Autophagy 8:8, 1267–1268; August 2012; © 2012 Landes Bioscience

Autophagy genes promote apoptotic cell corpse clearance

Wei Zou,[1,*] Xiaochen Wang,[1] Ronald D. Vale[2,3] and Guangshuo Ou[2,3,4]

[1]National Institute of Biological Sciences; Beijing, China; [2]Howard Hughes Medical Institute; Chevy Chase, MD USA; [3]Department of Cellular and Molecular Pharmacology; University of California; San Francisco; San Francisco, CA USA; [4]National Laboratory of Biomacromolecules; Institute of Biophysics; Chinese Academy of Sciences; Beijing, China

Autophagy is a catabolic process through which damaged organelles and protein aggregates are delivered to lysosomes for degradation. Autophagy genes are reported to promote exposure of "eat me" signals on the surface of apoptotic cells, but whether they function in engulfing cells is not clear. Recently, we found that the autophagy mutants *atg-18* and *epg-5* are defective in removing apoptotic cells derived from the *C. elegans* Q neuroblast, a phenotype that can be fully rescued by expression of ATG-18 and EPG-5 in the engulfing cell. Loss of ATG-18 or EPG-5 does not affect cell corpse engulfment but causes defects in phagosomal recruitment of RAB-5 and RAB-7 and formation of phagolysosomes. EPG-5, ATG-18 and LGG-1 are sequentially recruited to phagosomes, suggesting that they function at different steps of phagosomal maturation. Our studies indicate that autophagy genes function sequentially to promote apoptotic cell corpse degradation in the engulfing cell.

Keywords: autophagy gene, apoptotic cell corpse, engulfment, degradation

Submitted: 05/05/12

Revised: 05/15/12

Accepted: 05/16/12

http://dx.doi.org/10.4161/auto.20786

*Correspondence to: Wei Zou;
Email: zouwei@nibs.ac.cn

Punctum to: Li W, Zou W, Yang Y, Chai Y, Chen B, Cheng S, et al. Autophagy genes function sequentially to promote apoptotic cell corpse degradation in the engulfing cell. J Cell Biol 2012; 197:27–35; PMID:22451698; http://dx.doi.org/10.1083/jcb.201111053

Apoptotic cell degradation is important for animal development and tissue homeostasis. Defects in this process lead to inflammation, autoimmune diseases and neurodegenerative diseases. Degradation of apoptotic cells is mediated by fusions between apoptotic cell-containing phagosomes and intracellular organelles including early endosomes, late endosomes and lysosomes. How these processes are regulated is not well understood.

Autophagy is an evolutionarily conserved process through which protein aggregates and damaged organelles are degraded. As in apoptotic cell degradation, autophagosomes fuse with lysosomes to mediate cargo degradation. Previous studies by Dr. Beth Levine's group suggest that autophagy genes act in dying cells to promote apoptotic cell clearance by regulating exposure of engulfment signals, most likely through an energy-related mechanism. Autophagy proteins were recently shown to be recruited to apoptotic or necrotic cell-containing phagosomes or entotic vacuoles that harbor apoptotic cells in cultured mammalian cells, but whether they regulate phagocytic degradation of apoptotic cells in live animals remains unclear.

We asked whether autophagy genes contribute to clearance of apoptotic cell corpses in *C. elegans*. During Q neuroblast development, four Q cells undergo apoptosis and are removed by hyp7 cells. We developed fluorescent markers to label Q cells and monitor their engulfment and degradation in hyp7 cells. We found that apoptotic Q cell corpses were cleared ~2 h after their birth in wild type, but some Q cell corpses persisted in *atg-18* and *epg-5* mutants even 4 h post birth, indicating defective clearance of Q cell corpses. Expression of ATG-18 and EPG-5 controlled by engulfing cell-specific promoters fully rescued the persistent cell corpse phenotype, indicating that autophagy genes function in engulfing cells to promote Q cell corpse clearance.

To determine which step of apoptotic cell clearance was affected by loss of autophagy genes, we performed time-lapse analysis to follow both engulfment and degradation of Q cell corpses. Importantly, we found that engulfment of Q cell corpses was unaffected in *atg-18* and

epg-5 mutants, but their degradation in phagocytes was significantly delayed.

After engulfment, apoptotic cells are enclosed within phagosomes which undergo a maturation process to form phagolysosomes in which cell corpses are degraded. By time-lapse recording, we observed that phagosomal recruitment of RAB-5 and RAB-7, which function at early and late maturation stages, respectively, was significantly delayed in *atg-18* or *epg-5* mutants. Phagolysosome formation was also delayed in these mutants, indicating a role of ATG-18 and EPG-5 in phagosome maturation. We next found that EPG-5 was recruited to phagosomes at a very early stage when the actin halo was forming; ATG-18 was recruited at a similar stage with RAB-5, while LGG-1 was recruited when phagolysosomes were forming. The sequential recruitment of autophagy proteins to phagosomes was directly visualized by our newly developed time-lapse recording system using triple fluorescence proteins. Thus, EPG-5, ATG-18 and LGG-1 may function sequentially to promote apoptotic cell degradation.

How do autophagy genes function to promote apoptotic cell degradation in the engulfing cell? It is possible that they promote biogenesis of autophagosomes and autolysosomes, which may fuse with phagosomes and thus promote apoptotic cell corpse degradation. Alternatively, autophagy genes may function independently of autophagy to promote apoptotic cell corpse degradation. The second hypothesis may be more plausible because some autophagy mutants such as *unc-51/atg-1* and *atg-7*, do not affect degradation of apoptotic Q cell corpses, and ATG-5 and ATG-7 do not appear to associate with phagosomes. The independence of autophagy in apoptotic corpse removal is consistent with two other recent studies from Dr. Michael Overholtzer's group and Dr. Douglas Green's group. They report that the degradation of apoptotic, necrotic or entotic cells requires autophagy proteins, but does not involve the double-membrane structures that are characteristic of autophagosomes.

We provided evidence to show that loss of autophagy genes affects degradation but not engulfment of apoptotic Q cell corpses in *C. elegans*. As null or strong loss-of-function mutations in most autophagy genes cause lethality at larval stages, we mainly examined clearance of Q cell corpses in the two viable mutants, *atg-18* and *epg-5*. Therefore, a systematic analysis of the role of other autophagy gene products in clearance of apoptotic cells derived from different cell lineages is required for understanding how the autophagy pathway or autophagy proteins may be involved in removing apoptotic cell corpses.