ATG4 – More than a Protease?

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Abstract

The ATG4 proteases are key regulators of autophagy. Until recently it was thought their main function was to mediate the processing of ATG8 family members. A new study reveals a role for ATG4's independent of their catalytic activity, and proposes novel functions in mediating lipid transfer and mitophagy.

Main text

Autophagosomes are essential for removal of damaged organelles or protein aggregates from cells. It is thought that the later steps in autophagosome formation involves a series of ubiquitin-like conjugation reactions that result in the covalent attachment of phosphatidylethanolamine to the ATG8 family (LC3/GABARAPs in mammalian cells) which is inserted into the autophagosome membrane. Key regulators of this conjugation reaction are members of the ATG4 family cysteine proteases. In human cells there are 4 ATG4 members (ATG4A, B, C, D) that mediate the C-terminal processing of seven LC3/GABARAP isoforms (LC3A, B, B2 C, GABARAP, GABARAPL1, GABARAPL2) [1]. In the absence of six LC3/GABARAP family members, autophagosomes can form, but are deficient in fusion with the lysosome [2]. Until recently, it was thought that processing of LC3/GABARAPs by all four ATG4 family members is required for efficient autophagy.

In a recent article, Nguyen et al. discover that the ATG4 proteins can mediate the formation of autophagosomes upon induction of mitophagy in the absence of LC3/GABARAPs and independent of their protease activity [3]. First, the authors generated a quadruple knockout cell line of ATG4s and observed that ATG4 proteins, most notably ATG4B is required for mitophagy. Next, in order to investigate the role of ATG4's independent of LC3/GABARAPs, Nguyen et al. combined the quadruple knockout of ATG4s with a hexa-knockout HeLa cell line deficient in LC3A, B, C, GABARAP, GABARAPL1 and GABARAPL2. These cells display severe alterations in phagophore formation. This defect in autophagosome formation can be rescued by re-introduction of all four ATG4s. Strikingly, this defect can also be rescued by catalytically inactive ATG4s, suggesting that the protease activity and ATG4-mediated

processing of LC3/GABARAPs is dispensable for autophagosome formation upon induction of mitophagy. Intriguingly, the authors show that GABARAPL1 processing can occur in the absence of ATG4s, suggesting that additional proteases may exist to mediate this step. Finally, the authors confirm a previous notion that ATG4's can de-conjugate LC3-protein adducts in an autophagy-independent manner [1, 3]

These observations change our current view on the function of ATG4's in autophagy and raise the possibility that the role of ATG4's extends beyond being selective proteases for LC3/GABARAPs. A key question emerging is: how do ATG4s mechanistically promote autophagosome formation in the absence of LC3/GABARAP processing?

To understand this, the authors used proximity labelling to identify novel interaction partners of ATG4's during mitophagy and suggest that these proteins interact with proteins involved in vesicle trafficking and lipid transfer. Prominent among the list of proteins identified close to ATG4A were ARFIP2 (Arfaptin2) and PI4K2A (Phosphatidylinositol 4-kinase type 2-alpha), both of which were previously identified in ATG9A vesicles [4]. Intriguingly, also detected were proteins involved in lipid modification and transfer such as VPS13C, PI3 kinase complexes, as well as regulators of RAB proteins, small GTPases which direct vesicle traffic (DENND4C, RABGAP1, RABGAP1L, TBC1D5, TBC1D15).

ATG9A is a transmembrane protein resident in the Golgi/endosomal system which is mobilized into vesicles to initiate the formation of autophagosome membranes, including those around the mitochondria [5, 6]. Nguyen and colleagues focused in on the surprising possibility that ATG4s may have role in initiating the growth of the autophagosome membrane around the damaged mitochondria. Upon treatment with OA (Oligomycin and Antimycin), inducing PINK-PARKIN dependent mitophagy, ATG9A vesicles could be colocalized at the surface of mitochondria. This localization of ATG9A vesicles, which usually transiently associate with autophagosome initiation sites, was observed when the ULK kinase was inhibited, and dependent on ATG4s. Two factors, ARFIP2, and LRBA (Lipopolysaccharide responsive beige-like anchor protein), reported to be involved in autophagy in B cells [7], were also required for the localization of ATG9A to mitochondria. ARFIP2, required for ATG9A vesicle mobilization during amino acid starvation [4], now is shown to have a role in mitophagy (Figure 1). These results

also support a role for ATG4A through a direct interaction with ATG9A, and independent of its protease activity and ATG8s.

So, what happens without ATG4s during mitophagy? Nguyen and colleagues address this question by visualizing vesicles, membranes, and mitochondria involved in mitophagy using correlative confocal microscopy (CLEM) with high resolution FIB-SEM (Focused Ion Beam-Scanning electron microscopy) and AIVE (AI-directed Voxel Extraction) to extract the 3D tomographic data. OA damaged mitochondria are seen to be partially surrounded by autophagosome membranes which co-localize with ATG2B in cells lacking all ATG8s. ATG2B is a lipid transfer protein which makes contacts with ER membranes to transfer lipids. In cells treated identically but also lacking ATG4s the autophagosome structures are smaller, have a different appearance and less ATG2B. These data suggest a role for ATG4 in expansion of the growing autophagosome membrane during mitophagy, possibly through the ATG2-mediated transfer activity, but also possibly through regulation of vesicle trafficking given the abundance of Rab protein regulators found in the proximity of ATG4s.

This study raises many questions about the canonical role of both ATG8s and ATG4s. Together with the work from this lab in 2016, a picture is emerging that the previously assigned, essential function of both protein families in autophagy and mitophagy may not be complete. Many questions remain unanswered about the relationship between ATG8s, ATG4s, ATG2 and ATG9 vesicles, such as where and when does ATG4 meet ATG9A and what regulates this interaction, and ultimately how do ATG4's mediate these processes independent of their protease function?

Figure 1. A novel role for ATG4 mediated by interactions with ATG9A. ATG4 endoprotease activity (open orange circle) is essential for cleavage of LC3 and GABARAPs (GRP) and lipidation (LC3-II and GRP-II). Independent of its catalytic activity (closed orange circle), Lazarou and colleagues [3] propose ATG4 mediates expansion of the phagophore. An ATG4 interactome reveals previously unknown ATG4 interactors, including ATG9A, ARFIP2, and LRBA, which mediate ATG4's new role in phagophore formation around damaged mitochondria. CLEM and EM tomography support the association of ATG9A vesicles to phagophores surrounding the mitochondria., and the role for ATG4 in the size of the phagophore.

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Figure

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