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Interaction between Autophagy and EBV: Impacts on the Autophagic Pathway and EBV Infection

Man Wang[1]* and **Shuai Jiang**[2]

[1]Institute for Translational Medicine, Medical College of Qingdao University, Qingdao, 266021, China
[2]State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, 430072, China

***Corresponding Author**: Man Wang, Institute for Translational Medicine, Medical College of Qingdao University, Qingdao, 266021, China, Tel: +86-532-82991791, E-mail: wangman@qdu.edu.cn

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Abstract

Autophagy is known as an evolutionarily conserved cellular degradation pathway for the recycling of cytoplasmic organelles and proteins. The autophagic pathway is also stimulated by various stresses, such as starvation, oxidative stress and pathogen infection. Autophagy serves to a cellular defense mechanism against pathogens. Epstein-Barr Virus (EBV) is an oncogenic virus, associated with various malignancies of epithelial and lymphoid origin, in which the majority of cells harbor a latent infection *in vivo*. Increasing evidence has shown that the host cells try to control EBV infection by activating the autophagic machinery. EBV has evolved novel ways of modulating autophagy during latent and lytic phases of the virus life cycle. In this review, we present an overview of recent advances in our understanding of the complex interplay between autophagy and EBV. The comprehensive understanding of the interactions between autophagy and EBV may lead to novel therapeutic strategies against EBV-related malignancies.

Keywords: Autophagy; Cellular defense mechanism; EBV; Novel therapeutic strategies; EBV-related malignancies

1. Introduction

Autophagy, or 'self-eating', is an evolutionarily conserved process involving the degradation and recycling of cytoplasmic macromolecules. Autophagy is essential for maintenance of homeostasis in response to various forms of stresses, such as starvation, low energy levels, hypoxia and oxidative stress [1, 2]. Under these conditions, the activation of autophagy enables the cell to survive by providing amino acids and energy, by degrading the damaged organelles and proteins. Autophagy also plays an important role in the host immune responses to pathogens [3]. During autophagy, cytoplasmic components including damaged organelles are sequestered inside a membrane, called a phagophore or isolation membrane, that expands to form a double-membrane vesicle termed autophagosome. The autophagosome subsequently undergoes fusion with a late endosome or lysosome, to form an autolysosome, in which the sequestered components are degraded [4, 5]. Degradation of the sequestered components usually generates amino acids, nucleotides and free fatty acids that can be recycled for synthesis of macromolecules and ATPs. The origin of the autophagosomal membrane is still a question of debate. However, there is growing evidence that the Endoplasmic Reticulum (ER) provides lipids for growing autophagosomes [6, 7]. So far, over 30 autophagy-related (*ATG*) genes have been identified in yeast, and most of these genes have orthologs in higher eukaryotes [8]. Among them, 18 *ATG* genes were involved in the formation of autophagosomes [9, 10].

Epstein-Barr virus (EBV) is a common human herpes virus that infects over 90% of the world's population [11]. EBV is associated with several human malignancies such as nasopharyngeal carcinoma (NPC), gastric carcinoma (GC), Burkitt's lymphoma (BL), Hodgkin lymphoma (HL) and post-transplant lymphoproliferative disorder (PTLD) [12]. Like all herpes viruses, EBV has latent and lytic phases in its life cycle, the former maintaining the virus long-term in the host and the latter effecting viral production and spread [13]. As obligatory intracellular pathogens, virus survival is intimately linked to their ability to not only make use of host cell functions for their benefits but also to regulate cellular processes that prevent their replication. This review concentrates on recent major advances in our understanding of the complex interaction between autophagy and EBV.

2. Inhibition of Autophagy Contributes to EBV Evading Immune Responses

Autophagy provides a route for antigens to enter antigen-processing pathways within virus-infected cells, allowing them to be processed and presented to antigen-specific T cells. Epstein-Barr nuclear antigen 1 (EBNA1) is the only viral protein expressed in all EBV latency states and all types of EBV-induced malignancies [14]. EBNA1 is required for the persistence of EBV genomes during its latent phase [15]. As an endogenous antigen, EBNA1 was expected to be presented onto MHC class I after proteasomal processing. Rather, EBNA1 inhibits MHC class I-mediated antigen presentation through blocking proteasomal degradation [16]. EBNA1 is targeted to MHC class II compartments via autophagic uptake and fusion of autophagosomes with these compartments [17]. Previous studies indicated that EBNA1 was found in double membrane vesicles, consistent with its localization to autophagosomes [18, 19]. Inhibition of autolysosomal acidification resulted in the accumulation of EBNA1-positive autophagosomes [20]. Endogenous MHC class II antigen presentation may present an important mechanism to initiate $CD4^+$ T cell responses to pathogens. A number of studies have proven that EBNA1 elicited $CD4^+$ T cell responses after MHC class II processing [21 - 23]. MHC class II-restricted EBNA1 recognition by $CD4^+$ T cells was inhibited after suppressing the expression of the essential autophagy genes, *ATG7* and *ATG12* [18, 20]. The MHC class II-mediated presentation of EBNA1 by autophagy suggested that this process might be involved in the adaptive immune response to viruses [20]. However, EBNA1 is usually poorly presented on MHC class II molecules, contributing to the development of several EBV-positive malignancies [24, 25]. The inhibition of autophagy by EBV may limit adaptive immune response and thus help the virus evade the immune response of the host.

EBV encodes two proteins, *Bam*HI rightward fragment 1 (BHRF1) and *Bam*H1 A leftward fragment 1 (BALF1), which are orthologs of the cellular antiapoptotic protein, Bcl2 [26]. Kaposi's Sarcoma Herpes virus (KSHV)-encoded Bcl2 ortholog was able to inhibit Beclin1-dependent autophagy [27]. EBV-encoded orthologs of Bcl2, BHRF1 and BALF1, might also negatively regulate autophagy by binding to Beclin1. The potential inhibition of autophagy would not only block the presentation of EBNA1-derived peptides by MHC class II but also prevent other contributions to autophagy-mediated immune response.

3. EBV-Encoded Antigens Induce Autophagy

Several EBV-encoded antigens have been reported to induce the autophagic response in host cells. During its latent phase, EBV latent membrane protein 1 (LMP1) induced autophagy through its membrane-spanning domains in a dose-dependent manner [28]. Cells expressing low levels of LMP1 exhibited early stages of autophagy, autophagosomes. In contrast, cells expressing high levels of LMP1 exhibited late stages of autophagy, autolysosomes. Inhibition of autophagy in EBV-positive cells led to an accumulation of LMP1, indicating that LMP1 regulated autophagy to control its own degradation. LMP1 auto catalyzed its degradation to avoid immune detection and to relive the cytostasis it triggered [28]. It has been recently reported that EBV latent membrane protein 2A (LMP2A) promoted autophagosome formation and expression of proteins in the autophagosome pathway [29]. During the lytic phase, EBV immediate early product Rta enhanced autophagy, and ATG5, a key component of autophagy, mediated Rta-induced autophagic process in transfected 293T cells [30]. The expression of Rta increased the transcription of the genes that participate in the formation of autophagosomes, including *ATG9B*, *LC3A* and *LC3B*, as well as those that are involved in the regulation of autophagy such as *TNF*, *IRGM*, and *TRAIL*. Moreover, Rta induced autophagy via the ERK signaling pathway to promote viral lytic development.

4. EBV Manipulates Autophagy to Promote Viral Production

EBV has learned how to manipulate the autophagic machinery for its replication. The previous study showed that autophagy was activated and played an important role during the reactivation of EBV from latency in several EBV-positive cells [31]. The early autophagic phases could promote the expression of EBV lytic genes and viral production, indicating that the autophagic activation was instrumental for the early phases of EBV replication. However, the autophagic flux was blocked at the final steps, and the block occurred only when the complete set of EBV lytic genes was expressed. This strategy might allow EBV to hijack the autophagic vesicles for its intracellular transportation and viral production. EBV could also avoid its own degradation into the lysosomes by blocking the degradative phase of autophagy. The possible mechanism underlying the autophagic block during EBV lytic phase could be the down regulation of the essential protein for the autophagosome maturation, Rab7 [32, 33].

More recently, the role of autophagy during EBV lytic phase has been investigated in cellular models allowing the complete or partial expression of EBV genome. De Leo et al. [34] reported that inhibition of autophagy in the early or late phase of the process could largely promote EBV transcription and replication. EBV reactivation transiently stimulated autophagy. The stimulation of autophagy possibly represented a cellular response to viral reactivation rather than being enhanced by the virus to favor its early lytic infection. However, early in the lytic phase of infection, EBV was capable of counteracting autophagy. The molecular mechanisms underlying EBV-mediated suppression of autophagic process remained to be further elucidated. These results were contrary to the previous study [31], and the discrepancy might be due to the different strategies applied to block autophagy, the time frame of experiments or cell type specificity. Recent findings suggested that EBV could subvert autophagy and recruit autophagosomal membranes for efficient envelope acquisition during its productive infection [35]. Inhibition of autophagic membrane formation blocked the production of infectious particles and led to the accumulation of viral DNA in the cytosol. Conversely, stimulation of autophagic membrane formation enhanced viral production. Moreover, the lipidated form of cellular LC3, a key autophagy marker, was identified in EBV particles, demonstrating that autophagosomal membranes were incorporated into the viral envelopes.

Conclusion

Autophagy is an evolutionarily conserved and important homeostatic process for the degradation of cytoplasmic components. In this review, we have highlighted recent studies on the interplay between autophagy and EBV. EBV encodes viral effector proteins to evade the autophagy-associated immune response. EBV-encoded antigens can induce the autophagic response. EBV has evolved a variety of strategies to modulate the autophagic machinery for viral replication. Given its role in the life cycle of EBV, autophagy may be a potential target for the anti-viral therapy. However, the precise mechanisms of how EBV regulates the autophagic machinery during its infection still remain elusive. Therefore, elucidating the interaction between autophagy and EBV will lead to discovery of novel drug targets for better treatments of EBV-associated cancers.

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References

1. Dreux M, Chisari FV (2010) Viruses and the autophagy machinery. Cell Cycle 9(7):1295-1307.
2. Pratt ZL, Sugden B (2012) How human tumor viruses make use of autophagy. Cells 1(3):617-630.
3. Virgin HW, Levine B (2009) Autophagy genes in immunity. Nat Immunol 10(5):461-470.
4. Mizushima N (2007) Autophagy: process and function. Genes Dev 21(22):2861-2873.
5. Shimizu S, Yoshida T, Tsujioka M, Arakawa S (2014) Autophagic cell death and cancer. Int J Mol Sci 15(2):3145-3153.
6. Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, Griffiths G, Ktistakis NT (2008) Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol 182(4):685-701.
7. Yla-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL (2009) 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy 5(8):1180-1185.
8. Klionsky DJ, Cregg JM, Dunn WA, Jr., Emr SD, Sakai Y, Sandoval IV, Sibirny A, Subramani S, Thumm M, Veenhuis M, Ohsumi Y (2003) A unified nomenclature for yeast autophagy-related genes. Dev Cell 5(4):539-545.
9. Mizushima N, Yoshimori T, Ohsumi Y (2011) The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol 27:107-132.
10. Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 10(7):458-467.
11. Rubicz R, Yolken R, Drigalenko E, Carless MA, Dyer TD, Bauman L, Melton PE, Kent JW, Jr., Harley JB, Curran JE, Johnson MP, Cole SA, Almasy L, Moses EK, Dhurandhar NV, Kraig E, Blangero J, Leach CT, Goring HH (2013) A genome-wide integrative genomic study localizes genetic factors influencing antibodies against Epstein-Barr virus nuclear antigen 1 (EBNA-1). PLoS Genet 9(1):e1003147.
12. Khan G, Hashim MJ (2014) Global burden of deaths from Epstein-Barr virus attributable malignancies 1990-2010. Infect Agent Cancer 9(1):38.
13. Kenney SC, Mertz JE (2014) Regulation of the latent-lytic switch in Epstein-Barr virus. Semin Cancer Biol 26:60-68.
14. Mansouri S, Pan Q, Blencowe BJ, Claycomb JM, Frappier L (2014) Epstein-Barr virus EBNA1 protein regulates viral latency through effects on let-7 microRNA and dicer. J Virol 88(19):11166-11177.

15. Frappier L (2012) The Epstein-Barr Virus EBNA1 Protein. Scientifica (Cairo) 2012:438204.
16. Ning S (2011) Innate immune modulation in EBV infection. Herpesviridae 2(1):1.
17. Leung CS, Taylor GS (2010) Nuclear shelter: the influence of subcellular location on the processing of antigens by macroautophagy. Autophagy 6(4):560-561.
18. Leung CS, Haigh TA, Mackay LK, Rickinson AB, Taylor GS (2010) Nuclear location of an endogenously expressed antigen, EBNA1, restricts access to macroautophagy and the range of CD4 epitope display. Proc Natl Acad Sci U S A 107(5):2165-2170.
19. Nimmerjahn F, Milosevic S, Behrends U, Jaffee EM, Pardoll DM, Bornkamm GW, Mautner J (2003) Major histocompatibility complex class II-restricted presentation of a cytosolic antigen by autophagy. Eur J Immunol 33(5):1250-1259.
20. Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, Munz C (2005) Endogenous MHC class II processing of a viral nuclear antigen after autophagy. Science 307(5709):593-596.
21. Cavignac Y, Esclatine A (2010) Herpesviruses and autophagy: catch me if you can! Viruses 2(1):314-333.
22. Leskowitz RM, Zhou XY, Villinger F, Fogg MH, Kaur A, Lieberman PM, Wang F, Ertl HC (2013) CD4+ and CD8+ T-cell responses to latent antigen EBNA-1 and lytic antigen BZLF-1 during persistent lymphocryptovirus infection of rhesus macaques. J Virol 87(15):8351-8362.
23. Tsang CW, Lin X, Gudgeon NH, Taylor GS, Jia H, Hui EP, Chan AT, Lin CK, Rickinson AB (2006) CD4+ T-cell responses to Epstein-Barr virus nuclear antigen EBNA1 in Chinese populations are highly focused on novel C-terminal domain-derived epitopes. J Virol 80(16):8263-8266.
24. Long HM, Haigh TA, Gudgeon NH, Leen AM, Tsang CW, Brooks J, Landais E, Houssaint E, Lee SP, Rickinson AB, Taylor GS (2005) CD4+ T-cell responses to Epstein-Barr virus (EBV) latent-cycle antigens and the recognition of EBV-transformed lymphoblastoid cell lines. J Virol 79(8):4896-4907.
25. Paludan C, Bickham K, Nikiforow S, Tsang ML, Goodman K, Hanekom WA, Fonteneau JF, Stevanovic S, Munz C (2002) Epstein-Barr nuclear antigen 1-specific CD4(+) Th1 cells kill Burkitt's lymphoma cells. J Immunol 169(3):1593-1603.
26. Coleman CB, McGraw JE, Feldman ER, Roth AN, Keyes LR, Grau KR, Cochran SL, Waldschmidt TJ, Liang C, Forrest JC, Tibbetts SA (2014) A gammaherpesvirus Bcl-2 ortholog blocks B cell receptor-mediated apoptosis and promotes the survival of developing B cells in vivo. PLoS Pathog 10(2):e1003916.
27. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B (2005) Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 122(6):927-939.
28. Lee DY, Sugden B (2008) The latent membrane protein 1 oncogene modifies B-cell physiology by regulating autophagy. Oncogene 27(20):2833-2842.
29. Fotheringham JA, Raab-Traub N (2015) Epstein-Barr virus latent membrane protein 2 induces autophagy to promote abnormal acinus formation. J Virol 89(13):6940-6944.
30. Hung CH, Chen LW, Wang WH, Chang PJ, Chiu YF, Hung CC, Lin YJ, Liou JY, Tsai WJ, Hung CL, Liu ST (2014) Regulation of autophagic activation by Rta of Epstein-Barr virus via the extracellular signal-regulated kinase pathway. J Virol 88(20):12133-12145.
31. Granato M, Santarelli R, Farina A, Gonnella R, Lotti LV, Faggioni A, Cirone M (2014) Epstein-barr virus blocks the autophagic flux and appropriates the autophagic machinery to enhance viral replication. J Virol 88(21):12715-12726.
32. Ganley IG, Wong PM, Gammoh N, Jiang X (2011) Distinct autophagosomal-lysosomal fusion mechanism revealed by thapsigargin-induced autophagy arrest. Mol Cell 42(6):731-743.
33. Hyttinen JM, Niittykoski M, Salminen A, Kaarniranta K (2013) Maturation of autophagosomes and endosomes: a key role for Rab7. Biochim Biophys Acta 1833(3):503-510.
34. De Leo A, Colavita F, Ciccosanti F, Fimia GM, Lieberman PM, Mattia E (2015) Inhibition of autophagy in EBV-positive Burkitt's lymphoma cells enhances EBV lytic genes expression and replication. Cell Death Dis 6:e1876.
35. Nowag H, Guhl B, Thriene K, Romao S, Ziegler U, Dengjel J, Munz C (2014) Macroautophagy Proteins Assist Epstein Barr Virus Production and Get Incorporated Into the Virus Particles. EBioMedicine 1(2-3):116-125.