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Regulation of lysosomal phosphoinositide balance by INPP5E is essential for autophagosome–lysosome fusion

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ABSTRACT

Macroautophagy (autophagy) is a multistep intracellular degradation system. Autophagosomes form, mature, and ultimately fuse with lysosomes, where their sequestered cargo molecules are digested. In contrast to autophagosome formation, our knowledge of autophagosome-lysosome fusion is limited. In a recent study, we identified a novel regulator of autophagy, INPP5E (inositol polyphosphate-5-phosphatase E), which is essential for autophagosome-lysosome fusion. INPP5E primarily functions in neuronal cells, and knockdown of the corresponding gene causes accumulation of autophagosomes by impairing fusion with lysosomes. Some INPP5E molecules localize at the lysosome, and both lysosomal localization and INPP5E enzymatic activity are crucial for autophagy. In addition, INPP5E decreases PtdIns(3,5)P_2 levels on lysosomes, leading to activation of CTTN (cortactin) and stabilization of actin filaments, which are also essential for autophagosome-lysosome fusion. Mutations in *INPP5E* are causative for Joubert syndrome, a rare brain abnormality, and our results indicate that defects in autophagy play a critical role in pathogenesis.

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Phosphoinositides (PIs) have diverse functions in intracellular membrane trafficking, including autophagy. Phosphorylation at the 3-, 4-, and 5-position of the inositol ring of phosphatidylinositol or PIs generates different varieties of PIs, which have specific functions in cells. One of the best-characterized PIs, phosphatidylinositol 3-phosphate (PtdIns3P), is essential for autophagosome formation. However, it remains unclear whether other PIs are involved in this process, particularly at the stage of autophagosome-lysosome fusion.

To investigate this issue, we performed an siRNA screen focusing on phosphoinositide phosphatases. The screen identified INPP5E as a phosphatase that functions during autophagy. Knockdown of INPP5E causes impairment of autophagic flux, and LC3 and SQSTM1/p62 puncta accumulate to high levels in INPP5E-knockdown neuronal cells, indicating that the enzyme functions not in autophagosome formation but during a later stage of autophagy. In INPP5E-knockdown cells, LC3-II and SQSTM1 are not degraded upon proteinase K treatment, suggesting that the accumulated autophagosomes are properly enclosed. Furthermore, colocalization of LC3 puncta with lysosomes is impaired in INPP5E-knockdown cells. Taken together, these findings indicate that INPP5E is required for autophagosome-lysosome fusion. Exogenous expression of an inactive mutant of INPP5E fails to restore autophagic flux in INPP5E-knockdown cells, suggesting that enzymatic activity is essential for autophagy.

INPP5E is mainly distributed in the cytoplasm, but is also localized on lysosomes. Lysosomal localization is essential for INPP5E function during autophagy: a deletion construct lacking the CAAX domain, which is required for lysosomal localization of INPP5E, does not rescue the autophagic defect in INPP5E-deficient cells. Defects in lysosomal integrity and the endocytic pathway indirectly affect autophagy, making it difficult to dissect the molecular mechanisms underlying autophagosome-lysosome fusion. However, our study clearly demonstrated that knockdown of INPP5E affects neither lysosomal integrity nor fusion between endosomes and lysosomes, further highlighting the specific role of INPP5E during autophagosome-lysosome fusion.

INPP5E dephosphorylates PtdIns(3,5)P_2, PtdIns(4,5)P_2, and PtdIns(3,4,5)P_3 to generate PtdIns3P, PtdIns4P, and PtdIns(3,4)P_2, respectively. To determine the substrate of INPP5E during autophagosome-lysosome fusion, we monitored the expression of 3 protein probes, the ML1N domain of MCOLN1, PLCD1/PLCδ1-PH, and BTK-PH, which bind to PtdIns(3,5)P_2, PtdIns(4,5)P_2, and PtdIns(3,4,5)P_3, respectively. Only ML1N is localized on lysosomes, and knockdown of INPP5E increases the intensity of ML1N-positive puncta colocalized with lysosomes, suggesting that INPP5E dephosphorylates and decreases the level of PtdIns(3,5)P_2 on lysosomes by converting it to PtdIns3P. Consistent with this idea, the level of mCherry-2x FYVE, a well-known PtdIns3P probe that colocalizes with LAMP1-positive lysosomes, is

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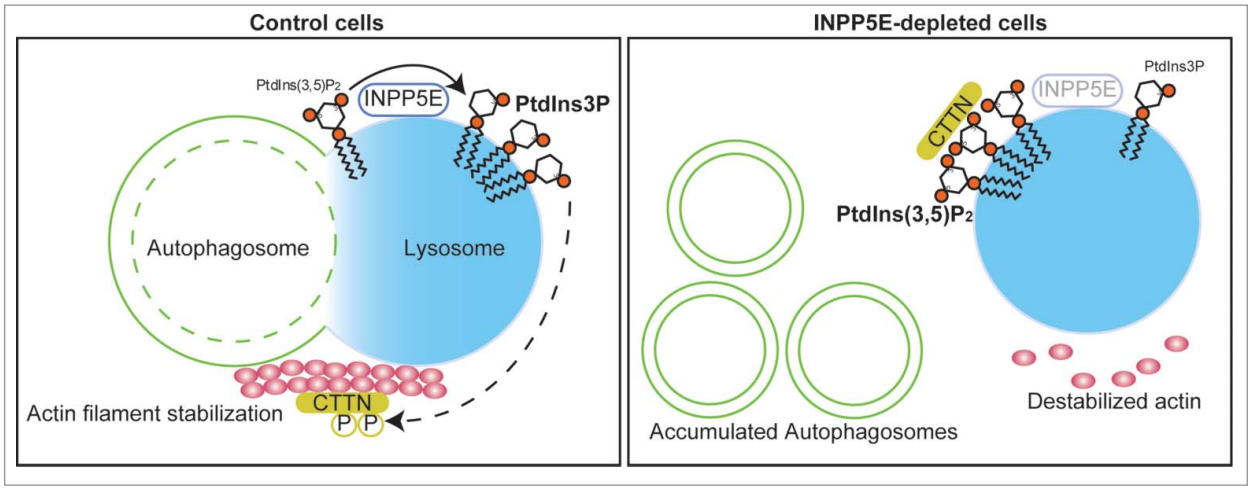

Figure 1. Model of INPP5E-mediated autophagosome-lysosome fusion in neuronal cells. In control cells (left), INPP5E is transiently localized on lysosomes, where it dephosphorylates PtdIns(3,5)P_2. A decrease in PtdIns(3,5)P_2 level and an increase in PtdIns3P level on lysosomes lead to activation of CTTN/cortactin, which can then bind and stabilize actin filaments. The stabilized actin filaments, in turn, facilitate autophagosome-lysosome fusion. In INPP5E-depleted cells (right), CTTN is trapped by elevated levels of PtdIns(3,5)P_2, which accumulates on lysosomes in the absence of INPP5E function, and remains inactive, leading to destabilization of actin filaments. Enclosed autophagosomes thus accumulate in INPP5E-depleted cells.

significantly elevated in INPP5E-depleted cells. By contrast, puncta containing the PtdIns3P effector WIPI2 on LC3-positive autophagosomes are unchanged, indicating that INPP5E is not involved in control of PtdIns3P on autophagosomes. These results strongly suggest that dephosphorylation of PtdIns(3,5)P_2 by INPP5E on lysosomes is required for autophagosome-lysosome fusion. Intriguingly, double knockdown of INPP5E and PIKfyve, a phosphoinositide kinase that converts PtdIns3P to PtdIns(3,5)P_2, abolishes PtdIns(3,5)P_2 on lysosomes, suggesting that the effect of PIKfyve is stronger than that of INPP5E. Double-knockdown cells still accumulate LC3 puncta, consistent with the previous finding that inhibition of PIKfyve causes defects in autophagy, possibly due to loss of lysosomal activity. Together, these results suggest that proper levels of lysosomal PtdIns(3,5)P_2 and PtdIns3P are essential during the late stage of autophagy.

How do the INPP5E-mediated decrease in PtdIns(3,5)P_2 level and increase in PtdIns3P level affect autophagosome-lysosome fusion? Recent work showed that PtdIns(3,5)P_2 controls endosomal actin dynamics by regulating CTTN-actin interactions. In this context, PtdIns(3,5)P_2 competes with actin filaments for binding to CTTN. Thus, reduction of the PtdIns(3,5)P_2 level allows CTTN to bind actin, leading to stabilization of actin filaments on endosomes. Moreover, CTTN knockdown and inhibition of actin polymerization also block autophagosome-lysosome fusion. These observations prompted us to investigate the roles of INPP5E on actin filament stabilization during autophagosome-lysosome fusion. We found that INPP5E knockdown decreases the intensity of F-actin on lysosomes. Phosphorylation of CTTN on Tyr421 and Tyr466, which activates the protein, is impaired in INPP5E-knockdown cells. These results indicate that reduced levels of PtdIns(3,5)P_2 lead to activation of CTTN and actin filament stabilization, thereby facilitating autophagosome-lysosome fusion (Fig. 1).

Mutation in *INPP5E* is one of the causes of Joubert syndrome, a rare brain abnormality. In patient genomes, several causative mutations have been identified in the C-terminal inositol polyphosphate phosphatase catalytic domain. Expression of INPP5E constructs with these mutations fail to fully rescue the defect in autophagic flux in INPP5E-depleted cells, and the degree of rescue conferred by each mutant construct correlates with their enzymatic activities. Thus, our results suggest that a previously unappreciated autophagic defect could underlie this syndrome, suggesting novel therapeutic strategies. Thus, Joubert syndrome can be added to the growing list of autophagy-related genetic diseases. Because the defect occurs at the lysosome, Joubert syndrome should also be considered as one of the many lysosomal diseases.

The results reported here demonstrate that modulation of PIs by INPP5E on the lysosome are critical for autophagosome-lysosome fusion, but does not affect lysosomal integrity or the endocytic pathway in neuronal cells. Our findings provide a conceptual advance not only in the field of autophagy but also in general cell biology: in particular, we have revealed a novel function of PIs in cellular activities. Our study also raises several important questions: Given that INPP5E knockdown in other cell types affects autophagy only mildly, why do neuronal cells depend so heavily on INPP5E for autophagosome-lysosome fusion? How is INPP5E recruited to lysosomes? Do INPP5E and PtdIns(3,5)P_2 also function in selective autophagy? Answering these questions will further contribute to our understanding of the molecular mechanisms of autophagy, including autophagosome-lysosome fusion.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.