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Class III PI3K Vps34: essential roles in autophagy, endocytosis, and heart and liver function

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Abstract

Mammalian phosphatidylinositol (PI) 3-kinases are a family of proteins that share the ability to phosphorylate phosphoinositides at the 3 position of the inositol ring. By doing so, these kinases produce phospholipid molecules that are involved in various cell signaling pathways, such as insulin signaling and endocytosis. The pathways regulated by PI3-kinases are crucial for maintaining cellular homeostasis and thus must be tightly regulated. Irregular PI3-kinase activity is observed in numerous human pathological conditions, such as diabetes, cancer, and inflammation. One family member, Vps34, is of particular interest because it is the only PI3-kinase identified in yeast and it has been evolutionarily conserved through mammals. Vps34 plays an essential role in the cellular process of autophagy, a process linked to human health and disease. Understanding the precise role of mammalian Vps34 will likely be integral to drug development for various diseases.

Keywords

Vps34; autophagy; endocytosis; PI3K; homeostasis

Vps34 in yeast

Vps34 was identified by a screen for genes involved in protein sorting to the vacuole in *Saccharomyces cerevisiae*. Subsequent studies revealed that the protein possessed PI3-kinase activity. Initial studies focused on the role of Vps34 in delivery of proteins such as hydrolases to the yeast vacuole, which is analogous to the mammalian lysosome. It was then discovered that yeast Vps34 forms at least two distinct macromolecular complexes, which separately regulate vacuolar protein sorting and macroautophagy.¹ Vps34 is believed to regulate these membrane trafficking events by producing PI(3)P, which recruits downstream effector proteins containing PI(3)P binding domains.

Macroautophagy (also simply referred to as autophagy) is a catabolic cellular process involving the *de novo* formation of an organelle termed the *autophagosome*, which

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Conflicts of interest

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sequesters cytoplasmic material and degrades it via fusion with the vacuole/lysosome. Breakdown products, such as amino acids and lipids, are exported from the lysosome/vacuole into the cytoplasm for metabolic use. Autophagy is constantly maintained at a basal level, whereby it functions as a quality control mechanism. However, it can also be enhanced in response to a number of stress signals, especially nutrient starvation. By degrading bulk cytoplasm or excess cellular material, autophagy provides the cell with required nutrients for survival. In addition, autophagy can be activated in response to hypoxia, DNA damage, and infection.² Because autophagy is essential for maintaining cellular homeostasis, its deregulation is observed in multiple diseases and may even contribute to pathogenesis.³ Autophagy has a tumor suppressive role by maintaining cell health and homeostasis. However, evidence also shows that autophagy aids tumor cell survival following chemotherapy, and current efforts focus on combining traditional chemotherapeutics with autophagy inhibitors.

Vps34 also regulates protein delivery to the vacuole in yeast and may have a role in endocytosis in mammals. Similar to autophagy, endocytosis is a dynamic membrane trafficking pathway that helps maintain cellular homeostasis.⁴ The early endosome serves to receive vesicular cargo from the plasma membrane and Golgi, and to sort the enclosed cargo for recycling or degradation. Once filled with cargo destined for degradation, the early endosome matures into a late endosome that then fuses with a lysosome to facilitate degradation. Vps34 is believed to produce PI(3)P on early endosomes and subsequently recruit downstream effectors that regulate vesicle docking and cargo sorting. Vps34 may also have a role in endosome maturation.

Discovery of a mammalian homolog

Efforts by Volinia *et al.* identified a human homolog of yeast Vps34p, with 58% sequence similarity.⁵ Similar to yeast, mammalian Vps34 utilizes only phosphatidylinositol (PI) as a substrate, thereby producing PI(3)P. Not long after, it was shown that feeding human colon cancer cells synthetic PI(3)P activates autophagy. It was also shown that PI3-kinase inhibitors wortmannin, LY294002, and 3-methyladenine (3-MA) block autophagy in mammalian cells.^{6–8} Thus it seemed likely that Vps34 controlled mammalian autophagy via the production of PI(3)P. Likewise, many studies used these PI3-kinase inhibitors to describe a role for Vps34 at the early endosome.

However, mammals have evolved to encode multiple PI3-kinases with various activities and functions. The PI3-kinase inhibitors wortmannin, LY294002, and 3-MA are not selective for Vps34, and can target these other kinases. Therefore, it is difficult to draw definitive conclusions about the precise role of mammalian Vps34 in autophagy or endocytosis. Moreover, a recent study using a conditional deletion of *Vps34* in mouse sensory neurons found that the autophagic pathway was not disrupted in Vps34-deficient neurons.⁹ These seemingly paradoxical observations prompted us to create our own conditional knockout model to further explore the precise role (or lack thereof) of mammalian Vps34 in autophagy.

The function of mammalian Vps34

Vps34 deletion in vivo

As whole-body knockout of Vps34 is embryonically lethal, we created a conditional knockout mouse using the Cre-lox system; Cre recombinase removes exon 4 of the Vps34 gene causing a frameshift and deletion of the majority of the protein. These animals were crossed with albumin-Cre or muscle creatine kinase (Mck)-Cre mice to delete Vps34 in the liver and heart, respectively (these organs were chosen because autophagy has an integral role in their function). Mice with Vps34-deficient livers developed hepatomegaly and hepatosteatosis, as well as impaired protein turnover (Fig. 1A); similarly, mice with Vps34-deficient hearts developed cardiomegaly and displayed decreased heart contractility (Fig. 1B). Both conditional knockout mice begin dying around eight weeks of age.¹⁰ Further characterization has shown that Vps34-null liver- and heart-specific mice resemble mice deficient in core autophagy proteins Atg5 or Atg7.

Effect on autophagy in vitro

To better understand the precise role of Vps34 in autophagy, we produced mouse embryonic fibroblasts (MEFs) from Vps34^{f/f} mice after addition of adenovirus-containing Cre, which deletes endogenous Vps34. Vps34-null MEFs are devoid of GFPFYVE puncta, an indicator of PI(3)P; we also observed a complete lack of autophagosomes in both untreated and serum-starved Vps34-null cells by electron microscopy (EM) (Fig. 1C); and the autophagic substrate p62 accumulates in Vps34-null cells. Compared with normal punctate structures in wild-type cells, the autophagosome marker LC3 surprisingly forms large aggregates in null cells. By immuno-gold EM, we determined that these LC3 structures are indeed cytoplasmic protein aggregates that are not associated with any membrane structures. These data suggest that autophagosome formation is completely blocked in the absence of Vps34.

Effect on endocytosis in vitro

We briefly explored the effect of Vps34 deletion on the endocytic pathway in our *in vitro* model. We found that endocytic recycling of transferrin, a function of the early endosome, is unaltered in Vps34-null cells. In comparison, endocytic degradation of the epidermal growth factor receptor (EGFR)—a function of the late endosome/lysosome—is severely abrogated. These data challenge the view that Vps34 is required to recruit sorting machinery to the early endosome.

Effect on mTOR signaling in vitro

Although it is known that mTOR is a potent inhibitor of autophagy, evidence also shows that Vps34 is required for the activation of mTOR in response to nutrient stimulation.⁸ To evaluate the capacity for mTOR signaling in Vps34-null MEFs, we have begun studies using phosphorylation of downstream target S6 as a readout to determine whether amino acid-induced mTOR signaling is severely reduced in Vps34-null MEFs; our initial results indicate that it is.

Summary

A member of the PI3-kinase family, Vps34 was originally identified in yeast by a screen for genes involved in protein sorting to the vacuole. It was later discovered that Vps34 also controls autophagy in yeast. The mammalian homolog was identified, and, based on the use of PI3-kinase inhibitors, a suggestion was made that the mammalian homolog also controls endocytic trafficking and autophagy in mammals. However, the PI3-kinase inhibitors currently available are not specific for Vps34, and it is therefore difficult to determine the precise contributions of Vps34 in these pathways. After a recent report that specific deletion of *Vps34* in mouse sensory neurons does not affect autophagy, we were prompted to further investigate the role of mammalian Vps34 in autophagy.

We have produced liver- and heart-specific Vps34 conditional knockout mice. Characterization of the mice thus far shows that Vps34 is essential for normal heart and liver function; additional work *in vitro* shows that autophagosome formation is completely blocked in the absence of Vps34. We have also observed a surprising preservation of early endosome function, and a severe defect in late endosome function in the absence of Vps34. Finally, our work in progress indicates that Vps34 is necessary for amino acid-induced mTOR signaling. From these data we conclude that Vps34 is an important PI3-kinase responsible for autophagy induction in mammals. However, the precise role of Vps34 in the endocytic pathway requires further elucidation, as there are multiple differences between the effects of wortmannin and stable Vps34 deletion.

Vps34 inhibitors are currently under investigation because defects in autophagy have been implicated in the pathogenesis of multiple diseases. However, our work indicates that caution should be taken in these efforts, as systemic and/or complete inhibition of Vps34 will likely lead to severe cellular and organ damage. Rather than global inhibition of Vps34, then, small molecules that block partial functions of Vps34 (i.e., autophagy but not endocytosis) may be a better solution.

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Figure 1.

(A) Wild-type and Vps34^{f/f}-Alb-Cre⁺ mice were fed normally or fasted for 24 hours. Liver to body mass ratio was determined and expressed as normalized to the respective fed animals. (B) Echocardiograms of wild-type and Vps34^{f/f}-Mck-Cre⁺ animals *in vivo* demonstrates an increase in left ventricular wall thickness and mass, and decreased cardiac contractility. (C) Representative EM images of untreated or serum-starved Vps34^{f/f} MEFs infected with empty vector or Cre. Serum starvation induces autophagosome formation in control but not Vps34-null cells. Large, single-membraned empty vacuoles are observed in both untreated and starved Vps34-null cells, likely representing enlarged late endosome/lysosomes. The data in this figure are from Ref. 10.