Virology 479-480 (2015) 450–456

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Viruses and the autophagy pathway

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ARTICLE INFO

Article history:
Received 13 January 2015
Returned to author for revisions
30 January 2015
Accepted 12 March 2015
Available online 6 April 2015

Keywords:
Autophagy
Virus
Amphisome
Endosome
Beclin 1
IFN
SNARE
AWOL

ABSTRACT

Studies of the cellular autophagy pathway have exploded over the past twenty years. Now appreciated as a constitutive degradative mechanism that promotes cellular homeostasis, autophagy is also required for a variety of developmental processes, cellular stress responses, and immune pathways. Autophagy certainly acts as both an anti-viral and pro-viral pathway, and the roles of autophagy depend on the virus, the cell type, and the cellular environment. The goal of this review is to summarize, in brief, what we know so far about the relationship between autophagy and viruses, particularly for those who are not familiar with the field. With a massive amount of relevant published data, it is simply not possible to be comprehensive, or to provide a complete "parade of viruses", and apologies are offered to researchers whose work is not described herein. Rather, this review is organized around general themes regarding the relationship between autophagy and animal viruses.

Contents

Observation of autophagosomes during infection

In 1965, George Palade"s group published electron microscopy images of poliovirus-infected HeLa cells (Dales et al., 1965). Late in infection, they identified vesicles with two lipid bilayers. Between the bilayers was an electron-light lumen, and the region within the inner bilayer appeared to be cytosol-like. Dales and Palade observed apparent virions near, and often within, these vesicles. The authors suggested these resembled "autolytic vesicles," which "appear to represent a secondary response to infection." Similar vesicles, termed "compound membrane vesicles," were later identified in images of Coxsackievirus-infected mouse pancreata, indicating that these structures were present during infection of multiple picornaviruses (Burch and Harb, 1979; Harb and Burch, 1975).

These unique, double-membraned, "autolytic vesicles" are now better known as autophagosomes, the hallmark organelles of the cellular autophagy pathway (Carlsson and Simonsen, 2015). Autophagy is a process of cellular homeostasis and stress response, in which

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http://dx.doi.org/10.1016/j.virol.2015.03.042
0042-6822/

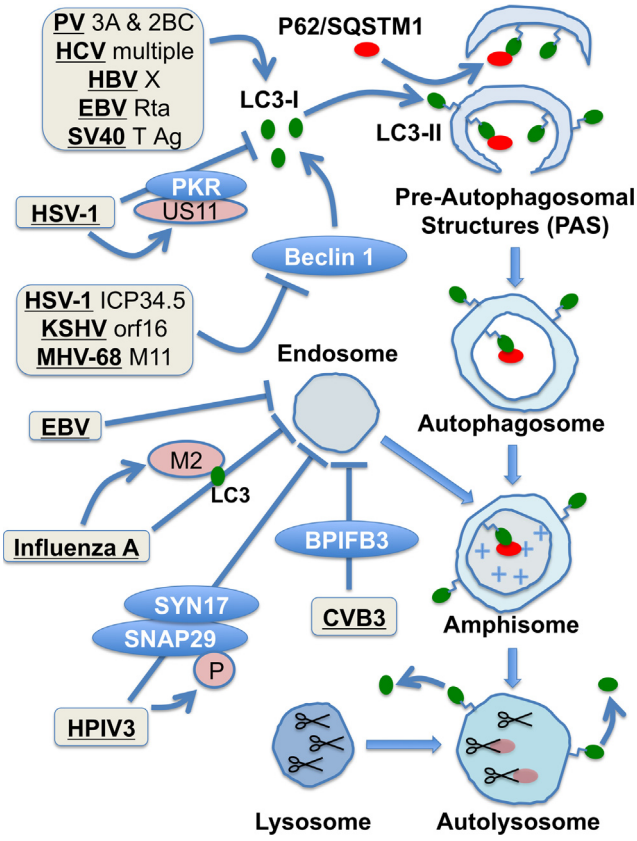

Fig. 1. Viral regulation of the autophagic pathway. Autophagy is initiated when a signal is sent, through the Beclin 1 complex and other signaling complexes, to convert the cellular LC3 protein from its non-lipidated LC3-I form to phosphotidylamine-conjugated LC3-II. The newly lipidated LC3-II localizes to cup-shaped Pre-Autophagosomal Structures (PAS), and is required for the PAS to self-fuse into double-membraned autophagosomes with cytosolic contents. Autophagosomes fuse with endosomes, which provide vacuolar ATPases and promote acidification of the newly formed vesicle, termed an amphisome. Acidic amphisomes fuse with lysosomes, delivering proteases for degradation of internal contents. LC3-II is recycled to LC3-I, so it is difficult to monitor flux through the pathway using LC3 lipidation. The autophagic cargo adapter p62/SQSTM1 directly interacts with LC3, which directs its localization to PAS. LC3-I and LC3-II can be distinguished by western blot, but the recycling pathway makes it difficult to use LC3-II levels to monitor autophagy. However, lower steady state levels of p62 indicate higher levels of active autophagic degradation. Many of the known virus-encoded activators of autophagic signaling, and in some cases active autophagy, are listed, although the mechanisms of activation are poorly understood. Many other viruses induce autophagic signaling, but the specific proteins which provide the signal are unknown. Several viruses are known to inhibit either autophagic signaling, or autophagic maturation, as shown. HSV-1 US11 protein inhibits autophagic signaling through inhibition of PKR. HSV-1 ICP34.5, KSHV orf16, and MHV-68 M11 inhibit autophagosome formation by binding to the Beclin 1 autophagy signaling molecule. Influenza A, CVB3, HPIV3, and EBV induce autophagosomes, but block autophagosome maturation and autophagic degradation. Influenza M2 protein binds to LC3 to inhibit amphisome formation. The HPIV3-encoded P protein binds to SNAP29, inhibiting endosome–autophagosome fusion. In the case of CVB3, the virus requires host BPIFB3 to inhibit vesicle maturation.

cytosolic cargo is engulfed by forming autophagosomes and degraded when these vesicles fuse with lysosomes (Fig. 1). Autophagy is a constitutive pathway, but the amount of degradation varies by cell type, availability of nutrients, environment, or other cellular stresses, including infection (Galluzzi et al., 2014). Autophagy itself provides a survival mechanism during cell starvation, and plays crucial roles in development, post-natal survival, and the immune response (Choi et al., 2013). Examination of the immune response led to early studies genetically linking the autophagic pathway and the cellular response to virus infection.

Autophagy as an anti-viral response

The autophagy field came to the spotlight with identification of mutant strains of *Saccharomyces cerevisae* which are sensitive to starvation (Harding et al., 1995; Tsukada and Ohsumi, 1993). The comprehensive set of genes identified in these studies moved autophagy from a largely descriptive field to one with the tools to dissect the genetics and molecular biology of the pathway. While the primary sequence of autophagy genes rarely suggested a cellular function, mammalian homologues began to be identified. In one case, a binding partner of the anti-apoptotic regulatory protein Bcl-2, Beclin 1, was identified as a homologue of the gene now known as yeast *ATG6* (Liang et al., 1999, 1998). Beclin 1, when expressed from a recombinant Sindbis Virus, reduced virus load and protected mice from fatal encephalitis.

Subsequent work extended the idea of Beclin 1 as a main regulator of an anti-viral response. Several viruses encode a Beclin 1-binding protein, such as a Herpes Simplex Virus 1 protein, ICP34.5, and viruses lacking this domain are less pathogenic in a mouse encephalitis model (Orvedahl et al., 2007). ICP34.5 binding to Beclin 1 inhibits the formation of autophagosomes in neurons, suggesting that the virus has evolved to actively inhibit autophagy. Other viral proteins inhibiting through Beclin 1 binding include Bcl-2 homologs, such as the KSHV orf16 protein and the MHV-68 M11 protein (Ku et al., 2008; Su et al., 2014). In addition to ICP34.5's Beclin 1 binding, HSV also encodes the US11 protein, which inhibits autophagy through direct interaction with the PKR kinase, indicating that HSV encodes at least two proteins capable of inhibiting autophagy, which may speak to the importance of autophagy as an antiviral against this particular virus (Lussignol et al., 2013).

The hypothesis that a cellular process dedicated to degrading cytosolic contents would also engulf and destroy pathogens has proven to be true for several pathogens, and the process has been termed "xenophagy" (Paulus and Xavier, 2015). One effector of xenophagy, the STimulator of INterferon Genes (STING), a transmembrane protein, senses dsDNA viruses and targets them for autophagic degradation. (Reviewed in Barber, 2014). Movement of STING itself within a cell is dependent on components of the autophagy machinery (Ishikawa et al., 2009). STING also induces type I IFN, which indicates a role for autophagy in cell-to-cell immune signaling. Degradation of viral antigens by xenophagy can feed into the MHC Class II presentation pathway as well, indicating that xenophagy can play a role in both clearing a cell of pathogens and prolonged presentation of peptides from those pathogens (Paludan et al., 2005).

Autophagy and the immune response

The anti-viral activity of autophagy in systemic immunity depends on the overall environment of the host. Toll-like receptor-dependent autophagy is required for an antiviral response against Rift Valley fever virus, Vesicular Stomatitis Virus, and others (Moy et al., 2014). The machinery of autophagy is required for a successful IFNγ response against murine norovirus (MNV), although this effect does not involve autophagic degradation (Hwang et al., 2012). Anti-viral autophagic states can be induced through cell to cell signaling as well. Evidence suggests that placental trophoblasts protect the fetus from viruses by secreting exosomes, which contain miRNAs from the chromosome 19 cluster that are capable of inducing autophagy in neighboring cells, conferring anti-viral resistance (Delorme-Axford et al., 2013). In this case, autophagy is anti-viral even for viruses which have been shown to be resistant to the anti-viral effects of autophagy,