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Review

Modulation of the autophagy pathway by human tumor viruses

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ABSTRACT

Autophagy is a highly conserved and regulated process in eukaryotic cells by which components of the cytoplasm, such as damaged organelles and foreign pathogens, become enveloped into double-membrane autophagosome vesicles that fuse with the lysosome for degradation. Viruses are adept at subverting host cellular pathways for their replication and survival. The human tumor viruses, Epstein-Barr virus (EBV), Kaposi's Sarcoma-Associated Herpesvirus (KSHV), Hepatitis B virus (HBV), and Hepatitis C virus (HCV), have evolved novel ways of modulating autophagy during productive and latent stages of the virus life cycle. This review will discuss how the autophagy pathway becomes activated upon viral infection and the role of viral proteins in regulating the autophagy pathway. Specifically, we will examine how virus-encoded homologs of autophagy proteins evade autophagy-mediated degradation by blocking the induction, elongation, or maturation steps in the autophagy pathway. We will also discuss how certain viruses enhance autophagy induction or usurp autophagic machinery for their own replication. A comprehensive understanding of the autophagic response to tumor viruses may enable the discovery of novel antiviral and/or anticancer drug therapies.

1. Introduction

Autophagy ("to eat itself") is an evolutionally conserved and highly regulated homeostatic process that involves the envelopment of cytoplasmic components into double-membrane autophagosome vesicles that fuse with the lysosome for degradation during nutrient starvation in a non-specific manner. This intracellular catabolic degradation system is tightly controlled by autophagy-related genes (*Atg*), which can initiate or suppress steps in the autophagy pathway in order to maintain cellular homeostasis. The serine/threonine kinase mammalian target of rapamycin (mTOR) is an important regulator of autophagy. Under normal conditions, mTOR represses autophagy induction by phosphorylating Unc-like kinase 1 and 2 (ULK1/2). Nutrient starvation conditions or treatment with the mTOR inhibitor rapamycin impedes mTOR kinase activity, leading to autophagy initiation and nucleation of a phagophore membrane. During the initiation step of autophagy, Beclin-1 forms a complex with Vps34, a class III phosphoinositide 3-kinase (C3 PI 3-kinase), which contributes to autophagosome nucleation and assembly [1]. During the elongation step of autophagy, light chain 3 (LC3-I) is proteolytically processed and conjugated with a lipidated phosphatidylethanolamine (PE) *via* an ubiquitin-like conjugation system. Lipidated LC3-II can serve as a marker for autophagosome formation since LC3-II is embedded within the lumen of the autophagosome [2]. Autophagosomes subsequently undergo a maturation step by fusion with endosomes or lysosomes. The Beclin-1/Vps34/UVRAG complex positively contributes to autophagosome maturation and endocytic trafficking [3,4]. Rab7 GTPase activity has also been shown to stimulate fusion of autophagosomes with late endosomes/lysosomes. The acidic environment in autolysosomes ultimately degrades the cargo by lysosomal hydrolysis.

Autophagy is an essential part of both the innate and adaptive immune systems. Xenophagy, a form of selective autophagy, specifically recognizes intracellular microbes and physically targets these pathogens to the autophagy pathway for degradation [5]. As a result, some pathogens have evolved multiple strategies to suppress autophagy, whereas others utilize autophagosome membranes and the autophagic machinery to enhance their replication. The gamma-herpesviruses Epstein-Barr virus (EBV) and Kaposi's Sarcoma-Associated Herpesvirus (KSHV) are oncogenic viruses that encode viral homologs of cellular autophagy proteins, which negatively regulate autophagy during latency, a stage in the life cycle where no progeny viruses are produced. In contrast, the tumor viruses Hepatitis B virus (HBV) and Hepatitis C virus (HCV) utilize components of the autophagy pathway to promote productive lytic replication. In this review we will focus on the function of autophagy as an intracellular defense mechanism and examine how human tumor viruses subvert multiple steps in the autophagy pathway to facilitate viral propagation and evade immune detection.

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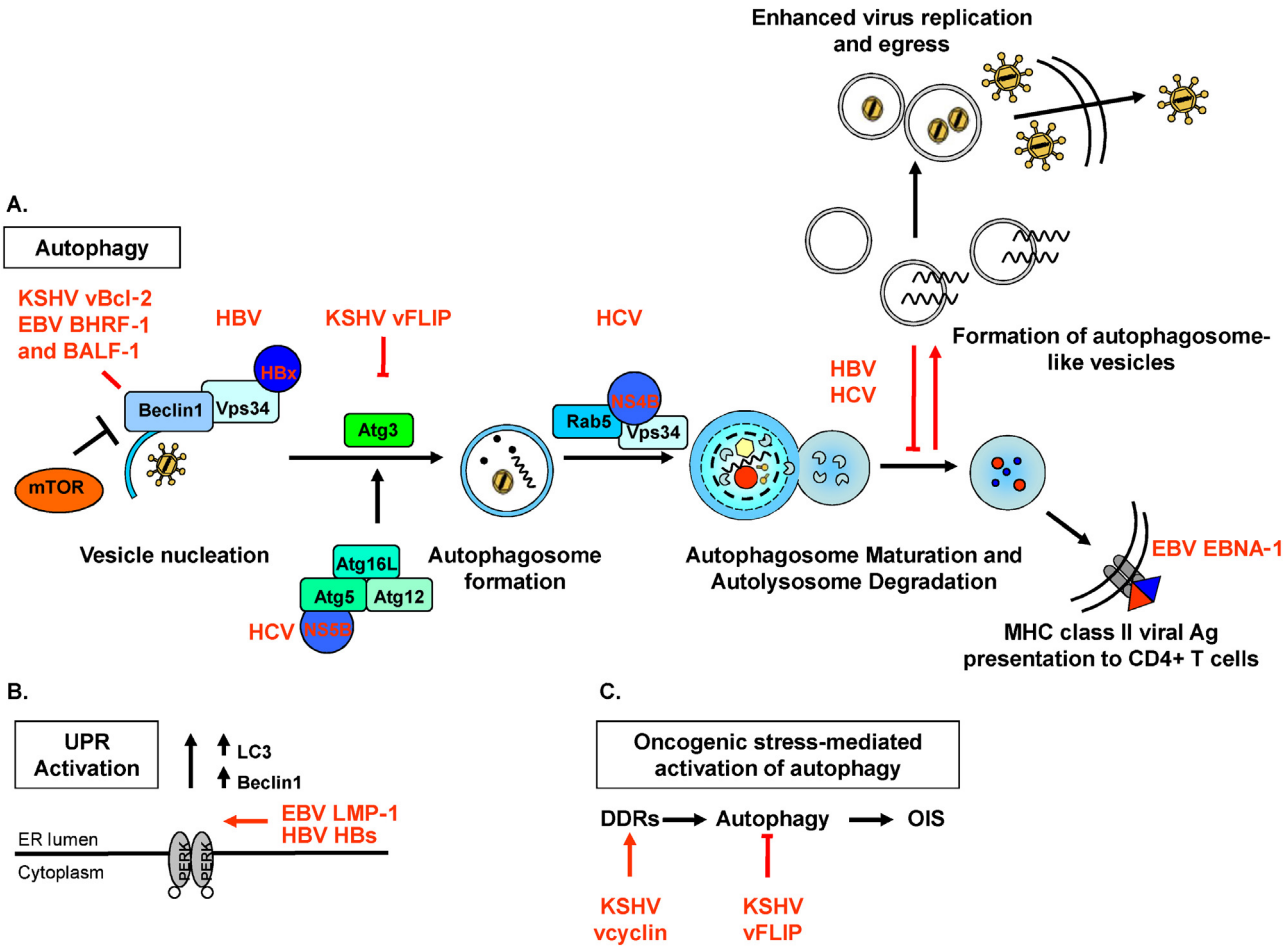

Fig. 1. Modulation of the host autophagy pathway by human tumor viruses. The autophagy pathway functions as an intrinsic antiviral defense mechanism by directly engulfing foreign pathogens for lysosomal mediated degradation. The human tumor viruses Epstein-Barr virus (EBV), Kaposi's Sarcoma-Associated Herpesvirus (KSHV), Hepatitis C virus (HCV), and Hepatitis B virus (HBV) have evolved multiple strategies to either subvert or enhance steps in the autophagy pathway for their replication and pathogenesis. (A) Upon autophagy initiation, Beclin-1 forms an activating complex with the class III PI3 kinase Vps34 for vesicle nucleation. The gamma-herpesviruses counteract autophagy initiation by encoding viral Bcl-2 homologs, which strongly bind to Beclin-1 to function as a repressor complex of vesicle nucleation. In contrast to gamma-herpesviruses, the HBV protein HBx stimulates autophagy induction by binding to Vps34. During vesicle elongation and autophagosome formation, LC3 undergoes lipid conjugation *via* an ubiquitin-like system. KSHV vFLIP blocks autophagosome formation by binding to Atg3, thereby preventing LC3 processing. HCV NS5B promotes autophagosome formation by enhancing LC3 lipid conjugation *via* its interaction with Atg5. HCV NS4B has been shown to complex with Vps34 and Rab5, but the mechanism of how HCV prevents the maturation of autophagosomes into autolysosomes remains unclear. Degradation of viral antigens in autolysosomes can be presented onto MHC molecules, such as the EBV EBNA1 epitope. (B) The unfolded protein response (UPR) pathway, which is commonly activated during viral replication (EBV LMP1 and HBV HBs), can indirectly contribute to autophagy activation. Phosphorylation of the protein kinase PERK leads to downstream transcription of LC3 and Beclin-1 *via* ATF4 translation, thereby contributing to autophagy induction. (C) Acute oncogenic stress activates DNA damage responses (DDRs), ultimately triggering autophagy and oncogene-induced senescence (OIS). Expression of KSHV v-cyclin activates an oncogenic stress response and DDRs. KSHV-infected cells remain refractive to OIS by vFLIP-mediated subversion of autophagy.

2. Activation of autophagy as a host response to viral infection

2.1. Induction of autophagy by cellular stress responses

Environmental stress responses, such as the accumulation of protein aggregates, damaged organelles, or intracellular bacteria and viruses, can induce selective autophagy. During productive viral replication, robust viral protein synthesis contributes to ER stress and activation of the unfolded protein response (UPR) pathway. UPR is characterized by the induction of the ER stress sensors ATF6, IRE1α, and PERK as an attempt by stressed cells to regain homeostasis [6]. ER stress and UPR upregulation ultimately activate the autophagy pathway and autophagosome formation [7]. Both DNA and RNA viruses can indirectly trigger autophagy *via* UPR activation (Fig. 1B). The DNA virus EBV encodes the oncogene latent membrane protein 1 (LMP1), which is required for proliferation in infected B cells. The six-transmembrane spanning domains (6TM) of LMP1 activate PERK, leading to UPR-mediated autophagy induction [8,9]. LMP1-induced autophagic degradation may serve as a mechanism to limit LMP1 accumulation in EBV-infected B cells. Expression of the small surface protein (HBs) of HBV can also induce the UPR pathway, leading to autophagy induction [10]. The positive-stranded RNA virus HCV induces the ER stress and UPR pathway, followed by autophagosome formation and accumulation [11]. HCV stimulates an incomplete autophagic response by inducing UPR-dependent autophagosome accumulation and suppression of autophagosome fusion with lysosomes [11]. Inhibition of ER stress by siRNA knockdown of ATF6, IRE1, or PERK suppressed LC3 lipidation, a marker of autophagosome formation, during HCV infection. The UPR-autophagy pathway positively contributes to HCV replication since siRNA knockdown of Atg5 or CHOP in human hepatoma Huh7 cells inhibited early-stage synthesis of incoming HCV RNA [12]. Thus, HCV might usurp the UPR-autophagy pathway to use it as a mechanism of enhancing HCV RNA replication while inhibiting