HS1BP3 inhibits autophagy by regulation of PLD1 and generation of PA

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Abstract

Macroautophagy (autophagy) is a membrane trafficking and intracellular degradation process involving the formation of double-membrane autophagosomes and their ultimate fusion with lysosomes. Much is yet to be learnt about the regulation of this process, especially at the level of the membranes and lipids involved. We have recently found that the PX domain protein HS1BP3 is a negative regulator of autophagosome formation. HS1BP3 depletion increased the formation of LC3-positive autophagosomes both in human cells and zebrafish. HS1BP3 localizes to ATG16L1- and ATG9-positive autophagosome precursors deriving from recycling endosomes, which appear to fuse with LC3-positive autophagosomes. The HS1BP3 PX domain interacts with phosphatidic acid (PA) and 3'-phosphorylated phosphoinositides. When HS1BP3 is depleted, the total cellular PA content is upregulated stemming from increased activity of the PA-producing enzyme phospholipase D (PLD) and increased localization of PLD1 to ATG16L1-positive membranes. We propose that HS1BP3 negatively

regulates autophagy by decreasing the PA content of the ATG16L1-positive autophagosome precursor membranes through inhibition of PLD1 activity and localization.

Several aspects of the role of specific lipids in autophagy are poorly understood, such as the lipid composition of the autophagosomal membrane and the involvement of lipid binding proteins and lipid-modifying enzymes in altering the properties of forming autophagosomes. To approach some of these questions we have focused on PX-domain containing proteins, which are known to bind phosphoinositides (PIs), but little is known about their function in autophagy.

To search for PX-proteins in autophagy, we performed an imaging-based siRNA screen, where HS1BP3 was identified as a putative negative regulator of autophagy. Knockdown of HS1BP3 increased LC3 puncta formation and LC3 lipidation, both under basal and starved conditions. Autophagic flux assays indicated that HS1BP3 functions during autophagosome formation. Accordingly, depletion of HS1BP3 increased the degradation of the autophagic cargo p62/SQSTM1 and long-lived proteins. For *in vivo* analysis, HS1BP3 was silenced in transgenic GFP-LC3 zebrafish and as observed in cells, the number of GFP-LC3 puncta increased in embryos lacking HS1BP3. This was partly rescued by co-injecting human HS1BP3-mRNA, indicating that HS1BP3 negatively regulates autophagy also in zebrafish.

HS1BP3 is generally cytosolic, but also enriched on structures containing autophagy proteins ATG9 and ATG16L1 as well as recycling endosome markers transferrin and transferrin receptor (TfR) (Figure 1). Live cell imaging revealed that these structures fuse with LC3-positive vesicles, indicating that they represent recycling endosome-derived membranes providing input to forming autophagosomes. This is in line with recent findings

showing that ATG9- and ATG16-positive vesicles traffic via the plasma membrane through recycling endosomes and that such trafficking is important for autophagosome biogenesis.

The PX domain is a lipid-binding domain and although most PX proteins bind phosphatidylinositol-3-phosphate (PtdIns3P), they can also bind other PIs. The N-terminal PX domain of HS1BP3 specifically bound phosphatidic acid and 3-phosphorylated PIs. Interestingly, lipidomic analysis of the total cellular lipid content from cells depleted of HS1BP3 revealed a twofold increase in PA compared to control cells.

To investigate whether there is a direct link between elevated PA levels and increased autophagy in HS1BP3 depleted cells, we treated cells with inhibitors against different PA producing enzymes. Autophagy was blocked upon drug-mediated inhibition of PLD and LPAATs (lysophosphatidic acid acyltransferases), which generate PA from phosphatidylcholine (PC) and lysophosphatidic acid (LPA), respectively. We asked whether the increased autophagy observed in cells lacking HS1BP3 could be blocked by inhibitors against these enzymes. Indeed, treating HS1BP3-depleted cells with a PLD inhibitor significantly blocked autophagy. Interestingly, the total PLD activity was increased in cells lacking HS1BP3, suggesting that HS1BP3 regulates PLD activity and as a result, the PA level.

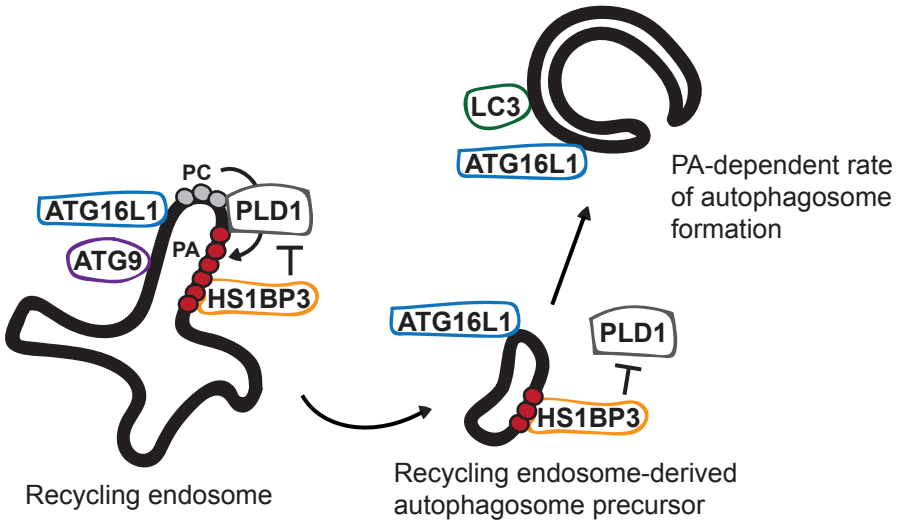
PLD1 has previously been implicated in autophagosome formation and accordingly we found PLD1, but not PLD2, to colocalize with HS1BP3 and ATG16L1. How and where does HS1BP3 regulate PLD1 activity? We were not able to detect an interaction between HS1BP3 and PLD1 and therefore asked whether HS1BP3 may regulate the activity of PLD1 present on recycling endosome-derived vesicles. Interestingly, the colocalisation between PLD1 and ATG16L1 was increased in cells depleted of HS1BP3. As both HS1BP3 and PLD1 contain a lipid-binding PX-domain, we hypothesized that the two proteins compete for membrane binding to ATG16L1-positive structures. Indeed, overexpression of either full length HS1BP3

or its PX-domain alone decreased the colocalisation between PLD1 and ATG16L1. This was not the case in cells expressing a deletion mutant of HS1BP3 lacking the PX-domain, supporting the idea that HS1BP3 membrane binding prevents access of PLD1 to ATG16L1-containing structures. Moreover, knockdown of both HS1BP3 and PLD1 blocked the increase in LC3 spot formation and lipidation observed in cells depleted of HS1BP3 alone, further supporting that HS1BP3 affects autophagosome formation through PLD1. The simultaneous binding of the PLD1 PX-domain to PA and PIs has been found to increase its membrane affinity. It is therefore tempting to speculate that the analogous lipid-binding specificity of HS1BP3 and PLD1 is responsible for their observed competitive binding to ATG16-positive membranes.

The exact role of PA in autophagy stimulation is not clear. Structurally, PA is a cone-shaped lipid which could have a role in forming the autophagosomes by inducing negative membrane curvature, which is important for recruiting autophagy proteins, such as ATG3 which senses highly curved membrane for LC3-lipidation. PA has also been implicated in fusion events during exocytosis and mitochondrial fusion, and it is possible that PA might stimulate homotypic fusion of ATG16L1-vesicles, which has been shown to occur upon autophagy induction. In line with such a model, PLD1 was recently found as an effector of the SNARE-protein VAMP7 which is required for homotypic fusion of ATG16L1-vesicles.

Taken together, our results demonstrate that HS1BP3 senses local PA-levels and provides negative feedback by its binding to PA, controlling the activity of PLD1 and its access to autophagic precursor membranes, thereby contributing to the negative regulation of autophagosome biogenesis. By increasing our knowledge about lipids and their role in autophagy we might be able to better answer important questions like the origin of the autophagosomal membrane and the regulation of its shape and size during autophagosome formation.

Figure 1: Model of HS1BP3-regulated PA production on recycling endosome derived autophagosome precursor membranes. PLD1 generates PA on ATG9 and ATG16L1-positive recycling endosome membranes . HS1BP3 is recruited to these membranes by binding of its PX domain to PA, thereby inhibiting PLD1 activity by displacing membrane-bound PLD1. HS1BP3 thus provides negative feedback on the generation of PA on autophagy-relevant membranes.


LC3
ATG16L1
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PLD1
ATG9
PA
HS1BP3
ATG16L1
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HS1BP3
Recycling endosome
Recycling endosome-derived
autophagosome precursor