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Minireview

Molecules and Cells

Autophagy in Viral Replication and Pathogenesis

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Autophagy is a catabolic process that is important for the removal of damaged organelles and long-lived proteins for the maintenance of cellular homeostasis. It can also serve as innate immunity to remove intracellular microbial pathogens. A growing list of viruses has been shown to affect this cellular pathway. Some viruses suppress this pathway for their survival, while others enhance or exploit this pathway to benefit their replication. The effect of viruses on autophagy may also sensitize cells to death or enhance cell survival and play a critical role in viral pathogenesis. In this article, we review the relationships between different viruses and autophagy and discuss how these relationships may affect viruses and their host cells.

INTRODUCTION

Autophagy is a catabolic process by which cells remove protein aggregates and damaged organelles for recycling. During autophagy, membrane crescents termed phagophores will form in the cytosol to sequester part of the cytoplasm. The edges of these phagophores will extend and eventually fuse to form enclosed double-membrane vesicles, known as autophagosomes. Autophagosomes will subsequently fuse with endosomes to form amphisomes and with lysosomes to form autolysosomes. The contents of autophagosomes will eventually be degraded by lysosomal enzymes (Levine and Kroemer, 2008). Autophagy occurs at a basal level in cells during normal conditions and is important for maintaining cellular homeostasis. However, during stress, such as nutrient starvation, autophagy can also be induced as a way to produce a limited amount of nutrients for the survival of cells.

The molecular pathways that regulate autophagy have been studied extensively. An important positive regulator of autophagy is the class III phosphatidylinositol 3-kinase (PI3KC3), which phosphorylates phosphatidylinositol to produce phosphatidylinositol 3-phosphate (PI3P). PI3P is a docking lipid important for the association of proteins with membranes. It is critical for the formation of autophagic vacuoles. PI3KC3 consists of a catalytic subunit hVps34 and a regulatory subunit p150. It has also been shown to complex with Beclin-1/Atg6, UVRAG, and Bif-1. This complex formation is necessary for the initiation of autophagy (Liang et al., 2006; Mari and Reggiori, 2007; Takahashi et al., 2007). Two ubiquitin-like conjugation systems are required for the formation of autophagosomes. The first conjugation system is responsible for the coupling of Atg12 to Atg5 to form a covalently linked heterodimer, which then recruits Atg16 to induce the formation of phagophores. The second conjugation system couples LC3/Atg8 to the phospholipid phosphatidylethanolamine. This lipidation of LC3 requires Atg4B, Atg7 and Atg3 enzymes and is critical for the formation of autophagosomes (Fujita et al., 2008a; 2008b; Hanada et al., 2007; Ichimura et al., 2000; Kabeya et al., 2000; Kirisako et al., 2000).

Autophagy may also be negatively regulated. A well-studied negative regulator of autophagy is mammalian target of rapamycin (mTOR). mTOR can inhibit autophagy through the inactivation of the Atg1-Atg13-Atg17 serine/threonine protein kinase complex and the suppression of the formation of the PI3KC3-Beclin-1/Atg6-UVRAG-Bif-1 kinase complex (Kim et al., 2002; Mizushima et al., 2001; Suzuki et al., 2001). mTOR can also interfere with the two ubiquitin-like conjugation systems mentioned above. Other negative regulators of autophagy include the PI3K class I-AKT pathway and the anti-apoptotic proteins Bcl-2 and Bcl-XL, which bind to Beclin-1 and suppress its interaction with PI3KC3 (Degenhardt et al., 2006; Degtyarev et al., 2008; Pattingre et al., 2005).

Autophagy plays important roles in many developmental pathways and disease processes (Levine and Klionsky, 2004; Shintani and Klionsky, 2004). It can also serve as innate immunity to remove intracellular microbial pathogens. Autophagy that is specific for the removal of viruses and bacteria is termed xenophagy. Although autophagy can suppress the replication of certain viruses, this cellular process has also been exploited by other viruses to benefit their replication. There is a growing list of viruses that have been shown to affect the autophagic pathway. In the following sections, we will review the relationships between viruses and autophagy.

DNA Viruses

Herpesviridae

Several herpesviruses have been shown to negatively regulate autophagy. One well studied example is the herpes simplex virus type 1 (HSV-1). The HSV-1 protein ICP34.5 can suppress autophagy via two different mechanisms: the dephosphorylation of eIF2α and the binding to Beclin-1 (Orvedahl et al., 2007; Talloczy et al., 2002). eIF2α is a translation initiation factor. It is

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inactivated after phosphorylation by the double-stranded RNA-dependent kinase PKR. Mouse embryonic fibroblasts defective in PKR or expressing a constitutively active eIF2α that contains a serine to alanine mutation at amino acid 51 cannot undergo autophagy upon nutrient starvation, indicating that the phosphorylation of eIF2α is important for the induction of autophagy (Talloczy et al., 2002). ICP34.5 can recruit protein phosphatase 1α (PP1α) to cause dephosphorylation of eIF2α and, as a result, suppress autophagy. ICP34.5 can also bind to Beclin-1 and prevent it from binding to the PI3KC3-UVRAG complex to initiate autophagy. Since HSV-1 mutants defective in the ICP34.5 gene are impaired in causing lethal encephalitis in mice (Orvedahl et al., 2007), autophagy apparently suppresses HSV-1 pathogenesis. Whether autophagy also suppresses HSV-1 replication is controversial, as while one study indicates that autophagy degrades HSV-1 (Talloczy et al., 2006), another study indicates that autophagy antagonism has no effect on HSV-1 replication in permissive cell cultures (Alexander et al., 2007). A more recent study suggests that the binding of ICP34.5 to Beclin-1 may preclude autophagy-mediated class II antigen presentation and thereby enhances the virulence and pathogenesis of HSV-1 (Leib et al., 2009).

Similar to HSV-1, the bovine herpesvirus type 1 (BHV-1) also encodes a protein named bICP0, which appears to inhibit autophagy. This is because the bICP0-null mutant of BHV-1, but not the wild-type virus, could induce autophagosomes (Geiser et al., 2008). How bICP0 may induce autophagosomes, however, remains unclear. Human cytomegalovirus (HCMV) can also inhibit autophagy in primary human fibroblasts, albeit with mechanisms different from those used by HSV-1. HCMV activates the mTOR signaling pathway (Chaumorcel et al., 2008), which is known to suppress autophagy. Interestingly, HCMV infection also suppresses lithium chloride-induced autophagy, which is mTOR-independent (Chaumorcel et al., 2008). Thus, HCMV can suppress autophagy via both mTOR-dependent and independent pathways (Chaumorcel et al., 2008; Geiser et al., 2008). Several other herpesviruses including Kaposi's sarcoma-associated herpesvirus (KSHV), Herpesvirus saimiri (HVS) and Molluscum contagiosum virus (MCV) can also suppress autophagy by expressing vFLIP, a homolog of cellular FLICE-like inhibitor protein (cFLIP) (Lee et al., 2009). vFLIP, similar to cFLIP, can bind to and prevent Atg3 from binding to LC3 (Lee et al., 2009). As mentioned above, Atg3 is an enzyme critical for the lipidation of LC3 and the formation of autophagic vacuoles (Ichimura et al., 2000). In addition, KSHV and other γ-herpesviruses, such as murine γHV68, encode homologs of Bcl-2. These viral homologs of Bcl-2, similar to Bcl-2, can bind to Beclin-1 to prevent it from binding to PI3KC3 and hence suppress the initiation of autophagy (E et al., 2009; Liang et al., 2006; Pattingre et al., 2005; Sinha et al., 2008).

Not all herpesviruses can suppress autophagy. In contrast to the herpesviruses mentioned above, Epstein-Barr virus (EBV) and varicella-zoster virus (VZV) induce rather than suppress autophagy. The EBV latent membrane protein 1 (LMP-1) can induce autophagy in a dose-dependent manner (Lee and Sugden, 2008b). Interestingly, the expression level of EBV LMP-1 also seems to be controlled by autophagy, as its level increases if autophagy is suppressed by short hairpin RNA (shRNA) targeting Beclin-1 or Atg7 (Lee and Sugden, 2008a; 2008b). How VZV induces autophagy is unclear. However, its induction of autophagy does not require its late gene products, since the suppression of viral late gene expression with phosphonoacetic acid does not abolish VZV-induced autophagy (Takahashi et al., 2009).

Thus, herpesviruses can affect autophagy either positively or negatively, depending on the virus. However, with the exception of HSV-1, which suppresses autophagy to enhance its replication and pathogenesis, the role of autophagy in the life cycle of other herpesviruses is not clear.

Adenoviridae

Conditional replicating adenoviruses have been produced to selectively replicate in cancer cells to cause cancer cell death. The study on such viruses has led to the finding that these viruses can suppress the mTOR signaling pathway and induce the dimerization of Atg5 and Atg12, the lipidation of LC3 and the accumulation of autophagic vacuoles (Alonso et al., 2008; Ito et al., 2006). This has led to the speculation that adenovirus may induce autophagic cell death to facilitate the release of progeny virus particles at the end of its infection cycle (Jiang et al., 2008).

Papillomaviridae

Human papillomavirus 16 (HPV16) is a high-risk papillomavirus that can cause cervical carcinoma. The E7 protein of HPV16 can enhance the lipidation of LC3 and increase the number of LC3 puncta (i.e., autophagic vacuoles) in human keratinocytes, indicating that HPV16 E7 may induce autophagy (Zhou and Munger, 2009). As keratinocytes expressing E7 are prone to cell death upon cell-to-cell contact or serum deprivation, autophagy induced by E7 may sensitize cells to death (Zhou and Munger, 2009; Zhou et al., 2009a). Further studies will be required to understand the relationship between HPV and autophagy.

Polyomaviridae

Simian virus 40 (SV40) small t antigen has recently been shown to induce autophagy (Kumar and Rangarajan, 2009). This protein binds to and inactivates protein phosphatase 2A (PP2A), which results in the enhanced phosphorylation and activation of AMP-activated protein kinase (AMPK), particularly during glucose deprivation. The activation of AMPK leads to the inactivation of mTOR. This inactivation of mTOR is likely responsible for the induction of autophagy by the small t antigen, since the treatment of control cells with rapamycin, which inhibits the mTOR activity, also increased autophagic vacuoles to a level similar to that of small t antigen-expressing cells. The expression of the SV40 small t antigen suppresses cell death caused by glucose deprivation. Thus, it has been proposed that the SV40 small t antigen may function to maintain energy homeostasis during glucose deprivation by activating AMPK, inhibiting mTOR, and inducing autophagy to provide an alternate energy source for the survival of tumor cells (Kumar and Rangarajan, 2009).

Parvoviridae

Studies on human parvovirus B19-infected cells revealed an increase in the ratio of lipidated LC3 to non-lipidated LC3 and the presence of mitochondria in autophagosomes. These observations indicate that human parvovirus B19 may enhance autophagy. The suppression of autophagy in B19-infected cells with 3-methyladenine enhanced cell death (Nakashima et al., 2006), suggesting a role of autophagy in the survival of infected cells.

Hepadnaviridae

Hepatitis B virus (HBV), a major causative agent of severe liver diseases, can also induce autophagy in cell cultures, mouse liver and an infected patient (Sir et al., 2010). The research of our laboratory indicates that HBV induces autophagy via its X

protein (HBx), which binds to PI3KC3 and enhances its enzymatic activity. This leads to enhanced autophagic flux, although without an increase of the autophagic protein degradation rate. The enhancement of autophagy is important for HBV DNA replication, since the suppression of autophagy using siRNA targeting hVps34 or Atg7 or using 3-methyladenine to inhibit PI3KC3 results in the suppression of HBV DNA replication. The effect of autophagy on HBV is mostly at the DNA replication step after the packaging of the viral pregenomic RNA, since autophagy does not significantly affect HBV RNA transcription or pregenomic RNA packaging. Since the suppression of fusion between autophagosomes and lysosomes with bafilomycin A1 has little effect on HBV DNA replication, the replication of HBV DNA likely does not involve autolysosomes. The observation that autophagy enhances HBV DNA replication is consistent with earlier reports that fasting enhances HBV DNA replication in the mouse model (Li et al., 2009; Shlomai et al., 2006).

A separate report indicates that HBV activates the expression of Beclin-1 via its HBx protein and sensitizes cells to starvation-induced autophagy (Tang et al., 2009). We were not able to observe such activation of the Beclin-1 gene by HBV. The reason for this discrepancy is unclear, although it is clear from our studies that HBV by itself is sufficient to induce autophagy without the need of nutrient starvation (Sir et al., 2010).

RNA Viruses

Picornaviridae

Several picornaviruses, most notably, the poliovirus, have been shown to affect the autophagic pathway. Poliovirus can induce the accumulation of autophagosomes and autolysosomes in infected cells. This induction of autophagic vacuoles and the lipidation of LC3 require the viral proteins 2BC and 3A, although how these viral proteins induce autophagic vacuoles remains to be determined (Suhy et al., 2000; Taylor and Kirkegaard, 2007). It is clear, though, that this induction of autophagy by poliovirus is important for polioviral RNA replication, as the suppression of autophagy with 3-methyladenine, which inhibits the activity of PI3KC3, or with siRNA targeting LC3 or Atg12 led to the suppression of viral RNA replication (Jackson et al., 2005). It is likely that autophagosomes provide the membranous support for viral RNA replication, since the polioviral RNA replication complex colocalizes with autophagosomes (Jackson et al., 2005). Besides its role in polioviral RNA replication, autophagy can also mediate the release of poliovirus from infected cells without cell lysis. This phenomenon was first noted when it was discovered that the suppression of LC3 or Atg12 expression with siRNA decreased the amount of extracellular viral particles even more than intracellular viral particles (Jackson et al., 2005). This observation was subsequently confirmed when it was found that there was a positive correlation between the motility of autophagosomes and the release of extracellular poliovirus particles (Jackson et al., 2005; Suhy et al., 2000; Taylor et al., 2009).

Other members of the picornavirus family, such as coxackieviruses, can also induce autophagy. Coxsackievirus B4 (CVB4), an important cause of infant meningitis and encephalitis, induces autophagy in a calpain-dependent manner (Yoon et al., 2008). Coxsackievirus B3 (CVB3), which causes myocarditis, induces the lipidation of LC3 and the accumulation of autophagosomes without enhancing autophagic protein degradation (Wong et al., 2008). For both coxsackieviruses, the inhibition of autophagosome formation resulted in the suppression of viral replication and, conversely, the induction of autophagy enhanced viral replication. Interestingly, blocking the fusion between autophagosomes and lysosomes with siRNA targeting the lysosomal protein LAMP2 also significantly enhanced the replication of CVB3 (Wong et al., 2008). As blocking the fusion between autophagosomes and lysosomes can lead to the accumulation of autophagosomes in cells (Sir et al., 2008a), these observations indicate that autophagosomes likely play a critical role in the replication of coxsackieviruses.

Whether rhinoviruses, another distinct group of the picornavirus family, can also induce autophagy is controversial. Although in one report it was demonstrated that human rhinovirus type 2 (HRV2) and type 14 (HRV14) could induce the accumulation of both early and late autophagic vacuoles (Jackson et al., 2005), in another report it was found that HRV2 could not induce these autophagic vacuoles (Brabec-Zaruba et al., 2007). In this latter report, it was also shown that drugs that induce or suppress autophagy had no effect on the replication of HRV2. The reason for this discrepancy is unclear.

Flaviviridae

Hepatitis C virus (HCV), which can cause acute and chronic hepatitis as well as other severe liver diseases, induces the accumulation of autophagosomes in cells (Ait-Goughoulte et al., 2008; Sir et al., 2008a). However, our studies indicate that HCV does not induce autolysosomes and, similar to CVB3, does not enhance autophagic protein degradation (Sir et al., 2008a). This led us to conclude that HCV may actually suppress the fusion between autophagosomes and lysosomes to enhance the accumulation of autophagosomes in cells (Sir et al., 2008a). Further studies indicate that HCV induces the accumulation of autophagosomes via the induction of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) (Sir et al., 2008a; 2008b). Similar to poliovirus and coxsackieviruses, this induction of autophagosomes plays an important role in HCV replication, as siRNA-knockdown of cellular genes including Atg7, LC3, Atg4B, Atg12 and Beclin-1, which are important for the formation of autophagosomes, significantly reduced the HCV RNA replication level (Dreux et al., 2009; Sir et al., 2008a; Tanida et al., 2009). By analyzing HCV entry, protein translation, RNA replication and viral maturation and release using cell cultures, it was suggested that the autophagy machinery might be required for the initiation of HCV replication, possibly by facilitating the translation of the incoming HCV genomic RNA (Dreux et al., 2009).

Besides HCV, dengue virus can also activate the autophagic pathway to enhance its RNA replication. However, unlike HCV, dengue virus does induce late autophagic vacuoles including amphisomes and autolysosomes (Lee et al., 2008; Panyasrivanit et al., 2009). Dengue virus-2 (DENV-2) and dengue virus-3 (DENV-3) are antigenically related but distinct viruses. DENV-2 double-stranded RNA (dsRNA) (i.e., the RNA replicative intermediate), NS1 protein and the ribosomal protein L28 colocalize with amphisomes, whereas DENV-3 dsRNA and NS1 colocalize with both amphisomes and lysosomes (Khakpoor et al., 2009; Panyasrivanit et al., 2009). The treatment of dengue virus-infected cells with the drug L-arginine, which inhibits the formation of autolysosomes and hence induces the accumulation of autophagosomes and amphisomes, has opposite effects on DENV-2 and DENV-3. It enhances DENV-2 replication and suppresses DENV-3 replication. Thus, it has been suggested that DENV-2 uses amphisomes, whereas DENV-3 uses both amphisomes and autolysosomes as the sites for their viral RNA translation and replication (Khakpoor et al., 2009; Panyasrivanit et al., 2009).

Bovine viral diarrheal virus (BVDV) is a pestivirus that also belongs to the flavivirus family. Curiously, cytopathic viruses

isolated from cattle with lethal mucosal disease had been found to contain the LC3 gene in the viral genome (Meyers et al., 1998). This insertion of the LC3 sequence into the BVDV genome was apparently a result of genetic recombination. The infection of cells by BVDV carrying the LC3 gene does not affect the expression of LC3 in cells (Fricke et al., 2004). The relationship between BVDV and autophagy is unclear (Wong et al., 2008).

Nidovirale

Coronaviridae and *Arteriviridae* are two different families of viruses that have been grouped into the order *Nidovirale*. Members of both virus families, which include mouse hepatitis virus (MHV) and SARS-CoV for the coronavirus family and equine arteritis virus (EAV) of the arterivirus family, have all been shown to induce double-membrane vesicles (DMVs) resembling autophagosomes. These viruses use DMVs for the formation of their RNA replication/transcription complexes and their induction of DMVs requires the expression of viral nonstructural proteins (Clementz et al., 2008; de Haan and Reggiori, 2008; Knoops et al., 2008; Posthuma et al., 2008; Prentice et al., 2004). Whether these DMVs are indeed autophagosomes, however, is not clear. Prentice et al. (2004) presented data to show the colocalization of the MHV replication complexes with LC3 and Atg12. However, such colocalization was not observed by others (de Haan and Reggiori, 2008). Furthermore, although Prentice et al. showed that the induction of DMVs by MHV required Atg5, Zhao et al. (2007) reported opposite results. In any case, if these DMVs are indeed autophagosomes, they are induced by unusual mechanisms because their induction by MHV is insensitive to 3-methyladenine, an inhibitor of PI3KC3 that is essential for the initiation of autophagy (Prentice et al., 2004). Clearly, the relationship between nidoviruses and autophagy will require further studies (de Haan and Reggiori, 2008).

Orthomyxoviridae

The infection by influenza virus A can also lead to the accumulation of autophagosomes in mouse embryonic fibroblasts (Gannage et al., 2009; Zhou et al., 2009b). Similar to HCV, this was due to the inhibition of fusion between autophagosomes and lysosomes (Gannage et al., 2009). The studies on individual influenza virus proteins indicate that the influenza virus M2 protein is sufficient and necessary to induce the accumulation of autophagosomes (Gannage et al., 2009). The M2 protein contains the proton channel activity. However, this proton channel activity is not required for blocking the fusion between autophagosomes and lysosomes. Since the M2 protein colocalizes with autophagosomes and can bind to Beclin-1, it is likely that M2 blocks the formation of autolysosomes through its interaction with Beclin-1 (Gannage et al., 2009), which has been shown to also regulate the fusion between autophagosomes and lysosomes (Matsunaga et al., 2009). This blocking does not affect viral replication but it enhances the retention of viral proteins and RNA in infected mouse embryonic fibroblasts. It has been suggested that this retention may reduce the antigenicity of influenza virus infection and prevent their presentation by MHC molecules. Whether this is indeed the case requires further studies. In A549 lung epithelial cells, however, it was reported that the inhibition of autophagy reduced influenza virus A replication (Zhou et al., 2009b), raising the possibility that the effect of autophagy on influenza virus may be cell type-specific (Rossman and Lamb, 2009). Cells deficient in autophagy have enhanced apoptotic cell death after influenza virus infection and cells infected by a MS2-deficient virus that is incapable of blocking the fusion between autophagosomes and lysosomes have an increased survival rate (Gannage et al., 2009). Thus, the autophagy pathway appears to be important for the survival of cells after influenza virus infection.

Rhabodoviridae

Vesicular stomatitis virus (VSV) has also been shown to induce autophagy (Shelly et al., 2009). This induction of autophagy by VSV, however, is a cellular innate immune response to suppress VSV infection, as the suppression of autophagy with siRNA targeting individual autophagy genes, such as Atg1 and Atg5, increased the yield of progeny viruses. The VSV G protein, which is the envelope protein, is sufficient to induce autophagy, indicating that this protein possesses the pathogen-associated molecular pattern (PAMP) that is recognized by an unknown pattern-recognition receptor (PRR), which then stimulates this innate immune response (Shelly et al., 2009).

Reoviridae

Rotavirus can also induce autophagic vacuoles via its NSP4 protein. The rotavirus NSP4 protein colocalizes with LC3, although such colocalization with the lysosomal marker LAMP1 was not detected. This suggests that NSP4, similar to HCV and influenza virus, binds to autophagosomes and suppresses their fusion with lysosomes (Berkova et al., 2006). In rotavirus-infected cells, NSP4 and LC3 colocalize with viroplasm, the site of rotaviral RNA replication. This raises the possibility that NSP4 may recruit autophagosomal membranes to viroplasm to enhance viral RNA replication. Further studies will be required to confirm this possibility (Berkova et al., 2006).

Retroviridae

Human immunodeficiency virus-1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), interacts with the autophagic pathway in different ways. This virus induces the accumulation of autophagic vacuoles in macrophages and this induction is dependent on its Nef protein. The autophagic pathway augments HIV-1 replication, as the suppression of autophagy with 3-methyladenine or by siRNA-knockdown of Beclin-1 or Atg7 expression reduced the yield of progeny viruses, and the induction of autophagy with rapamycin, a drug that suppresses mTOR, enhanced HIV-1 yield. These effects of autophagy on HIV-1 appear to be specific to macrophages, as similar effects were not observed in T cell lines (Espert et al., 2009; Kyei et al., 2009). Although HIV-1 Nef induced the lipidation of LC3 and increased the LC3 puncta (i.e., autophagosomes), it did not increase autophagic protein degradation but rather, suppressed autophagic protein degradation induced by nutrient starvation (Kyei et al., 2009). Similar to HCV and influenza virus A, HIV-1 Nef blocks the maturation of early autophagic vacuoles into acidified, degradative autolysosomes. This blocking prevents the degradation of HIV-1 Gag proteins, which bind to LC3 and are localized to autophagic vacuoles, indicating an important role of Nef in preventing autophagic removal of HIV-1 assembly intermediates or virions (Kyei et al., 2009). Nef binds to Beclin-1 and causes redistribution of hVps34, the catalytic subunit of PI3KC3, to membranes (Kyei et al., 2009). How this interaction between Nef and Beclin-1 or whether other activities of Nef prevents the maturation of early autophagic vacuoles into autolysosomes remains to be determined. It is noteworthy that, in two separate reports, HIV-1 was shown to suppress the induction of autophagic vacuoles in CD4+ T cells (Espert et al., 2009; Zhou and Spector, 2008). Thus, HIV-1 seems to have different effects on autophagy in different cell types (Brass et al., 2008; Espert et al., 2009; Kyei et al., 2009).

In addition to inducing the accumulation of early autophagic vacuoles and preventing the maturation of these vacuoles into autolysosomes in macrophages, HIV-1 envelope proteins gp120 and gp41 expressed on the cell surface can also induce autophagy of uninfected cells via a bystander effect. This bystander effect requires the binding of HIV-1 gp120 to CD4 and CXCR4 on the surface of uninfected cells and is dependent on the fusogenic activity of HIV-1 gp41 (Denizot et al., 2008; Espert et al., 2006). This induction of autophagy triggers apoptotic cell death and likely plays an important role in the depletion of CD4+ T cells and the development of immunodeficiency in HIV-infected patients.

CONCLUSION

Autophagy can serve as an innate immune response to suppress viral infections, as demonstrated by the studies on VSV. The infection of cells by VSV induces the autophagic response, and the suppression of autophagy enhances the VSV yield. Similarly, the HSV-1 mutant unable to suppress autophagy is attenuated, indicating that the suppression of autophagy is important for the survival of HSV-1.

To enhance their survival, viruses have involved different mechanisms to subvert this innate immune response. A common pathway shared by several herpesviruses, including HSV-1, KSHV, γHV68, to suppress autophagy is to produce gene products that prevent Beclin-1 from binding to the PI3KC3 complex. Several other viruses, such as coxsackieviruses, HCV, influenza virus, HIV-1 and perhaps rotaviruses, have also evolved mechanisms to perturb autophagy by suppressing the fusion between autophagosomes and lysosomes. The suppression of this membrane fusion can prevent the degradation of viral particles by lysosomal enzymes. The molecular mechanisms for this suppression of membrane fusion are largely unknown, although both influenza virus A and HIV-1 apparently target Beclin-1, a protein factor that is important for the fusion between autophagosomes and lysosomes.

Although some viruses suppress autophagy, other viruses enhance it. Poliovirus, dengue viruses and HBV enhance autophagy to enhance their replication. Although EBV, VZV, HPV16, SV40 and parvovirus B19 are also reported to induce autophagy, whether their induction of autophagy plays any role in their life cycle is not clear. With the exceptions of HBV, which enhances the PI3KC3 activity, and SV40, which inactivates PP2A to activate AMPK to inactivate mTOR, the molecular mechanisms of how viruses induce autophagy remain largely unclear.

The induction of autophagy as well as the suppression of fusion between autophagosomes and lysosomes can lead to the accumulation of autophagic vacuoles in cells. Several RNA viruses including poliovirus, dengue viruses, rotaviruses and nidoviruses use the membranes of autophagic vacuoles for their RNA replication. For other viruses, such as coxsackieviruses, HCV, HIV-1, HBV and perhaps also influenza virus A, autophagosomes also enhance viral replication, although the molecular mechanisms of these enhancements remain unclear.

Besides enhancing viral replication, autophagic vacuoles may also be used by viruses as delivery vehicles for the release of viral particles from infected cells. Examples are poliovirus and HIV-1.

The induction of autophagy by different viruses may also cause different effects on their host cells. Autophagy induced by adenovirus causes cell death, possibly for the release of progeny viral particles. Autophagy induced by HPV16 sensitizes cells to death upon cell-to-cell contact or serum deprivation, and autophagy induced by HIV-1 via a bystander effect causes CD4+ T cells to undergo apoptosis. In contrast, autophagy induced by parvovirus B19 and SV40 enhances cell survival under stress. These effects of viruses on their host cells can have profound consequences on viral pathogenesis. For example, the depletion of CD4+ T cells by HIV-1 can lead to the development of acquired immunodeficiency syndrome (AIDS).

Many viruses have been shown to affect autophagy. It is likely that this list of viruses will continue to grow. Autophagy may be used by cells to suppress viral replication. However, it may also be exploited by viruses to enhance their replication and survival. A better understanding of how different viruses affect and respond to autophagy will lead to a better understanding of viral pathogenesis and the development of antiviral drugs that target the autophagic pathway.

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