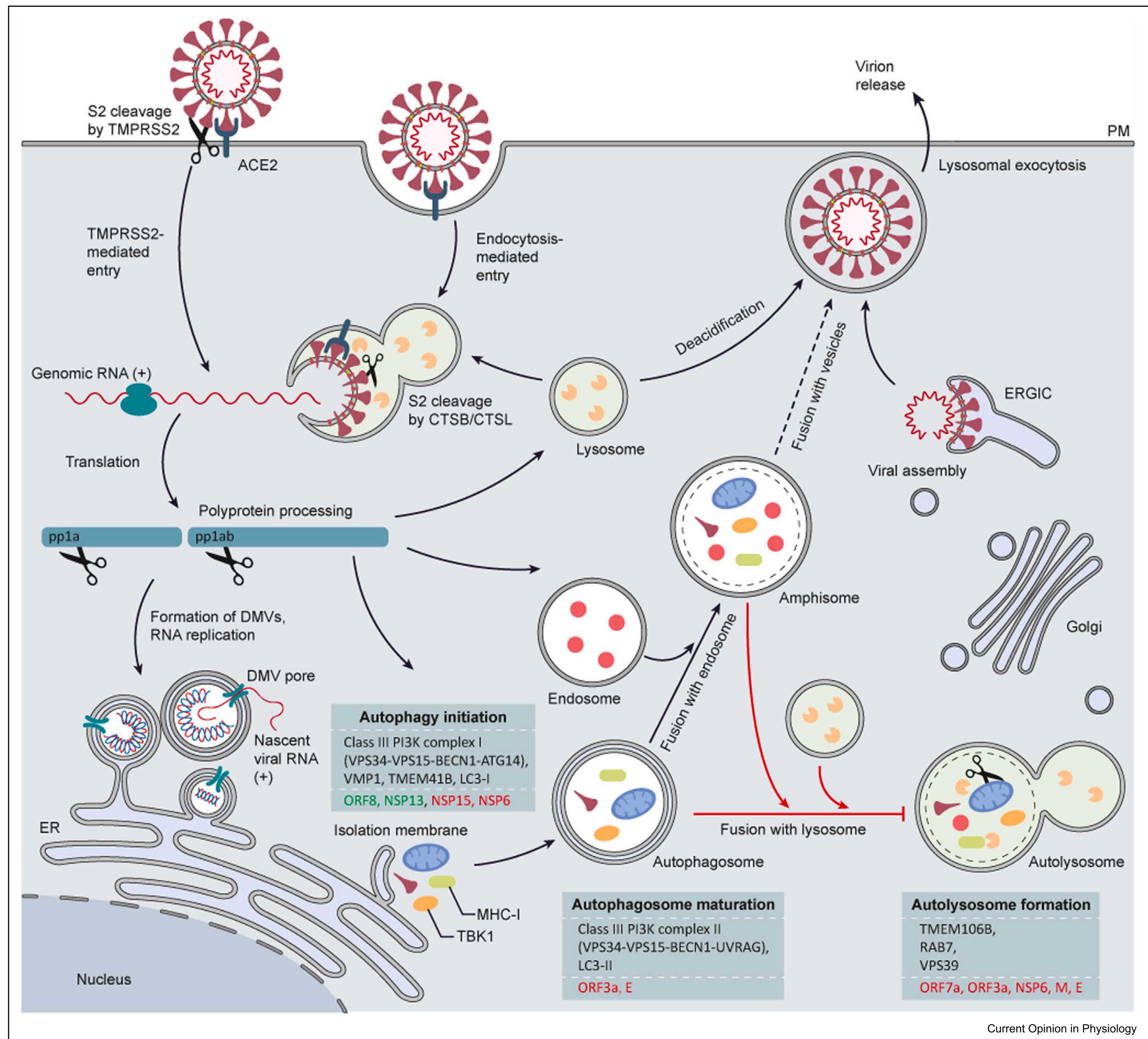
Introduction

As a catabolic process essential for cellular homeostasis maintenance, autophagy sequestrates a portion of the cytosolic constituents and delivers them to lysosomes for degradation [1]. In response to different stresses such as nutrient deprivation, oxidative effects, organelle damage, and pathogen invasion, cargos are either nonselectively or selectively engulfed by double-membraned autophagosomes, which eventually fuse with lysosomes to become degradative autolysosomes to digest and recycle sequestrated materials [1–3]. Autophagy requires the concerted actions of specific autophagy proteins and factors involved in endocytic trafficking. Upon autophagy induction in mammalian cells, the FIP200–ATG13–ULK1 Atg1 complex is firstly assembled on the ER, followed by targeting of the class-III phosphatidylinositol 3-phosphate kinase (PtdIns3-kinase) complex I (VPS34–VPS15–BECN1/Beclin1–ATG14) to generate phosphatidylinositol 3-phosphate (PtdIns3P)-enriched ER subdomains, called omegasomes [4]. Omegasomes recruit downstream autophagy proteins for nucleation and initiation of isolation membranes (IMs). IMs are labeled by ubiquitin-like Atg8/LC3 family proteins that are conjugated to phosphatidylethanolamine (PE), a process catalyzed by the E1-like enzyme ATG7, the E2-like enzyme ATG3, and the E3-like enzyme ATG12–ATG5 [5]. IMs expand and close to form double-membrane autophagosomes, which then mature by fusing with early endosomes, late endosomes, and lysosomes to become single-membraned amphisomes. These eventually form degradative autolysosomes [1]. Fusion of autophagosomes/amphisomes with late endosomes/lysosomes requires Rab7, tethering factors such as the homotypic fusion and protein sorting (HOPS) complex and EPG5, and the STX17–SNAP29–VAMP8 SNARE complex [1]. Maturation is also facilitated by autophagosome-localized PtdIns3P generated by the class-III PI3K complex II (VPS34–VPS15–BECN1–UVRAG) [6]. Autophagic cargos are degraded by lysosomal hydrolases and then recycled [6,7].

Coronaviruses are a group of spherical, enveloped viruses with an ~30-kb, positive-sense, single-stranded RNA genome [8]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of the global COVID-19 pandemic, belongs to the *betacoronavirus* genus along with mouse hepatitis virus (MHV), severe acute respiratory syndrome coronavirus (SARS-CoV), and Middle East respiratory syndrome coronavirus [8,9]. The genomic RNA of SARS-CoV-2 encodes four viral structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N), and also 16 nonstructural proteins (NSP 1–16) and at least 9 accessory proteins (ORF proteins) [10,11]. Coronavirus replication and release involve extensive endomembrane remodeling, trafficking, and fusion [12]. After engaging with the cell surface-localized receptor (e.g. ACE2 for SARS-CoV-2), coronaviruses enter host cells through endocytosis-mediated internalization or direct fusion with the plasma membrane (PM) (Figure 1) [13,14]. ER-derived double-membrane vesicles (DMVs) serve as replication

Figure 1



The interplay between SARS-CoV-2 and autophagy. SARS-CoV-2 enters host cells through direct fusion with the cell membrane or endocytosis. Surface-localized TMPRSS2 or late endosomal/lysosomal cathepsin B/cathepsin L recognizes and cleaves a specific site on the S2 subunit of S protein (S2 cleavage), which leads to membrane fusion and viral genome release. ER-derived DMVs serve as ROs to provide viral RNA replication platforms. The molecular pore embedded in DMVs exports nascent viral RNA for translation or assembly. Virions are assembled at the ERGIC or SMVs (not shown) and released through lysosomal exocytosis. A series of autophagy proteins are essential for SARS-CoV-2 infection, including VPS34 complexes, VMP1, TMEM41B, LC3, TMEM106B, RAB7, and VPS39. After translation and processing, SARS-CoV-2 viral proteins manipulate multiple steps of the autophagy pathway to benefit viral replication and egress. NSP6, ORF8, and NSP13 promote, while NSP15 inhibits autophagy initiation/autophagosome formation; NSP6 and E inhibit autophagosome maturation; ORF7a, ORF3a, NSP6, and M inhibit formation of autolysosomes. Specifically, ORF8 mediates autophagic degradation of MHC-I, and NSP13 mediates autophagic degradation of TBK1. ORF3a inhibits the fusion between amphisomes/ autophagosomes and lysosomes through sequestration of the HOPS component VPS39. ORF3a, ORF7a, and NSP6 lead to lysosome deacidification. ORF3a also promotes lysosomal exocytosis-mediated viral egress. Proteins encoded by autophagy genes that are required for SARS-CoV-2 infection are listed in black. SARS-CoV-2 viral proteins involved in autophagy inhibition and autophagy induction are in red and green, respectively. LC3-I, unlipidated LC3; LC3-II, lipidated LC3.

organelles (ROs) for coronaviral RNA synthesis (Figure 1) [15,16••]. The virions are assembled at the ER–Golgi intermediate compartment (ERGIC) or single-membrane vesicles (SMVs) and released through the secretory pathway and/or the lysosome exocytic pathway [17–19]. In virus-infected cells, autophagy provides an innate defensive mechanism by selectively degrading virions or viral proteins/genomes, and by activating the immune system for virus clearance [20,21]. On the other hand, viruses hijack the autophagic machinery to facilitate critical processes such as replication and egress [22,23]. In this review, we will briefly summarize the 'proviral' and 'antiviral' roles of autophagy in positive-stranded RNA virus infection, then we will focus on the latest progress in understanding the intersection of SARS-CoV-2 and autophagy for remodeling of endomembrane compartments for viral replication and egress. Potential therapeutic interventions targeting autophagy in SARS-CoV-2 infection will also be discussed.

Dual functions of autophagy during positive-stranded RNA virus infection

Positive-stranded RNA virus infection can induce autophagy via multiple mechanisms. Toll-like receptors (TLRs) such as TLR3, TLR7, TLR8, and TLR10 sense viral constituents and induce autophagy [24,25]. TLR signaling triggers the recruitment of BECN1 and TRAF6 through the TLR downstream adaptor MyD88/TRIF to activate autophagosome biogenesis [24]. Viral proteins and/or genomes can also activate autophagy by eliciting cellular stresses such as ER stress and oxidative stress [26,27]. Autophagy defends cells against viral infection by delivering viruses/viral proteins to lysosomes for degradation [3,21]. Autophagy also activates innate and adaptive immune responses. For example, during virus infection, autophagy contributes to major histocompatibility complex class I and II (MHC-I and MHC-II) antigen presentation by facilitating viral antigen processing and transport [28,29]. Autophagy also regulates cytokine production, inflammation, and cell survival to defend against virus invasion [20,30–32].

Positive-stranded RNA viruses have evolved diverse mechanisms to subvert autophagosomes and/or amphisomes for replication and extracellular release. Some viruses such as dengue virus, Zika virus, hepatitis-C virus (HCV), and Chikungunya virus exploit DMVs with hallmarks of autophagosomes as ROs to anchor viral RNA replication complexes [23]. Poliovirus and enteroviruses also use non-lytic secretory autophagy for egress [23]. The autophagosomes formed in cells infected with positive-stranded RNA viruses are able to fuse with late endosomes, but cannot further fuse with lysosomes [33–37]. Inhibiting the assembly of STX17–SNAP29–VAMP8 complexes appears to be a mechanism widely employed by viruses to block autolysosome formation. For example, the viral proteinase 3C of coxsackievirus B3 and enterovirus 68 mediates cleavage of SNAP29 to inhibit autophagosome–lysosome fusion [33,36].

Coronavirus infection also promotes autophagosome formation but blocks fusion of autophagosomes/amphisomes with lysosomes [38–41]. Coronaviruses induce the formation of ER-derived DMVs to provide platforms for genome replication and to protect viral RNAs from degradation and immune defenses (Figure 1) [42]. Despite the morphological similarities between DMVs and autophagosomes, the intact autophagic machinery is not required for DMV formation and viral replication in coronavirus-infected cells. LC3, an Atg8 family member, is enriched in viral RNA-containing DMVs during MHV infection [43]. However, the PE-conjugated form of LC3, which labels autophagic structures, fails to label DMVs. The DMVs in MHV-infected cells are labeled with the ER-associated degradation (ERAD) components EDEM1 and OS-9, indicating that the machinery mediating the formation of the unlipidated LC3-coated EDEMosomes, which deliver the short-lived ERAD regulators to endosomes/lysosomes, is involved in forming DMVs [43]. LC3 participates in DMV formation or genome replication of MHV and SARS-CoV [43,44]. Depleting LC3 inhibits DMV formation as well as MHV infection [43]. Consistent with the idea that conjugation of LC3 with PE is not involved in DMV formation, the autophagic genes required for LC3-PE conjugation, such as *ATG7*, *ATG5*, and *ATG16L*, are dispensable for coronavirus infection. The replication of MHV is not affected in *Atg7*-knockout (KO) mouse embryonic fibroblasts (MEFs) [43], and *Atg5*-deleted bone marrow-derived macrophages or MEFs [45]. Depletion of *ATG5* or *ATG16L* also has no effect on SARS-CoV-2 infection in Huh7–Lunet/T7 cells and in Vero E6 cells [41,46]. KO of *Atg5* in MEF cells expressing human ACE2 (MEF–hACE2) has no effect on SARS-CoV infection [47]. SARS-CoV-2 replication was, however, reported to be slightly decreased in *Atg5* KO MEF–hACE2 cells but dramatically blocked by *Atg3* depletion [48]. In addition to autophagy, the LC3/Atg8 conjugation system acts in multiple vesicle trafficking and secretion processes [49]. The differential involvement of the LC3/Atg8 conjugation system in autophagy-independent processes under different experimental settings may account for the discrepancies. Therefore, the autophagic structures play distinct roles during the infection of different positive-stranded RNA viruses.

Severe acute respiratory syndrome coronavirus 2 hijacks the autophagic machinery for endomembrane remodeling

Genome-wide CRISPR/Cas9 screens and proteomics have identified a subset of genes involved in autophagy as essential for SARS-CoV-2 infection [50–55]. The ER-

localized transmembrane autophagy proteins VMP1 and TMEM41B are required for autophagosome formation (Figure 1) [55,56]. During autophagosome biogenesis, VMP1 regulates the establishment and disassociation of ER–IM contacts, while TMEM41B interacts with VMP1 [56,57]. Depletion of VMP1 or TMEM41B significantly inhibits infection by SARS-CoV-2 and other coronaviruses at post-entry steps [52–54,58]. TMEM41B contributes to viral replication complex formation by mobilizing ER lipids for endomembrane remodeling [59,60].

Autophagy genes involved in PtdIns3P synthesis were also identified in the screens [50,51]. Depletion of the PtdIns3-kinase complex-I and complex-II components VPS34/PIK3C3, ATG14, BECN1, and UVRAG dramatically prevents SARS-CoV-2 infection [41,51,55]. Treatment with VPS34 inhibitors, including 3-methyladenine (3-MA), Wortmannin, and SAR405, effectively inhibits SARS-CoV-2 replication in multiple cell lines, lung tissues from hACE2 transgenic mice, and xenografted human lung tissues [38,41,51]. The expression level of the VPS34–VPS15–BECN1 complex is gradually increased along with SARS-CoV-2 infection up to 72 hours post infection (hpi), while the ATG14 expression level is temporarily increased [41]. Thus, a subset of genes essential for autophagosome formation is also involved in the endomembrane remodeling required for SARS-CoV-2 infection.

Lysosome function is essential for degrading autophagic cargoes and also for lysosomal regeneration from autolysosomes [1,61]. RAB7 is a late-endosome/lysosome-localized small GTPase essential for endosomal maturation, lysosome function, and autophagy [62]. Depleting RAB7 or inhibiting RAB7 activity inhibits SARS-CoV-2 and MHV infection [18••,55]. TMEM106B is a lysosomal membrane protein involved in acidification, transportation, and exocytosis of lysosomes [63]. TMEM106B depletion impairs autophagosome–lysosome fusion [64], leading to accumulation of autophagosomes as well as accelerating frontotemporal lobar degeneration pathologies in a progranulin (GRN)-deficient mouse model [65,66]. The coronaviruses SARS-CoV-2 and MHV exploit the late endosome/lysosome exocytic pathway for egress (Figure 1) [18••]. SARS-CoV-2 or MHV virions are delivered into or assembled at RAB7/LAMP1-labeled single-membrane structures [18••]. During lysosomal exocytosis, the BORC–ARL8b complex mediates the retrograde transport of late endosomes/lysosomes to the PM, and the STX4–SNAP23–VAMP7 SNARE complex mediates fusion with the PM [67]. SARS-CoV-2 infection facilitates recruitment of the SNARE complex and significantly elevates lysosomal exocytosis [18••,40•]. SARS-CoV-2 infection also causes lysosomal deacidification, which may contribute to the enhanced lysosomal exocytosis [18••,39]. The nature of these LAMP1-labeled single-membrane structures in SARS-CoV-2-infected cells has yet to be determined. SARS-CoV-2-infected cells accumulate amphisome-like structures, in which viral particles are detected [39••]. It is likely that SARS-CoV-2 induces promiscuous fusion of intracellular vesicles, resulting in formation of hybrid structures that mediate virus egress. Lysosomal exocytosis requires TRPML-mediated lysosomal Ca^{2+} release [67]. The TRPML inhibitor berbamine inhibits SARS-CoV-2 infection [68]. In addition to the large, SMVs that contain multiple viral particles, numerous small vesicles that usually contain a single viral particle are also observed at 10 hpi in SARS-CoV-2-infected cells [69]. These small vesicles also traffic to and fuse with the PM [69]. Thus, multiple pathways may be employed for viral release.

Severe acute respiratory syndrome coronavirus 2 viral proteins manipulate the autophagy pathway

The SARS-CoV-2 viral proteins modulate autophagy at multiple steps to benefit virus production (Table 1). SARS-CoV-2 infection effectively blocks autolysosome formation and concurrently causes accumulation of numerous autophagosomes/amphisomes [39••,70]. Mechanistically, late endosome/lysosome-localized ORF3a of SARS-CoV-2 interacts with and sequestrates the

Table 1

The role of SARS-CoV-2 viral proteins in the autophagy pathway.

NSP6	● Inhibits *cis*-Golgi- and endosome-derived HyPAS formation and autophagosome biogenesis. ● Inhibits lysosomal acidification and acidic autolysosome formation. ● IBV/MHV/SARS-CoV NSP6 promotes autophagosome formation but inhibits autophagosome maturation.	[72–75]
ORF3a	● Inhibits acidic autolysosome formation through sequestration of the HOPS component VPS39. ● Inhibits PI3K complex-II assembly through interaction with and sequestration of UVRAG. ● Inhibits lysosomal acidification and promotion of lysosomal exocytosis.	[39••,40•,48]
ORF8	● Promotes autophagic degradation of MHC-I.	[81]
NSP13	● Promotes autophagic degradation of TBK1.	[82]
ORF7a	● Inhibits lysosomal acidification and acidic autolysosome formation.	[78]
NSP15	● Inhibits autophagy initiation.	[78]
M	● Inhibits autolysosome formation.	[39••,78]
E	● Inhibits autophagosome maturation and autolysosome formation.	[78]

HOPS component VPS39 to prevent the assembly of STX17–SNAP29–VAMP8 SNARE complexes that are required for fusion between autophagosomes/amphisomes and lysosomes [39••,70]. Expression of SARS-CoV-2 ORF3a also leads to deacidification of lysosomes and significantly elevates lysosomal exocytosis (Table 1) [18••,39]. SARS-CoV-2 ORF3a promotes SARS-CoV-2 infection and MHV egress [40•,48]. Interestingly, SARS-CoV ORF3a, which displays 72.4% amino acid identity with SARS-CoV-2 ORF3a, fails to inhibit autophagy or promote lysosomal exocytosis [39••,40•]. Ser171 and Trp193 in SARS-CoV-2 ORF3a are essential for sequestrating VPS39, promoting lysosomal exocytosis and blocking autophagy [40•]. Mutating the corresponding residues (i.e. E171S and R193W) endows SARS-CoV ORF3a with these functions [40•]. SARS-CoV-2 ORF3a, but not SARS-CoV ORF3a, interacts with UVRAG, whose sequestration inhibits PtdIns3-kinase complex-II assembly for autophagosome maturation [48]. Compared with SARS-CoV, the presence of a Furin cleavage site (PRRAR) in the S protein and also the ability of ORF3a to enhance lysosomal exocytosis endows SARS-CoV-2 with more efficient virus entry and release, contributing to its increased infectivity and pathogenicity [40•,71].

Autophagosomes in SARS-CoV-2-infected cells are smaller than those in starved cells [39••]. Resembling SARS-CoV-2 infection, expression of NSP6 from infectious bronchitis virus (IBV), MHV, and SARS-CoV inhibits autophagic flux and causes accumulation of smaller autophagosomes than the ones formed under starvation conditions [39••,72–74]. NSP6 expression as well as IBV infection induces the formation but restricts the expansion of autophagosomes [73,74]. SARS-CoV-2 NSP6 inhibits the formation of hybrid pre-autophagosomal structures (HyPAS), which are derived from cis-Golgi and endosomal membranes essential for autophagosome biogenesis [75]. NSP6 expression, similar to SARS-CoV-2 infection, induces inflammatory cell death, known as pyroptosis, which is associated with blocked autophagy and lysosomal deacidification [72]. SARS-CoV-2 NSP6 also inactivates a lysosomal ATPase proton-pump component, ATP6AP1, to impair lysosomal acidification, and thus blocks autophagosome maturation (Table 1) [72]. It will be interesting to determine whether the NSP6 mutations found in the SARS-CoV-2 Omicron variant affect its role in autophagy [76,77]. ORF7a, E, M, and NSP15 of SARS-CoV-2 have also been shown to impair autophagy (Table 1). ORF7a prevents autophagic degradation by reducing lysosomal acidification, while NSP15 inhibits autophagy initiation [78]. Expression of M also inhibits the formation of acidified autolysosomes [39••,78]. Lysosomal neutralization has been shown to promote their exocytosis [79,80]. Therefore, multiple SARS-CoV-2 viral proteins cause lysosomal deacidification to block autophagy while facilitating exocytosis for viral release.

SARS-CoV-2 viral proteins also promote autophagic degradation of host factors essential for immune responses. SARS-CoV-2 ORF8 promotes autophagic degradation of MHC-I to downregulate viral antigen presentation [81]. NSP13 recruits TANK-binding kinase 1 (TBK1) to p62 and promotes its autophagic degradation, thereby inhibiting type-I interferon (IFN-I) production [82]. Depletion of BECN1 restores MHC-I expression in ORF8-expressing cells and IFN-β production in NSP13-expressing cells [81,82]. Therefore, autophagy activity may be temporally modulated during SARS-CoV-2 infection. At the early stage of infection, selective autophagy is activated to remove host factors, thus allowing the virus to escape immune surveillance. At late stages, viral proteins, which have been abundantly translated, inhibit autophagy by reducing the autophagosome size (e.g. NSP6) and blocking formation of degradative autolysosomes (e.g. ORF3a, NSP6, and ORF7a). Formation of deacidified lysosomes/hybrid structures promotes exocytosis-mediated viral egress.

Potential interventions targeting autophagy

SARS-CoV-2 infection elicits cytokine storm syndrome, an important contributor to severe COVID-19 cases [83]. Autophagy stagnation also triggers massive proinflammatory cytokine production [84]. Depletion of core autophagy genes such as *BECN1* and *ATG7*, or treatment with autophagy inhibitors, dramatically promotes cytokine secretion [84,85]. The abnormal cytokine response upon SARS-CoV-2 infection probably involves autophagy dysregulation. Autophagy is required for recovery from neuroinflammation [86]. COVID-19 patients show meningoencephalitis with ectopic cytokine production in the cerebrospinal fluid [87,88]. Dysfunctional autophagy may be responsible for the vulnerability of neuronal cells during SARS-CoV-2 infection.

Therapeutic approaches targeting autophagy have been shown to prevent SARS-CoV-2 replication and egress, and also have the potential to alleviate the exaggerated inflammation caused by cytokine storms in COVID-19 patients. Using computational approaches, anti-SARS-CoV-2 compounds were designed and verified by cell culture virus infection assays [89]. Remarkably, most of these compounds are viral entry inhibitors and/or autophagy modulators [89], which suggests that autophagy modulators can play antiviral roles at post-entry steps. Many pharmacological autophagy modulators have also been identified as potential therapies for COVID-19. Some of them are FDA-approved drugs, while others are undergoing clinical trials. Chloroquine (CQ) and hydroxychloroquine (HCQ), which are well-known inhibitors of autolysosome formation, effectively prevent infection by HIV, HCV, SARS-CoV, and SARS-CoV-2 [90]. By increasing the pH of intracellular acidic organelles, CQ and HCQ affect multiple steps in the SARS-CoV-2 life

cycle and exhibit contrasting antiviral activities in different cell lines, which could be due to their differential functions in viral entry and release [90,91]. Various VPS34 inhibitors, which inhibit autophagy initiation, prevent SARS-CoV-2 replication and show promise for COVID-19 treatment [47]. Autophagy activators, such as vitamin D3, mitigate uncontrolled inflammatory responses and have potential for combination drug therapy [85]. As the existing antiviral drugs targeting the autophagy pathway often have nonspecific effects, such as lysosomal dysfunction induced by CQ/HCQ treatment, caution is needed in their clinical application.

Conclusions and perspectives

During SARS-CoV-2 infection, the endomembrane system is extensively remodeled to generate DMVs for viral replication and multiple nondegradative lysosomes for viral egress. Autophagy also intersects with the endomembrane compartment at multiple sites. Not surprisingly, there is intricate interplay between SARS-CoV-2 infection and autophagy. SARS-CoV-2 viral proteins enhance autophagic degradation of pivotal immune proteins, enabling the virus to escape surveillance. Viral proteins also interfere with lysosomal acidification and function, thus impairing the fusion of autophagic vesicles with lysosomes in order to hijack the end-stage autophagic structures for viral egress. Future studies must investigate how autophagy activity is differentially modulated at different stages of viral infection and how autophagy induction and autophagosome maturation are coordinately controlled by viral proteins. The mechanism by which SARS-CoV-2 viral proteins interact with ER-associated autophagy proteins (such as VMP1, TMEM41B, and the VPS34 complex) for DMV generation also requires investigation. Given the important role of autophagy in cytokine production and inflammation, it will be important to determine the impact of autophagy on the abnormally elevated cytokine levels in SARS-CoV-2 patients.

Elucidating the interplay between SARS-CoV-2 viral proteins and the autophagic machinery helps us to develop new intervention strategies. The main focus for COVID-19 treatment has been blocking SARS-CoV-2 entry, such as by inhibiting ACE2 binding and inactivating cell surface-localized proteases that cleave S proteins. The emergence of the Omicron variant shows that the receptor and spike proteins can mutate to enhance viral entry while evading neutralizing antibodies. The Omicron variant enters host cells largely via endocytosis rather than by transmembrane protease serine 2 (TMPRSS2)-mediated membrane fusion due to mutations in the spike protein [92]. Just two different amino acids are responsible for the differential function of SARS-CoV-2 ORF3a and SARS-CoV ORF3a in autophagosome maturation and lysosomal exocytosis. It will be interesting to investigate whether mutations in different SARS-CoV-2 variants also modulate the autophagy pathway and its effect on viral infectivity. The autophagic machinery is generally hijacked to facilitate DMV generation and lysosomal egress, which are essential for coronavirus infection. Therefore, therapeutic approaches to precisely manipulate autophagy at different stages of virus infection are promising for combating COVID-19 and yet-to-emerge coronaviruses.

Conflict of interest statement

The authors declare no conflict of interest.

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