AUTOPHAGY
2016, VOL. 12, NO. 11, 2258–2259
http://dx.doi.org/10.1080/15548627.2016.1212790

Taylor & Francis
Taylor & Francis Group

AUTOPHAGIC PUNCTUM

Primary cilium and autophagy: The avengers of cell-size regulation

Idil Orhon[a,b,†,*], Nicolas Dupont[a,b,†], and Patrice Codogno[a,b]

[a]Institut Necker-Enfants Malades (INEM), INSERM U1151-CNRS UMR 8253, Paris, France; [b]Université Paris Descartes-Sorbonne Paris Cité, Paris, France

ABSTRACT
The maintenance of cellular homeostasis in response to extracellular stresses by autophagy is vital for the health of various tissues. Extracellular stimuli may include nutrient starvation, endoplasmic reticulum stress, hypoxia, cytotoxic agents, or mechanical stress. The primary cilium (PC) is a microtubule-based sensory organelle that regulates the integration of various extracellular stimuli. The interconnection between macroautophagy/autophagy and the PC is beginning to be illuminated. In this punctum, we discuss our recent study of PC-dependent autophagy in response to fluid flow in kidney epithelial cells. Urinary flow in kidney tubules creates a shear stress that regulates epithelial cell volume. PC-mediated autophagy is necessary for the regulation of cell size. The signal from the PC is transduced by the activation of STK11/LKB1 and by MTOR inhibition. Our results clarify the physiological role of PC-dependent autophagy in the kidney and suggest that autophagy manipulation may provide a route to the treatment of ciliopathies.

ARTICLE HISTORY
Received 4 July 2016
Revised 5 July 2016
Accepted 10 July 2016

KEYWORDS
epithelial cell; kidney; macroautophagy; proximal tubule; shear stress

Autophagy is of key importance in the maintenance of cellular homeostasis in different tissues in the face of various stress conditions. In vitro and in vivo, different extracellular chemical stimuli, including nutrient starvation, cytotoxic agents, and hypoxia, induce autophagy. The role of autophagy in response to mechanical stresses, such as compression, stretching, or shear stress has been poorly understood. Clearly, however, autophagy is a vital mediator of the dialog between the cell and its extracellular milieu.

The PC are essentially sensory antennae and are found on most vertebrate, polarized cells. The PC integrate and transduce extracellular stimuli to trigger various cellular pathways via the receptors and signaling molecules enriched along the ciliary axoneme. Various signaling cascades are regulated by the PC including SHH (sonic hedgehog), PDGFR (platelet-derived growth factor receptor), PKD1/polycystin 1-PKD2/polycystin 2-mediated Ca^{2+} signaling, and AMPK-STK11-mediated MTOR and WNT pathways.

The connection between PC and autophagy was first described in studies of the response of cultured cells to serum deprivation. This functional interaction between the PC and autophagy is mediated by the activation of the SHH pathway via the intraflagellar transport machinery, which recruits various autophagy-related (ATG) proteins to the PC. The Kuhn laboratory showed that in kidney tubules epithelial cell size is regulated by the PC; in this tissue, the PC serve as urine flow sensors and activate the STK11-AMPK-MTOR signaling cascade. This regulation is important as in polycystic kidney diseases defects in PC function result in the disruption of cell size and kidney organization.

In our study, we investigated the role of the interplay between PC and autophagy upon mechanical stress of kidney epithelial cells in vitro and in vivo. The regulation of cell size by autophagy was proposed some time ago in studies that altered amino acid concentrations and osmolarity. We hypothesized that shear stress caused by constitutive urine flow stimulates the activation of autophagy by the PC and regulates epithelial cell volume. To confirm this, we first used an in vitro model to stimulate long-term fluid flow. We subjected cultured ciliated epithelial cells to a 1 dyn/cm^2 flow and followed autophagic activity. The induction of autophagosome formation and autophagic flux in cells under fluid flow correlates with a decrease of cell volume. This activation is abolished in cilia-defective cells derived from kidneys of mice with a mutation in *Ift88* that have no cell-volume regulation under fluid flow. We also used a pharmacological approach to inhibit the class III phosphatidylinositol 3-kinase, and a lentiviral approach to decrease levels of autophagy proteins ATG5 and ATG16L1. These experiments showed that in the absence of a functional autophagy, kidney epithelial cells lose their ability to control their volume under fluid flow. ATG16L1 was previously shown to be critical for PC-dependent activation of autophagy. ATG16L1 is recruited to the ciliary base, a significant signaling hub, upon nutrient deprivation and triggers autophagosome induction. Our data show that ATG16L1 is also recruited to the ciliary base upon mechanical stress, suggesting that translocation of ATG16L1 is a hallmark for PC-dependent autophagy induction.

We used several approaches to study the link between the PC and autophagy in vivo. We altered autophagic activity by

CONTACT Patrice Codogno patrice.codogno@inserm.fr INSERM U1151, 14 rue Maria Helena Vieira da Silva, CS 61431, 75993 Paris cedex 14, France.
[†]These authors contributed equally to this work.
*Present affiliation: University Medical Center Groningen, Groningen, The Netherlands.
Punctum to: Orhon I, Dupont N, et al. Primary cilium-dependent autophagy controls epithelial cell volume in response to fluid flow. Nat Cell Biol 2016; 18(6):657-67; PMID:27214279; http://dx.doi.org/10.1038/ncb3360

chemical blocking via the administration of the lysosomotropic agent chloroquine; we disrupted urinary flow via a unilateral ureteral obstruction; and we used a *Kif3a*-mutant mouse that has impaired ciliogenesis. Studies in each of these systems led us to the conclusion that autophagy is induced by the urinary flow in the mouse kidney tubules and is required for epithelial cell size regulation via a functional PC.

Previously, the MTOR pathway was shown to be involved in kidney cell homeostasis in ciliopathies and in cell-size regulation via the STK11-AMPK cascade. Therefore, we speculated that the mechanism regulating autophagy activation by the PC might depend on the MTOR pathway. Indeed, we observed that long-term fluid flow stimulated autophagy via MTOR inhibition through STK11 activation. This mechanism was independent of nutrient deprivation-induced autophagy, which occurs via the PC upon SHH activation. As the initial stimulation of autophagy by mechanical stress depends on ciliary calcium channel PKD2/polycystin 2 signaling, we hypothesize that the cilium serves as an evolutionarily conserved signaling hub for autophagy under different environmental stresses.

In various tissues, upon different extracellular stimuli, several signaling pathways can be transduced by the PC to activate autophagy. These include the SHH/hedgehog pathway, stimulated by serum deprivation; the STK11-AMPK-MTOR cascade stimulated by prolonged fluid flow and required for the maintenance of kidney tubule cell size; and the PKD2/polycystin 2 pathway stimulated by mechanical stress. The function of autophagy that depends on the calcium-specific cation channel PKD2/polycystin 2 in response to fluid flow is independent of cell-size regulation and is poorly understood. Although it was recently demonstrated that calcium signaling at the PC does not induce a calcium influx into the cytoplasm, mutations in the gene that encodes PKD2/polycystin 2 are linked to the development of cysts in patients with the ciliopathy autosomal dominant polycystic kidney disease. The possible functional role of autophagy in kidney disease development remains to be investigated. Another interesting question concerns the functional relevance of the trafficking of various ATGs, specifically ATG16L1, to the axoneme or basal body of the cilium upon stimulation of cells. Whether or not the recruitment of autophagy proteins induces autophagosome biogenesis in the proximity of the cilium remains to be investigated. Finally beyond these results, the interplay between autophagy and the primary cilium is also important to consider during the differentiation of embryonic stem cells. These findings suggest that the crosstalk between autophagy and the primary cilium has more to tell us in the future.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

PC's lab was funded by INSERM, CNRS, University Paris-Descartes, INCa and ANR; ND was funded by the Association pour la Recherche sur le Cancer.