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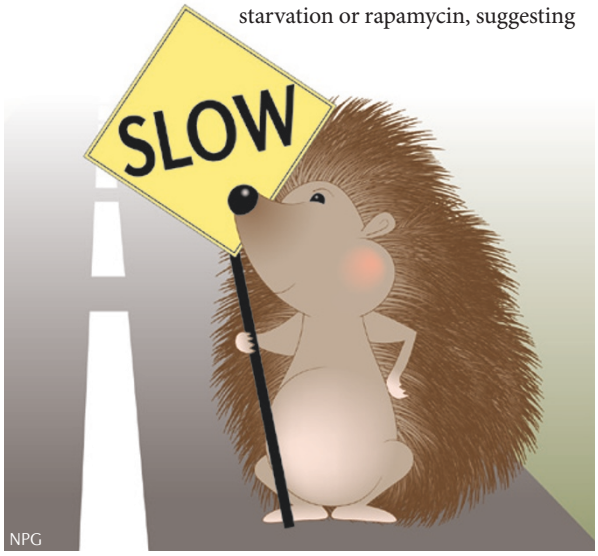
CELL SIGNALLING

Hedgehog puts a damper on autophagy

> autophagy is impaired by canonical Hedgehog signalling

Cellular homeostasis is maintained by autophagy, a process that degrades damaged organelles and misfolded proteins and is activated in response to stress. Autophagy has also been implicated in embryogenesis, but the signalling pathways that regulate autophagy during embryonic development are unclear. Now, Jimenez-Sanchez *et al.* show that Hedgehog signalling, which has a key role during development, regulates autophagy.

To determine whether Hedgehog signalling plays a part in autophagy, the authors investigated the effects of Hedgehog agonists. First, they showed that Hedgehog peptides reduced autophagosome numbers (as measured by decreased levels of LC3-II, a marker of autophagosomes) and decreased protein clearance in cultured mammalian cells compared with untreated cells. This inhibitory effect was also observed when autophagy was induced by starvation or rapamycin, suggesting that Hedgehog signalling impairs autophagy in both basal and induced conditions.



The transmembrane Hedgehog receptor Patched (PTCH) inhibits Smoothened in the absence of ligand, thereby inhibiting downstream signalling. Upon ligand binding, Smoothened inhibition is released, which leads to activation of glioma (GLI) transcription factors and Hedgehog-induced gene expression. The authors investigated the roles of PTCH1 and PTCH2 in autophagy inhibition and found that overexpression of these receptors, which inhibits Hedgehog signalling, resulted in increased autophagosome numbers and decreased levels of protein aggregates in both cultured mammalian cells and mouse embryonic fibroblasts (MEFs). In agreement with this, knockdown of PTCH1 and PTCH2 decreased autophagosome numbers in the presence of autophagy inducers. Moreover, the authors showed that PTCH2 depletion impaired autophagosome formation (but did not affect maturation), which is consistent with previous results using Hedgehog agonists. Using a similar approach, the authors showed that small interfering RNA-mediated knockdown of Smoothened increased autophagic flux, whereas overexpression of a constitutively active from of Smoothened decreased stimuli-induced autophagy.

Next, the authors tested the roles of GLI transcription factors, which are the downstream targets of Smoothened, and found that in *Gli2*-knockout MEFs treated with Hedgehog ligands autophagy was not impaired. This effect could be rescued by overexpressing GLI2, implicating GLI2 as a key modulator in this pathway. These results were confirmed in *Drosophila melanogaster* larvae fat body cells, in which autophagy was increased when Hedgehog signalling was inhibited through overexpression of a constitutively repressive form of the fly orthologue of mammalian GLI, Cubitus interruptus (CI).

So, does Hedgehog signalling affect the transcription of autophagy modulators? To test this, the authors used a PCR array to examine the expression profile of autophagy genes in response to Hedgehog ligand and found that PKR-like ER kinase (PERK) levels were decreased, suggesting that this kinase is as a potential target. Moreover, activation of the Hedgehog pathway resulted in decreased phosphorylation of the downstream target of PERK, eukaryotic transcription factor 2α (eIF2α), suggesting that Hedgehog signalling negatively regulates the PERK–eIF2α pathway to impair autophagy. However, activating Hedgehog signalling in MEFs that expressed a non-phosphorylatable form of eIF2α still resulted in decreased autophagosome numbers, which indicates that other pathways may also be involved in Hedgehog signalling-mediated control of autophagy.

This work shows that autophagy is impaired by canonical Hedgehog signalling and implicates this pathway in the control of protein homeostasis.

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ORIGINAL RESEARCH PAPER Jimenez-Sanchez, M. *et al.* The Hedgehog signalling pathway regulates autophagy. *Nature Commun.* 13 Nov 2012 (doi:10.1038/ncomms2212)