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A tethering coherent protein in autophagosome maturation

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Autophagy is a cellular pathway that degrades damaged organelles, cytosol and microorganisms, thereby maintaining human health by preventing various diseases including cancers, neurodegenerative disorders and diabetes. In autophagy, autophagosomes carrying cellular cargoes fuse with lysosomes for degradation. The proper autophagosome-lysosome fusion is pivotal for efficient autophagy activity. However, the molecular mechanism that specifically directs the fusion process is not clear. Our study reported that lysosome-localized TECPR1 (TECtonin β-Propeller Repeat containing 1) binds the autophagosome-localized ATG12–ATG5 conjugate and recruits it to autolysosomes. TECPR1 also binds PtdIns3P in an ATG12–ATG5-dependent manner. Consequently, depletion of TECPR1 leads to a severe defect in autophagosome maturation. We propose that the interaction between TECPR1 and ATG12–ATG5 initiates the fusion between the autophagosome and lysosome, and TECPR1 is a TEthering Coherent PRotein in autophagosome maturation.

Keywords: autophagy, TECPR1, ATG12–ATG5, autophagosome maturation

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ATG5, a 32 kDa protein essential for autophagy, is covalently modified by ATG12. The ATG12–ATG5 conjugate interacts non-covalently with ATG16 to form the ATG12–ATG5-ATG16 complex that localizes to autophagosome precursors and plays an essential role in autophagosome formation. Besides ATG16, another ATG12–ATG5T-associated protein, TECPR1, has been discovered recently. TECPR1 was first described as a component of the autophagy interaction network and reported to associate with the ATG12–ATG5-ATG16 complex, CCT chaperonin complex and TRAPP vesicle-tethering complex. However, our immunoprecipitation (IP) result showed that TECPR1 could pull down ATG12–ATG5 but not ATG16, suggesting that TECPR1 and ATG16 form two mutually exclusive protein complexes with ATG12–ATG5. With deletion mutagenesis and co-IPs, we identified a region of TECPR1 spanning from amino acids 566 to 610 as the ATG12–ATG5 interacting region (AIR), which is necessary and sufficient to bind to ATG12–ATG5.

To investigate the subcellular localization of TECPR1, we set up an inducible cell line in which tagged TECPR1 is expressed at a physiological level controlled by doxycycline addition. We found that tagged TECPR1 mainly localizes to lysosomes but not early autophagic membranes. And once autolysosomes are accumulated by treatment with chloroquine (CQ), a lysosomal inhibitor, the colocalization between TECPR1 and LC3 is significantly increased. Using immuno-EM, we observed that EGFP-TECPR1 signals are present on the membrane of the large autolysosomes in CQ-treated cells. These results indicate that TECPR1 resides on the autolysosome membrane. Most importantly, we found that ATG5 is recruited to the autolysosome membrane by TECPR1, which extends the current autophagy model that ATG5 localizes exclusively to phagophores.

We also investigated the function of TECPR1 in autophagy by RNAi knockdown experiments. The autophagy phenotypes we observed in TECPR1-deficient cells suggest a role of TECPR1 in autophagosome maturation. In TECPR1-deficient cells, two well-known autophagic

substrates, the conjugated form of LC3 (LC3-II) and SQSTM1/p62, are both accumulated; autophagosomes are also piled up. The LC3-II augmentation might be caused by either autophagy activation or a maturation block. These two possibilities could be distinguished by an autophagy flux assay because treatment of lysosome inhibitors will further increase LC3-II in the prior case but remain unchanged in the latter case. In TECPR1-deficient cells, the autophagy flux is blocked, suggesting that the autophagosome maturation is likely defective without TECPR1.

LC3 tandemly fused with both GFP and mRFP is often employed to monitor autophagosome maturation. GFP, but not mRFP, is quenched in the acidic environment, and therefore a lack of green fluoresence serves as a marker for mature autophagosomes. Phagophores and autophagosomes decorated by LC3 display both green (GFP) and red (mRFP) fluorescence, while LC3-positive autolysosomes are only labeled with red fluorescence. In TECPR1 wild-type cells, red-only autolysosomes are dramatically increased upon starvation, suggesting an increase of mature autophagosomes. However, in TECPR1-deficient cells, nearly all the autophagic structures are marked by both green and red signals indicating accumulation of phagophores and/or early autophagosomes. These results further confirm that autophagosome maturation is blocked in the absence of TECPR1.

Finally, we found the pleckstrin homology (PH) domain of TECPR1, at the close proximity of AIR, is critical for its autophagic function. This PH domain alone binds to PtdIns3P in vitro. However, the full-length TECPR1 displays no PtdIns3P binding. Upon either binding of ATG12–ATG5 or deletion of AIR, the full-length TECPR1 regains the ability to interact with PtdIns3P. This is probably because the ATG12–ATG5 interaction at the AIR region helps to expose the PH domain of TECPR1, which is otherwise concealed by AIR. Importantly, both full-length and AIR-deleted TECPR1 can rescue those autophagy phenotypes that we observed in TECPR1-deficient cells while PH-deleted TECPR1 could not. These results suggest that the PtdIns3P binding activity of TECPR1 is dependent on the association with ATG12–ATG5 and is essential for its function in autophagosome maturation.

ATG12–ATG5-ATG16 has long been proposed to have a major role in phagophore formation. This complex decorates the outer membrane of the forming autophagosome, where ATG12–ATG5-ATG16 recruits LC3 for its lipid conjugation. Interestingly, our study suggests that, in addition to its function in autophagosome formation, ATG12–ATG5 may also have an important role in autophagosome maturation by forming another complex with TECPR1. Since ATG5 is essential for autophagosome formation, it is very difficult to directly and specifically investigate its requirement for autophagosome maturation through either conventional knockdown or knockout studies. Future experiments should be performed in an in vitro system using purified recombinant proteins to further confirm this notion.

Autophagosome maturation is implicated in a wide spectrum of human diseases. If this process goes wrong, damaged organelles, cytosolic proteins and bacterial pathogens could bypass lysosomal degradation. Our study suggests that TECPR1 offers the fusion specificity between autophagosomes and lysosomes by interacting with ATG12–ATG5 and PtdIns3P. Therefore, TECPR1 is likely an ideal drug target, and dissecting its regulation should provide new insights into the autophagosome maturation field and therapeutic tools for human diseases.

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