Satoshi Waguri

ISOLATION MEMBRANES-ASSOCIATED TUBULES: A KEY ORGANELLE FOR THE FORMATION OF AUTOPHAGOSOME

Department of Anatomy and Histology, Fukushima Medical University School of Medicine, 1 Hikarigaoka, Fukushima, Fukushima 960-1295, Japan

Autophagy is a lysosomal degradation system where an isolation membrane (IM, also known as a phagophore) with double-membrane structure sequestrates a cytoplasmic portion to become an autophagosome, which then fuses with lysosomes in order to degrade its contents. This process is triggered by several stimuli, including nutrient and energy depletion, which generally leads to the suppression of mechanistic target of rapamycin (mTOR) activity. The uncoordinated 51-like kinase 1 (Ulk1) complex is then activated and nucleated as the autophagosome formation site. Atg9-containing vesicles and the class III phosphatidylinositol 3-kinase (PI3K)-complex that produces the phosphatidylinositol 3-phosphate (PI3P)-rich domain are recruited. The PI3P-binding protein the WD-repeat protein interacting with phosphoinositides (WIPIs) and Atg2 then promote the expansion of the IM, which eventually closes to become the autophagosome. The Atg12-5-16L complex is also recruited to the IM, producing the lipidated form of microtubule-associated protein light chain 3 (LC3-II) on the membrane, which is considered to be involved in the closure and fusion of the autophagosome with the lysosome and/or the selective engulfment of large substrates through binding to autophagy receptors (or adaptors) [1].

In the past decade, we and others have suggested that the IM is formed though an intermediate structure adjacent to endoplasmic reticulum (ER) called “omegasome”. To visualize fine structures of omegasome, we developed fixation protocols for correlative light-electron microscopy (CLEM) and electron tomography [2]. These techniques were then applied to mouse embryonic fibroblasts (MEF) expressing a omegasome marker, GFP-tagged double FYVE domain-containing protein 1 (GFP-DFCP1), which is recruited on a PI3P-rich domain. As a result, we observed a cluster of thin tubular structures between the edge of IM and ER profiles. Moreover, part of them were continuous with the IM and/or ER. These IM-associated tubular structures (IMATs) were observed in several cell lines and MEFs deficient for *Atg5*, *Atg7*, or *Atg16L1*, but not in MEFs deficient for a Ulk1 component, *FIP200*. These results suggest that they are relevant to earlier events in autophagosome formation. Taken together, our findings indicate that the IMATs represent a part of omegasome mediating biogenesis of autophagic isolation membranes from the ER [3]. Recently, we are exploring the IMAT in a certain type of mitophagy, which will also be introduced in this talk.

1. Galluzzi L et al., Molecular definitions of autophagy and related processes. ***EMBO J.*** 36:1811-1836 (2017)

2. Arai R, Waguri S. Improved Electron Microscopy Fixation Methods for Tracking Autophagy-Associated Membranes in Cultured Mammalian Cells. ***Methods Mol Biol.*** 1880:211-221 (2019)

3. Uemura T, et al., A cluster of thin tubular structures mediates transformation of the endoplasmic reticulum to autophagic isolation membrane. ***Mol. Cell. Biol***. 34(9):1695-1706 (2014)