Unveiling the Secrets of the Ancestral PI3 Kinase Vps34

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Vps34 is the primordial member of the PI3 kinase family involved in vesicular trafficking, nutrient signaling, and autophagy. A report in *Science* unveils the Vps34 structure, providing new insights into the catalytic mechanism, explaining why Vsp34 is so difficult to inhibit, and facilitating design of chemical tools and potential drugs.

The oldest members of the family are not always the easiest to understand and manage. So it is with the phosphatidylinositide 3-kinase (PI3K) brood. In an exciting new paper, Roger Williams and colleagues have provided us with the first molecular comprehension of the behavior of the relatively poorly understood ancestral enzyme, Vps34 (*v*acuolar *p*rotein *s*orting 34), encouraging hope that its activity may be controlled pharmacologically. The studies shed new light on how the whole PI3K dynasty operates and on the interfamilial differences between the members (Miller et al., 2010). There are significant implications for understanding and treating cancer.

PI3Ks are important lipid kinases that catalyze phosphorylation of the 3′-hydroxy position of the inositol ring of phosphatidylinositides, generating products that act as second messengers in the cell. These lipid kinases are grouped into three main classes (Vanhaesebroeck et al., 2001). The class I PI3Ks— comprising the class IA catalytic isoforms p110α, p110β, and p110δ and the class 1B member p110γ— are the most studied. Indeed, the p110α isoform is one of the most frequently mutated kinases in human malignancies (www.sanger.ac.uk/genetics/CGP/cosmic/). p110α and the other class I siblings, as well as the more distant class IV PI3K-related protein kinase relatives or PIKKs (including mTOR), are being actively pursued as cancer therapeutic targets, with drugs now in the clinic (Workman et al., 2010). In contrast, the sole class III ancestral isoform Vps34 and the class II isoforms are much less understood and not so strongly linked to oncogenesis (see Backer, 2008). Moreover, whereas the crystal structures of the α, δ, and γ isoforms have been solved (Walker et al., 1999; Huang et al., 2007; Berndt et al., 2010), facilitating the design of potent and selective inhibitors, this had been lacking for Vps34 before the report by Miller et al.

The gene encoding Vps34 was first cloned by Herm and Emr in 1990 following a *Saccharomyces cerevisiae* screen that revealed its involvement in vesicular trafficking (Backer, 2008). It is expressed ubiquitously in all eukaryotes. Vps34 was subsequently demonstrated to be the primordial PI3 kinase with a particular substrate and product profile, converting phophatidylinositol (PI) to phosphatidylinositol-3-phosphate (PI3P). The PI3P product in turn recruits proteins containing FYVE or PX domains, thereby initiating various complexes at the membranes of endosomes, phagosomes and autophagosomes (Backer, 2008). Autophagy is the critical catabolic lysosomal/vacuolar self-digestion pathway that is activated by nutrient deprivation and other stresses and controls the balance of damage mitigation versus survival promotion (White and DiPaola, 2009). There appears to be a core autophagy-regulating complex containing: (1) Vps34 itself; (2) the N-terminally myristoylated putative kinase Vps15, which stimulates the PI3K activity of Vps34 at membranes; and (3) Beclin 1, which shows allelic loss in some cancers. Recruitment of ATg14L to this core complex is thought to promote induction of the first stages of autophagy, whereas the alternative binding of UVRAG and/or Rubicon to the Beclin 1/Vps34 complex controls the later stages of autophagosome maturation (Funderbunk et al., 2010). Thus, it is clear that the orchestration of the Vps34 PI3K activity by its associating protein partners lies at the heart of the autophagic process (Figure 1A). In addition to vesicular trafficking, Vps34 has been implicated in nutrient sensing and protein synthesis through the mTOR pathway and in signal transduction downstream of G protein-coupled receptors (Backer, 2008). However, there are many outstanding questions about the cellular roles of Vps34 under homeostatic and pathological conditions, including cancer, which structural biology and improved chemical probes—used alongside genetic methods—could help us to answer.

This is where Miller et al. rode to the rescue. First, they solved the crystal structure of Vps34 from *Drosophila melanogaster* at 2.9Å resolution, revealing several important insights. A striking difference with the class I PI3Ks is the fully ordered activation loop in Vps34. This loop controls phosphoinositide substrate preferences and is disordered in class I PI3K structures (Figure 1B), The understanding of this part of the Vps34 structure allowed a detailed proposal for its catalytic mechanism, supported by mutagenesis of key residues. Comparison of Vps34 with p110γ structures showed that the catalytic loop of Vps34 is possibly captured in an active conformation, whereas p110γ structures could correspond to an inactive conformation (Walker et al., 1999). The difference between the two suggests a transition from inactive to active forms that may represent a fascinating activation

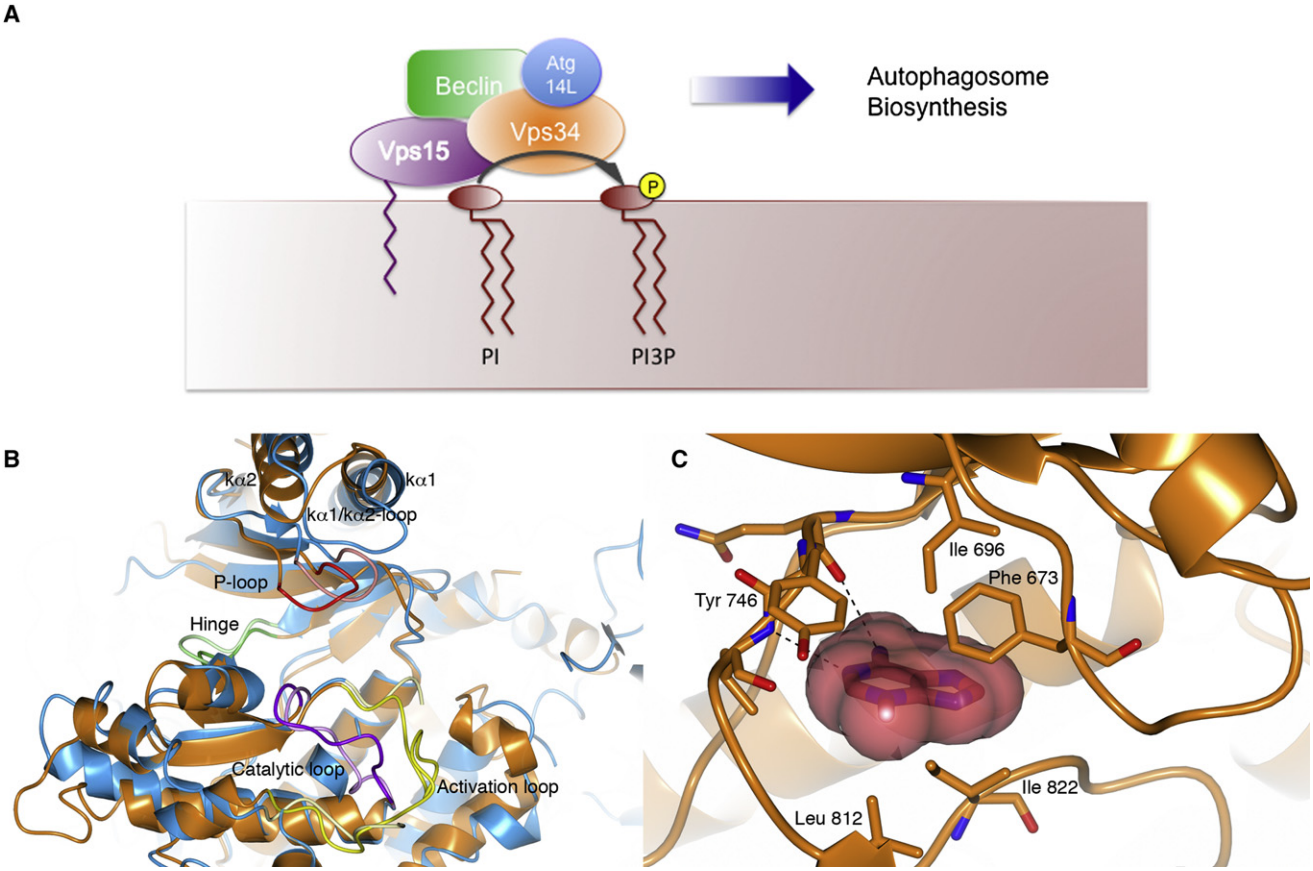

Figure 1. Structure, Function, and Inhibition of Vps34

(A) Schematic of Vps34 in complex with Beclin 1, Atg14L, and Vps15. Vps15 anchors this multiprotein complex to the membrane via its N-terminally attached myristoyl group (purple) and activates the essential PI3K activity of Vps34. The Vps34 substrate, phosphatidylinositol (PI), and its product, phosphatidylinositol-3-phosphate (PI3P), are shown in red. The complex with ATg14L is involved in the induction of the first stages of autophagosome formation, whereas alternative complexes with UVRAG and/or Rubicon (not shown, see text) are involved in the later stages of autophagosome maturation.

(B) An overlay of p110γ (PDB code 3 dbs) shown in blue and Vps34 (PDB code 2x6h) shown in orange with the P loop that binds the phosphates of ATP in pink and red, respectively, the hinge in green/light green, the activation loop in yellow/lemon, and the catalytic loop in purple/light purple. Also indicated are helices kα1 and kα2 and the loop connecting them. Important structural differences with p110γ include a shift in the P loop in Vps34, the lack of a bulge in the hinge region in Vps34, the different conformation of the catalytic loop and the ordering of the activation loop.

(C) The binding of the fragment-like 3-methyladenine (3-MA) in Vps34 (PDB code 2x6f). 3-MA is shown with carbon atoms in red and a solvent accessible surface superimposed. The two hydrogen bond interactions with the hinge are indicated as dashed lines. The hydrophobic residues that snugly surround 3-MA and appear to be important for its specificity against Vps34 are labeled.

mechanism on membranes that applies to all PI3Ks.

Of particular importance for the action of PI3K inhibitors is the smaller ATP-binding pocket in Vps34 (volume 800 Å^3) compared to that of the class I p110γ (volume 1200 Å^3). This is caused by an inward curling of the loop that binds the phosphates of ATP (P loop), together with a similar shift in the loop connecting the so-called kα1/kα2 helices (Figure 1B). In addition, the one residue shorter hinge region between the N and C lobes of the kinase domain of Vps34 results in the absence of the bulged-out space in this region that is common to class I PI3Ks. It is clear that the smaller ATP cavity of Vps34 restricts the binding of typical PI3K inhibitors, which is consistent with the weak affinities for Vps34 of the commonly used class I PI3K tool inhibitors PIK-90 and PI-103. In addition, Miller et al. argue that whereas some class I PI3Ks, notably p110δ (Berndt et al., 2010), can form an induced allosteric specificity pocket when binding propeller-shaped inhibitors such as PIK-39, this is prevented in the more rigid and constrained ATP pocket of Vsp34.

Lacking a potent Vps34 inhibitor, 3-methyladenine (3-MA) has been used at very high concentration (e.g., 10 mM) in cells to inhibit this class III PI3K and autophagy (Kondo et al., 2005). Miller et al. determined the structure of the complex between Vps34 and 3-MA (Figure 1C), which has a very low molecular weight and so fits well into the smaller ATP pocket. Furthermore, although not potent, 3-MA shows some selectivity for Vps34 versus class I PI3Ks, which is explained by the conserved hydrophobic residues that snugly surround 3-MA in Vps34.

Using these new structural insights, Miller et al. were able to reverse the modest selectivity of PIK-93 for p110γ over Vps34 (IC_{50} values 36 nM and 4 nM, respectively) as a proof of concept for structure-based design against Vps34. They synthesized the analog PT210 that

was 10-fold less potent for Vps34 (450 nM) but 1000-fold less potent for p110γ (4,428nM) giving a 10-fold selectivity in favor of Vps34. At this stage there are no structural data confirming the binding mode of PT210 in Vps34 or p110γ, but the results suggest that even more potent and selective inhibitors may be within reach.

So how might such emerging inhibitors be used? First, they could be employed as improved tools to ask outstanding questions about the precise roles of Vps34 in cells. With respect to therapy, since autophagy is a double-edged sword in cancer (White and DiPaola, 2009) the jury is still out as to whether inhibiting autophagy would be a good or a bad thing. The potential therapeutic effects of pharmacologic Vps34 modulation may well be context-dependent, and thus there could perhaps be a need for biomarkers for patient selection. Improved Vps34 inhibitors would allow us to determine in a better way than before whether it is time for the newly unveiled ancestral PI3K to join some of the upstart younger generation as a new cancer drug target. Furthermore, the recent emergence of GOLPH3 as an oncoprotein involved in vesicular trafficking (Scott and Chin, 2010) suggests that this area might be of broader therapeutic significance, giving rise to an even more extended target family.

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Loss of 53BP1 Is a Gain for BRCA1 Mutant Cells

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Mutations in *BRCA1* predispose to tumorigenesis presumably from the inability to accurately repair DNA double-strand breaks by homologous recombination. Two new papers shed light on how loss of the DNA damage response protein 53BP1 reverses phenotypes of BRCA1 mutant cells, with potential clinical implications.

Defects in homologous recombination (HR) cause chromosome instability and are associated with tumor predisposition (Moynahan and Jasin, 2010). The inability to accurately repair DNA double-strand breaks (DSBs) by HR ultimately forces cells to rely on alternative nontemplate-based repair pathways, including nonhomologous end joining (NHEJ), resulting in the accumulation of chromosome aberrations, a hallmark of tumor cells. *BRCA1*, mutations of which are associated with a markedly increased risk of breast and ovarian cancer, was the first tumor suppressor gene identified to have an important role in HR.

Recently, a surprising observation was reported for mice deficient in BRCA1 and the DNA damage response protein 53BP1, in that loss of 53BP1 rescued the embryonic lethality, tumor susceptibility, and premature aging of mice homozygous for *Brca1* exon 11 deletion without fully eliminating the chromosome instability (Cao et al., 2009). Although deficiency of other DNA damage response factors such as p53 and Chk2 had