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Review

Interplay between the cellular autophagy machinery and positive-stranded RNA viruses

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Autophagy is a conserved cellular process that acts as a key regulator in maintaining cellular homeostasis. Recent studies implicate an important role for autophagy in infection and immunity by removing invading pathogens and through modulating innate and adaptive immune responses. However, several pathogens, notably some positive-stranded RNA viruses, have subverted autophagy to their own ends. In this review, we summarize the current understanding of how viruses with a positive-stranded RNA genome interact with the host autophagy machinery to control their replication and spread. We review the mechanisms underlying the induction of autophagy and discuss the pro- and anti-viral functions of autophagy and the potential mechanisms involved.

Keywords autophagy; positive-stranded RNA viruses; autophagosome; p62/SQSTM1; double-membrane vesicle; xenophagy

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Introduction

Viruses are intracellular pathogens that depend solely on host cells to replicate their genome and assemble intact viral progeny. Positive-stranded RNA viruses are well known for their ability to induce the remodeling of intracellular membranes to form a scaffold for viral replication complexes [1,2]. However, the exact origins of the membranous structures and the pathways responsible for their formation remain to be fully elucidated. Although endoplasmic reticulum (ER) is utilized by a large number of viruses, other membranous organelles can also be exploited by viruses. Recent studies have shown that the membranous structures contain markers for autophagosomes, suggesting a pivotal role for autophagy in controlling replication of positive-stranded RNA viruses [3,4].

Autophagy is a conserved cellular process responsible for removing damaged organelles and misfolded proteins to maintain cellular homeostasis under both normal and stress conditions [5]. Autophagy was previously regarded to be non-selective. However, increasing evidence has suggested the selectivity of autophagy in recycling unwanted organelles, removing aggregate-prone proteins, and clearing specific viral proteins [6–8]. The importance of selective autophagy in different physiological and pathological states has been increasingly recognized. Recently, autophagy has emerged as a critical player in the control of viral infection and immunity [9–13]. On the one hand, autophagy can serve as a host defense mechanism for some pathogens, such as sindbis virus and herpes simplex virus, by clearing them out of the cells [14–16]. On the other hand, many positive-stranded RNA viruses have been reported to subvert this cellular machinery to favor their own replication and release.

In this review, we discuss the current understanding as to how positive-stranded RNA viruses interact with the host autophagy machinery to control their replication and spread. We review the mechanisms underlying the induction of autophagy. We also discuss the pro- and anti-viral functions of autophagy and the potential mechanisms involved. Understanding how the autophagy pathway is activated and the biological significance of autophagy in the control of viral life cycle is essential for exploration of new anti-viral targets.

Autophagy: Mechanism and Regulation

Among the three types of autophagy (macroautophagy, microautophagy, and chaperon-mediated autophagy), macroautophagy (hereafter referred to as autophagy) is the most extensively studied in both yeast and mammalian cells [6,17,18]. The process of autophagy can be divided into four sequential steps (**Fig. 1**). Initially, a crescent-shaped double-membrane vesicle (DMV) called isolation membrane or phagophore is formed to sequester misfolded

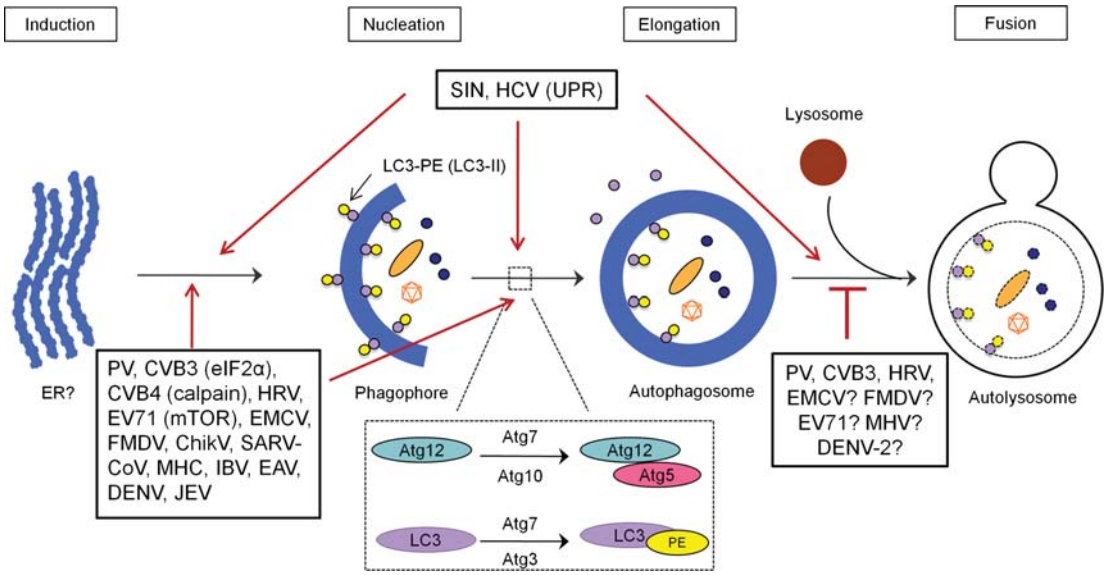

Figure 1 The activation of autophagy pathway by different positive-stranded RNA viruses The process of autophagy consists of four steps: induction, nucleation, elongation, and fusion with lysosomes. Two ubiquitination-like conjugation systems, Atg12–Atg5 and LC3-PE, are essential for the formation of autophagosomes. Positive-stranded RNA viruses induce either complete or incomplete autophagy as indicated. ER stress-induced UPR, eIF2α phosphorylation, and mTOR/p70S6K signaling pathway have been associated with the activation of autophagy in HCV, EV71, and CVB3 infection, respectively. Calpain pathway is required for the activation of autophagy in CVB4 infection. ER, endoplasmic reticulum; UPR, unfolded protein response; eIF2α, eukaryotic initiation factor 2α; mTOR, mammalian target of rapamycin; p70S6K, p70 ribosomal protein S6 kinase; PE, phosphatidylethanolamine; PV, poliovirus; CVB, coxsackievirus; HRV, human rhinovirus; EV71, enterovirus 71; EMCV, encephalomyocarditis virus; FMDV, foot and mouth disease virus; ChikV, chikungunya virus; SARS-CoV, severe acute respiratory syndrome-coronavirus; MHV, mouse hepatitis virus; IBV, infectious bronchitis virus; EAV, equine arteritis virus; DENV, dengue virus; JEV, Japanese encephalitis virus; SIN, sindbis virus; HCV, hepatitis C virus; ?, implicated by indirect experimental evidence but direct evidence is still missing.

proteins and damaged organelles (induction and nucleation step). Subsequently, the two ends of phagophore fuse to form a complete DMV termed as autophagosome (elongation step). Finally, the outer membrane of the autophagosomes fuses with lysosomes to form autolysosomes while the inner membrane and the cargo enwrapped in autophagosomes are degraded by hydrolyses (fusion step). Autophagosomes can also fuse with early or late endosomes to form amphisomes [19].

More than 30 autophagy-related genes (*Atg*) have been identified to participate in the autophagic process [5,20,21]. The key proteins involved in the formation of autophagosomes include: (i) uncoordinated (UNC)-51-like kinase (ULK) complex, composed of ULK1, ULK2, Atg13, focal adhesion kinase family interacting protein of 200 kDa (FIP200), and Atg101; (ii) class III phosphatidylinositol 3 (PI3)-kinase complex, comprising of Vps34, p150, beclin-1, Atg14, and Ambra1 (activating molecule in Beclin-1-regulated autophagy protein 1); (iii) two ubiquitination-like conjugation systems, composed of Atg4, Atg12, Atg5, Atg16L1, Atg7, Atg10, Atg3, and microtubule-associated protein light chain (LC3) [5,20,21].

The two conjugation systems are essential for the formation of autophagosomes (**Fig. 1**). For Atg5–Atg12 conjugation, Atg12 is first activated by Atg7 and then transferred to Atg10. Atg12 finally forms a conjugate with Atg5 to activate the formation of autophagosomes [22]. For LC3–phosphatidylethanolamine (PE) conjugation, nascent LC3 is first cleaved by Atg4 to become LC3-I, which is subsequently activated by Atg7 and then transferred to Atg3. Finally, LC3 is conjugated to PE to form a LC3–PE complex (LC3-II), which participates in the formation of autophagosomes [22]. Recruitment of LC3 protein to autophagic vesicles has been considered a common trait of autophagosome formation. In addition, conversion from LC3-I to LC3-II has been widely accepted as a marker of autophagic signaling [23].

Autophagosome formation is also tightly controlled by multiple signaling pathways. Autophagic protein beclin-1 forms the class III PI3-kinase complex with Vps34, a class III PI3-kinase, to facilitate autophagosome formation by providing phosphatidyliositol 3-phosphates to isolation membrane [24,25]. The mammalian target of rapamycin (mTOR) and the eukaryotic initiation factor 2α (eIF2α) kinases also participate in autophagy process by negatively and positively regulating the formation of autophagosomes, respectively [16,26]. The adaptor protein p62/SQSTM1 has been revealed to be essential in mediating selective autophagy [7,8]. It binds to both ubiquitin and LC3 and targets ubiquitinated proteins to autophagosomes for degradation [27]. p62/SQSTM1 can also be selectively degraded by autophagy [28].

Autophagy functions as a protein quality control system. Thus, defects in autophagy have been associated with several pathological conditions, such as neurodegenerative diseases, myopathy, cancer, and aging [29–31]. Autophagy has also been implicated in the modulation of infection and immunity. Autophagy serves as a critical component of innate immune response by removing bacteria, viruses, and protozoans from the host cells through xenophagy, whose targets are foreign bodies rather than self-molecules [32,33]. The antigens are then presented through MHC class II molecules, initiating adaptive immune response [34]. In this process, autophagy participates and assists both innate and adaptive immunity to clear the pathogens out of the body. However, as opposed to the anti-viral activity, many positive-stranded RNA viruses have successfully developed strategies to hijack autophagy to foster their replication.

Activation of Host Autophagy

Since the early reports that coronavirus and poliovirus subvert the host autophagy machinery to support their replication [3,35], growing numbers of studies indicate that the interaction between autophagy and positive-stranded RNA viruses is widely present. In addition to poliovirus, the other members in picornaviridae family, including coxsackievirus group B (CVB) [36–39], enterovirus 71 (EV71) [40,41], foot and mouth disease virus (FMDV) [42], encephalomyocarditis virus (EMCV) [43], and human rhinovirus (HRV) [3,44], also activate the cellular autophagy pathway. Moreover, sindbis virus (SIN) [15,45] and chikungunya virus (ChikV) [46] in the family of togaviridae, severe acute respiratory syndrome-coronavirus (SARS-CoV), mouse hepatitis virus (MHV), and infectious bronchitis virus (IBV) in the family of coronaviridae [35,47–50], equine arteritis virus (EAV) in the family of arteriviridae [51,52], as well as dengue virus (DENV) [53–60], hepatitis C virus (HCV) [54,61–67], and Japanese encephalitis virus (JEV) [68] in the family of flaviviridae have also been found to induce the activation of autophagy (**Fig. 1**).

Incomplete and complete autophagy activation

The aforementioned viruses have been demonstrated to activate the autophagic process as measured by increased accumulation of autophagosomes or autophagosome-like vesicles, augmented conversion from LC3-I to LC3-II, and elevated number of punctate LC3-expressing cells, which are widely accepted criteria for monitoring autophagy [69,70]. However, since autophagy is a dynamic process composed of autophagosome formation and degradation, the increased autophagosomes can be a result of increased formation, decreased fusion with lysosomes, or both. Autophagic flux is a measurement of the balance between the rate of autophagosome formation and degradation. Thus, it is more meaningful to determine the rate of autophagic flux to have an integrated view of the complete process [69,70].

Recent study has shown that infection with sindbis virus induces autophagosome formation with an increased autophagic flux [15]. Sindbis virus promotes the formation of autophagosomes, as evidenced by increases in the percentage of cells with GFP-LC3 dots and LC3-II conversion and by visualization of DMVs in virus-infected cells [15]. It was further found that the protein levels of p62, a marker for autophagic flux, are reduced without the changes of its mRNA expression [15]. These results suggest that sindbis virus triggers a complete autophagic response.

Unlike sindbis virus, poliovirus in picornaviridae family has been proposed to block the fusion of autophagosomes with lysosomes [3]. In consistent with this proposition, it was demonstrated that protein expression of p62 is unaltered [37] or increased [36] during CVB3 infection, suggesting that CVB3 infection triggers increased autophagosome formation, but with a reduced autophagic flux. These studies suggest that blockage of autophagosome–lysosome fusion may be a viral strategy to ensure the existence of optimal number of autophagosomes for viral benefits. Further study is needed to clarify the mechanisms by which virus blocks the maturation of autophagosomes into autolysosomes.

Incomplete autophagic flux has also been reported in HCV infection [65,71]. Although autophagosome formation is induced during HCV infection, it was shown that the degradation rate of long-lived proteins and autophagic substrate protein p62 is not significantly changed [65,71]. However, these studies are conflicting with the report by Ke and Chen [54], which showed that the process of autophagy is complete during HCV infection. Using a tandem reporter construct (mRFP-GFP-LC3) [72] and by immunogold-electron microscopy analysis of initial- and late-stage autophagic vacuoles, it was demonstrated that HCV-induced autophagosomes fuse with lysosomes [54]. This observation was further supported by the evidence that disruption of autolysosome maturation inhibits HCV RNA replication and protein expression [54]. Further work is required to clarify this apparent discrepancy.

The experimental data about the effects of positive-stranded RNA viruses on autophagic flux are still sparse. Development of new research technologies will allow for a more reliable and sensitive measurement for monitoring autophagic flow. Understanding the dynamic status of autophagosome formation and degradation after viral infection will not only provide novel insights into the mechanisms by which viruses exploit the autophagic machinery but also assist in the development of anti-viral therapies.

Intracellular signaling pathways trigger autophagy

How does virus infection trigger autophagy? HCV infection has been demonstrated to induce autophagy by activating the unfolded protein response (UPR) [54,65]. The accumulation of misfolded proteins in the ER causes ER stress and activates UPR via three sensors: protein kinase R-like ER kinase (PERK), inositol-requiring kinase 1 (IRE1), and activating transcription factor 6 (ATF6) [73]. It has been shown that HCV infection can induce ER stress to activate all three sensors of UPR [65,71]. Gene silencing of either PERK, IRE1, or ATF6 leads to significant reduction of LC3 lipidation induced by HCV infection, suggesting that all three UPR signaling pathways are required for the induction of autophagy [54,65].

During CVB3 infection, phosphorylation of mTOR remains unchanged whereas the phosphorylation of eIF2α is increased [37], suggesting that eIF2α is a potential signaling pathway responsible for CVB3-induced autophagy. The upstream signaling leading to eIF2α phosphorylation is still unknown. It may be due to the activation of protein kinase R by double-stranded RNA (dsRNA) during CVB3 replication, or it is the downstream effector induced by the activation of PERK as mentioned above. In addition to the eIF2α pathway, it was found that calpain activity is required for CVB4-induced autophagy, as calpain inhibitors were shown to reduce the formation of autophagosomes [39].

The mTOR pathway plays a central role in negatively regulating autophagy activity. The activation of mTOR complex 1 (mTORC1) results in phosphorylation of two effectors, p70 ribosomal protein S6 kinase (p70S6K) and eukaryotic initiation factor 4E binding protein 1 (4E-BP1), which promote protein translation and inhibit autophagy [74]. In EV71-infected SK-N-SH cells, decreased expression of mTOR and p70S6K has been associated with activation of autophagy [40]. But this result was not seen in EV71-infected RD cells, which suggests that EV71 may utilize different signaling pathways in different cell types. The upstream signaling molecules of mTOR in EV71 infection remain unclear. But the activation of mTOR/p70S6K is independent of the activation of class I PI3-kinase/Akt and Erk1/2 signaling pathways [40].

Viral proteins activate autophagy

In addition to cellular components, some viral proteins have been shown to contribute to the biogenesis of autophagosomes. For example, some viral non-structural proteins are able to induce membrane rearrangement. Although expression of either poliovirus non-structural protein 2BC or 3A alone is insufficient to induce autophagosome structure, co-expression of 2BC and 3A has been shown to facilitate the modification of LC3-I to LC3-II and to induce the formation of DMVs that closely resemble the ones induced by poliovirus [3,75]. Further investigation demonstrated that covalent modification of LC3 by expression of polioviral protein 2BC targets LC3 to cellular membranes [76]. The expression of HCV non-structural 4B (NS4B) protein was reported to induce autophagosome-like vehicles similar to those induced in the cells expressing the whole polyprotein or harbor HCV sub-genomic replicons [66]. Similarly, expression of flavivirus non-structural protein NS4A is sufficient to induce autophagy and promote cell survival [57]. It was also reported that co-expression of NSP2 and NSP3 of EAV induces the formation of autophagosome-like DMVs [52].

In summary, despite these positive reports on the activation of autophagy, not all viruses in the family of positive-stranded RNA viruses were shown to activate the cellular autophagy. For example, there was a report showing that autophagy is not induced after HRV2 infection and modulation of the autophagy pathway does not affect viral propagation [77]. In addition, it was demonstrated that infection with HRV-1A, another serotype of HRV, does not induce autophagy and modulation of autophagy has no effects on its replication [44].

Pro-viral Function of Autophagy

Autophagy has been initially identified as a cellular defense mechanism to clear the invading viruses; however, a growing body of research evidence demonstrated that this host machinery can be evolved by numerous positive-stranded RNA viruses, including poliovirus [3], CVB3 [37], CVB4 [39], EV71 [40], HRV [44], FMDV [42], EMCV [43], DENV [53,56], HCV [54,61,62,65,78], MHV and SARS-CoV [35], ChikV [46], and JEV [68] to support their life cycle. Pharmacological inhibition or gene silencing of autophagy pathway *in vitro* has been demonstrated to inhibit growth and/or spread of these viruses, whereas induction of autophagy results in increased viral yield. Multiple mechanisms have been suggested to be involved in this pro-viral function of autophagy (**Fig. 2**).

Serve as viral replication sites

One of the common features shared by positive-stranded RNA viruses is to assemble and replicate on intracellular membranes. The functions of the membranous structures are proposed to provide a scaffold for anchoring and concentrating the replication complexes to prevent the immune response triggered by dsRNA intermediates and to afford certain lipids required by genome synthesis [2]. The replication complexes are usually composed of viral RNA-dependent RNA polymerase, accessory non-structural proteins with helicase and nucleotide triphosphate activity, viral RNA, and host cell factors. As parts of the intracellular membranous structures, autophagosomes or

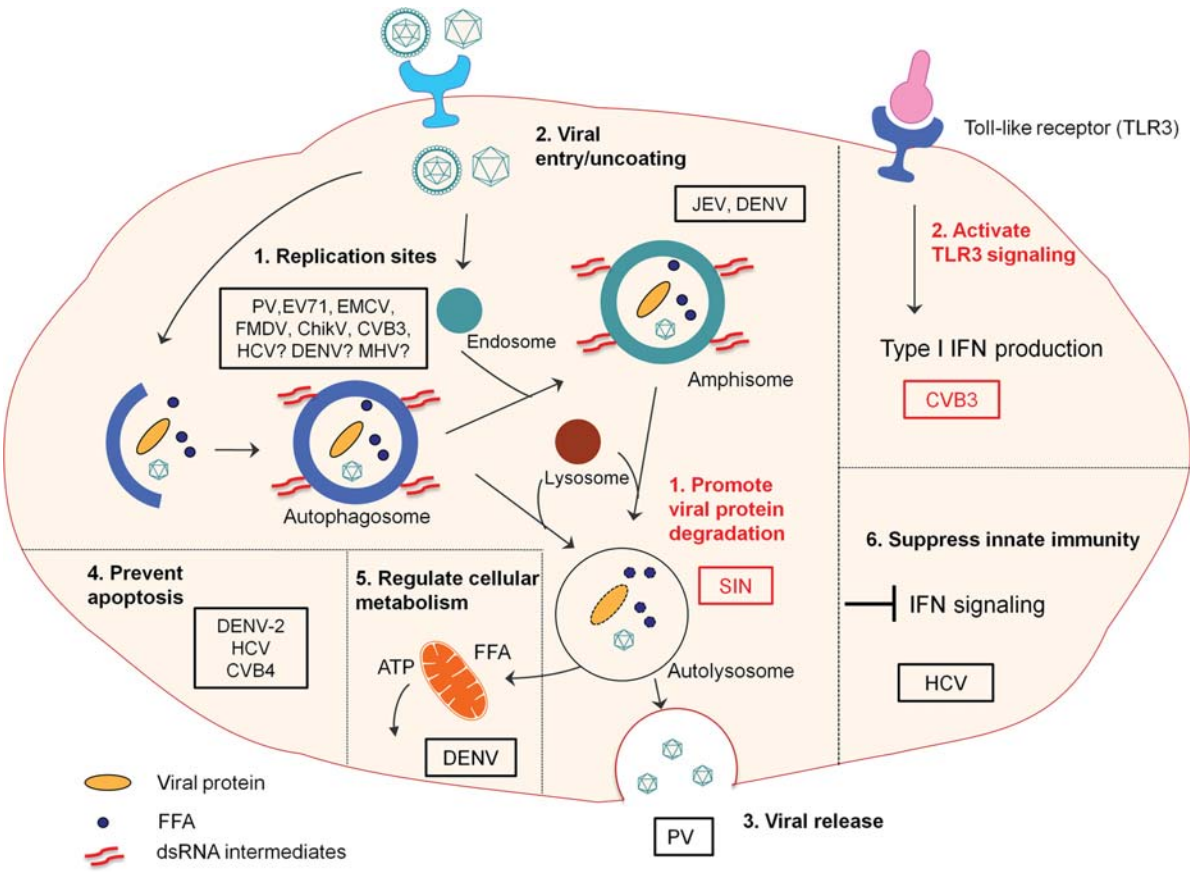

Figure 2 The pro-viral and anti-viral functions of autophagy during positive-stranded RNA viral infection Pro-viral functions of autophagy (viruses are circled in black rectangles): (1) autophagosomes (PV, EV71, CVB3, EMCV, FMDV, ChikV) or amphisomes (DENV) serve as sites for viral replication; (2) amphisomes are also linked to the entry/uncoating of JEV and DENV; (3) the topological structure of autophagosmes is associated with non-lytic egress of PV particles; (4) autophagy prevents premature cell death to maintain favorable cellular environment for viral replication (DENV-2, HCV, and CVB4); (5) autophagy favors DENV replication by selectively degrading lipid droplets to generate ATP for viral replication; (6) suppression of IFN signaling is related to the pro-viral function of autophagy in HCV infection. Anti-viral functions of autophagy (viruses are circled in red rectangles): (1) autophagy inhibits SIN replication by promoting the clearance of viral capsid protein; (2) autophagy is required for TLR3-medicated type-I IFN production during CVB3 infection. PV, poliovirus; CVB, coxsackievirus; EV71, enterovirus 71; FMDV, foot and mouth disease virus; ChikV, chikungunya virus; HCV, hepatitis C virus; HRV, human rhinovirus; EMCV, encephalomyocarditis virus; SIN, sindbis virus; DENV, dengue virus; MHV, mouse hepatitis virus; JEV, Japanese encephalitis virus; FAA, free fatty acid; IFN, interferon; TLR3, toll-like receptor 3; ATP, adenosine triphosphate; ?, implicated by indirect experimental evidence but direct evidence is still missing.

amphisomes have been shown to function as scaffolds required for the replication and assembly of certain positive-stranded RNA viruses.

Replication of several positive-stranded RNA viruses has previously been linked to cellular membranous structures [49,51,79,80]. Recent studies provided the direct evidence of the association between viral replication complexes and autophagosome structures. Confocal microscopy showed the co-localization of polioviral protein 3A, a critical component of the poliovirus RNA replication complex, with the autophagosome marker LC3 [3]. DENV non-structural protein NS1 and dsRNA were reported to co-localize with LC3 and ribosomal protein L28 [55,60]. Immuno-electron microscopy also showed co-localization of EV71 capsid protein VP1 with autophagosomes in virus-infected mouse neurons [40]. Confocal and immune-electron microscopy revealed that both non-structural protein 3A and capsid protein VP1 co-localize with autophagosomes during EMCV infection [43]. Co-localizations of non-structural proteins 2B, 2C, and 3A with LC3 and between structural protein VP1 and Atg5 were also reported in FMDV-infected cells [42]. The ultrastructural analysis showed that the ChikV virions locate in the lumen of autophagomes-like vacuoles [46].

Similar to other positive-stranded RNA viruses, HCV infection induces intracellular membrane redistribution. However, controversy exists as to whether autophagosomes serve as sites for HCV replication. By sucrose gradient analysis, LC3-II was found to co-sediment with HCV RNA and non-structural proteins NS3 and NS5A [63]. However, confocal microscopy showed little evidence of co-localization of LC3 or Atg5 with HCV core, NS3,

NS4A/4B, and NS5A proteins [61,62,65,78]. Moreover, it was demonstrated that knockdown of either LAMP2 or Rab7, two critical proteins responsible for the fusion of autophagosomes with lysosomes, inhibits HCV viral replication [54]. These studies suggest that autophagosomes may not be major sites for HCV genome replication.

Although MHV replication complexes were found to be associated with LC3 and Atg12 [35], conflicting results were also reported with regard to the role of autophagy in MHV replication. As opposed to the findings in embryonic stem cell lines that autophagy induced by MHV enhances viral replication, likely through providing a replication site [35], using primary macrophages and murine embryonic fibroblasts it was found that MHV replication does not require the autophagy gene Atg5 [81].

Although direct evidence of the association of viral replication complexes with autophogasomes is lacking for CVB3, blockage of the fusion between autophagosomes and lysosomes using pharmacological inhibitors or knockdown of the genes critical for this fusion increases the accumulation of autophagosomes in virally infected cells and consequently leads to enhanced viral replication [37]. This study provides indirect evidence that autophagosomes are critical components during CVB3 replication, likely by serving as virus anchoring and replication sites. Similar to the observation in CVB3, inhibition of the fusion between autophagosomes or amphisomes and lysosomes was found to increase the viral yield of DENV-2 [60]. These data together with the discoveries that DENV-2 replication complexes co-localize with LC3 and an endosome marker imply that DENV-2 may use the amphisomes as sites for viral replication [60]. However, this effect seems to be viral serotype-specific. It was found that inhibition of lysosome fusion reduces DENV-3 yields and results in an accumulation of viral NS1 [55]. The mechanisms by which autophagy favors DENV-3 replication remain elusive.

Promote viral entry/uncoating and release

Recent evidence has suggested that autophagy may participate in viral life cycle at the early phase of viral infection [59,60,68]. Both DENV and JEV are enveloped RNA viruses in the family of flaviviridae. They enter the host cells primarily via receptor-mediated endocytosis, followed by pH-dependent fusion with endosomes and subsequent release into the cytoplasm. Recent studies demonstrated the co-localization of viral replication complexes or inoculated viral particles with both autophagosome and endosome markers [59,60,68]. These observations imply a potential role for autophagosome–endosome fusion in viral entry/uncoating.

Autophagy has also been suggested to facilitate non-lytic egression of some positive-stranded RNA viruses. Poliovirus, a non-enveloped positive-stranded RNA virus, is often considered as a lytic virus releasing from the cells by cell lysis. However, non-lytic release of poliovirus was also reported [82]. In the study of the biological relevance of autophagy in poliovirus infection, it was found that the decreases in extracellular virus are always more profound than intracellular virus when important autophagic proteins are knocked down [3]. The lower levels of viral particles in extracellular virus in autophagy-suppressing cells have been correlated with reduced non-lytic release of cytoplasmic contents that can be mediated by autophagy [3,83]. This hypothesis is supported by the observation that both LC3 and polioviral VP1 are present in extracellular matrix adjacent to the infected cells [3]. Further investigation is needed to provide direct evidence that autophagy provides a topological mechanism to facilitate viral release.

Suppress innate anti-viral immunity

Recent studies by Ke and Chen [54] and Shrivastava *et al*. [84] have shed light on how autophagy benefits for HCV infection. They provided evidence that complete autophagic process is required to promote HCV replication and this is largely due to the suppressive effect of autophagy on anti-viral innate immune response [54,84]. Upon HCV infection, the innate immune response is initiated by activation of interferon-β (IFN-β) production mediated by HCV-derived pathogen-associated molecular pattern (PAMP) [54]. Inhibition or activation of UPR-mediated autophagy has been shown to increase or reduce IFN-β production mediated by HCV-derived PAMP, respectively [54]. Similar observation was also made with DENV-derived PAMP, suggesting that both viruses may share the same mechanism to evade the innate immune response [54]. Moreover, Shrivastava *et al*. [84] also reported that inhibition of autophagy by knocking down beclin-1 or Atg7 reduces HCV replication, which is accompanied by the activation of IFN signaling pathway, as measured by increased levels of IFN-regulated genes, including IFN-β, 2′5′-oligoadenylate synthetase 1, IFN-α, and IFN-α-inducible protein 27 mRNAs. Together, these studies indicate an important mechanism by which HCV avoids the innate immune response through activating the host autophagy pathway.

Regulate cellular metabolism

Autophagy has been demonstrated to be involved in the regulation of cellular metabolism. It regulates lipid metabolism through modulating the degradation of triglycerides stored in lipid droplets, a process called lipophagy [85,86]. Autophagy therefore represents a new cellular process for abnormalities in lipid metabolism and accumulation. Recent study has shown a novel mechanism responsible for autophagy-mediated pro-viral properties toward DENV replication [53]. It was reported that DENV-induced

autophagosomes deliver the lipid droplets to lysosomes where triglycerides are depleted and the free fatty acids are released [53]. The released free fatty acids undergo β-oxidation in mitochondria to generate ATP affording the energy for DENV replication [53]. Although the exact signaling pathway contributing to this selective autophagy and the detailed mechanisms responsible for ATP regulation of DENV replication remain unclear, the findings in this research provide the first evidence linking viral infection to autophagy-mediated metabolic regulation. Whether this mechanism also applies to other viruses requiring the host cellular metabolism for their replication warrants further investigation.

Prevent premature cell death

Premature cell death has been considered as an anti-viral host mechanism by providing an unfavorable environment for viral propagation. The cross-talk between autophagy and apoptosis has become evident [87–90]. Induction of autophagy has often been linked to inhibition of apoptosis. DENV-2-induced autophagy has been demonstrated to prevent cells from apoptosis [57]. It was shown that knockdown of autophagy abolishes the protective role of autophagy against cell death and leads to reduced viral replication [57]. In exploring the mechanisms underlying the pro-viral role of autophagy in HCV life cycle, it was found that HCV infection in autophagy-knockdown cells promotes cell death, suggesting a role of autophagy in prolonging cell survival for the establishment of successful viral infection [84]. The cross-talk between autophagy and apoptosis has also been reported in CVB4 infection [38]. Suppression of autophagy by 3-methyladenine (3-MA), a selective class III PI3-kinase inhibitor, triggers caspase activation and inhibition of apoptosis using a pan-caspase inhibitor increases autophagosome formation [38].

Degradation of intracellular or viral proteins

Degradation of intracellular proteins that are against viral replication is a common strategy virus has developed to support its replication [91]. As alluded to earlier, the significance of selective autophagy in many cellular processes has been increasingly appreciated [6,8]. It is therefore conceivable that autophagy-mediated proteolysis of host anti-viral factors may also play a role in promoting viral growth. In addition, appropriate concentrations of viral proteins have been reported to be critical for the optimal replication of viruses and too much viral proteins could be a drawback for positive-stranded RNA viral replication [92–94]. Several lines of evidence have demonstrated that viruses can exploit the host ubiquitin-proteasome system for viral protein degradation to provide the proper stoichiometric ratio of structural and non-structural viral proteins during viral life cycle [95–98]. Thus, it is tempting to postulate that autophagy may also be advantageous for viral replication by degrading excess viral non-structural proteins. Indeed, lysosome-mediated proteolysis has been suggested to be involved in the rapid turnover of HCV NS2 [99]. p62-mediated selective autophagic degradation has also been reported for SIN capsid protein although this role was demonstrated to be anti-viral [15].

In summary, the pro-viral functions of autophagy in positive-stranded RNA viral life cycle apparently involve multiple pathways, either direct effects on viral replication or indirect influences on the host immune and non-immune-related activities. Given the importance of autophagy in regulating diverse cellular functions, it is speculated that other functions of autophagy, for example cell cycle regulation, cell differentiation, and gene transcription, may also contribute to enhanced viral replication and this requires further investigation.

Anti-viral Effects of Autophagy

The autophagy machinery is not always beneficial for positive-stranded RNA viruses. It has been shown that autophagy functions as an anti-viral host defense against SIN infection (**Fig. 2**) [14,15]. In response to SIN infection, mice with beclin-1 overexpression have improved survival rate, reduced viral loads, and attenuated viral pathogenesis as compared to control mice [14]. Study using neuron-specific Atg5 knockout mice showed that disruption of Atg5 gene leads to enhanced susceptibility of mouse central nervous system to SIN infection [15]. Further investigation demonstrated that loss of Atg5 in SIN-infected neurons results in impaired viral capsid protein clearance, increased p62 accumulation, and accelerated cell death, without affecting viral replication and type I IFN production [15]. *In vitro* study showed that p62 binds directly to viral capsid protein and transports it to autophagosomes for lysosome-mediated degradation [15]. Electron microscopic analysis provided the direct evidence that SIN virions are captured inside the autophagosomes or autolysosomes [15]. This study suggests that Atg5 plays a crucial role in protecting against SIN infection in mouse central nervous system by promoting p62-mediated clearance of viral proteins, rather than modulating innate immune response or viral replication [15]. Further study is required to elucidate the exact mechanisms by which p62 mediates selective autophagic degradation of SIN capsid protein.

Toll-like receptors (TLRs) play an important role in innate anti-viral immunity against CVB3 infection (**Fig. 2**) [100]. It was recently reported that autophagy plays a significant role in TLR-mediated type I IFN signaling during CVB3 infection [101]. It was found that blockage of autophagy by either gene-silencing LC3-II, beclin-1 or Atg5, or using pharmacological inhibitor 3-MA inhibits TLR3

signaling in response to dsRNA. Interestingly, in contrast to the earlier observation that incomplete autophagy increases CVB3 replication [37], it was demonstrated that complete autophagy is required for the activation of signaling triggered by TLR3, as inhibition of lysosome activity by bafilomycin A or chloroquine results in decreased type I IFN signaling [101]. The detail mechanisms in relation to a dual function of autophagy in supporting CVB3 replication by providing replication scaffolds and suppressing viral replication by triggering TLR3-mediated innate immune response require further investigation.

Conclusion

The available evidence highlighted in this review points to a crucial role for autophagy in regulating viral infection and/or in manipulating host anti-viral defense. Many positive-stranded RNA viruses interplay with autophagy to optimize their infection. They affect multiple aspects of viral life cycle, through promoting viral entry/uncoating, serving sites for viral replication, preventing cell death, regulating cellular metabolisms, escaping innate immunity, and controlling viral progeny release. Although the reported pro-viral function is dominant for positive-stranded RNA viruses, autophagy also plays an anti-viral role via facilitating the clearance of some viral structural proteins.

Despite significant progress in recent years, many of the details in this field remain to be elucidated, such as the signaling pathways responsible for virus-induced autophagy, the underlying mechanisms by which viruses inhibit the fusion of autophagosomes with lysosomes, and the molecular basis of virus-specific selective autophagy. A better understanding of these questions will be critical for developing novel autophagy-based treatment to control viral infection and viral pathogenesis.

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