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REVIEW

Cross-talking between autophagy and viral infection in mammalian cells

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Abstract Autophagy is a cellular process in degradation of long-lived proteins and organelles in the cytosol for maintaining cellular homeostasis, which has been linked to a wide range of human health and disease states, including viral infection. The viral infected cells exhibit a complicated cross-talking between autophagy and virus. It has been shown that autophagy interacts with both adaptive and innate immunity. For adaptive immunity, viral antigens can be processed in autophagosomes by acidic proteases before major histocompatibility complex (MHC) class II presentation. For innate immunity, autophagy may assist in the delivery of viral nucleic acids to endosomal TLRs and also functions as a part of the TLR-or-PKR-downstream responses. Autophagy was also reported to suppress the magnitude of host innate antiviral immunity in certain cases. On the other hand, viruses has evolved many strategies to combat or utilize the host autophagy for their own benefit. In this review we discussed recent advances toward clarifying the cross-talking between autophagy and viral infection in mammalian cells.

Keywords cross-talking, autophagy, viral infection

1 Introduction

Autophagy was first described in mammalian cells as early as 50 years ago (Kundu and Thompson, 2008), which regulates the processes of degradation and the recycling of cellular constituents. There are three different autophagic pathways: microautophagy, macroautophagy and chaperone-mediated autophagy (CMA) (Kunz et al., 2004; Klionsky, 2005; Massey et al., 2006).

Macroautophagy which referred as autophagy in the rest paragraphs is the major type to digest cytoplasmic constituents. Macroautophagy is mediated by the formation of the autophagosome, which is a double membrane vesicle and capable fusing with lysosome to form autolysosome to degrade and recycle the proteins (Klionsky, 2005; Cuervo, 2006).

Generally, the autophagic process is divided into three steps: signaling and autophagosome formation, targeting to and fusion with lysosomes, proteins degradation and recycling (Wang and Klionsky, 2003). In the first step, autophagy was activated by the induction of stress stimulation such as viral infection, starvation or others to initiate the autophagosome formation (Yin and Thummel, 2005; Heymann, 2006; Kadowaki et al., 2006), after which, autophagosome fuses with the lysosome to form autolysosomes. In that way, cytoplasmic materials in autophagosomes are released into the lysosome and subsequently consumed by hydrolases. Followed along with breakdown, the constituents are recycled through lysosomal transporters to the cytosol.

As a critical cellular process, autophagy interacts with many signal pathways. In the initial stage, the Beclin 1/class III phosphoinositide 3-kinase (PI3K) complex acts as an important member of autophagic pathway, promoting the autophagosome formation (Kihara et al., 2001; Zeng et al., 2006) (Fig. 1A). In contrast, mammalian target of rapamycin (mTOR) is known as the key downregulator of autophagy induction (Yang et al., 2005), which can be inhibited by rapamycin and activated by amino acids. Several cascades are involved in mTOR pathway as well. Class I PI3K/Akt lies on the upstream of mTOR and acts positively on it to inhibit autophagy, whereas the phosphatase and tension homolog (PTEN) acts antagonistically to the class I PI3K to induce autophagy (Dan et al., 2008). Besides, the UNC-51-like kinase 1 (ULK1)-autophagy-related protein 13 (Atg13)-FIP200 (a focal adhesion kinase [FAK] family-interacting protein of 200 kDa) complex is a direct target of mTOR and important regulator of autophagy in response to mTOR signaling. In detail, mTOR phosphorylates a mammalian homolog of Atg13 and the mammalian Atg1 homologs

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ULK1 and ULK2, and then the Atg13 binds to both ULK1 and ULK2 and mediates the interaction of the ULK proteins with FIP200. In these processes, the binding of Atg13 stabilizes and activates ULK and facilitate the phosphorylation of FIP200. FIP200 is a novel mammalian autophagy factor which plays essential role in autophagosome formation (Har et al., 2008). mTOR also interacts with p53 family. Recently, it was demonstrated that mTOR can inhibit the p53 family member p73, which is capable inducing cellular autophagy and multiple autophagy-associated genes downstream of mTOR (Rosenbluth and Pietenpol, 2009). In addition the mTOR pathway is necessary for nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) mediated repression of autophagy in tumor necrosis factor-alpha (TNFα)-treated cell. In cells lacking NF-κB activation, TNFα treatment upregulates the expression of the autophagy-promoting protein Beclin 1 and subsequently induces the accumulation of autophagic vacuoles (Dan et al., 2008; Rosenbluth and Pietenpol, 2009). Most importantly, autophagy also intersect with adaptive and innate immunity, this correlation will be discussed in the following paragraphs. Additionally, UV radiation resistance associated gene product (UVRAG) has been identified as a part of the PI3K/Beclin1 complex and representing an important signaling checkpoint in autophagy (Liang et al., 2006), and BCL-2 is able to inhibit autophagy by binding to Beclin 1 as a negative regulator of Beclin 1/classIII PI3K complex (Fig. 1B).

The functions of autophagy were first described as a cellular process of degradation long-lived proteins and organelles in the cytosol for maintaining cellular homeostasis (Levine and Klionsky, 2004; Boya et al., 2008; Cecconi and Levine, 2008; Gajewska et al., 2008; Tsukamoto et al., 2008), and it was demonstrated that malfunction of autophagy contributes to neurodegeneration and cancer (Ellinger, 2005; Komatsu et al., 2006a, 2006b; Mathew et al., 2007; Levine, 2007; Bursch, and Jaboin et al., 2009; Jaakkola and Pursiheimo, 2009). More interestingly, a bunch of researches showed that autophagy appears to be the defense against cellular pathogens in host cells, and certain viruses strategically take over the autophagic machinery to promote their infection and replication.

This review may render easier the comprehension of the complex interactions between the host cells and the viruses and might suggest to the reader new ideas for the discovery of new targets for antiviral therapy capable not only of killing pathogens but also of regulating the pathogen-host interactions involved in virus infection and replication and/or immune escape.

2 Autophagy helps antigen presentation to combat viral infection

Presentation of antigenic peptides by major histocompatibility complex (MHC) class I and II molecules is a crucial component of adaptive immunity. Several studies have unraveled the potential important roles that autophagy plays in the MHC class II antigen presentation process. When investigating the MHC class II-restricted presentation of Epstein-Barr virus nuclear antigen-1 (EBNA-1), it was observed that blocking lysosomal acidification led to accumulation of EBNA-1 in intracellular vesicles positive for LAMP-1 and co-staining with MDC (a fluorescent dye that labels autophagosomes). MHC class II-restricted presentation of endogenous EBNA-1 to T cells was reduced by 30%–70% when autophagy was blocked (Paludan et al., 2005; Strawbridge and Blum, 2007). Similarly, in another study, targeting the influenza matrix protein 1 (MP1) to the autophagosome by fusing with the ubiquitin-like protein Atg8 (known as LC3 in mammalian cells) significantly enhanced the delivery of the antigen for MHC class II presentation to MP1-specific CD4 T cells. And the Atg12-targeted siRNA is able to reduce T cell response. Obviously, cytosolic antigens can be delivered to the MHC class II compartment via autophagy for enhanced MHC class II presentation to CD4 T cells. However, the MHC class I presentation was not affected in these studies (Schmid et al., 2007). More studies are still needed to clarify the mechanisms of autophagy in MHC class II presentation. Such knowledge will be very helpful for designing more effective vaccines in the prevention of infectious diseases.

3 Autophagy and innate immune system regulate each other in response to virus invasion

Under the stress of viral infection, cells need to recognize the virus immediately to stimulate initial antiviral responses (Takeuchi and Akira, 2007). Therefore, the innate immune system encodes several classes of pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)–like helicases (RLHs) and double-stranded RNA (dsRNA)-dependent protein kinase (PKR) to perform this recognition. As sensors that discriminate self from non-self, PRRs can recognize viruses and trigger certain signaling pathways that induce antiviral mediators such as type I IFNs and pro-inflammatory cytokines.

As an important family of PRRs, the Toll-like receptors (TLRs) family includes more than 10 members that have been identified so far. TLRs mainly expressed on antigen-presenting cells, such as macrophages or dendritic cells, and play critical roles to provoke innate immunity and establish adaptive immunity. Besides the common effects, such as inflammatory cytokine induction or upregulation of costimulatory molecule expression, each TLR has its specific functions, which are not only critical in anti-microbial immunity but also involved in manifestations of

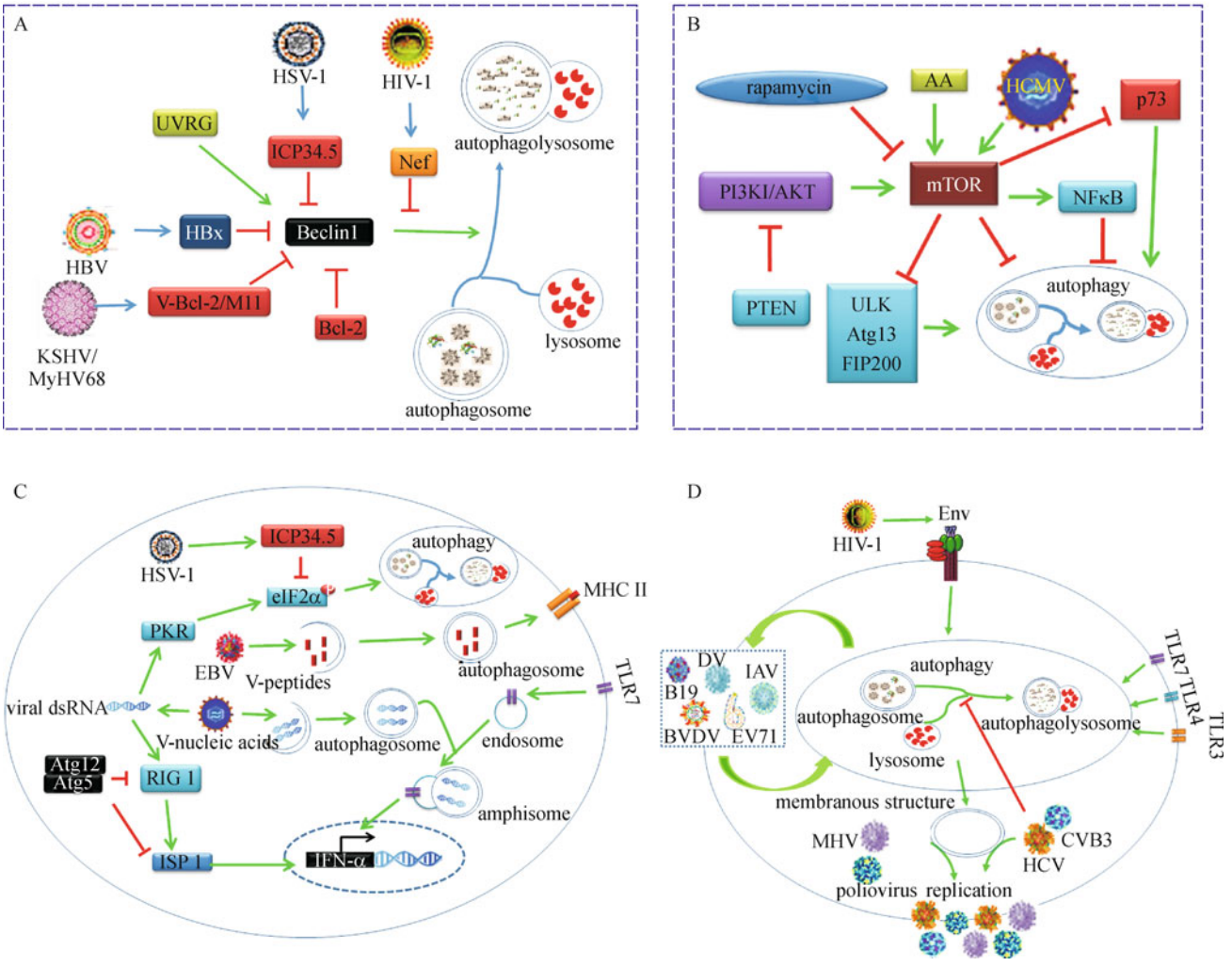

Fig. 1 The cross-talking between viruses and host cells. A: Beclin1 is a component PI3K/Beclin1 complex which acts an important role in the initial stage of autophagy. UVRAG is a part of the PI3K/Beclin1 complex and representing an important signaling checkpoint in autophagy, while BCL-2 is able to inhibit autophagy by interacting with Beclin 1 as a negative regulator. It is because the essential roles of PI3K/Beclin1 complex, several viruses evolve some strategies to combat or employ it for their own benefits. HSV-1 expresses protein ICP34.5, HIV-1 has Nef, Kaposi's sarcoma-associated herpesvirus (KSHV) produces vBCL-2 and the murine γ-herpesvirus 68 encodes protein M11, as well as HBV express protein HBx to suppress autophagy by blocking the Beclin1. B: mTOR is known as the key down-regulator of autophagy induction, which can be inhibited by rapamycin and activated by amino acids. Class I PI3K/Akt lies on the upstream of mTOR and acts positively on it to inhibit autophagy, whereas the phosphatase and tension homolog (PTEN) acts antagonistically to the class I PI3K to induce autophagy indirectly. Besides, the ULK-Atg13-FIP200 complexe is a direct target of mTOR and important regulator of autophagy in response to mTOR signaling. Additionally, mTOR activity inhibits p73, which is the inducer of cellular autophagy, and in TNFα-treated cell, mTOR is necessary for NF-κB activation mediated repression of autophagy. In HCMV infected cells, mTOR can be activated to suppress autophagy for its own benefits. C: When viruses replicating in cells, the viral dsRNA activates PKR to phosphorylate eIF2α and then mediate viruses degradation through autophagy. However, HSV-1 has possibility encoding ICP34.5 to suppress cellular autophagy by dephosphorylating eIF2α in PKR pathway. In pDCs, the autophagic vesicles can wrap the replication intermediate of escaped VSV in cytoplasmic and then fuse with endosome who contain TLR7 to recognize viral replication intermediate to produce IFN- α. As we know, the viral dsRNA also stimulates IFN-α production via RIG-1 and ISP-1. Interestingly, in mouse embryonic fibroblasts (MEFs), the IFN-α production can be down-regulated in response to VSV by Atg5-Atg12 direct association with both RIG-I and IPS-1 through the caspase recruitment domains. For adaptive immunity, autophagy can deliver antigens into autophagosomes for processing by acidic proteases before MHC class II presentation. For example, autophagy helps presentation of endogenous EBNA-1 to T cells in EBV infected cells. D: Besides, TLR3, TLR4 and TLR7 are able to induce autophagy in certain cells. MHV, Poliovirus and CVB3 as well as HCV are capable inducing autophagosomes to generate sites and support their replication. Besides, HCV and CVB3 can suppress autophagic protein degradation by blocking the fusion of autophagosome and lysosome. Certain viruses such as enterovirus 71, influenza A virus, BVDV, human parvovirus B19 and dengue virus-2 are able to activate autophagy to enhance the efficiency of viral replication, but detail mechanisms are still unclear. More interestingly, Env of HIV is able to induce autophagy and accumulate the Beclin 1 in uninfected cells via CXCR4

autoimmunity (Kaisho and Akira, 2006). Recently, TLRs has been described to cooperate with cellular autophagy to support delivering viral nucleic acids and producing certain cytokines. In vesicular stomatitus virus (VSV) infected plasmacytoid dendritic cells (pDCs), autophagy was found to be required for the recognition of VSVs by TLR7 and the production of IFN-α by pDCs. After treating with the inhibitors of autophagy, 3-methyladenine and Wortmannin, the production of IFN-α was significantly decreased. Furthermore, Atg5 deleted pDCs and ATG5 −/− chimera mice failed to secrete IFN-α in response to VSV infection in vitro and in vivo. The author speculated that pDCs can utilize autophagic vesicle to wrap the replication intermediate of escaped VSV in cytoplasmic, and fuse with endosome who contain TLR7, and then the replication intermediate could be recognized by TLR7 to start production of cytokines such as IFN-α (Lee et al., 2007).

The link between autophagy and innate immune system is not one-sided. Some TLRs were reported having the ability to activate autophagy, such as TLR3, TLR4, TLR7 and other TLR family members. The autophagy induction by TLR ligands was dependent of the expression of Beclin 1, a key factor in autophagosome formation, toll-like receptors adapter myeloid differentiation primary response gene (88) (MYD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF). When knocking-down the expression of Beclin 1, MYD88 or TRIF by shRNA, the induction of autophagy was also inhibited. MyD88 and TRIF could co-immunoprecipitate with Beclin1. It was proposed that by recruiting Beclin 1 into the TLR-signaling complex, MYD88 and/or TRIF reduced the binding of Beclin 1 to Bcl-2 which is an important autophagy inhibitor and then upregulate cellular autophagy (Delgado et al., 2008; Shi and Kehrl, 2008).

Besides TLRs, PKR-eIF2α pathway can also trigger the autophagy induction. As a family of evolutionarily conserved serine/threonine kinases, the eIF2α kinases were described as a regulator of stress-induced translational arrest. In yeast, the eIF2α kinase GCN2 and the GCN2-dependent tanscriptional transactivator GCN4 play a vital role in starvation-induced autophagy. GCN2 and the IFN-inducible eIF2α kinase PKR are essential for virus-induced autophagy in mammalian cells (Tallóczy et al., 2002; Cuervo, 2004). It was found that eIF2α phosphorylation could activate phosphorylation of eukaryotic elongation factor 2 (eEF-2), and then eEF-2 may serve as an integrator of various cell stresses for autophagy signaling (Py et al., 2009).

In conclusion, in responses of viral infection, host cell takes "two-pronged" approach to prevent further viral spread. First, the replication intermediate dsRNA activate the PKR by binding to N-terminal domain to sweep the viruses by triggering the cellular autophagy, additionally, stimulating apoptosis in the infected cells.

Interestingly, recent studies suggested that autophagy may not only cooperate with innate immunity, it may also function as downregulator of innate immunity. RIG-I is another class of PRR which detect cytoplasmic viral RNA and initiate innate immune responses. In non-pDCs innate recognition of VSV in mouse embryonic fibroblasts (MEFs) via RIG-I pathway was downregulated by autophagy (Jounai et al., 2007). Deletion of Atg5 gene and Atg7 gene in MEFs can increase the production of type I interferons in response to VSV, and this effect was attributable to Atg5-Atg12 by direct association with both RIG-I and IFN-beta promoter stimulator 1 (IPS-1) through the caspase recruitment domains (Fig. 1C). These findings suggested that autophagy could perform as an immuno-suppressive pathway that increases host susceptibility to viral infection, or rather, merely provides negative feed-back to dampen immune/inflammatory signaling so as to avoid excessive and potentially harmful inflammatory host responses.

Further investigation of this correlation might give new idea to develop the therapeutic maneuvers against viral infection, allergy and autoimmune diseases, even cancer.

4 Viruses arouse formation of autophagosome-like vesicle to support viral infection

As described above, the cellular autophagy set several defense lines to combat virus. First, viruses are phagocytosed and digested in autophagosomes by hydrolases. Secondly, autophagic process is able to deliver certain viral antigens to MHC class II-loading compartments for assisting the antiviral function of adaptive immunity. Thirdly, autophagy can cooperate with innate immunity to combat virus. So, autophagy seems more likely to be a potential antiviral mechanism. However, paradoxically, there are lines of evidence showed that some virus can utilize autophagy process for their own benefit.

In poliovirus-infected cells, an accumulation of membranous structures was found in the cytoplasm early after infection. These double-membrane structures contain markers of the secretary pathway (Dales et al., 1965), which indicated that these structures originate from a process analogous to the formation of autophagic vacuoles. Further research identified several hallmarks of cellular autophagosomes in poliovirus-induced vesicles, including co-localization of LAMP1 and LC3. Different from that autophagy helps antigen presentation in EBV infected cells as described above, the autophagic process supports the poliovirus replication. Inhibition of the autophagosomal pathway by 3-methyladenine or RNAi treatment to reduce LC3 or Atg12p concentrations reduced poliovirus yield (Kirkegaard and Jackson, 2005). How the autophagic vesicles affect the virus multiplication? The researchers proposed that poliovirus may exploit the autophagic machinery for viral RNA replication and/or the non-lytic

release of the cytoplasmic viruses. There are some observations supporting this hypothesis. LC3 and the poliovirus capsid protein VP1 present in extracellular structures adjacent in poliovirus-infected cells, and the RNA replication complexes co-localize with autophagosomes. The reduction of extracellular virus yield is more than intracellular virus yield after treating with RNAi to LC3 or Atg12p (Kirkegaard and Jackson, 2005). However, the details remain unclear. The poliovirus proteins 2BC and 3A may play an important role in the induction of autophagy, since 2BC and 3A are sufficient to induce double-membraned vesicles (Suhy et al., 2000; Jackson et al., 2005; Taylor and Kirkegaard, 2007) (Fig. 1D). What an effective strategy to employ the cellular autophagic pathway to support viral RNA replication and viral release. Facing the immune pressure, the virus use the way as smart as "Taichi" to avoid being killed and get the power of the enemy. These strategies are used by other viruses as well.

The murine hepatitis virus (MHV), a member of the Coronaviridae is enveloped positive sense RNA viruses that replicate entirely in the cytoplasm of cells. Recent studies showed that the viral RNA replication occurs on cytoplasmic double membrane vesicles derived from the autophagic pathway, and the viral yield of extracellular virus is significantly diminished in clonal isolates of Atg5 −/− mouse embryonic stem cells (Kirkegaard et al., 2004; Prentice et al., 2004), which indicate that the MHV requires the autophagic pathway to form infectious virions. There are two hypotheses on the mechanism of the activation of autophagic pathway during the MHV infection. First, the double-stranded RNA generated during viral replication may activate protein kinase R and stimulate autophagy. Alternatively, MHV bind directly to the cellular receptor, Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM-1), and induce signaling events that result in autophagy. Encouragingly, in another member of Coronaviridae, the severe acute respiratory syndrome (SARS) virus infected cells; the double membrane vesicles were also observed (Snijder et al., 2006). The relationship between SARS infection and autophagy is interesting and should be focused more. If the components of the autophagic pathway are required for formation of a viral replication complex and for efficient viral growth, the new target for treatment to SARS will be brought.

Similar to poliovirus and MHV, Coxsackievirus B3 (CVB3), is able to take over host antimicrobial autophagy machinery to facilitate their own replication. In CVB3 infected cells, the number of double-membrane vesicles increased, accompanied by an increase of the LC3-II/LC3-I ratio and an accumulation of punctate GFP-LC3-expressing cells, two markers of cellular autophagosome formation. The inhibition and induction autophagy can reduce and increase viral replication. But p62, the marker for autophagy-mediated protein degradation, showed no apparent changes, which suggested that CVB3 infection triggers autophagosome formation without promoting protein degradation by the lysosome. Furthermore, the blockage of autophagosome-lysosome fusion significantly promoted viral replication (Wong et al., 2008). To support their replication, viruses may use the membrane structure of autophagosome, but they have to escape the digestion in lysosome. How to do these contradictory things? The strategy of CVB3 virus to block the autophagosome-lysosome fusion is an efficient way. Using the stimulator of autophagosome-lysosome fusion will be a method to suppress CVB3 replication.

Coincidentally, hepatitis C virus (HCV) can also result the accumulation of autophagosome and suppress autophagic protein degradation by blocking the fusion between autophagosomes and lysosomes to enhance its RNA replication (Ait-Goughoulte et al., 2008). It is suggested that the induction of autophagosomes by HCV is dependent on the unfolded protein response (UPR), a cellular stress response related to the endoplasmic reticulum which is activated in response to an accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum. The inhibitor of UPR is able to suppress HCV-induced autophagosome accumulation. At the same time, the replication of HCV can be decreased by UPR's inhibitor (Sir et al., 2008a). That is to say, autophagy is important to support infection of HCV. In fact, cellular autophagic proteins, such as Beclin-1, Atg4B, Atg5, and Atg12, act as proviral factors, which initiate the infection of HCV. These autophagy-related proteins are required for translation of viral RNA (Dreux and Chisari, 2009; Dreux et al., 2009). Possibly, autophagic factors help viral RNA binding to translation complex, and may recruit cellular factors for translation of HCV. As expected, silencing of autophagy-related genes by siRNA transfection significantly slowed down the replication of HCV replicon (Sir et al., 2008b) (Fig. 1D).

Autophagy can also be induced in EV71 infected cells, and further enhances virus replication. This induction was inhibited by the autophagy inhibitor 3-MA with decreased LC3 aggregation and decreased virus titer. In contrast, autophagy in EV71 infected cells was further enhanced by tamoxifen and rapamycin, LC3 aggregation and virus titer accordingly increased (Huang et al., 2009). Besides, two other human RNA viruses, dengue virus (DV) and influenza A viruses (IAV) also reported as a autophagy inducer. The autophagic process is triggered after infecting, and the autophagic machinery is favorable for viral replication (Lee et al., 2008, Zhou et al., 2009). However, how viruses stimulate autophagy and which part of autophagic process support viral infection are still obscure. Maybe there is a common mechanism to utilize the cellular autophagy for viral infection, such as using autophagosome to replicate viral nuclear acid, or to release virion.

Not only RNA virus, but also DNA virus applies certain strategy to take over autophagic process. Human parvovirus B19 is one of the smallest single-stranded DNA virus,

which lacks an envelope and infects erythroid cells to cause several diseases. B19 infection can induces cell cycle arrest at G1 and G2/M phases, and apoptosis. Morita et al. has reported a typical kinetics of B19 infection, in which most of the infected cells got arrested at G2 phase within 12 h after the infection, then survived up to 72 h, and started dying accompanied with viral replication (Morita and Sugamura, 2002). Recently, it was demonstrated that B19 infection also induced an intracellular autophagosome as judged by endogenous LC3 staining. B19-induced autophagy occurs in the later time point, mostly at 48 to 96 h after the infection. Moreover, inhibition of autophagy by 3-MA significantly facilitated B19-infection-mediated cell death that suggested a novel mechanism by which B19-infected cells survived (Nakashima et al., 2006) (Fig. 1D). We can speculate that autophagy-mediated survival of B19-infected cells may potentially leave more time periods for the viral replication. However, further studies are still needed to verify the true effects of autophagy on the replication of B19.

5 Pathways through which virus crosstalk with autophagy

As the gate-keeper of autophagy, mTOR always attacked by viruses for their own benefits. When primary human fibroblasts were infected with Human cytomegalovirus (HCMV), the autophagosome formation was obviously inhibited. This inhibition occurred early in the infection by a mechanism involving viral protein(s). Indeed, only infected cells expressing viral protein(s) displayed a striking decrease of autophagy; whereas bystander, non-infected cells displayed a level of autophagy similar to that of control cells. It was observed that infection with HCMV induced rapid phosphorylation of 4E-BP1 and p70S6K who are two of mTOR substrates. Therefore the mTOR signaling pathway was stimulated by HCMV and rendered infected cells resistant to autophagy. To be noted, infected cells also became resistant to the stimulation of autophagy by lithium chloride, an mTOR-independent inducer of autophagy. So other step(s) should also be targeted by HCMV to block autophagy (Chaumorcel et al., 2008) (Fig. 1B). Recently, it was found that Enterovirus 71 (EV71) infection-induced autophagy was through mTOR/p70S6K signaling pathway, this induction is cell-type specific in SK-N-SH cells, however, class I PI3K/Akt was not the upstream transducer (Huang et al., 2009).

The PKR-eIF2α pathway which positively regulates autophagy was also the target of certain virus to block autophagy. Herpes simplex virus type 1 (HSV-1) is known to be able to antagonize the host autophagy response in multiple cell types via the neurovirulence protein ICP34.5 (infected cell protein 34.5) which is encoded by the HSV-1 RL1 gene (Chou and Roizman, 1986), ICP34.5 contains an N-terminal region, followed by the 68-87 domain, and a C-terminal GADD34 homology domain (Chou et al., 1990; Chou and Roizman, 1992, 1994). Previous researches have shown that the GADD34 domain could recruit a host phosphatase, PP1α, to dephosphorylate eIF-2α. An ICP34.5 deletion mutant virus has been shown to promote autophagy in murine embryonic fibroblasts (MEFs) compared with the wild type virus, but it no longer triggers autophagy in the PKR −/− and Ser-51 nonphosphorylatable mutant eIF2α MEFs, which suggest that PKR-eIF2α signaling is the target of ICP34.5 for antagonizing autophagy. However, ICP34.5 also modulates autophagy through binding to Beclin1. The binding of ICP34.5, either directly or indirectly, to Beclin 1 is required for its autophagy-inhibitory function (Leib et al., 2000; Tallóczy et al., 2006; Alexander et al., 2007; Orvedahl et al., 2007). This viral evolution is compatible with Darwin's theory of evolution, and the HSV-1 who expresses the ICP34.5 is the survival of the fittest. Otherwise, it will be cleared.

Besides HSV-1, Beclin1 is also targeted by other viruses. BCL-2, the prototypic cellular antiapoptotic gene, is able to decrease Sindbis virus replication and Sindbis virus-induced apoptosis in mouse brains, resulting in protection against lethal encephalitis. To investigate potential mechanisms which BCL-2 protects against central nervous system by Sindbis virus infection, Beclin1 was identified using yeast two-hybrid screen. Beclin1 was found interacting with BCL-2 in mammalian cells and plays an important role in antiviral host defense. Beclin1 lacking the putative BCL-2 binding domain or Beclin1 containing a premature stop cordon near the 5′ terminus significantly decreased the survival of mice infected with Sindbis virus and increased the viral titers (Liang et al., 1998), so the protected function of BCL-2 against sindbis virus depends on Beclin1.

In another herpesvirus family, γ-herpesviruses, it was reported that certain members can encode BCL-2-like proteins targeting autophagic protein Beclin 1 to inhibit autophagy, such as the vBCL-2 of Kaposi's sarcoma-associated herpesvirus (KSHV) and the murine γ-herpesvirus 68-encoded protein, M11 (Pattingre et al., 2005; Liang et al., 2006). At present, it is unknown whether autophagy evasion by vBCL-2 is important for γ-herpesviruses replication and pathogenesis (Levine, 2007), but it will be interesting to compare the interaction between BCL-2-Beclin1 and vBCL-2-Beclin1 (Fig. 1A). In that case, we can see if vBCL-2 act as a competitor of BCL-2, and to investigate the details.

Hepatitis B virus X (HBx) protein is known as an oncogenic transactivator, which may cause HBV-infected cells to grow continuously in the development of hepatocellular carcinoma by inhibiting the pRb tumor suppressor and increasing E2F1 activity (Choi et al., 2001). Until not long ago, a novel function of HBx in increasing autophagy through the upregulation of Beclin1 expression were found. Overexpression of HBx results in

the upregulation of the endogenous mRNA and protein levels of Beclin 1 in the tested cells (Tang et al., 2009). Why HBV upregulate autophagy is still unclear. But there is very useful information that HBx might bind the promoter of Beclin1 to upregulate its expression. Investigating the structure of HBx is a potential way to design or find an autophagy stimulator which can be utilized to study the cellular autophagy (Fig. 1A).

6 Special strategies for virus to exploit autophagy

It's an expectation to find the common mechanisms of viruses involving in autophagy to improve antiviral research. But things always evolve in different way from our hope. Some viruses can exploit autophagy with special ways, for example, Bovine viral diarrhea virus (BVDV) which belongs to the flavivirus family. Interestingly, the genome of the cytopathogenic BVDV contains a cellular insertion coding for microtubule-associated protein-1 light chain-3(LC3) which is one of the autophagic markers. This genome insertion has been reported to enhance the pathogenicity of the virus and the insertion is somehow associated with the expression of the viral nonstructural protein NS3, in most cases by induction of proteolytic processing at the LC3/NS3 fusion site, exactly at the amino acid of the LC3 sequence glycine 120. The mammalian protease Atg4 which can process LC3 for its coupling to autophagosomal membranes can also efficiently cleaves the LC3/NS3 fusion expressed in LC3-positive cytopathogenic BVDV. This virus may use proteolytic event of the autophagy machinery to facilitate the expression of viral protein and enhance its own replication (Fricke et al., 2004) (Fig. 1D).

Different from BVDV, Human immunodeficiency virus type1(HIV-1) which is the pathogen of AIDS uses more powerful strategies to employ autophagy. HIV-1 express envelope glycoproteins (Env) in infected cells, and then the Env bind to CXCR4 on the surface of uninfected cells to induce autophagy and accumulate the Beclin 1. This Env-mediated autophagy is required to trigger the apoptosis of bystander $CD4^+$ T cell since blockade of autophagy at different steps, by either drugs or siRNAs specific for Beclin 1 and Atg7 genes totally inhibited the apoptotic process (Espert et al., 2006; Denizot et al., 2008). But what's the benefit of inducing bystander $CD4^+$ T cells death? Obviously, HIV-1 killed their enemy cells to pave their way. The power of HIV-1 is not limited to kill bystander $CD4^+$ T cells; recently, it was found that cellular autophagy can be controlled by HIV-1 to promote its yields in infected cells. First, HIV Gag-derived proteins colocalized and interacted with the autophagy factor LC3, and autophagy is able to promote productive Gag processing. Secondly, to avoid degradation in maturation stages of autophagy, HIV-1 protein Nef acts as an anti-autophagic maturation factor through interactions with the autophagy regulatory factor Beclin 1, thus protecting HIV from degradation (Kyei et al., 2009). In conclusion, HIV-1 evolved diverse strategies to support its infection and replication by taking over autophagy: first, the protein Env leads to the apoptosis of bystander $CD4^+$ T cells via autophagy pathway, and then HIV-1 can enhance viral yields by using the early stages while inhibiting the late stages of autophagy (Fig. 1A and D).

7 Conclusions

The relationship between autophagy and virus attracts more and more attention after its finding. First, autophagy was identified to be involved in the infection of many common viruses, such as HIV, IAV and HBV. Some of these viruses can inhibit autophagy to evade the clearance of host immune system, and others are able to take over certain autophagic steps, thus contributing to viral infection and replication. Secondly, autophagy interacts with both adaptive and innate immunity. Here, we would like to ask: if the similar viruses have a common cross-talking with autophagy? Could we find this common relation to support potential antiviral therapy? Is there any relationship between autophagy and the carcinogenicity of certain virus? Understanding how autophagy defends against viral infection and how virus infection stimulates autophagy-related cellular pathways for its own replication is a challenge and opportunity for the future. To uncover these mechanisms may open up great potential of autophagy for vaccines and antiviral drugs development.

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