Autophagy 8:4, 707–708; April 2012; © 2012 Landes Bioscience

Mammalian PIK3C3/VPS34

The key to autophagic processing in liver and heart

Nadia Jaber,[1] Zhixun Dou,[1] Richard Z. Lin,[2,3] Jianhua Zhang[4,5] and Wei-Xing Zong[1,*]

[1]Department of Molecular Genetics & Microbiology; Stony Brook University; Stony Brook, NY USA; [2]Department of Physiology and Biophysics; Stony Brook University; Stony Brook, NY USA; [3]Northport VA Medical Center; Northport, NY USA; [4]Department of Pathology; University of Alabama; Birmingham, AL USA; [5]Birmingham VA Medical Center; Birmingham, AL USA

© 2012 Landes Bioscience. Do not distribute

PIK3C3/Vps34 is the class III PtdIns3K that is evolutionarily conserved from yeast to mammals. Its central role in mammalian autophagy has been suggested through the use of pharmacological inhibitors and the study of its binding partners. However, the precise role of PIK3C3 in mammals is not clear. Using mouse strains that allow tissue-specific deletion of *PIK3C3*, we have described an essential role of PIK3C3 in regulating autophagy, and liver and heart function.

PIK3C3 belongs to the PtdIns3K family that phosphorylates the 3'-hydroxyl group of phosphatidylinositol and phosphoinositides. The substrate of PIK3C3 is limited to phosphatidylinositol, and thus it produces PtdIns3P. Vps34 was first identified during a yeast genetic screen for vacuolar protein sorting mutants, and its role in endocytic trafficking was subsequently studied.

The involvement of PIK3C3 in autophagy was discovered by using PtdIns3K inhibitors and yeast genetic studies. Pharmacological PtdIns3K inhibitors wortmaninn and 3-methyladenine (3-MA) strongly inhibit autophagy. In yeast, Vps34 is in a complex with Vps15, Vps30/Atg6, and Atg14 that regulates autophagy, and loss of Vps34 leads to defective autophagy. Subsequent studies in mammalian cells showed that BECN1, a mammalian homolog of Vps30, associates with PIK3C3. These pioneer studies suggested a critical role of Vps34/PIK3C3 in regulating autophagy, and provoked the identification of new partners in the Vps34/PIK3C3 complex. In addition, the inhibitory compounds provided a convenient experimental tool to pharmacologically block autophagy.

Despite the emerging knowledge on PIK3C3, little was known about its physiological role and how it precisely regulates autophagy in the mammalian system. Unlike yeast, mammalian cells evolved more sophisticated mechanisms to control autophagy. The widely used PtdIns3K inhibitors lack specificity and cannot precisely interrogate the role of PIK3C3 in this biological process. To address these questions, we have generated a mouse strain where the exon 4 of the *Pik3c3* gene is deleted in the presence of Cre recombinase, resulting in a frame shift and loss of 755 of the 887 amino acids of PIK3C3. Consistent with it being the main producer of PtdIns3P, deletion of *Pik3c3* in MEFs leads to a decrease in PtdIns3P levels, as detected by the disappearance of the punctate distribution of the PtdIns3P-binding domain FYVE conjugated with GFP. *Pik3c3*$^{-/-}$ MEFs accumulate large-sized translucent vacuoles as a result. These vacuoles appear to be single-membraned and lack cargo content. Furthermore, they are decorated with late endosome marker RAB7A and lysosome marker LAMP1, and are acidic. In agreement with the established role of PIK3C3 in endocytosis, endocytic degradation of EGFR is severely blocked in *Pik3c3*$^{-/-}$ cells.

Electron microscopy reveals that the *Pik3c3*$^{-/-}$ MEFs are deficient in autophagosome formation, since double-membraned autophagic vacuoles are not observed in

Keywords: VPS34, PIK3C3, autophagy, endocytosis, PtdIns3K, PtdIns3P, liver, heart, cancer

Submitted: 01/31/12

Revised: 02/06/12

Accepted: 02/06/12

http://dx.doi.org/10.4161/auto.19627

*Correspondence to: Wei-Xing Zong;
Email: wzong@notes.cc.sunysb.edu

Punctum to: Jaber N, Dou Z, Chen JS, Catanzaro J, Jiang YP, Ballou LM, et al. Class III PI3K Vps34 plays an essential role in autophagy and in heart and liver function. Proc Natl Acad Sci USA 2012; 109:2003–8; PMID:22308354; http://dx.doi.org/10.1073/pnas.1112848109

either fed or starved conditions. In agreement with this notion, the formation of GFP-DFCP1 and ATG12 puncta, which are associated with early autophagosomes, is blocked in *PIK3C3*$^{-/-}$ cells. Measurement of long-lived protein degradation and SQSTM1/p62 levels shows that the *Pik3c3*$^{-/-}$ MEFs have impaired autophagy flux. These data strongly imply that *Pik3c3*$^{-/-}$MEFs are deficient in autophagy. When testing the autophagosome marker LC3 (MAP1LC3), however, we find that *Pik3c3*$^{-/-}$cells display elevated LC3-II and large-sized LC3-containing structures even at the nonstarved level. A careful investigation of the LC3-containing cellular structures reveals that they are not bona fide autophagosomes. First, the number and size of these structures do not appear to change in response to starvation or lysosomal inhibitors. Second, the LC3-positive cellular structures are morphologically distinct from starvation-induced LC3 puncta. Third, immunogold labeling of GFP-LC3 reveals that these structures do not associate with any membranes, but are instead protein aggregates. Lastly, the GFP-mCherry-LC3 expressed in *Pik3c3*$^{-/-}$ cells display large yellow aggregates with few red puncta. Taken together, we conclude that the LC3 cellular structures are not autophagosomes, but are formed as a result of severely blocked autophagic degradation.

To observe the effect of *Pik3c3* deletion in vivo, we have generated mice with conditional deletion of *Pik3c3* in the liver and the heart. In both organs, deficient autophagy is observed. The *Pik3c3*$^{-/-}$ liver displays hepatomegaly, steatosis, lack of glycogen storage, and impaired starvation-induced protein degradation, which are highly consistent with the autophagy-deficient *Atg5* and *Atg7* knockout livers. The *Pik3c3*$^{-/-}$ heart shows cardiomegaly, misalignment of mitochondria, swollen endosomes, and is accompanied by contractile dysfunction. In both organs, double-membraned autophagic vacuoles do not form, and levels of LC3-II, SQSTM1, and polyubiquitinated proteins are elevated. These observations are consistent with those we observed in *Pik3c3*$^{-/-}$MEFs. It is noteworthy that *Pik3c3* has been genetically deleted in mouse sensory neurons and T-lymphocytes, albeit with a different genetic deletion strategy. It was found that the mutant neurons and T-cells possess deficient endosomal activity but are capable of autophagy. Our finding that PIK3C3 is essential for autophagy in MEFs, heart, and liver raises a discrepancy between the two models, which could result from the difference in genetic background, manipulation, and/or tissue-specific variations. A detailed comparison of the two models may help to resolve the issue.

While an essential role of Vps34 in autophagy is established in yeast and now as indicated by our findings, in mammals, the detailed mechanisms are still not fully understood. It is not entirely clear how Vps34/PIK3C3 is recruited to, and potentially activated at the precursor autophagosomal membranes. In addition, we know very little about how PtdIns3P regulates autophagosome formation. The study of WIPI family proteins and other PtdIns3P binding proteins will facilitate the understanding of this question, and help to address the recruitment of the ATG12–ATG5-ATG16L1 complex to the precursor membranes. In addition to autophagy, PIK3C3 is also implicated in TOR signaling. We find that while the basal level of TOR remains intact, amino acid-stimulated TOR activation is severely impaired. Mechanistic studies are needed to further clarify how PIK3C3 regulates the TOR pathway. Lastly, our data suggest that systematic and profound inhibition of PIK3C3 may have severe detrimental physiological consequences and therefore, caution should be taken if PIK3C3 inhibitors are to be used clinically.

©2012 Landes Bioscience.
Do not distribute