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Crosstalk between cilia and autophagy: implication for human diseases

Manuela Morleo^{a,b}, Helena L.A. Vieira^{c,d,e}, Petra Pennekamp^{f,g}, Alessandro Palma^h, Liliana Bento-Lopes^c, Heymut Omran^{f,g}, Susana S. Lopes^{c,g}, Duarte C. Barral^c, and Brunella Franco^{a,i,j}

^aTelethon Institute of Genetics and Medicine (TIGEM), 80078, Pozzuoli, Italy; ^bDepartment of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples, Italy; ^cCEDOC, NOVA Medical School, NMS, Universidade NOVA de Lisboa, Lisboa 1169-056, Portugal; ^dUCIBIO, Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal; ^eAssociate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal; ^fDepartment of General Pediatrics, University Hospital Münster, University of Münster, Münster 48149, Germany; ^gMember of the European Reference Networks ERN-LUNG, Lisbon, Portugal; ^hDepartment of Onco-hematology, Gene and Cell Therapy, Bambino Gesù Children's Hospital - IRCCS, Rome, Italy; ⁱMedical Genetics, Department of Translational Medical Science, University of Naples "Federico II", Naples, Italy; ^jScuola Superiore Meridionale, School for Advanced Studies, Naples, Italy

ABSTRACT

Macroautophagy/autophagy is a self-degradative process necessary for cells to maintain their energy balance during development and in response to nutrient deprivation. Autophagic processes are tightly regulated and have been found to be dysfunctional in several pathologies. Increasing experimental evidence points to the existence of an interplay between autophagy and cilia. Cilia are microtubule-based organelles protruding from the cell surface of mammalian cells that perform a variety of motile and sensory functions and, when dysfunctional, result in disorders known as ciliopathies. Indeed, selective autophagic degradation of ciliary proteins has been shown to control ciliogenesis and, conversely, cilia have been reported to control autophagy. Moreover, a growing number of players such as lysosomal and mitochondrial proteins are emerging as actors of the cilia-autophagy interplay. However, some of the published data on the cilia-autophagy axis are contradictory and indicate that we are just starting to understand the underlying molecular mechanisms. In this review, the current knowledge about this axis and challenges are discussed, as well as the implication for ciliopathies and autophagy-associated disorders.

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Autophagy: the basics

Macroautophagy (hereafter referred as autophagy) is an evolutionarily conserved process used by cells to sequester intracellular components inside double-membrane organelles, called autophagosomes. These organelles fuse with lysosomes to degrade their content and recycle nutrients back to the cytoplasm (Figure 1(a)). Autophagy is a tightly regulated pathway that maintains cellular homeostasis, and it is crucial for defense against extracellular agents (e.g., pathogens)[1]. Underscoring its essential role, autophagy dysfunction has been linked to a variety of human diseases (e.g., cancer, degenerative and immune disorders), as well as aging [2–4].

Autophagosomes are trafficked via microtubules and fuse with lysosomes, generating a single membrane organelle, the autolysosome [5] (Figure 1(a)). Lysosomes, the destination of cargoes processed by autophagy, have an acidic pH and contain hydrolases with the capacity to degrade macromolecules, including lipids, proteins and sugars. The acidic pH is maintained by a proton pump, the multicomplex vacuolar-type H⁺-translocating ATPase (V-ATPase) [6]. Upon fusion of autophagosomes with lysosomes, cargoes are degraded, and the resultant building blocks are subsequently released for anabolic reactions.

Autophagosome biogenesis is a process orchestrated by autophagy-related (ATG) proteins, and involves several steps

including phagophore nucleation, expansion, and closure to allow sequestration of cytoplasmic cargoes [7]. A number of upstream signaling complexes regulate autophagy, including MTOR (mechanistic target of rapamycin kinase) complex 1 (MTORC1), a serine-threonine kinase that positively regulates cell growth and proliferation by promoting anabolic activities, and by limiting catabolic processes such as autophagy [8]. Under nutrient rich conditions, MTORC1 activity prevents autophagy by inhibition of the mammalian autophagy-initiating kinase ULK1 through phosphorylation; conversely, in instances of nutrient scarcity, ULK1 is activated by AMP activated protein kinase (AMPK) and promotes autophagy [9]. Briefly, upon autophagy initiation, the ULK1 kinase complex translocates to the phagophore and recruits the class III phosphatidylinositol 3-kinase (PtdIns3K) complex which produces the signaling lipid phosphatidylinositol-3-phosphate (PtdIns3P) in the phagophore membrane. The signal generated coordinates the recruitment and activation of the autophagic cascade machinery to orchestrate autophagosome formation [7].

Importantly, although autophagy was initially viewed as primarily a nonselective intracellular degradation pathway, recent studies have shown that autophagy selectively degrades substrates. Indeed, selective autophagy is required to maintaining the number and integrity of cellular organelles, as well as to protect cells from pathogens. This process consists of the

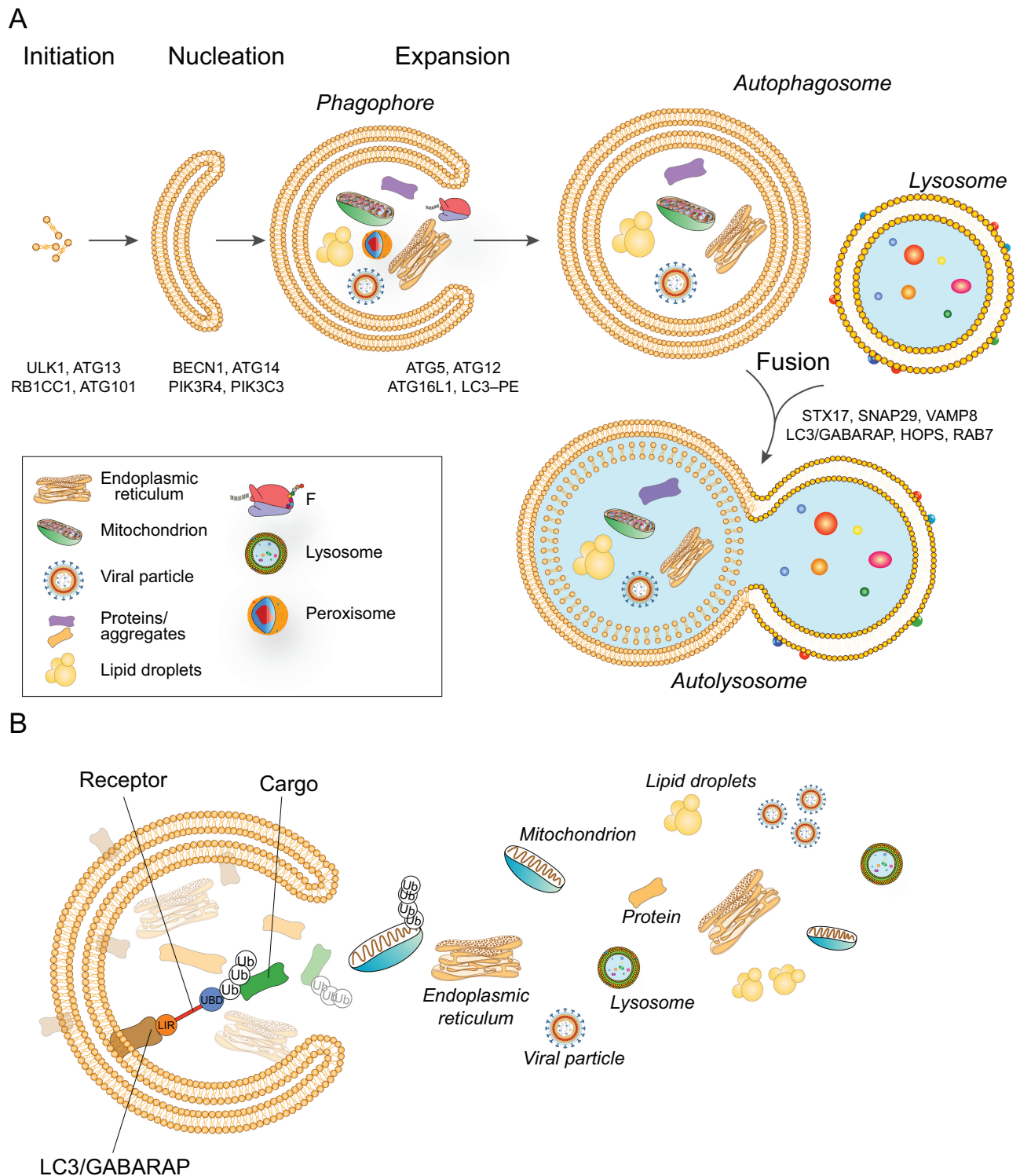


Figure 1. Schematic representation of the autophagy process. (a) The phagophore expands while engulfing intracellular components, such as mitochondria, viral particles, protein aggregates, endoplasmic reticulum, to be degraded. The phagophore then closes to become an autophagosome characterized by a typical double membrane. The autophagosome later fuses with the lysosome to form an autolysosome that degrades the sequestered cytoplasmic material. (b) Selective cargoes are recognized by the autophagic receptors which are characterized by the ability to simultaneously interact with LC3/GABARAP and selective cargoes, thus bridging the growing phagophore and the targeted cargoes. Cargoes can be ubiquitinated. Autophagy receptors bind the ubiquitinated cargoes through ubiquitin-binding domains (UBD) (blue) and bind LC3 and/or GABARAP subfamily proteins through the LIR domain (LIR, Orange). Ub: ubiquitin.

specific targeting of cargoes for degradation, including proteins and unwanted/damaged organelles such as mitochondria, portions of the ER, ribosomes, peroxisomes, lysosomes among others [10] (Figure 1(b)). Most cargoes are recognized

by the Atg8-family proteins. These are core autophagic proteins associated with the autophagosome membranes via covalent conjugation to phosphatidylethanolamine. Family members include the MAP1LC3 (microtubule associated

protein 1 light chain 3; LC3A, LC3B, LC3B2, LC3C) and the GABARAP (GABA type A receptor-associated protein; GABARAP, GABARAPL1, and GABARAPL2) sub-families (Figure 1(b)). Atg8 proteins are characterized by 15- to 20-amino-acid-long sequence motifs, termed LC3-interacting regions (LIRs) [10]. In some cases, the cargo is recognized by selective autophagy receptors, which are characterized by the ability to bind cargoes and facilitate the recruitment of autophagic machinery and the formation of the growing phagophore [11] (Figure 1(b)). Autophagy receptors bind Atg8-family proteins through a LIR domain and are finally degraded with the cargo. In mammals, over 30 autophagy receptors, such as SQSTM1/p62 (sequestosome 1), NBR1 (NBR1 autophagy cargo receptor), CALCOCO2 (calcium binding and coiled-coil domain 2), OPTN (optineurin) and TAX1BP1 (Tax1 binding protein 1) have been described. Their action is regulated by protein modifications, predominantly phosphorylation, ubiquitination, acetylation and oligomerization in order to operate in a specific type of autophagy, and/or to facilitate binding to other proteins [10,12]. Furthermore, selective autophagy can be classified as ubiquitination-dependent or -independent. In the first, cargoes are ubiquitinated: the ubiquitin is usually exposed to the cytosol and is captured by the receptors through a ubiquitin-binding domain. Receptors in turn bind LC3/GABARAP proteins through the LIR [11] (Figure 1(b)).

Cilia biology

Cilia are microtubule-based organelles protruding from the cell surface and come in two flavors: motile and immotile. Motile cilia are present in single or multiple copies (> 100) per cell, beating in coordinated waves to propel liquids and/or mucus across the tissue surface [13–15]. Motile cilia display different functions depending on the organ context and according to the cell type. One example is the respiratory epithelium where cilia on epithelial cells constantly move mucus up from the lungs to the back of the throat. Another example is given by the fallopian tubes, where cilia move the eggs toward the uterus. In the embryonic node, also known as the left-right organizer in vertebrates, motile cilia generate a directional fluid flow responsible for determination of the left-right (L-R) axis [16]. Such flow has been well studied in animal models (mice, frogs and zebrafish) and has been found to promote asymmetric gene expression ultimately leading to asymmetric activation of specific developmental signaling pathways that lead to vertebrate visceral asymmetry [16]. In addition to organ-specific motile cilia, most cells of the body also display a single immotile cilium known as the primary cilium with mechanosensory and chemosensory properties that allows the detection, integration, and transduction of a variety of extracellular signals. These signaling activities are carried out by transmembrane receptors embedded in the cilium membrane and by a myriad of signaling molecules localized to the primary cilium, which allow the cell to respond to various external stimuli and control the signaling pathways. The extracellular signals conveyed by primary cilia include mechanical stresses (e.g., bending of

the cilium by fluid flow and tissue deformation), paracrine signaling molecules (e.g., HH [hedgehog], WNT, NOTCH, Hippo), growth factors and hormones. In specialized cells (e.g., kidney epithelial cells and photoreceptors), primary cilia can respond to specific stimuli, including force, light, temperature, gravity, and osmolarity. Moreover, regulation of primary ciliogenesis is coordinated with mitosis, implying the association of primary cilia with control of cell proliferation. Indeed, ciliogenesis is tightly correlated with the cell cycle, as the formation of a primary cilium occurs post-mitotically in G₀ or early G₁ phase, while its reabsorption occurs in S or G₂ phase.

Both motile and immotile cilia have a microtubule-based structure formed by an axoneme composed of 9 microtubule doublets, anchored to the cell through a basal body (Figure 2). Most motile cilia have typically a central pair of microtubules with a “9 + 2” structure (nodal cilia lack the central pair) associated with dynein arms that power axonemal beating through ATP hydrolysis. Primary cilia lack this central pair and dynein arms; they have a “9 + 0” structure and therefore lack motility (Figure 2).

The basal body originates from the centrosome [17], a cytoplasmic organelle composed of two centrioles (mother and daughter) and centriolar satellites, which are granular structures of pericentriolar material (PCM) (reviewed in [18]) (Figure 2). A specialized ciliary domain, the transition zone, is localized at the base of cilia and exerts a gate function for protein and lipid exit and entry, thus controlling the protein and lipid composition of the ciliary compartment [19]. Cilia rely on intraflagellar transport (IFT), which uses kinesin and dynein motors for the supply of ciliary components synthesized in the cytoplasm and transported from the cell body to the ciliary tip and backwards [20] (Figure 2).

For a detailed description of cilia structure, assembly, maintenance and recent advances in the understanding of the cell biology of this organelle, readers can refer to other excellent reviews [21,22]. Hundreds of proteins have been linked to ciliogenesis. Moreover, genes encoding some of these proteins have been associated with inherited conditions known as ciliopathies. Ciliopathies involving primary cilia often present multi-organ phenotypes that affect the central nervous system, eye and skeleton as well as visceral organs including the kidneys (primary ciliopathies) [23]. These disorders include more common conditions such as autosomal dominant polycystic kidney disease (ADPKD) [24] and rare disorders including oral-facial-digital type I (OFDI) [25–27], Joubert (JBTS) [28], Meckel-Grouber (MKS) [29], Birt-Hogg-Dubé (BHD) syndromes [30] and nephronophthisis (NPHP) [23,31]. Moreover, a large body of evidence has demonstrated that primary cilia are dysfunctional in human cancers, including melanoma, breast, pancreatic and renal cell cancers, cholangiocarcinoma and Hurthle cell carcinoma [32–34]. Furthermore, dysfunction of motile cilia results in motile ciliopathies ranging from isolated laterality defects or subfertility in males, to primary ciliary dyskinesia (PCD), which is a multisystemic disorder that represents the most common motile ciliopathy with a global estimated prevalence of 1:10,000 [35].

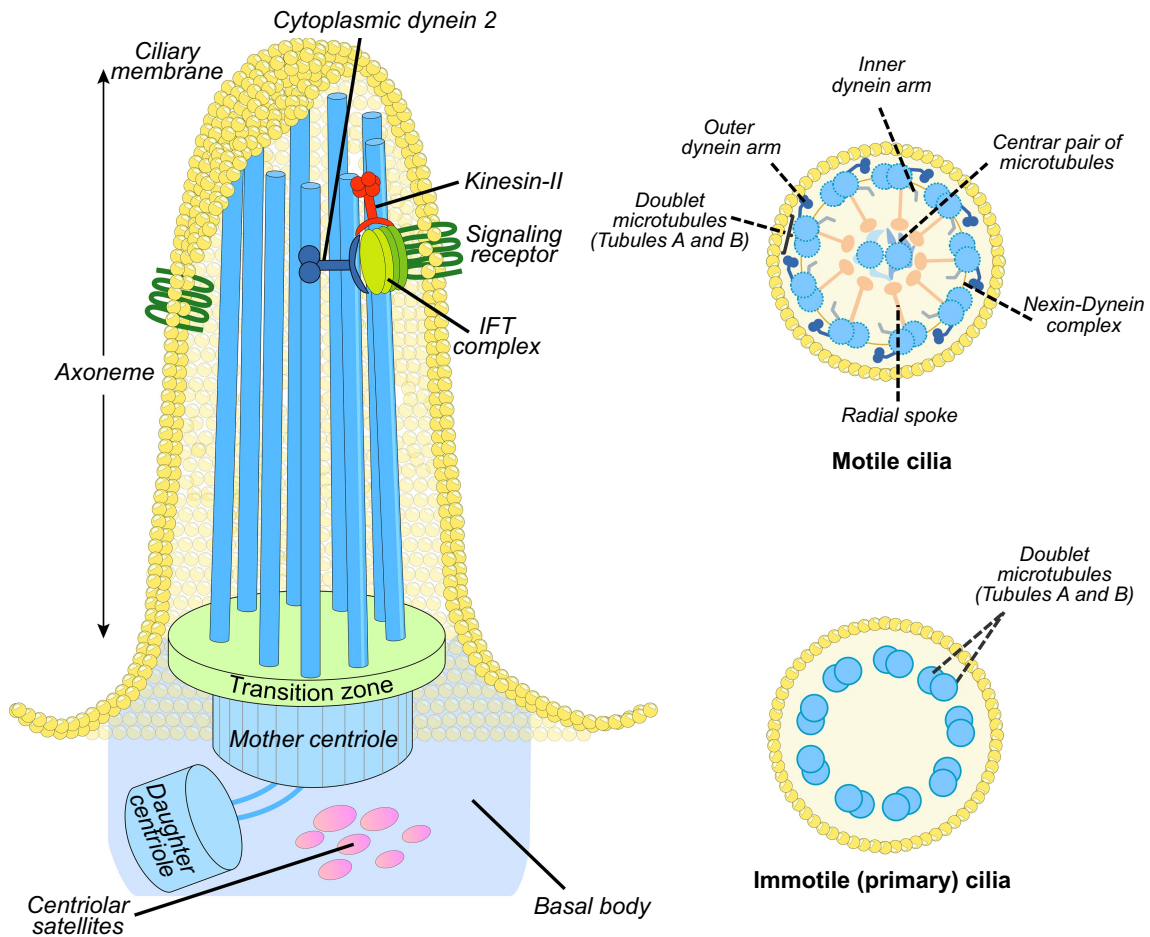


Figure 2. Cilia structure. Left: the ciliary axoneme is composed by nine microtubule doublets of microtubules (blue rods), which nucleate from the basal body (mother centriole). The axoneme protrudes from the cell membrane (ciliary membrane). The movement of IFT particles to the tip of the flagellum is powered by kinesin-II, a microtubule-based molecular motor. The movement of IFT particles back to the base of the flagellum is driven by the cytoplasmic dynein 2, another microtubule-based molecular motor. The mother and daughter centrioles are indicated by the blue cylinders and the transition zone by green at the bottom of the axoneme. The transition zone mediates interactions with the ciliary membrane. Right, top: motile cilia display a central microtubule pair connected with the outer microtubular doublets by radial spokes. The outer microtubules are connected by the nexin-dynein complex. Other structures include the axonemal inner and outer dynein arms. Right, bottom: Immotile primary cilia display nine peripheral microtubular doublets with no central pair and without dynein arms.

Autophagy and cilia come together

A tight link between primary cilia and autophagy was recently established. Indeed, autophagy has been shown to regulate ciliogenesis, and conversely, cilia-dependent signaling pathways and ciliary proteins are now known to control autophagy [36–39].

Selective autophagy takes action in ciliogenesis

The first indication that autophagy controls ciliogenesis was provided in 2013 by Tang and colleagues, who demonstrated that serum starvation-induced autophagy promotes ciliogenesis through selective degradation of the satellite pool of the OFD1 protein, encoded by the gene responsible for OFD type I syndrome [40]. OFD1 localizes to centrosomes/basal bodies, as well as centriolar satellites. In particular, it has been shown that the centrosomal/basal body pool of OFD1 is instead necessary for the formation of distal appendages of the mother centriole, a process required for IFT88 (Intraflagellar

transport 88 protein) recruitment, which in turn is necessary for cilia formation [41]. Moreover, characterization of the *ofd1* knockout mouse model, which recapitulates the human disorder, established a role for OFD1 in primary cilia assembly [42]. In contrast to data reported by Tang *et al.*, in the same issue, Pampliega and colleagues showed that basal autophagy inhibits ciliogenesis in mouse embryonic fibroblasts (MEFs) by the degradation of IFT20 (intraflagellar transport 20), an essential component of ciliary transport [43]. To reconcile these observations, the authors proposed that basal autophagy limits ciliogenesis by removing IFT20, while autophagy induced by serum starvation enhances ciliogenesis by removing the satellite pool of OFD1 (Figure 3(a)).

Following these initial observations, various laboratories demonstrated that autophagic degradation can control ciliogenesis [32,38,44]. Another player in the selective autophagy-cilia association is the cytosolic HDAC6 (histone deacetylase 6). HDAC6 has been identified as an important cytoplasmic tubulin deacetylase that influences mitosis and chemotaxis through the regulation of tubulin stability [45] and as an

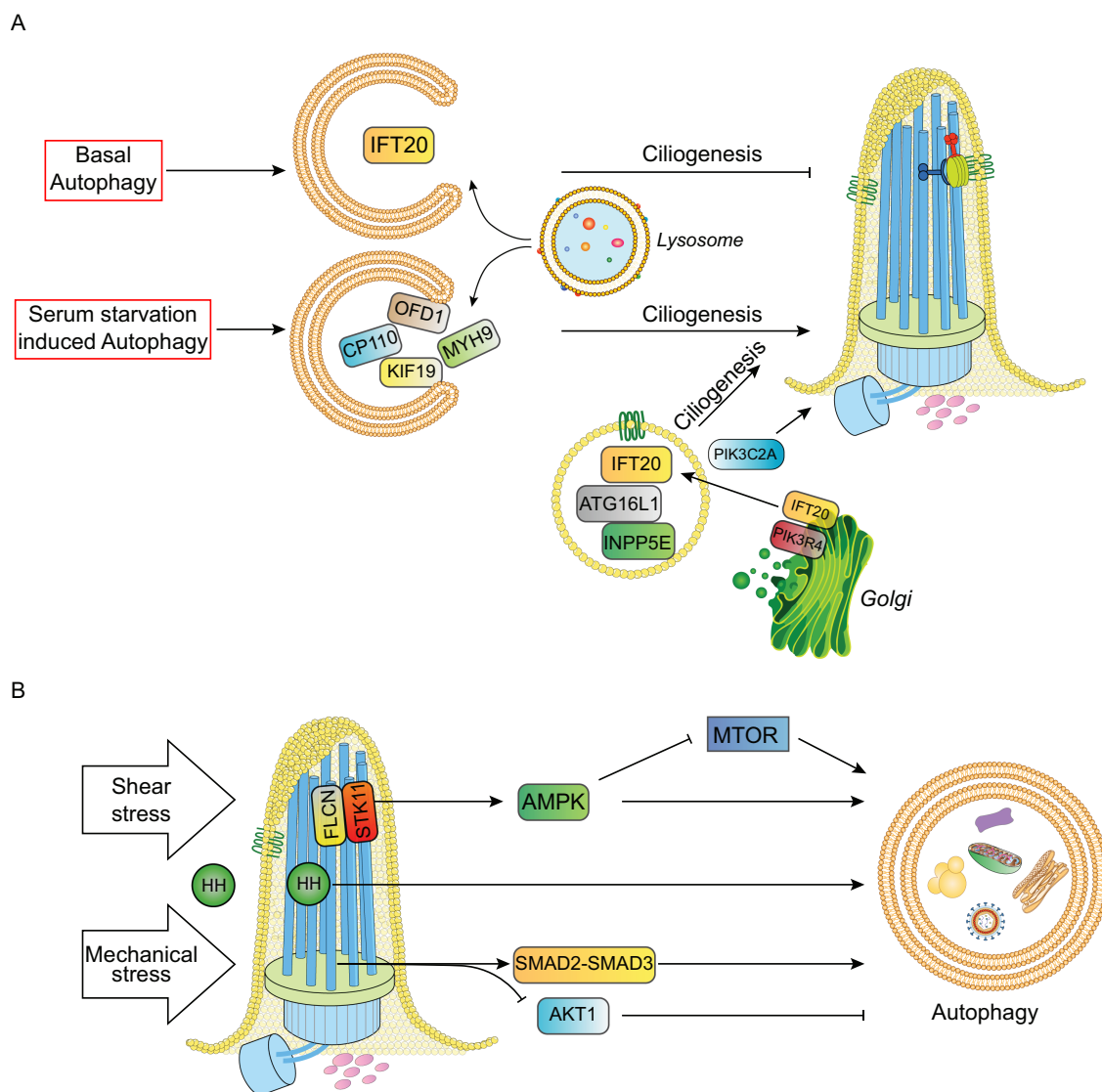


Figure 3. Cilia-autophagy crosstalk models. (a) Under serum starvation selective autophagy degrades ciliary proteins such as OFD1, MYH9, CP110 and KIF19 to promote ciliogenesis and cilia elongation. Conversely, basal autophagy inhibits ciliogenesis by removing IFT20. ATG16L1, INPP5E and IFT20 trafficking to cilia affects primary cilia organization and signaling independently of their functions in autophagy. PIK3C2A and ATG16L1 control ciliogenesis via PtdIns3P. (b) HH (hedgehog) ligands activate HH signaling transduction and autophagy; shear stress in kidney epithelial cells activate the FLCN-STK11-AMPK-MTORC1 cascade and the mechanical stress activates the autophagy stimulatory SMAD2-SMAD3 pathway and the inhibitory AKT1 signaling in trabecular meshwork cells.

enhancer of autophagosome-lysosome fusion [46]. Lam *et al.* reported that increased autophagy in cultured primary airway epithelial cells under chronic cigarette smoke exposure is associated with HDAC6-mediated motile cilia shortening and increased amounts of ciliary proteins (*e.g.*, IFT88, ARL13, CETN1 [centrin 1] and PCNT [pericentrin]) localized to autophagosome fractions in the mouse airway. Indeed, the motile cilia phenotype caused by cigarette smoke exposure results in bronchitis associated with airway inflammation, mucus obstruction and emphysema [47].

Three additional studies were recently published, further advancing the field. A decade ago it was shown that the removal of the CP110 (centriolar coiled-coil protein 110) from mother centrioles is required for ciliogenesis initiation after serum starvation [48,49]. However, the precise mechanism was unknown. Liu *et al.* have now identified NUDCD2/ NudCL2 (NudC domain containing 2) as a novel selective

autophagy receptor which mediates the removal of CP110 from mother centrioles through autophagy. The authors demonstrated that NudCL2 contains a LIR motif mediating the association of CP110 with LC3, and that this interaction occurs specifically upon serum deprivation to initiate ciliogenesis [50].

The second very recent example of selective autophagy with a role in ciliogenesis is given by NEK9 (NIMA related kinase 9) which functions as a selective autophagy receptor for MYH9 (myosin heavy chain 9), a negative regulator of ciliogenesis. NEK9-mediated autophagic degradation of MYH9 induces ciliogenesis by promoting actin dynamics. Interestingly, Yamamoto and colleagues showed that NEK9 contains a LIR domain that, when mutated, impairs cilia formation in murine kidneys [39].

In the third report, Arora *et al.*, discovered that autophagy also modulates motile cilia growth, a highly regulated process

important for flow generation [51]. Cilia length is crucial for mucociliary clearance [52,53], in *Chlamydomonas reinhardtii* where the beating of cilia occurs only after a critical length is reached [54], and in zebrafish, where altered cilia length results in perturbation of fluid flow generation [55]. It was previously shown that KIF19/KIF19A (kinesin family member 19) residing at the ciliary tip, was essential for cilia length control [56]. Arora and colleagues reported that ADCY6 (adenylate cyclase 6), known as a major supplier of cAMP in the modulation of primary cilia length [57,58], inhibits degradation of KIF19 by inhibiting autophagy [51] (Figure 3(a)). Specifically, they showed that KIF19 interacts with AMPK, and that ADCY6 inhibits AMPK-KIF19 binding. In airway epithelial cells, in the absence of ADCY6, the activation of AMPK mobilizes KIF19 into autophagosomes for degradation. Indeed decreasing KIF19 levels correlate with elongated cilia and viceversa [51]. Interestingly, experimental evidence suggests a length-dependent mechanical model for primary cilia. These findings add an additional layer of complexity in the autophagy-cilia interplay [59].

Core autophagic proteins with an autophagy-independent role in ciliogenesis

The first example of a protein involved in autophagic processes with an autophagy-independent role in cilia comes from PIK3R4/VPS15, a regulatory subunit of the class III PtdIns 3-kinase PIK3C3/VPS34, belonging to the PtdIns3K complex [60,61]. Pampliega *et al.*, showed that PIK3R4/VPS15 localizes to ciliary basal bodies and axonemes [43]. Some years later, Stoetzel and colleagues demonstrated that, besides being required for autophagy, PIK3R4/VPS15 regulates primary cilium length in human fibroblasts, as well as ciliary processes in zebrafish [62]. They showed that patients with mutations in *PIK3R4/VPS15* display a ciliopathy phenotype characterized by skeletal abnormalities, retinitis pigmentosa and renal cysts. They also demonstrated that fibroblasts from patients have shorter cilia due to defective formation and/or release of IFT20-positive vesicles from the cis-Golgi to the primary cilium [62].

Interestingly, ATG16L1 (autophagy related 16 like 1) which plays crucial roles in the conjugation of ATG8 protein, was also found to have non-autophagic functions. It was shown to interact with IFT20 both in nutrient-rich and serum deprivation conditions in ciliated and non-ciliated cells [43,63,64]. Moreover, ATG16L1 was found to be co-transported with IFT20 to the cilium during ciliogenesis, and, just recently, the absence of ATG16L1 was shown to lead to the formation of giant cilia that are at least partially dysfunctional [64]. The formation of this giant ciliary structures is caused by the absence of INPP5E (inositol polyphosphate-5-phosphatase E) in cilia with consequent accumulation of PtdIns(4,5)P₂ instead of PtdIns4P. Thus, the authors demonstrated that ATG16L1 controls ciliary phosphoinositides (PIs) by regulating INPP5E-IFT20 trafficking to cilia, independently of its functions in autophagy, and

affecting primary cilia organization and signaling functions [64,65].

Cilia influence autophagy

The first evidence that cilia act on autophagy was provided in 2013 by Ana Maria Cuervo's group, who demonstrated that some ATG proteins display a ciliary or periciliary localization. In particular, cilia stain positive for proteins involved in the early steps of autophagosome formation (*i.e.*, PIK3R4/VPS15, ATG16L1), components of the phagophore expansion complex (*i.e.*, PIK3C3/VPS34) or markers of mature autophagosomes (*i.e.*, LC3 and GABARAP), displaying a discrete punctate pattern at basal bodies and axonemes [43]. Based on these results, it was proposed that the ciliary membrane represents a specialized site for autophagosome formation [43]. However, BECN1 (beclin 1), a core component of the PtdIns3K complex, and ULK1, both key players in phagophore nucleation, do not localize to cilia, suggesting the existence of different autophagosome initiation complexes. Moreover, the same authors showed that autophagy activation upon serum starvation (which induces both ciliogenesis and autophagy), requires the presence of a functional primary cilium as Ift20- and Ift88-depleted cells (MEFs and kidney epithelial cells [KECs]) show defective ciliogenesis and decreased autophagy upon serum removal [43]. In addition, it was shown that stimulation of cells with HH ligands concomitantly activates transcription of HH signaling target genes and autophagy [43]. Autophagy overactivation was also observed in two additional experimental conditions in which HH signaling is constitutively active: in knockout cells for the negative regulator PTCH (patched), in which the HH signaling is constitutive activated, and in cells overexpressing GLI1, the main transcription factor for HH signaling target genes. Conversely, treatment with HH antagonists reduces autophagy [43]. In addition, HH stimuli are unable to induce autophagy in cells with defective ciliogenesis, while GLI1 overexpression partially rescues autophagy loss in cilia-defective cells [43]. After this initial report, a large body of experimental evidence has demonstrated that primary cilia-dependent autophagy can be activated by a variety of external stimuli (*e.g.*, starvation, growth factors, mechanical stress) [43,66–70]. Jang and colleagues identified a primary cilium-autophagy-Nrf2 control axis that directs human embryonic stem cells (hESC) toward neuroectoderm (NE) fate. Indeed, they showed that increased ciliation in NE precursors induced autophagy resulting in inactivation of NFE2L2/Nrf2. Inhibition of NFE2L2, which directly binds to upstream regions of the pluripotency genes *POU5F1* and *NANOG* to promote their expression, induces NE differentiation, thus demonstrating a key role for primary cilia-dependent autophagy in early steps of hESC differentiation [71].

One of the first examples of mechanical stress acting on primary cilia and stimulating autophagy was described by Orhon and colleagues in 2016. In this first report, the authors demonstrated that in renal epithelial cells primary cilia, which transduce extracellular shear stress induced by fluid flow,

ultimately induce STK11/LKB1 (serine/threonine kinase 11)-mediated AMPK activation and inactivate MTORC1. This stimulus results in autophagy upregulation and cell size reduction and, accordingly, cells with defective primary cilia show cellular hypertrophy [67,72]. A few years later, the same group demonstrated that FLCN (Folliculin), a component of the primary cilium, is upregulated upon shear stress and functions upstream of AMPK, orchestrating cell size reduction in KECs in an autophagy-dependent manner [66]. Intriguingly, in KECs submitted to shear stress, primary cilium-dependent autophagy has been demonstrated to act independently of ULK1 and BECN1. PtdIns3P, the key lipid involved in autophagosome biogenesis, is produced by the basal body localized class II PIK3C2A [73], which regulates ciliogenesis [74] and promotes the synthesis of a local pool of PtdIns3P upon shear stress [73]. Renal tubular epithelial cells from the *Pik3c2a*[±] mouse model show decreased autophagic activation and loss of cell-size control [73]. However, how primary cilia control AMPK-mediated autophagy induction in the absence of phosphorylation of its targets – ULK1 and BECN1 – remains an open question. Another recent study showed the importance of primary cilia-mediated autophagy in physiological adaptation to stress in the context of pressure-induced autophagy in trabecular meshwork (TM) cells located in the anterior segment of the eye. Changes in pressure gradients and fluid flow associated with eye movement, circadian rhythm or the ocular pulse are translated in continuous cycles of tissue deformation and relaxation; TM cells can sense these deformations as mechanical forces and respond by reorganizing the actin cytoskeleton, modifying gene expression, secreting cytokines, and remodeling the extracellular-matrix (reviewed in [75]). The activation of autophagy is one of the responses elicited in TM cells following application of mechanical stress [76,77]. Shim and colleagues reported that the primary cilium acts as a mechanosensor for mechanical stress-induced autophagy in TM cells, and identified AKT1 and SMAD2-SMAD3 as signaling pathways regulating the activation of this process which plays a critical role in intraocular pressure homeostasis [76] (Figure 3(b)). A further demonstration of cilia acting on autophagy was provided by Xiang *et al.*, in a study analyzing viability, proliferation and differentiation capacities of chondrocytes treated with cyclic tensile strain of different intensities. The authors found that mechanical stress promoted ciliogenesis and activated autophagy during cartilage development, and demonstrated that the integrity of primary cilia was of crucial importance for mechanical stress-regulated signal transduction and autophagy pathway [68].

Core ciliary proteins with cilia-independent autophagy functions

A variety of proteins involved in ciliogenesis and mutated in ciliopathies, act directly in different stages of the autophagic cascade, independently of cilia. OFD1 represents a paradigmatic example of the level of complexity underlying the selective autophagy-cilia link. Indeed, it has been recently demonstrated that the OFD1 protein is not only degraded by autophagy to promote ciliogenesis, but also

plays a direct role in the control of the autophagic cascade. Morleo and coauthors showed that OFD1 controls autophagosome biogenesis by promoting autophagic degradation of ATG13, a member of the ULK1 autophagy initiation complex, and that OFD1 acts as a novel autophagy receptor for ATG13 [78]. The central portion of OFD1 specifically binds ATG13 while the LIR domain at the C terminus of the OFD1 protein mediates the direct interactions with ATG8 proteins [78]. Furthermore, OFD1-mediated autophagic degradation of ATG13 inhibits autophagy independently of OFD1 cilia-related functions [78]. However, it cannot be ruled out that OFD1 could also selectively recognize ciliary cargoes besides ATG13, and thus influence ciliogenesis through their selective degradation in a cell context-dependent fashion. This hypothesis is supported by recent evidence showing that different cilia components are selectively degraded through autophagy (see above) [47]. Thus, OFD1 could be one of the players coordinating the intricate bidirectional crosstalk between autophagy and cilia, explaining the cellular need to compartmentalize autophagic and ciliary proteins to the same intracellular space [38,79]. Additionally PCM1 (pericentriolar material 1), which is necessary for ciliogenesis [80,81], is involved in GABARAP-autophagosome biogenesis independently of cilia [82]. Joachim and colleagues showed that PCM1 physically interacts with GABARAP through a LIR motif and controls GABARAP localization and degradation at centriolar satellites thus influencing GABARAP-autophagosome formation [82]. The experiments were performed in non-ciliated cells, leading the authors to hypothesize that also the role of GABARAP-PCM1 on autophagosome biogenesis is cilia-independent [82].

Another ciliary protein that intervenes at early steps of autophagy in a cilia-independent manner is IFT20, an integral component of the IFT system. Finetti *et al.*, showed that IFT20 regulates autophagy in non-ciliated T-cells, for which cell homeostasis, activation and differentiation relies on basal autophagy [83]. The authors showed that IFT20 interacts with ATG16L1 and is required for the association of ATG16L1 with the Golgi complex and early endosomes, both of which have been identified as membrane sources for phagophore elongation. Moreover, they demonstrated that under basal conditions, IFT20 participates in autophagosome formation at the early endosome stage, by allowing recruitment of ATG16L1 and downstream autophagy regulators to early endosomes in T cells [83].

Controversies in the cilia/autophagy interplay

The cilia-autophagy interplay has been controversial since its discovery [40,43] and contradictory findings demonstrating opposing roles for induced autophagy in ciliogenesis, have been described [38]. Liu and coauthors reported that the transcription factors PPARA and NR1H4, which are nutrient-sensing receptors, induce ciliogenesis under fasting conditions or negatively-regulate cilia formation under nutrients, by controlling the expression of autophagy genes [84]. PPARA activation facilitates autophagy by inducing expression of *LC3*, *SESN2*, *ULK1*, *ATG2A*, and *ATG12*

and by promoting ciliogenesis. In contrast, NR1H4 suppresses cilia formation by inhibition of autophagic gene expression [84]. Chemical compounds such as sertraline and thioridazine, induce ciliogenesis through autophagy activation. In particular, treatment with sertraline or thioridazine increases the number of LC3 puncta in retinal pigmented epithelial (RPE) and cancer cells, and decreases SQSTM1 protein levels suggesting activation of the autophagic flux [85,86]. These findings are in line with those described by Tang in 2013. Conversely, Lam *et al.* suggested the term “ciliophagy” for the autophagy-mediated cilia disassembly through degradation of ciliary components such as IFT88 [47]. Accordingly, Struchtrup showed that MEFs depleted for RPGRIP1L, a transition zone component, have reduced autophagic activity, which leads to cilia elongation [87]. These findings were confirmed by treating cells with 3-methyladenine (3-MA), an inhibitor of early stages of autophagy, which leads to increased cilia length; conversely reduced cilia length was observed after treatment with the autophagy activators rapamycin or ABT-737 [87].

Altogether, the abovementioned findings suggest that autophagic degradation could exert opposing effects on ciliogenesis in a context-dependent manner and that different players could be involved according to the cell type. However, contrasting findings have also been reported for experiments performed in the same cell line, hinting that culture conditions are important. An example is given by Wang, who showed that treatment with the autophagy inhibitors 3-MA, chloroquine or bafilomycin A₁ (Baf) decreases cilia length, while treatment with autophagy inducers such as Tat-Beclin 1 and trehalose promotes ciliogenesis in human kidney 2 (HK2) cells [88]. In contrast, Iaconis *et al.* demonstrated that treatment with Baf A1 and Tat-Beclin 1 exerts the opposite effect to those described by Wang *et al.* even though the experiments were performed in the same cell line [89]. These observations suggest that autophagy may have a cell context-dependent role in cilia formation and maintenance. Indeed, autophagy players and ciliary cargoes may differ according to the cell-type, the experimental conditions used and the stage of the cilia cycle (formation, elongation, or maintenance). Therefore, expression analysis and interactome profiles of autophagy players and co-factors in the same experimental conditions, could help understanding why the same biological process acts differently, even in the same cell type, and could provide information about the context-dependent role of autophagy in the control of ciliogenesis.

The ciliary compartment may represent a specialized site for effective autophagy control in response to cilia-mediated extracellular stimuli (e.g., fluid flow, pressure, and nutritional needs). Thus, cilia-mediated autophagy could control intracellular metabolic responses and govern physiological adaptations of organs during differentiation and development. On the flip side of the same coin, the autophagic-lysosomal pathway may influence ciliogenesis through the selective degradation of cilia regulators. Tables 1, 2 and 3 summarize proteins and molecules with a role both in autophagy and ciliogenesis.

Novel players in the cilia-autophagy game: lysosomes and mitochondria

Lysosome-cilia dual crosstalk

Besides autophagic proteins, lysosomal proteins are also involved in ciliogenesis, and conversely, ciliary proteins are involved in lysosomal functions. An example is the lysosomal v-ATPase accessory protein ATP6AP1 (ATPase H⁺ transporting accessory protein 1). Indeed, it has been shown that depletion of both maternal and zygotic *atp6ap1b* expression in zebrafish reduces the size of the Kupffer's vesicle (the L-R organizer in fish), alters cilia length and disrupts the establishment of proper L-R asymmetry in early developmental stages [90]. In addition, SNX10 (sorting nexin 10), a phosphoinositide-binding protein implicated in cargo sorting in the endosomal/lysosomal pathway, is required for the formation of primary cilia [91]. Mechanistically, SNX10 interacts with the v-ATPase complex and targets it to the centrosome where both synergistically regulate the ciliary trafficking of RAB8A, a critical regulator of ciliary membrane extension [91]. Finally, it has been reported that the lysosomal anion transporter SLC26A2 localizes to the cilia of human efferent duct cells where it may have a role in male fertility [92].

Finetti *et al.* recently showed that IFT20 is required for the correct targeting of lysosomal hydrolases, by regulating mannose-6-phosphate trafficking. *IFT20*-deficient cells display an increased lysosomal size and impairment to CTSB (cathepsin B) activity, suggesting lysosomal dysfunction. Furthermore, the authors observed activation of TFEB (transcription factor EB), a master regulator of lysosome biogenesis, which likely compensates for lysosome dysfunction in *IFT20*-deficient cells. Importantly, the lysosome-related function of IFT20 is shared by non-ciliated and ciliated cells [93].

Crosstalk between mitochondria and cilia

Mitochondria are involved in bioenergy production, biomolecule synthesis and cell death regulation. Controlled levels of reactive oxygen species (ROS) are constantly produced by mitochondria and act as important signaling molecules [94]. Mitochondria continuously undergo balanced fission and fusion events, which reflect mitochondrial dynamics. These processes highly affect mitochondrial functions, as well as their morphology and are under tight control to maintain proper mitochondrial homeostasis. The main mitochondrial quality control processes include mitophagy (selective mitochondrial autophagy) and mitochondrial biogenesis [95]. Thus, mitochondrial quality control plays a key role in the cellular stress responses, as well as during cancer progression and its associated cellular metabolic shift [95].

Mitochondrial proteins have recently been found to also localize to the ciliary basal bodies and the transition zone, with either demonstrated or suggested roles in ciliogenesis. An example is provided by XPNPEP3/NPHPL1 and SLC41A1/NPHPL2 proteins, which localize to mitochondria and modulate cilia function, and when mutated, result in nephronophthisis [96–98]. Another example of cilia-mitochondria potential interplay is given by the localization to

Table 1. Proteins involved in autophagy and acting as positive modulators of ciliogenesis.

PROTEINS	DEFINITION	SUBCELLULAR LOCALIZATION	Phenotype MIM#	MOLECULAR DETAILS	MODEL	REF
IFT20	intraflagellar transport 20; intraflagellar transport 20 homolog (Chlamydomonas)	Ciliary axoneme	-	Basal autophagy limits ciliogenesis by IFT20 degradation. Moreover, IFT20 participates in autophagosome formation by allowing recruitment of ATG16L1 and downstream autophagy regulators to early endosomes in a cilia-independent manner	MEFs; T-cells	[43,65,83]
PIK3R4/VPS15	phosphoinositide-3-kinase regulatory subunit 4	Golgi apparatus Endosome Ciliary basal body and axoneme Cytoplasm	Ciliopathy phenotype (skeletal abnormalities, retinitis pigmentosa and renal cysts)	It promotes formation and/or release of IFT20-positive vesicles from the cis-Golgi to the primary cilium (autophagy independent role)	Fibroblasts	[62]
ATG16L1	autophagy related 16 like 1	Ciliary axoneme and basal body Centrosome and ciliary basal body Nucleus	611,081	It controls ciliary PIs by regulating INPP5E-IFT20 trafficking to cilia, and affecting primary cilia organization and signaling functions (autophagy independent role).	MEFs	[43,65]
PCM1	pericentriolar material 1	basal body Centrosome and ciliary basal body Nucleus	-	It physically interacts with GABARAP through a LIR motif and controls GABARAP localization and degradation at centriolar satellites thus influencing GABARAP-autophagosome formation in cilia-independent manner.	HEK293A	[82]
PPARA	peroxisome proliferator activated receptor alpha	Nucleus	-	Induces expression of autophagy genes in fasting condition and induces ciliogenesis	RPE; MEFs; HK2; A549	[84]
NR1H4/FXR/bile acid receptor	nuclear receptor subfamily 1 group H member 4	Nucleus	617,049	Inhibits expression of autophagy genes under nutrient-rich conditions and negatively regulates cilia formation	RPE; MEFs; HK2	[84]

Abbreviations. XLR: X-linked recessive; AR: autosomal dominant; RPE: retinal pigmented epithelial cells; MEFs: mouse embryonic fibroblasts; HK2: human kidney-2 cells; LC3: microtubule-associated protein 1 light chain 3; PIs: phosphoinositides

Table 2. Proteins involved in autophagy and acting as negative modulators of ciliogenesis.

PROTEINS	DEFINITION	SUBCELLULAR LOCALIZATION	Phenotype MIM#	MOLECULAR DETAILS	CELL LINE	REF
OFD1/Cxorf5	OFD1 centriole and centriolar satellite protein	Basal body and transition zone of primary cilium; Nucleus	300,424 (XLR) 300,804 (XLR) 311,200 (XLD) 300,209 (XLR) 300,863 (XLD)	Autophagy degrades the satellite pool of OFD1 to promote cilia formation. On the other side OFD1 controls autophagosome biogenesis through ATG13 degradation.	RPE; MEFs; HK2	[40,78]
HDAC6	histone deacetylase 6	Nucleus Cytoplasm		Inhibition of HDAC6-mediated autophagy restores cilia in cholangiocarcinoma; HDAC6 controls cilia disassembly through autophagy-mediated degradation of ciliary components	CCA; MTECs; MEFs	[47,165,166]
CP110	centriolar coiled-coil protein 110	Centrosome and ciliary basal body	-	Selective autophagy removes CP110 from mother centrioles through NUDCD2 which mediates association of CP110 with LC3 to initiate ciliogenesis	MEFs	[50]
MYH9/Nonmuscle Myosin IIA	myosin heavy chain 9	Cytoplasm Nucleus	603,622 (AD) 155,100 (AD)	NEK9-mediated autophagic degradation of MYH9 induces ciliogenesis by promoting actin dynamics. NEK9 contains a LIR domain that, when mutated, impairs cilia formation	MEFs	[39]
KIF19	kinesin family member 19	Ciliary tip Cytoplasm	-	The activation of AMPK mobilizes KIF19 into autophagosomes for degradation. Selective autophagic degradation of KIF19 correlates with elongated cilia and vice versa	Airway epithelial cells MEFs	[51]
RPGRIP1/Nephrocystin 8	RPGRIP1 like	Ciliary basal body and transition zone	611,560 (AR) 611,561 (AR)	Depletion of <i>Rpgrip1l</i> reduces autophagic activity leading to elongation of cilia		[87]
VPS39	VPS39 subunit of HOPS complex	Endosome Lysosome Cytoplasm	-	VPS39 overexpression results in a decreased number of ciliated cells through enhanced autophagy while its depletion increases cilia length through autophagy inhibition	HK2	[89]

Abbreviations. CCA: cholangiocarcinoma cell line; MTECs: murine tracheal epithelial cells; RPE: retinal pigment epithelial cells; MEFs: mouse embryonic fibroblasts; HK2: human kidney-2 cells.

Table 3. Chemicals modulating autophagy and acting on ciliogenesis.

CHEMICALS MODULATING AUTOPHAGY	DEFINITION	EFFECTS ON AUTOPHAGY	MECHANISM OF ACTION ON AUTOPHAGY	EFFECTS ON CILIogenesis	CELL LINE	REF
Krebs-Ringer bicarbonate buffer	Buffer without serum and glucose	Activator	Nutrient starvation	Induces ciliogenesis	HK2	[88]
Sertraline	Selective serotonin reuptake inhibitor	Activator	Targets the AMPK-MTOR-RPS6KB/S6K signaling pathway	Induces ciliogenesis	RPE	[85]
Thioridazine	Member of the phenothiazine family	Activator	ND	Induces ciliogenesis	A549	[86]
Trehalose	Sugar	Activator	Promotes autophagy via TFEB	Induces ciliogenesis	HK2	[88]
Tat-Becn1	BECN1-derived peptide	Activator	Induces autophagosome biogenesis	Induces ciliogenesis	HK2	[88]
Rapamycin	Macrolide with immunosuppressant activity	Activator	MTOR inhibitor	Reduces cilia length	HK2	[89]
ABT-737	Small molecule, chemotherapy agent	Activator	Disrupts BCL2-BECN1 binding, a negative regulatory mechanism of autophagy	Reduces cilia length	MEFs	[87]
3-Methyladenine (3-MA)	Chemotherapy agent	Inhibitor	Inhibits pre-autophagosome assembly by blocking class III PtdIns3K	Reduces cilia length	HK2	[88]
Chloroquine	Used as antimalarial	Inhibitor	Impairs autolysosome formation	Reduces cilia length	MEFs; XTC.	[87,167]
Bafilomycin A ₁	Antibiotic of the macrolide family	Inhibitor	Impairs autolysosome formation	Induces ciliogenesis	UC1	[88]
					HK2	[88]
					HK2	[89]

Abbreviations. RPE: retinal pigment epithelial cells; MEFs: mouse embryonic fibroblasts; HK2: human kidney-2 cells; A549: human lung cancer cells; XTC: cells derived from Hürthle cell carcinoma; AMPK: AMP-activated protein kinase; MTOR: mechanistic target of rapamycin kinase; RPS6KB/S6K: ribosomal protein S6 kinase B; TFEB: transcription factor EB; PtdIns3K: phosphatidylinositol 3-kinase; BCL2: BCL2 apoptosis regulator; BECN1: beclin 1.

centrosomes of the voltage-dependent anion channel family members VDAC1, VDAC2 and VDAC3, which are porins present in the mitochondrial outer membrane that share non-redundant functions in ciliogenesis. VDAC1 and VDAC3 are found on mother centrioles, while VDAC2 localizes to centriolar satellites. VDAC3 modulates centriole assembly by recruiting TTK/MPS1 (TTK protein kinase), an important regulator of centriole assembly, to centrosomes [99]. The VDAC3-TTK module promotes ciliary disassembly during cell cycle entry and suppresses cilia assembly in proliferating cells [100]. Moreover, VDAC1 negatively regulates ciliogenesis, since its downregulation leads to inappropriate ciliogenesis in cycling RPE cells, and its overexpression suppresses cilia formation [100,101]. Instead, VDAC2 appears to promote ciliogenesis [100,101]. Whether VDACS levels in centrosomes are controlled by mitochondria is an open question.

Recently, it was shown in cancer cells that hypoxia induces cleavage of VDAC1 to VDAV1- Δ C, which reprogrammes cells by increasing glycolysis and mitochondrial respiration thus favoring cell survival [102]. Intriguingly, VDAV1- Δ C seems to lose its putative interaction site with tubulin and microtubules, which is thought to bring about repression of ciliogenesis, indicating a link between cancer-associated metabolic adaptation and cilia impairment [102]. In summary, VDAC emerges as a potential player in the crosstalk between mitochondria and ciliogenesis, and eventually cell proliferation and survival. Nevertheless, more studies are needed to shed light on this crosstalk.

A connection between mitochondria and motile cilia has also been described. Indeed, Burkhalter *et al.* showed that mitochondria influence cilia-dependent processes such as cilia length and motility in L-R asymmetry establishment in zebrafish embryos. Along these lines, whole exome analysis of heterotaxic patients revealed an increased burden of rare damaging variants in mitochondria-associated genes [103]. Another link between motile cilia and mitochondria came from work of Hofherr *et al.*, who identified the Ca^{2+} -regulated mitochondrial ATP-Mg/ P_i SLC25A25 (solute carrier family 25 member 25), which acts downstream of the ciliary PKD2/TRPP2 (polycystin 2, transient receptor potential cation channel) as an essential component of cilia-dependent pathways controlling the determination of L-R asymmetry in zebrafish [104]. This connection needs to be confirmed in other animal models but could provide a missing link for the molecular mechanisms behind the first asymmetric gene expression that occurs at the L-R organizer in zebrafish.

Crosstalk between mitochondria and the autophagy-cilia axis

Despite the scarcity of published data, an inter-dependency between ciliogenesis, mitochondrial function and autophagy seems to exist and be related with cellular stress responses. Bae and colleagues showed that mitochondrial stress induced by uncoupling agents (inhibitors of respiratory complexes) or depletion of the pro-fusion *OPA1* gene, which induces fission, stimulates ciliary growth in neuronal SH-SY5Y cells, thus demonstrating a novel regulatory function for mitochondria

dynamics in ciliogenesis [105]. Moreover, these authors showed that mitochondrial stress-induced ciliogenesis is dependent on ROS generation and AMPK activity, triggering autophagy by inhibiting the MTOR pathway (Figure 4(a)). Likewise, blockade of mitochondria-mediated autophagy by silencing Autophagy-Related 5 (*Atg5*) expression, prevents mitochondrial stress-related cilia elongation [105]. In the same study, it was shown that autophagy induced by mitochondrial stress increases the formation of primary cilia through enhancement of OFD1 degradation under nutrient-rich conditions (Figure 4(A)) [105]. In addition, treatment of mice with the mitochondrial toxin 1-methyl-4-phenylpyridinium (MPTP) elicits ciliary elongation and autophagy in dopaminergic neurons of the substantia nigra. Indeed, blockade of cilia formation in these neurons attenuates MPTP-induced autophagy and facilitates dopaminergic neuron loss and motor disability, suggesting a cytoprotective effect for the mitochondrial stress-induced ciliogenesis [105]. Accordingly, a transcriptomic study revealed that whenever the mitochondrial biogenesis control gene *TFAM* (transcription factor A, mitochondrial) is depleted, there is an upregulation of cilia and CFAP65 (cilia and flagella associated protein 65) [106], which may indicate once more that some sort of mitochondrial stress promotes ciliogenesis. In summary, mitochondrial stress appears to promote ciliogenesis, which in turn is a cytoprotective process.

However, cilia-autophagy-mitochondria communication is not limited to mitochondrial stress/autophagy-mediated ciliogenesis. Miceli and colleagues recently showed that shear stress induced by biological fluids in KECs stimulates AMPK-mediated lipophagy (*i.e.*, selective degradation of lipid droplets by autophagy), contributing to the production of fatty acids that provide mitochondrial substrates to generate ATP and produce energy (Figure 4B). Moreover, in KECs fluid flow stimulates AMPK-mediated mitochondrial biogenesis as assessed by the increased levels of mitochondrial proteins and DNA, and these flow-induced processes are dependent on primary cilia (Figure 4B). Finally, the concomitant cilia-dependent lipophagy and mitochondrial biogenesis contribute to ATP production, which is required to support energy-consuming cellular processes such as glucose reabsorption, gluconeogenesis and cytoskeletal remodeling in renal epithelial cells, demonstrating that the primary cilium, autophagy and mitochondrial metabolism are strictly interconnected (Figure 4B) [69,70].

One can speculate that ciliogenesis acts as a cytoprotective process as it is stimulated by cellular stress. In fact, in response to a certain stress (below the threshold of causing cell death) cells stop proliferation (senescence), which may be associated with ciliogenesis. Likewise, ciliogenesis could also be involved in the activation of endogenous mechanisms of defense leading to tolerance or a preconditioning state. Nevertheless, whether ciliogenesis is a cytoprotective response against stress needs further investigation.

In glial cells primary cilia regulate the survival of astrocytes under unfavorable conditions such as starvation. In contrast, disruption of ciliogenesis associated with increased cell proliferation occurs in human astrocytoma/glioblastoma cells. Thus, one can speculate that ciliogenesis disruption might

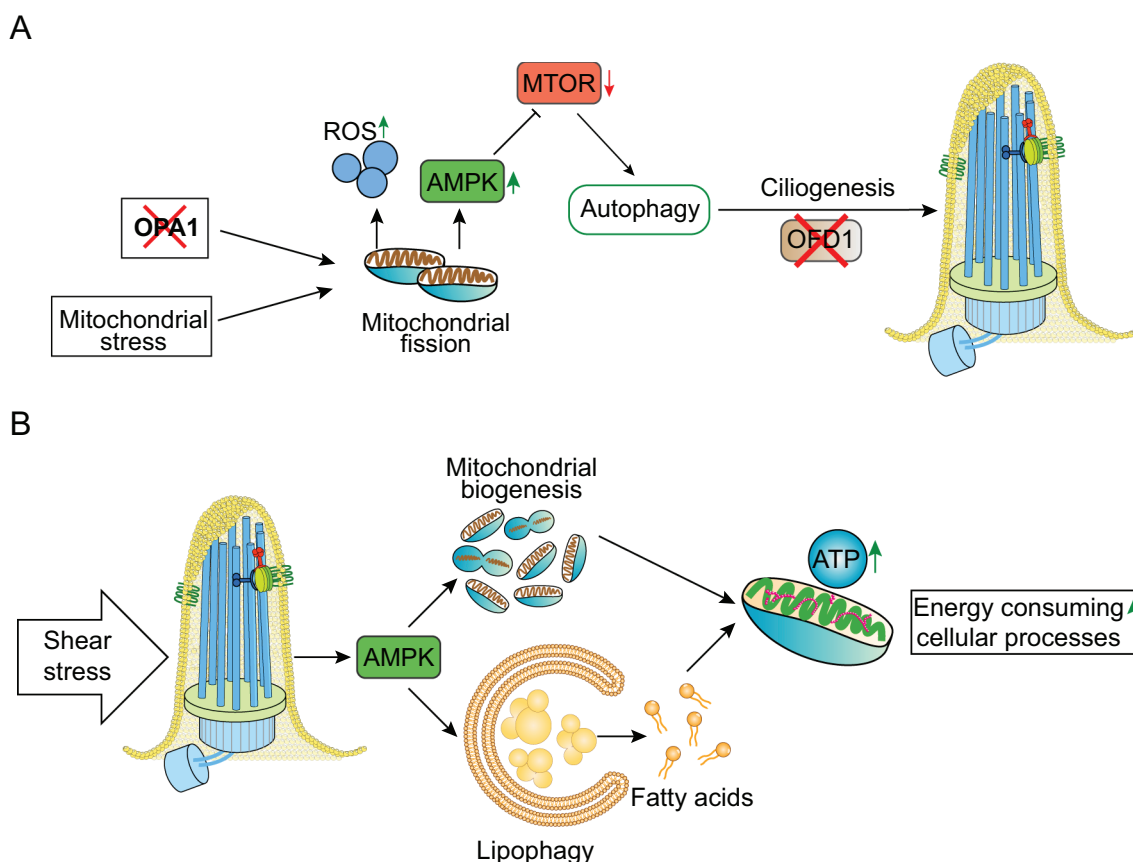


Figure 4. Mitochondria-autophagy-cilia crosstalk models. (a) Mitochondrial stress, such as depletion of the pro-fusion *OPA1* gene, and ROS generation stimulate autophagy by activating AMPK and inhibiting the MTOR pathway. Mitochondrial stress-induced autophagy acts as a cytoprotective process by promoting ciliogenesis through enhanced degradation of OFD1. (b) Shear stress induced by biological fluids stimulates AMPK-dependent selective degradation of lipid droplets by autophagy (lipophagy), contributing to the production of fatty acids that provide mitochondrial substrates to generate ATP and produce energy. Moreover, AMPK stimulates mitochondrial biogenesis, which contributes to ATP production. The concomitant mitochondrial biogenesis and lipophagy contribute to control ATP-dependent cellular mechanisms.

be associated with the reduction of mitochondrial metabolism and stimulation of autophagy (mitophagy), presenting increased levels of glycolysis, *i.e.*, the Warburg effect.

Autophagy- and cilia-related genetic diseases

Recently, cilia function and autophagy have been recently functionally linked with a possible impacts on human

health and disease [38,107]. At least some of the clinical manifestations observed in ciliopathies may be the result of perturbed autophagy and, conversely, cilia dysfunction could play a role in the increasing number of autophagy-related disorders.

Below, we present examples of pathways, genes and diseases associated with both cilia abnormalities and autophagic defects (Table 4).

Table 4. Ciliary- and autophagy-associated genes and human diseases.

GENES	DISEASES	OMIM	CHROMOSOMAL LOCALIZATION	INHERITANCE	GENE IDENTIFICATION/ ASSOCIATION TO DISEASES	REFERENCES AUTOPHAGY/CILIA
<i>OFD1</i>	Joubert syndrome type 10 (JBTS10)	300,804	Xp22.3-p22.2	XLR	[168]	[36,40,42,78,79]
	Oral-facial-digital syndrome 1 (OFD1)	311,200		XLD	[169,170]	
	Primary ciliary dyskinesia	300,933		XLR	[171,172]	
	Retinitis pigmentosa 23 (RP23)	300,424		XLR	[173]	
	Simpson-Golabi-Behmel syndrome type 2 (SGBS2)	300,209		XLR	[27,174]	
<i>PKD1</i>	Polycystic kidney disease 1 (ADPKD1)	601,313	16p13.3	AD	[175]	[67,118,119]
<i>PKD2</i>	Polycystic kidney disease 2 (ADPKD2)	173,910	4q22.1	AD	[176]	
<i>MTOR</i>	Focal cortical dysplasia type II, somatic	607,341	1p36.22	AD	[125]	[88,177–179]
	Smith-Kingsmore syndrome	616,638		AD		
<i>TSC1</i>	Tuberous sclerosis 1 (TSC1)	191,100	9q34.13	AD	[127]	[131–137]
<i>TSC2</i>	Tuberous sclerosis 2 (TSC2)	613,254	16p13.3	AD	[180]	
<i>INPP5E</i>	Joubert syndrome 1	213,300	9q34.3	AR	[181]	[141,144–146]
<i>OCRL1</i>	Lowe oculocerebrorenal syndrome	309,000	Xq26.1	XLR	[182]	[147–149]
<i>FLCN</i>	Birt-Hogg-Dube syndrome	135,150	17p11.2	AD	[183]	[30,156,157]

Oral-facial-digital syndrome 1 (OFD type I)

OFD type I syndrome is characterized by renal, hepatic and pancreatic cysts, skeletal defects, central nervous system involvement (malformations and intellectual disabilities) and orofacial abnormalities [26,108]. As discussed above, the OFD1 protein was recently shown to act as a novel autophagy receptor controlling autophagosome biogenesis [78]. In particular, it has been reported that dysregulated autophagy is associated with the pathogenesis of renal cystic disease in a mutant model of OFD type I syndrome in which autophagy modulation improves renal cystogenesis, suggesting that autophagy modulation might be a therapeutic target for renal cystic disease [36,78,79,109].

Autosomal dominant polycystic kidney disease (ADPKD)

PKD1 (polycystin 1, transient receptor potential channel interacting) and PKD2 localize to primary cilia in kidneys [110,111] where they are thought to form a complex that mediates mechanosensation through sensing cilia bending in response to fluid flow and triggering a calcium influx [112]. Mutations in *PKD1* or *PKD2* are associated with ADPKD, characterized by the cardinal manifestations of renal cysts, liver cysts, and intracranial aneurysm. In 2006, it was shown for the first time that the MTOR pathway, the major suppressor of catabolic processes such as autophagy, is inappropriately activated in renal cysts in ADPKD patients and murine models [113,114]. Treatment with the MTOR inhibitor rapamycin was shown to reduce renal cystogenesis in murine models [113,114]. However, treatment with sirolimus, an MTOR inhibitor, cannot halt polycystic kidney growth in ADPKD patients [115,116].

Several studies firmly linked PC1 and PC2 with autophagy regulation [67,117–119]. For example, in kidney epithelial cells and in response to shear stress, autophagy is stimulated not only by the MTORC1-AMPK pathway, as mentioned above, but also by calcium influx dependent on PC2 [67]. However, the role of PC2-dependent autophagy remains to be clarified since it is not involved in cell size regulation, as for MTORC1-AMPK dependent autophagy. Moreover, PC2 regulates autophagy independently of the location in the primary cilium (for a review see [120]).

In 2011, altered autophagy was demonstrated in rat and murine models of PKD [121]. Moreover, defective glucose metabolism in ADPKD has been linked with abnormal autophagy [122] and increased autophagy has been shown to improve the renal cystic phenotype in *pkd1* and *pkd2* deficient zebrafish models [123]. The hypothesis that suppression of autophagy may play a role in cyst formation and growth in ADPKD [124] has been put forward. However, the experimental evidence to support the role of autophagy in PKD remains limited and controversial.

Focal malformations of cortical development (FMCDs)

Brain somatic mutations in *MTOR* gene are associated with FMCDs, which are major etiologies of pediatric intractable epilepsies exhibiting cortical dyslamination [125]. Park *et al.*

showed that neuronal ciliogenesis is defective in FMCDs due to loss of autophagy-mediated degradation of the centriolar satellite portion of the OFD1 protein [126]. These findings were confirmed in murine models and in brain tissues from patients. Furthermore, the authors demonstrated that disruption of autophagy-mediated ciliogenesis leads to FMCD by abrogating Wnt signaling transduction [126].

Tuberous sclerosis complex (TSC)

TSC is an autosomal dominant multisystemic disease caused by mutation in either the *TSC1* [127] or *TSC2* [128] tumor suppressor genes and characterized by a variety of symptoms such as nonmalignant brain or heart tumors, kidney and lung cysts, as well as neurocognitive defects (reviewed in [129]). The *TSC1* and *TSC2* gene products, also known as hamartin and tuberin respectively, form a stable functional complex *in vivo* [130] and impact MTOR mediated downstream signaling [131,132], including autophagy [133,134]. Indeed, it was shown in several studies that *TSC1* and *TSC2* regulate primary cilia length, structure and function [135–138] (reviewed in [139]). Additionally, a recent study demonstrated that brains of TSC patients and murine models have reduced ciliated neurons and that MTORC1 activity inversely correlates with ciliation in *TSC1*- and *TSC2*-deficient neurons, which is restored by rapamycin treatment. The authors also identified inhibitors of the heat shock protein that suppress MTORC1 activity and rescue ciliation, leading to the identification of the heat-shock machinery as a druggable signaling node to restore MTORC1 activity and cilia impairment due to loss of *TSC1* and *TSC2* [140].

Joubert syndrome (JBTS)

Joubert syndrome is a ciliopathy characterized mainly by a distinctive cerebellar and brain stem malformation called the molar tooth sign, hypotonia, retinal involvement and developmental delay. *INPP5E*, which is one of the genes mutated in JBTS [141] encodes an PtdIns(1,4,5)P₃ 5-phosphatase localized to primary cilia, where it controls the ciliary PIPs, which impacts on the proper trafficking of primary cilia-associated signaling proteins and HH transduction. As a consequence of *INPP5E* inactivation, cells display shorter cilia [141] and suppression of the cilia-mediated HH signaling pathway [142,143]. Hasegawa *et al.* demonstrated that *INPP5E* also localizes to lysosomes and is required for autophagosome-lysosome fusion in a cilia-independent manner [144]. Similarly, the same authors showed that patients with *INPP5E* mutations affecting the phosphatase activity of the enzyme are associated with impaired autophagy [144]. Future studies should focus on determining whether autophagy defects underlie some of the clinical manifestations observed in JBTS.

Lowe oculocerebrorenal syndrome and dent-2 disease

OCRL/OCRL1 encodes a PtdIns(4,5)P₂ 5-phosphatase, which localizes to basal bodies and along axonemes, and plays a role in cilia biogenesis [145]. *OCRL* is mutated in Lowe

oculocerebrorenal syndrome and Dent-2 disease, two rare X-linked conditions [146] in which patient fibroblasts display shorter cilia [145,147,148]. De Leo and colleagues demonstrated that OCRL plays a direct functional role in autophagy, since it is recruited to lysosomes and is required for autophagosome-lysosome fusion [149]. Moreover, loss of OCRL catalytic activity causes accumulation of autophagosomes and lysosomal dysfunction in cells isolated from Lowe syndrome patient cells [149].

Birt-Hogg-Dubé (BHD) syndrome

FLCN (folliculin) gene mutations are associated with BHD syndrome, which predisposes patients to benign hair follicle tumors, pneumothorax, and renal cancer. *FLCN* encodes a GTPase-activating protein (GAP) localized on lysosomal membranes that plays a key role in the cellular response to amino acids through the MTORC1 signaling cascade [150–154]. *FLCN* GAP activity is inhibited during starvation and activated in the presence of nutrients, stimulating MTORC1 [155]. In addition, *FLCN* promotes autophagy following phosphorylation by ULK1 and physical interaction with GABARAP [156]. In clear cell tumors from a BHD patient, loss of *FLCN* was shown to increase GABARAP and LC3 puncta, as well as SQSTM1/p62 levels, demonstrating that autophagy is impaired in BHD-associated renal tumors. Besides its role in nutrient sensing and autophagy, *FLCN* also localizes to primary cilia and its inactivation results in impaired ciliogenesis and dysregulation of cilia-dependent WNT and PCP signaling pathways [30]. Recently, Zhong and colleagues showed that *FLCN* is part of a flow sensory mechanism that regulates MTORC1 through primary cilia [157]. In response to flow stress, *FLCN* recruits STK11/LKB1 to primary cilia for the activation of AMPK localized to basal bodies, resulting in MTORC1 downregulation. In cells depleted for *FLCN*, STK11/LKB1 fails to accumulate in primary cilia and AMPK at the basal bodies remains inactive, thus nullifying the inhibitory effect of flow stress on MTORC1 activity [157].

Other conditions associated with autophagic defects and possibly involving also cilia dysfunction include Huntington disease (HD) (reviewed in [81,158–160] and disorders associated with mutations in *PTEN* (phosphatase and tensin homolog) gene [161–164]. However, for these diseases a clear causal relation with cilia dysfunction has not been clearly established.

Conclusions and future perspectives

Experimental evidence suggests that primary cilia represent a specialized site for effective autophagy control and that selective autophagic degradation removes positive and/or negative regulators of ciliogenesis, thus influencing cilia formation and elongation.

However, contradictory findings have been reported since autophagic degradation can exert an opposite role on cilia biology in a context-dependent manner. One critical point

would be to establish standard culture conditions and methodologies to compare results from different laboratories worldwide. Moreover, the picture is getting more complex as new players have arisen, such as mitochondria and lysosomes, although our knowledge of them is still very limited.

The list of genes associated with ciliopathies or ciliary dysfunction and encoding proteins controlling autophagy keeps growing, suggesting far-reaching implications for cilium- and autophagy-related disorders. It is tempting to speculate that modulation of autophagy might represent a potential therapeutic opportunity to treat ciliopathies and, conversely, analysis of ciliogenesis might represent a good readout for therapeutic approaches involving modulators of autophagy in diseases not currently associated with primary cilia dysfunction [38].

Disclosure statement

The authors declare that they have no competing interests.

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ORCID

Manuela Morleo  <http://orcid.org/0000-0002-7553-3245>

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