Autophagy

ISSN: 1554-8627 (Print) 1554-8635 (Online) Journal homepage: https://www.tandfonline.com/loi/kaup20

Delivery of cytoplasmic proteins to autophagosomes

Kazufumi Ohshiro, Suresh K. Rayala, Adel K. El-Naggar & Rakesh Kumar

To cite this article: Kazufumi Ohshiro, Suresh K. Rayala, Adel K. El-Naggar & Rakesh Kumar (2008) Delivery of cytoplasmic proteins to autophagosomes, Autophagy, 4:1, 104-106, DOI: 10.4161/auto.5223

To link to this article: https://doi.org/10.4161/auto.5223

Published online: 09 Nov 2007.

[Autophagy 4:1, 104-106; 1 January 2008]; ©2008 Landes Bioscience

Addendum

Delivery of cytoplasmic proteins to autophagosomes

Kazufumi Ohshiro,[1] Suresh K. Rayala,1 Adel K. El-Naggar[2] and Rakesh Kumar[1,3,*]

[1]Molecular and Cellular Oncology and [2]Pathology; The University of Texas MD Anderson Cancer Center; Houston, Texas USA; [3]Molecular and Cellular Biology; Baylor College of Medicine; Houston, Texas USA

Key words: PELP1, HRS, ERβ, autophagy, selective degradation, resveratrol

Autophagy represents a signaling-dependent regulated process that allows the degradation of some cellular proteins in autophagosomes, and plays a critical role in the management of cellular homeostasis under various stress conditions. In recent years, selective degradation of cytoplasmic proteins during stress has attracted considerable scientific interest. Here we examined the ability of resveratrol to induce autophagy in a variety of human cancer cell lines. We found that resveratrol-induced autophagy is accompanied by colocalization of proline-, glutamic acid-, and leucine-rich protein-1 (PELP1) with the green fluorescent protein-microtubule-associated protein 1 light chain 3 (GFP-LC3) in autophagosomes. In addition, we found that hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), a previously shown PELP1-interacting protein, is co-recruited to autophagosomes in the presence of resveratrol. Although autophagy has been assumed to be a bulk and non-selective degradation process, in recent years, evidence of selective degradation of cytosolic proteins and organelles by autophagy is mounting. These observations suggest that the interaction of the target protein(s) with the delivery protein or proteins such as HRS facilitates the transport of certain cytoplasmic proteins to autophagosomes for their selective degradation, and thus, could influence the cytoplasmic as well as nuclear functions of nuclear receptor coregulators. Since PELP1 and, perhaps, other nuclear receptor coregulators are widely dysregulated in human cancers, these findings highlight the significance of the autophagic selective degradation of PELP1 following resveratrol (or other phytoestrogens) treatment in developing future strategies to use resveratrol under cancer prevention and therapeutic settings.

The formation of autophagosomes is initiated by the formation of double-membrane-bound structures inside an intact cell, upon encounter of environmental stress such as nutrient starvation, pathogen infection or chemical and radiation treatment. In mammalian cells, microtubule-associated protein 1 light chain3 (LC3) is recruited to the membranous structure during the mature process, which includes envelopment and sequestration of cytosolic proteins and organelles such as the Golgi apparatus, mitochondria and endoplasmic reticulum (ER). Subsequently, matured autophagosomes fuse with endosomes and lysosomes, converting into autolysosomes to degrade the captured cytoplasmic components. Although such a degradation process, commonly known as macroautophagy, has been assumed to be bulk and non-selective, accumulating evidence suggests a potential selective degradation of organelles by autophagy.[1,2] For example, peroxisomes that participate in hydrogen peroxide and fatty acid metabolism are proliferated in lower eukaryotes such as *Saccharomyces cerevisiae*, *Hansenula polymorpha* and *Pichia pastoris* and in mammalian cells during growth on fatty acids.[3-5] Alterations in nutrient conditions induce rapid and extensive peroxisome degradation whereas other organelles such as mitochondria, Golgi and cytosolic proteins show much lower levels of degradation, suggesting that the elimination of peroxisomes is selective.[3] The ER is selectively sequestered in autophagosomal structures induced when the unfolded protein response is overwhelmed.[6] Under ER stress conditions, ER clearance is controlled by autophagy, and autophagy-related proteins such as Atg1 and Atg8 are involved in the onset of autophagy and the survival of yeast cells.[7,8] In addition, autophagy may also be involved in the selective degradation of mitochondria[9,10] as suggested by the autophagic degradation of the mitochondrial protein Uth1 in yeast.[9] However, data from electron microscopy studies suggest that mitochondrial sequestration during autophagy is regulated in two temporally distinct manners in both Uth1-dependent and -independent manners.[10] In brief, selective sequestration and degradation of ER, peroxisomes and mitochondrial components plays a pivotal role in the processes important in cellular stress management.[5,8,10]

In recent years, selective degradation of cytoplasmic proteins has attracted considerable scientific interest.[11-14] For example, Onodera and Ohsumi reported[12] the loss of acetaldehyde dehydrogenase in yeast cells and its sequestration into autophagosomes under nitrogen starvation conditions; Yu et al. showed selective degradation of catalase under caspase 8 inhibitor-induced autophagy in a mouse cell line;[13] and Qing et al have linked the induction of autophagy to the suppression of the 90 kDa heat shock protein (Hsp90) by geldanamycin, which in turn allows the proteasome-independent degradation of IκB kinase (IKK).[14]

Proline-, glutamic acid- and leucine-rich protein 1 (PELP1/also known as MNAR) is a novel nuclear receptor coregulator that

*Correspondence to: Rakesh Kumar; Molecular and Cellular Oncology; The University of Texas MD Anderson Cancer Center; Houston, Texas 77030 USA; Email: rkumar@mdanderson.org

Submitted: 10/17/07; Revised: 10/26/07; Accepted: 10/31/07

Previously published online as an *Autophagy* E-publication: www.landesbioscience.com/journals/autophagy/article/5223

Addendum to: Ohshiro K, Rayala SK, Kondo S, Gaur A, Vadlamudi RK, El-Naggar AK, Kumar R. Identifying the Estrogen Receptor Coactivator PELP1 in Autophagosomes. Cancer Res 2007; 67:8164-71.

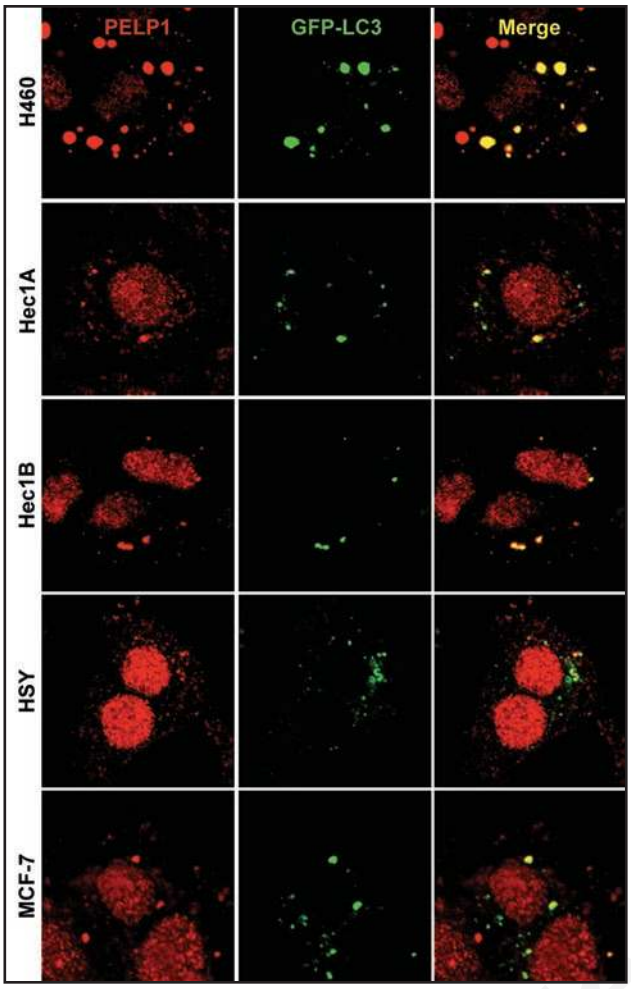

Figure 1. Colocalization of PELP1 and GFP-LC3 in resveratrol-induced autophagosomes in cancer cells. Lung cancer H460 cells, endometrial cancer Hec1A and Hec1B cells, salivary gland carcinoma HSY cells, and breast cancer MCF7 cells were transfected with GFP-LC3, treated with 100 μM resveratrol for 24 hrs, fixed, and immunolabeled with anti-PELP1 antibody. Colocalization of PELP1 and GFP-LC3 was assayed by confocal microscopy.

modulates genomic and nongenomic signaling of the estrogen receptor. Earlier work from Kumar's lab has reported a mechanistic role of an early endosomal protein, the hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), in the focal sequestration of PELP1 in the cytoplasm.[15] To define the precise nature of such a sequestration focal point, Ohshiro et al.[16] use a green fluorescent protein-LC3 (GFP-LC3) chimera and show that PELP1 and GFP-LC3 are co-recruited and degraded in autophagosomes in a variety of cancer cell types including lung, salivary gland, breast, and endometrial cancer cells upon treatment with the phytoestrogen resveratrol (Fig. 1). Since resveratrol also induces the arrest of the cell cycle at the G_1-phase,[16] we have now shown that resveratrol-induced autophagy of the lung cancer H460 cells was accompanied by inhibition of cyclin D1, a key regulator of the G_1 to S-phase transition (Ohshiro K, Kumar R, unpublished observations). Since PELP1 interacts with the retinoblastoma protein (pRb) and its overexpression leads to accumulation of hyperphosphorylated pRb,[17] we also found a reduction in the status of pRB phosphorylation upon resveratrol treatment of H460 cells, suggesting the blockade of pRB inactivation by resveratrol and that PELP1 sequestration into autophagosomes further disrupts the interaction between PELP1 and pRb and causes the inhibition of pRb phosphorylation. Collectively, these findings illustrate an example of a modifying role of autophagy upon the status and functions of the cell cycle machinery.

Selective capture of organelles during autophagy could be promoted by projections of the vacuole membrane, followed by contact and fusion, or engulfment by the vacuole of mitochondria[10] and by the enclosure of ER by autophagosomes in an envelope derived from the ER itself.[6] In addition, the actin cytoskeleton is reported to play an essential role in the elimination of excess peroxisomes by autophagy and the transport of precursor aminopeptidase I in the cytoplasm to vacuole targeting pathway.[18] However, we do not have sufficient understanding of the processes involved in the selective sequestration of non-organelle cytosolic proteins in autophagosomes. Although literature exists about the recruitment to, and roles of autophagy-related proteins at, the autophagosome,[19] it is still unknown exactly how target proteins selectively degradaed or are delivered to autophagosomes. In this context, Ohshiro et al.[16] found that resveratrol stimulation of cancer cells triggers a distinct colocalization of HRS with PELP1 in autophagosomes. Since HRS marks the early endosomes in the cytosol and plays a pivotal role in vesicular trafficking and signaling,[15] the above findings implicate a potential role of HRS in transporting PELP1 to the autophagosomes. Interestingly, in *Drosophila*, HRS controls signaling by sorting and degradation of receptors in a ligand-independent manner.[20] A recent study has found that an ortholog of HRS, CeVPS-27 is required in the endosomal and autophagic pathways and play a crucial role in endocytic trafficking of low-density lipoprotein receptor-related protein 1 in *Caenorhabditis elegans*.[21] Furthermore, at the same time of the publication of our current study,[16] HRS was found to play a crucial role in the maturation of autophagosomes in mammalian cells.[22] This study stems from the idea that phosphatidylinositol 3-phosphate (PI3P) plays crucial roles in endocytic and autophagic membrane trafficking and also binds to HRS via a FYVE domain.[23,24] Further, since class I and class III PI3-kinases inhibit and activate the autophagic pathway, respectively, these kinases are expected to be involved at the initial stages of the pathway.[25] It is possible that PI3P might also interact with HRS during resveratrol-induced autophagy and might participate in the delivery of its interacting protein, PELP1 to endosomes/autophagosomes. However, this has not yet been formally demonstrated. Emerging results illustrate that in addition to cancer, autophagy is likely to be involved in many human diseases and could be exploited for therapeutic gains.[26,27] For example, diseases caused by the accumulation or the aggregation of cytosolic proteins are likely to involve autophagy; α-synuclein accumulates and aggregates in neurons of patients with Parkinson's disease,[11] whereas mutant alpha-1-antitrypsin accumulates in liver cells of patients with liver inflammation and carcinoma.[28] And thus, targeting the activation of the step(s) responsible for selective degradation of target proteins by autophagy may be of therapeutic value. Further, as described above, IKK, which is essential for the activation of the NFκB pathway, is selectively degraded in an autophagic process induced by geldanamycin.[14] It is possible that the combination of geldanamycin and agents targeting the steps involved in the IKK-selective degradation pathway are likely to be of

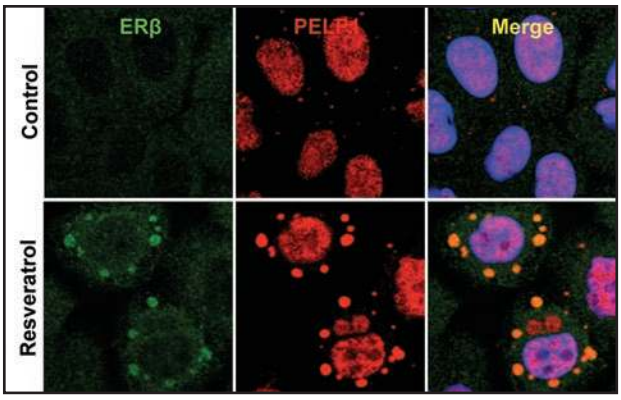

Figure 2. Localization of ERβ to vacuole-like compartments containing PELP1. H460 cells were treated with 100 μM resveratrol for 24 hrs, fixed, and immunolabeled with anti-PELP1 or anti-ERβ antibodies. Colocalization of PELP1 and ERβ was assayed by confocal microscopy.

therapeutic value in tumors driven by the NFκB pathway. In the same vein, since PELP1 is overexpressed in a variety of human tumors,[29] one potential way to inhibit the growth of PELP1-overexpressing estrogen receptor (ER) α-positive cancer cells is to use resveratrol to trigger autophagy in such cells. Furthermore, we have also found that resveratrol also promotes the accumulation of ERβ to vacuole-like structure in H460 cells (Fig. 2). Since PELP1 is known to interact with both ERα and ERβ,[30] it is tempting to speculate that the ERβ might be also recruited by its interaction with PELP1and degraded in autophagosomes under autophagic conditions induced by resveratrol. In brief, the reported autophagic selective degradation of PELP1 following treatment with resveratrol may offer new strategies to use resveratrol for cancer prevention in a therapeutic setting.

Acknowledgments

This study was supported by National Institutes of Health grants CA90970 and CA98823 to R.K.

References

1. Nair U, Klionsky DJ. Molecular mechanisms and regulation of specific and nonspecific autophagy pathways in yeast. J Biol Chem 2005; 280:41785-8.
2. Kim I, Rodriguez-Enriquez S, Lemasters JJ. Selective degradation of mitochondria by mitophagy. Arch Biochem Biophys 2007; 462:245-53.
3. Hutchins MU, Veenhuis M, Klionsky DJ. Peroxisome degradation in *Saccharomyces cerevisiae* is dependent on machinery of macroautophagy and the Cvt pathway. J Cell Sci 1999; 112:4079-87.
4. Kiel JA, Komduur JA, van der Klei IJ, Veenhuis M. Macropexophagy in *Hansenula polymorpha*: Facts and views. FEBS Lett 2003; 549:1-6.
5. Iwata J, Ezaki J, Komatsu M, Yokota S, Ueno T, Tanida I, Chiba T, Tanaka K, Kominami E. Excess peroxisomes are degraded by autophagic machinery in mammals. J Biol Chem 2006; 281:4035-41.
6. Bernales S, McDonald KL, Walter P. Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response. PLoS Bio 2006; 4:e423.
7. Yorimitsu T, Nair U, Yang Z, Klionsky DJ. Endoplasmic reticulum stress triggers autophagy. J Biol Chem 2006; 281:30299-304.
8. Yorimitsu T, Klionsky DJ. Eating the endoplasmic reticulum: Quality control by autophagy. Trends Cell Biol 2007; 17:279-85.
9. Kissova I, Deffieu M, Manon S, Camougrand N. Uth1p is involved in the autophagic degradation of mitochondria. J Biol Chem 2004; 279:39068-74.
10. Kissova I, Salin B, Schaeffer J, Bhatia S, Manon S, Camougrand N. Selective and non-selective autophagic degradation of mitochondria in yeast. Autophagy 2007; 3:329-36.
11. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. α-Synuclein is degraded by both autophagy and the proteasome. J Biol Chem 2003; 278:25009-13.
12. Onodera J, Ohsumi Y. Ald6p is a preferred target for autophagy in yeast, *Saccharomyces cerevisiae*. J Biol Chem 2004; 279:16071-6.
13. Yu L, Wan F, Dutta S, Welsh S, Liu Z, Freundt E, Baehrecke EH, Lenardo M. Autophagic programmed cell death by selective catalase degradation. Proc Natl Acad Sci USA 2006; 103:4952-7.
14. Qing G, Yan P, Xiao G. Hsp90 inhibition results in autophagy-mediated proteasome-independent degradation of IκB kinase (IKK). Cell Res 2006; 16:895-901.
15. Rayala SK, Hollander P, Balasenthil S, Molli PR, Bean AJ, Vadlamudi RK, Wang RA, Kumar R. Hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) interacts with PELP1 and activates MAPK. J Biol Chem 2006; 281:4395-403.
16. Ohshiro K, Rayala SK, Kondo S, Gaur A, Vadlamudi RK, El-Naggar AK, Kumar R. Identifying the estrogen receptor coactivator PELP1 in autophagosomes. Cancer Res 2007; 67:8164-71.
17. Balasenthil S, Vadlamudi RK. Functional interactions between the estrogen receptor coactivator PELP1/MNAR and retinoblastoma protein. J Biol Chem 2003; 278:22119-27.
18. Reggiori F, Monastyrska I, Shintani T, Klionsky DJ. The actin cytoskeleton is required for selective types of autophagy, but not nonspecific autophagy, in the yeast *Saccharomyces cerevisiae*. Mol Biol Cell 2005; 16:5843-56.
19. Xie Z, Klionsky DJ. Autophagosome formation: Core machinery and adaptations. Nat Cell Biol 2007; 9:1102-9.
20. Jekely G, Rorth P. Hrs mediates downregulation of multiple signalling receptors in *Drosophila*. EMBO Rep 2003; 4:1163-8.
21. Roudier N, Lefebvre C, Legouis R. CeVPS-27 is an endosomal protein required for the molting and the endocytic trafficking of the low-density lipoprotein receptor-related protein 1 in *Caenorhabditis elegans*. Traffic 2005; 6:695-705.
22. Tamai K, Tanaka N, Nara A, Yamamoto A, Nakagawa I, Yoshimori T, Ueno Y, Shimosegawa T, Sugamura K. Role of Hrs in maturation of autophagosomes in mammalian cells. Biochem Biophys Res Commun 2007; 360:721-7.
23. Simonsen A, Wurmser AE, Emr SD, Stenmark H. The role of phosphoinositides in membrane transport. Curr Opin Cell Biol 2001; 13:485-92.
24. Raiborg C, Bremnes B, Mehlum A, Gillooly DJ, D'Arrigo A, Stang E, Stenmark H. FYVE and coiled-coil domains determine the specific localisation of Hrs to early endosomes. J Cell Sci 2001; 114:2255-63.
25. Lindmo K, Stenmark H. Regulation of membrane traffic by phosphoinositide 3-kinases. J Cell Sci 2006; 119:605-14.
26. Kondo Y, Kanzawa T, Sawaya R, Kondo S. The role of autophagy in cancer development and response to therapy. Nat Rev Cancer 2005; 5:726-34.
27. Huang J, Klionsky DJ. Autophagy and human disease. Cell Cycle 2007; 6:1837-49.
28. Perlmutter DH. The role of autophagy in alpha-1-antitrypsin deficiency: A specific cellular response in genetic diseases associated with aggregation-prone proteins. Autophagy 2006; 2:258-63.
29. Vadlamudi RK, Kumar R. Functional and biological properties of the nuclear receptor coregulator PELP1/MNAR. Nucl Recept Signal 2007; 5:e004.
30. Bowers JL, Tyulmenkov VV, Jernigan SC, Klinge CM. Resveratrol acts as a mixed agonist/antagonist for estrogen receptors alpha and beta. Endocrinology 2000; 141:3657-67.