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Review

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Autophagy and Autosomal Dominant Polycystic Kidney Disease

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ABSTRACT

Autophagy is a protective mechanism that ensures cell survival. The mammalian target of rapamycin is the main regulator of autophagy, and mammalian target of rapamycin activation suppresses autophagy. Mammalian target of rapamycin inhibitors, like sirolimus, activate autophagy. Disorders in autophagy regulation are of central importance in many pathophysiological conditions. In the kidney, autophagy is dysregulated in autosomal dominant polycystic kidney disease, acute kidney injury, podocytopathies, transplant rejection, cold preservation ischemia, kidney aging, glomerular disease, and diabetic nephropathy. There are reasons to suspect that autophagy is dysregulated in autosomal dominant polycystic kidney disease. Pkd1 and 2 genes can control autophagy. There is abnormal autophagy in Pkd1 cells and polycystic kidney disease. Mammalian target of rapamycin inhibitors that activate autophagy slow cyst growth. Evidence emerging in polycystic kidney disease cells and polycystic kidney disease animal models shows that direct autophagy inhibition/activation affects cyst growth. The review will focus on the autophagy process, pathways that regulate autophagy, autophagy and kidney pathophysiology, and autophagy and autosomal dominant polycystic kidney disease.

Keywords: Clinical nephrology, autophagy, autosomal dominant polycystic kidney disease

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INTRODUCTION

What Is Autophagy?

Christian de Duve first described autophagy as "the degradation that occurs after the delivery of cytoplasmic materials to the lysosome." However, the acceleration of studies in this field occurred in the 1990s. In these years, Yoshinori Ohsumi et al.'s identification of a gene (autophagy-related gene (ATG)) and its protein belonging to autophagy-defective mutant yeast fungi is considered the first significant discovery in this field. Yoshinori Ohsumi,[1] who later continued his studies on autophagy, was granted the 2016 Nobel Prize in Medicine and Physiology "for his contributions to our understanding of the autophagic mechanisms operating in the cell under conditions of hunger, disease, and stress."

CLINICAL AND RESEARCH CONSEQUENCES

Autophagy is a dynamic balance and a highly evolutionarily conserved mechanism vital in maintaining cell homeostasis. The term originated from the ancient Greek words auto: self and phaji: eating (autophagy: self-eating). Autophagy is a catabolic process that provides the necessary energy and building blocks to the cell during fasting. However, autophagy has been shown to establish complex anabolic relationships with apoptosis and cell cycle control due to its close relationship with synthesis and degradation processes.[2] It is now accepted that autophagy is a protective mechanism that ensures cell survival and that disorders in autophagy regulation are of central importance in many pathophysiological conditions[2] (Table 1). In addition to kidney diseases,[3] there is evidence of defective autophagic signaling in many conditions, including neurodegenerative

conditions, muscular disorders, infectious and autoimmune events, and cardiovascular and pulmonary diseases.[4] Similarly, there is evidence that pathophysiological conditions such as infective, toxic, ischemic, oxidative stress, circadian rhythm, and aging are closely associated with defective autophagy. In addition, autophagy is critical in removing toxic substances from cells in the case of intracellular stress (starvation and growth-factor deprivation).[5]

Autophagy is a lysosomal degradation operation. Thanks to autophagy, intracellular wastes formed in the cell under physiological or pathological conditions, intracellular organelles, especially mitochondria, which show loss of function or dysfunction, are removed from the environment and separated into building blocks in lysosomes. Thus, these residues that can damage the cell and organelles that may play a role in initiating a series of processes that will result in destruction, including apoptosis, are removed from the environment. Thus, damaged organelles are recycled inside the cell and used as raw materials to synthesize other molecules[4] (Figure 1).

In cells that have entered the autophagic process, damaged organelles are first separated into double-membrane autophagosomes for degradation and recycling, and then these autophagosomes fuse with lysosomes. Three different types of autophagy have been described in detail.[5]

1. **Macroautophagy:** It is found in almost all eukaryotic cells. Double-membrane autophagosomes containing cytoplasmic components, damaged organelles, and their cargo fuse with lysosomes for breakdown and recycling.
2. **Microautophagy or endosomal microautophagy:** The cytoplasmic load to be degraded is caught straight by the invagination of the vacuolar membrane.
3. **Chaperone-mediated autophagy:** In this type of autophagy, vesicle or membrane invagination is not needed for substrate carriage to lysosomes. The cytosolic proteins to be degraded are taken into the lysosome via a special protein translocation complex localized into the lysosomal membrane. In addition, other types of autophagy have been described for other specific types of organelle or substrates, such as mitochondria (mitophagy), peroxisomes (pexophagy), endoplasmic reticulum (reticulophagy), protein aggregates (aggrephagy), lipid droplets (lipophagy), and inactive proteasomes (proteaphagy).

MAIN POINTS

- Autophagy is an evolutionarily conserved mechanism that ensures cell survival.
- Pkd genes can control autophagy.
- Defects in autophagic flux have been reported in polycystic kidney disease (PKD).
- The therapeutic effect of autophagy-inducer agents in PKD is controversial.

Table 1. Diseases Associated with Autophagy

Adult neurodegenerative disorders	Parkinson's disease
	Amyotrophic lateral sclerosis
	Frontotemporal dementia
	Neuronal ceroid lipofuscinosis
	Fulminant neurodegeneration
	Dementia with Lewy bodies
Kidney diseases	ADPKD, AKI, podocytopathies, transplant rejection, cold preservation ischemia, kidney aging, glomerular disease, diabetic nephropathy kidney surgical procedures
Hereditary neuropathies	Sensory and autonomic neuropathy type II
	Charcot-Marie-Tooth disease
	Distal hereditary motor neuronopathy
Ophthalmological diseases	Primary open-angle glaucoma
	Cataracts
Cardiac and skeletal myopathies	Danon's cardiomyopathy
	Distal myopathy with rimmed vacuole
	Dilated cardiomyopathy
	Sporadic inclusion body myositis
Inflammatory disorders	Crohn's disease
	Ulcerative colitis
Autoimmune diseases	Systemic lupus erythematous
	Diabetes
Infectious diseases	*Mycobacterium tuberculosis*
	Mycobacterium leprae
Cancer	Breast cancer
	Colorectal cancer
	HBV-related hepatocellular carcinoma
	Hematopoietic malignancies

ADPKD, autosomal dominant polycystic kidney disease; AKI, acute kidney injury; HBV, hepatitis B virus.

The first stage of autophagy is the ingestion of the damaged organelle or intracellular debris into a vesicle called a phagophore. This vesicle, which is initially cup-shaped, elongates over time (elongation) and matures into a spherical phagosome with a double-layered membrane (maturation). The structures that need to be removed (damaged organelles, cytoplasmic waste, etc.) are trapped in this spherical phagosome. This phagosome then fuses with lysosomes in the cytoplasm (fusion) to form autophagolysosomes where phagosome contents are degraded (degradation)[5] (Figure 1).

The most accepted measure of monitoring the intracellular autophagic processes is "autophagic flux." There are 2 possible explanations if an increase in autophagosomes is detected in

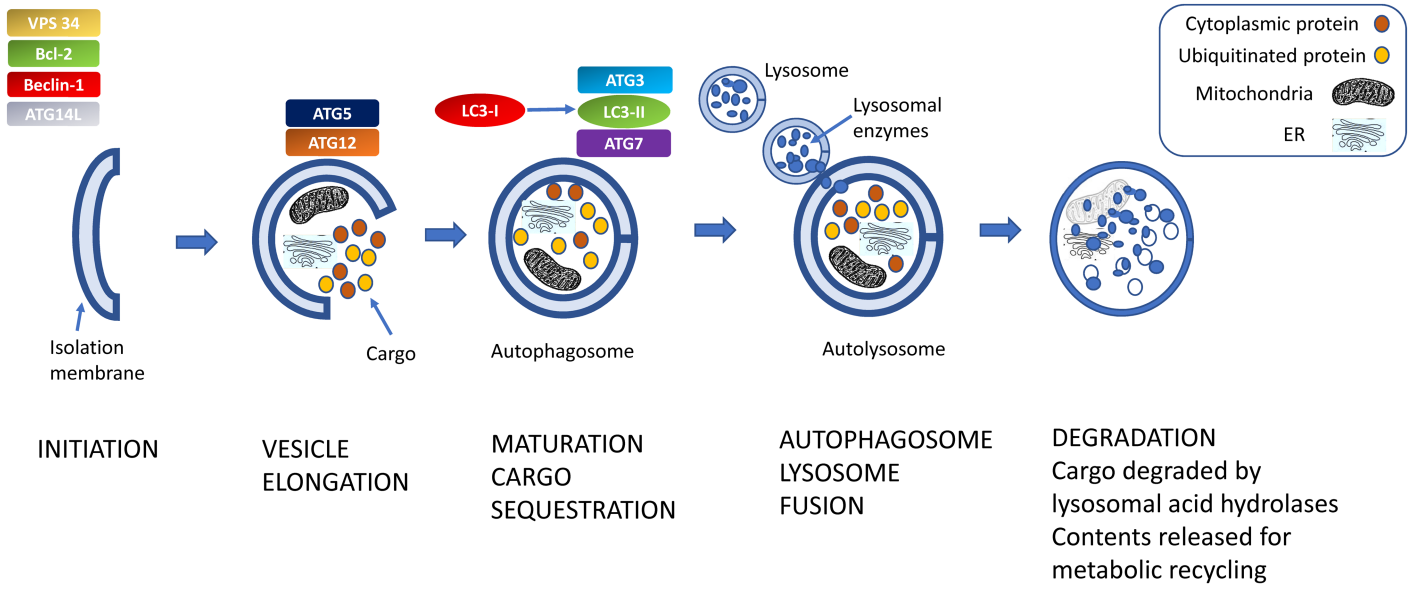

Figure 1. Stages of the autophagic pathway (essential autophagy proteins for each stage are shown in brackets). ATG, autophagy-related gene; BcL-2, B-cell lymphoma 2; ER, endoplasmic reticulum; LC3, microtubule-associated protein 1A/1B light chain 3; VPS34, Class III phosphoinositide 3-kinase

the cell. Either the production of autophagosomes is increased or the lysosomal clearance of autophagosomes is decreased. A guide to using and interpreting autophagic processes was published in 2016.[6] Evaluation of autophagic flux mandates measurement of autophagosomes, for example, microtubule-associated protein 1A/1B-light chain 3-II (LC3-II), an autophagosome marker in existence and lack of lysosomal inhibition. If an increase in LC3-II, a protein major in the autophagy pathway, is due to raised autophagosome production, then a lysosomal inhibitor, for example, bafilomycin A1 or chloroquine will further boost LC3-II levels. However, if the rise in LC3-II is due to a blockade of autophagic flux due to a lysosomal defect, there will be no change in LC3-II levels after lysosomal inhibition.[6] This technique is used to measure "autophagic flux."

KEY PATHWAYS THAT REGULATE AUTOPHAGY

Autophagy-related genes (ATGs) are of central importance in regulating autophagic processes. More than 30 different ATGs have been discovered so far.[7] Activation or inhibition of the autophagic cascade is mediated by the phosphorylation or dephosphorylation of ATG proteins, primarily via the mTOR pathway.[8] The mammalian target of rapamycin phosphorylates the autophagy regulatory complex containing unc-51-like kinase 1 (ULK1), the mammalian ATG13 protein, and focal adhesion kinase interacting protein 200 kD (FIP200). Under normal conditions, phosphorylation of the ULK1/2-mATG13-FIP200 complex functions as rate limiting in autophagic processes. However, mTOR is blocked in intracellular stress states such as cellular starvation and growth-factor deficiency. The result is ULK1 dephosphorylation. This dephosphorylation initiates processes that phosphorylate FIP200. With FIP200 phosphorylation, several ATGs (ATG3, ATG7) and mediator proteins (Beclin1, VPS34), and ubiquitin-like enzymes mediate the lipidation of LC3 with phosphatidylethanolamine (PE) to form LC3-PE. Several ATGs (ATG5-ATG12-ATG16L1 complex and VMP1-ATG9) are responsible for the elongation of the phagosome and sequestration of its cargo. The final mechanisms of autophagy are less well understood.[8] However, recent evidence reveals that the mTOR pathway also has an important role in autophagy termination.[8]

MAMMALIAN TARGET OF RAPAMYCIN SIGNALING AND AUTOPHAGY

Mammalian target of rapamycin, a serine/threonine kinase, is important in maintaining mammalian homeostasis by regulating cell survival, cell growth, protein synthesis, autophagic processes, and transcription mechanisms. Mammalian target of rapamycin functions as 2 different chemical and functional complexes in the cell, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Mammalian target of rapamycin complex 1 is formed by mTOR and the regulatory-related mTOR protein (Raptor), while mTORC2 is formed by mTORC2 and the rapamycin-independent companion of mTOR (Rictor). Activation of the mTORC1 pathway induces proliferation and suppresses apoptosis and autophagy.[9]

Although both components of the phosphatase mTOR complex (TORC1 and TORC2) have essential roles in regulating autophagy, the role of TORC1 has been most elucidated. The most important substrates of TORC1 in autophagic processes are ATG13 and ULK1. Initiation of autophagy requires these 2 molecules to be dephosphorylated. Under nutrient-rich conditions, the ULK1 complex results in the phosphorylation of ULK1 and ATG13 while directly binding mTORC1 via the Raptor subunit. Under starvation conditions, ULK1 is dephosphorylated

and dissociated from mTORC1 to phosphorylate ATG13 and FIP200 and accumulates at the specific endoplasmic reticulum site to initiate autophagosome formation.[10]

The function of the mTORC2 pathway in autophagic flux is less well-defined. Unlike mTORC1, mTORC2 can stimulate autophagic processes. Knockout of mTORC2 and downstream serum and glucocorticoid-inducible kinase 1 potently enhances both autophagy and autophagic degradation of mitochondria in *Caenorhabditis elegans*.[11]

AUTOPHAGY AND KIDNEY PATHOPHYSIOLOGY

Autophagy is critical in the regulation of kidney homeostasis. The main cell groups where autophagic pathways operate are mesangial cells, podocytes, tubular cells, and interstitium.

Autophagy in Mesangial Cells

Autophagy appears to be a mechanism that protects mesangial cells from apoptosis. There is evidence that autophagy is activated in mesangial cells exposed to advanced glycation products (AGEs), which have an essential function in the pathogenesis of diabetic nephropathy. Autophagy reduces the harmful effects of AGEs on mesangial cells. Inhibition of ATG5 has been shown to increase mesangial toxicity of AGEs.[12]

It has been shown that there is a relationship between kidney aging and the deposition of AGEs. In addition, Janus kinases/signaling transducers and activators of the transcription (JAK/STAT) pathway are related to a rise in cellular senescence.[13] Advanced glycation products tie to their specific receptors (RAGE) on mesangial cells and activate autophagy through the Akt/mTOR pathway.[14] A study investigating the relationship between senescent mesangial cells and autophagy and the role played by STAT5 in this relationship reported higher levels of pSTAT5 and RAGE in aged kidneys. The autophagy markers LC3-II and LC3-II/I and autophagic vacuoles were significantly higher in younger subjects than in older ones. Compared with the control group, senile mesangial cells treated with AGE had higher levels of RAGE and pSTAT5. However, autophagy indices were partially restored by the inactivation of STAT5 by losartan or shRNA silencing.[15]

Autophagy in Podocytes

Autophagy is also vital for podocytes. Rat podocytes have been shown to harbor large autophagosomes even in basal conditions.[2] It has been reported that severe proteinuria, glomerulosclerosis, and kidney failure developed in mutant rats for the Vps34 gene, one of the proteins regulating autophagy.[2] Damage to the ATG5 gene results in podocyte loss and glomerulosclerosis.[16] Disruptions in the balance of the autophagic cycle result in podocyte damage. Mice having a podocyte-specific knockout of the mTOR gene were shown to develop proteinuria and kidney failure at 3 weeks postnatally. Podocytes from these mice showed accumulation of LC3 as well as autophagosomes, autophagolysosomal vesicles, and damaged mitochondria. In addition, similar findings were found in podocytes treated with rapamycin, a pharmacological mTOR inhibitor. These findings suggest that although mTOR inhibition activates autophagy, increased autophagic processes result in autophagic vesicle accumulation and may cause cell toxicity.[17]

Autophagy in Tubules

Genetic deletion of ATG5, one of the important regulatory proteins of autophagy, results in severe proximal tubule damage.[18] Proximal tubule cells are susceptible to drugs/substances such as cisplatin, cyclosporine, and cadmium. It has been shown that by autophagy activation, tubule cells can quickly clear organelles and DNA fragments damaged by these agents and protect themselves from apoptosis.[19] Severe tubular damage was observed in mice lacking the ATG5 and ATG7 genes in models of ischemia-reperfusion injury.[20] Another study showed that autophagosome inhibitors cause severe kidney failure in mice, and this situation was reversed with rapamycin, an mTOR inhibitor.[21]

AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE AND AUTOPHAGY

Autosomal dominant polycystic kidney disease (ADPKD), the most common inherited kidney disease, develops due to mutations in the Pkd 1 or 2 gene. Mutations in the Pkd1 gene are responsible for 85% of cases, and mutations in the Pkd2 gene for the remaining 10-15%. The products of these genes, polycystin 1 (PC1) and polycystin 2 (PC2), are localized in the primary cilia of kidney tubule epithelial cells and function as an ion pump that regulates intracellular calcium influx. The flow effect of the intratubular filtrate results in the bending of the cilia. As a result of the conformational change resulting from this bending, calcium channels formed by PC1 and PC2 open, and intracellular calcium levels increase.[5] However, with mutations in the Pkd gene, the calcium channel structure is disrupted, and the bending of the cilia by the flow of tubular fluid results in an insufficient opening of the calcium channels. The result is decreased cilia-mediated intracellular calcium flux, resulting in phosphorylation-mediated activation of multiple proliferative cascades and cyst formation and growth.[5]

There are many causes to suspect that autophagy may be erratic in ADPKD (Table 2).

Pkd1 and 2 Genes Can Control Autophagy

Pkd proteins play essential roles in the initiation of autophagy and the advanced stages of autophagy. Lysosomal-associated membrane protein (LAMP) degradation, lysosomal acidification, and impaired cathepsin B processing and activity were detected in kidneys from Pkd1 knockout mice. This lysosomal dysfunction resulted in impaired autophagosomal-lysosomal fusion and reduced ubiquitin cargo delivery from multivesicular bodies/exosomes to lysosomes. The defect in lysosomal acidification and lysosomal biogenesis was due to the breakdown of lysosomal proteins. Calpain, a calcium-dependent cysteine

Table 2. Reasons to Suspect That Suppressed Autophagy May Play a Role in Cyst Growth in ADPKD
Pkd1 and 2 genes can control autophagy
There is suppressed autophagy in Pkd1 cells and PKD kidneys
Autophagy activators can decrease cyst growth
There is a link between autophagy, cilia, and cyst growth
Direct autophagy inhibition/activation affects PKD

ADPKD, Autosomal dominant polycystic kidney disease; PKD, polycystic kidney disease.

protease, is responsible for this degradation, yielding lysosomal membrane permeability and loss of lysosomal function by degrading LAMP1 and LAMP2. Thus, Pkd1 affects lysosomal functions that are responsible for accurate autophagic flux.[22]

Mitochondrial damage is associated with abnormalities in PC1 expression. Morphological analysis of cardiomyocyte mitochondria of heterozygous PC1 mice (Pkd1+/−) was compatible with mitochondrial fission. PC1 knockdown human-induced pluripotent stem cells (iPSC) and cultured rat cardiomyocytes had lesser functional mitochondria (reduced mitochondrial respiration, mitochondrial membrane potential, and ATP production). PC1 silencing caused deregulated mitochondrial dysfunction by the AKT-FoxO1 signaling pathway.[23] Pkd2 modulates AMP-activated protein kinase (AMPK) and mTOR activity by interacting with RyR2 to alter Ca2+ release from the sarcoplasmic reticulum in glucose-starved cardiomyocytes, inducing autophagy and protecting cardiomyocytes from apoptotic cell death.[24]

Pkd proteins are directly implicated in the regulation of autophagy. Pkd2/PC2/TRPP2, a non-selective Ca2+ permeable cation channel belonging to the family of transient receptor potential channels (polycystin 2, transient receptor potential cation channel), appears to be required for autophagy. It has been reported that Pkd2 is a scaffold that enters into a physical and functional relationship with beclin1 (BECN1), one of the critical molecules of autophagy, with one of the 2 CCD domains located in its carboxy-terminal tail. It appears to be dependent on intracellular Ca2+ mobilization by Pkd2.[25]

PC2 is a novel autophagy regulator acting to control intracellular Ca2+ homeostasis. A recent study determined that a calcium trafficking disorder caused by defects in Pkd2 proteins also disrupts autophagic flux in cardiomyocytes. In PC2-deficient cells where autophagy is activated pharmacologically with sirolimus or via nutrient depletion, the autophagy flux was blunted by intracellular Ca2+ chelation.[26]

Unusual interaction between PC1 and PC2 has recently been described, with autophagic processes at their core. PC1 dose-dependently down-regulates PC2 expression via its C-terminal helix domain, regulating PC2 expression in vitro and in vivo. The histone deacetylase 6 (HDAC6) enzyme, which transports proteins not breakdown by proteasomes and precipitated in the cytoplasm to aggresomes, ties to PC2. Interestingly, PC1 overexpression speed up the transport of non-PC1-dependent PC2-HDAC6 complex to aggresomes, leading to increased breakdown of PC2 via autophagy.[27]

There Is Abnormal Autophagy in Pkd1 Cells and PKD Kidneys

The first proof of autophagosomes in PKD kidneys was shown in 2011. In this study, autophagosomes, autolysosomes, and mitophagy were seen in cells surrounding cysts in polycystic kidneys in both Han: SPRD rats and congenital polycystic kidney (cpk) mice. Specific to the Han: SPRD rat, autophagosomes were detected by electron microscopy in the tubular cells lining the cysts. Immunofluorescence examination revealed an increase in LC3-II in epithelial cells lining the cysts.[28]

There is disruption of autophagic flux in both human and mouse Pkd−/− cell cultures. Autophagic flux disruption, mTOR activation, and cystic kidneys develop in zebrafish carrying the pkd1 mutation orthologous to the mammalian Pkd1 gene. In Pkd1−/− cells, no increase in the expression of LC3-II, one of the most significant markers of autophagic flux, was detected after lysosomal inhibition, indicating a significant defect in autophagic flux.[29] Consistent with this finding, by immunofluorescence, the count of autolysosomes and autophagosomes in Pkd1−/− cells are both low in basal conditions and cannot be increased by lysosomal inhibitor treatment.[29] Cpk mice and Han: SPRD rats show increased expression of LC3-II, possibly resulting from a reduced autophagosome-lysosome fusion. The expression of LC3II, the marker of autophagy that is expected to be activated in response to glucose deprivation, is not activated in Pkd1 knockout mouse embryonic fibroblasts.[28,30] In a Pkd1-hypomorphic mouse model (Pkd1 miRNA transgenic mice), kidney expression of ATG12, ATG3, beclin1, and p62 genes was significantly lower than in wild-type controls.[31,32]

Autophagy Activators Can Decrease Cyst Growth

Activating the mTORC1 pathway results in decreased apoptosis and increased proliferation while suppressing autophagy. The mTORC1 pathway is activated in cyst-lined epithelial cells in ADPKD patients and 3 different mouse models. $Pkd1^{RC/RC}$ [33,34] and $Pkd2^{WS25/-}$ mice have increased mTORC1 (4E-BP1) and mTORC2 (p-Akt Ser473) signaling in the kidney.[35]

There is strong evidence for mTOR activation in human PKD kidneys. In liver and kidney tissues from autosomal recessive polycystic kidney disease (ARPKD) patients, it was shown that molecules such as S6K, which indicate that the mTOR pathway is active, are strongly expressed in kidney cyst lining cells and bile ducts.[36] In another study, overexpression of molecules such as Akt and S6K, implying that the mTOR pathway is active, was detected in human kidney samples from ARPKD.[37] These studies suggest that mTOR inhibitors may be effective in ADPKD. Human and animal studies conducted with molecules such as

curcumin, triptolide, roscovitine, metformin, and tolvaptan, whose autophagy-inducing effects are known, can slow cyst growth.[5] The suppressive effect of autophagy induction on apoptosis and cell proliferation may reduce cyst growth.[38-40]

Under normal conditions, intracellular mTOR activation is known to suppress autophagy. Similarly, mTOR inhibitors are strong autophagy activators in many other cell subsets.[41] Rapamycin, a drug that inhibits the mTOR complex, has been found to reduce cyst size in experimental animal models.[42-44] Indirect or direct-acting autophagy activators such as cyclin-dependent kinase inhibitors, mTOR inhibitors, tyrosine kinase inhibitors, metformin, caspase inhibitors, triptolide, and curcumin have been shown to provide varying levels of protection against PKD.[5]

Many molecules have the potential to restore the dysregulated autophagic flux in PKD. However, in a study investigating the effect of trehalose, an autophagy-inducing disaccharide independent of mTOR, on autophagy-related proteins and cyst growth, no effects were found on kidney function and cyst growth.[45]

There Is a Link Between Autophagy, Cilia, and Cyst Growth

As mentioned earlier, PC1 and PC2 are localized to the cilia. The link between cilia and mTOR signaling has also been partially elucidated. A recent study showed that primary cilia manage mTORC1 activity through the tumor suppressor protein liver kinase B1.[46] Similarly, cilia abnormalities are associated with increased mTOR activity in PKD. These findings explain a significant rise in mTOR activity in 3-week-old cilia knockout mice.[47] An interaction between autophagy and cilia via mTOR signaling and the ubiquitin-proteasome system has been reported. In this study, autophagy was suppressed in cilia-suppressed cell lines, and this situation was reversed with rapamycin, an mTOR inhibitor. Consistent with this finding, in human kidney proximal tubular cells, autophagy activation caused cilia elongation, whereas autophagy inhibition by 3-methyladenine, chloroquine, or BAF1 resulted in cilia shortening. In cells in which autophagy was blocked genetically (ATG5 or ATG7 knockout), a shortening of cilia was detected. Restoration of cilia length in ATG5-knockout cells with a proteasome inhibitor suggested that cilia and autophagy are mutually regulated via the mTOR signaling pathway and the ubiquitin-proteasome system.[48]

Healthy ciliogenesis and optimal cilia functions are autophagy-dependent processes.[49] It has been shown that autophagy directly stimulates ciliogenesis.[50] Some signals from cilia directly induce autophagy. Autophagic processes regulate the growth of cilia by providing the degradation of proteins required for intraciliary transport.[51]

Autophagy Inhibition/Activation Affects Polycystic Kidney Disease

The central importance of autophagy in PKD was found when examining kidney plasticity in a mouse model that allows both Pkd genes to be inactivated and activated. By activating the gene, the cysts regressed rapidly, and inflammation and cystic cell proliferation regressed, while autophagy activation was detected.[52]

Studies in both Pkd1−/− cell and Pkd1−/− zebrafish models have revealed a close connection between autophagy and apoptosis. Cystic kidneys in pkd1 mutants have mTOR activation, increased apoptosis, and impaired autophagic flux. Autophagy inhibition by knockdown of ATG5, one of the significant autophagy genes, increases cystogenesis , while autophagy activation using Beclin-1 peptide reduces cysts. In addition, autophagy activation by both mTOR-independent (minoxidil and carbamazepine) and mTOR-dependent (sirolimus) techniques reduces cyst formation.[29] Saicosaponin-d, a sarcoplasmic/endoplasmic reticulum Ca2+ ATPase pump inhibitor, can repress proliferation in ADPKD through intracellular inhibition of mTOR signaling, calcium deposition, and activation of the calcium/calmodulin-dependent protein kinase β-AMPK, and activation of autophagy. Treatment with an AMPK inhibitor (Compound C), autophagy inhibitor (3-methyladenine), an intracellular calcium chelator (BAPTA/AM), or CaMKKβ inhibitor (STO-609) reverses the impacts of Saicosaponin-d.[53] Similarly, in vitro models of ADPKD have reported positive effects of trichostatin A, a histone deacetylase inhibitor, in preventing cyst formation and inducing autophagy.[54] MicroRNA studies have also demonstrated dysregulation of autophagic flux in PKD. Inhibition of miR-25-3p in PKD mice increased LC3-II/LC3-I ratio and Ulk1 expression while reducing cyst size in kidney tissues. These findings are evidence that inhibition of miR-25 activates autophagy. Moreover, inhibition of miR-25-3p not only suppressed autophagy but also suppressed the proliferation of kidney cells. Silencing ATG14 canceled the inhibitory effect of the miR-25-3p inhibitor on kidney cell proliferation, suggesting that the effect of miR-25-3p in PKD cells was due to the inhibition of ATG14 expression.[55]

Metformin reduces pronephric cyst formation by 42%-61% compared to untreated controls in a zebrafish model of Pkd2 deficiency, in part through induction of autophagy. Metformin also suppresses cell proliferation in pronephric ducts, phosphorylation of adenosine monophosphate-activated protein kinase, and activates autophagy.[56]

Although many studies describe the defects in autophagic flux in PKD and report the beneficial effects of autophagy-inducing agents, studies that contradict this hypothesis have been published recently.

A recent study examined mTOR and autophagic pathways in a rodent model with an aggressive PKD course in which Ift46 is deleted specifically in kidney collecting duct cells. Although hyperactivation of mTOR signaling and subsequent inhibition of autophagy flux was observed in these mice, autophagy induction (starvation and LC3 overexpression) was ineffective

in reducing cyst growth. This detrimental effect was related to increasing Erk phosphorylation by LC3, which is associated with PKD progression.[57]

In conclusion, Pkd genes can control autophagy. Mammalian target of rapamycin inhibitors that are known to suppress autophagy slow cyst growth, but whether this effect to slow cyst growth is mediated by suppressed autophagy is not known. Knockout of autophagy slows cyst growth in Pkd cells and zebrafish. Autophagy knockout in mouse models of PKD has shown contradictory results. The "jury is still out" as to whether autophagy is good or bad in in vivo models of PKD and in humans.

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REFERENCES

1. Ohsumi Y. Historical landmarks of autophagy research. *Cell Res*. 2014;24(1):9-23. [CrossRef]
2. Wang Z, Choi ME. Autophagy in kidney health and disease. *Antioxid Redox Signal*. 2014;20(3):519-537. [CrossRef]
3. Huber TB, Edelstein CL, Hartleben B, et al. Emerging role of autophagy in kidney function, diseases and aging. *Autophagy*. 2012;8(7):1009-1031. [CrossRef]
4. Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med*. 2013;368(7):651-662. [CrossRef]
5. Nowak KL, Edelstein CL. Apoptosis and autophagy in polycystic kidney disease (PKD). *Cell Signal*. 2020;68:109518. [CrossRef]
6. Klionsky DJ, Abdelmohsen K, Abe A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy*. 3rd ed. 2016;12(1):1-222. [CrossRef]
7. Motley AM, Nuttall JM, Hettema EH. Pex3-anchored Atg36 tags peroxisomes for degradation in Saccharomyces cerevisiae. *EMBO J*. 2012;31(13):2852-2868. [CrossRef]
8. Choi ME. Autophagy in kidney disease. *Annu Rev Physiol*. 2020;82(1):297-322. [CrossRef]
9. Kim HJ, Edelstein CL. Mammalian target of rapamycin inhibition in polycystic kidney disease: from bench to bedside. *Kidney Res Clin Pract*. 2012;31(3):132-138. [CrossRef]
10. Itakura E, Mizushima N. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy*. 2010;6(6):764-776. [CrossRef]
11. Aspernig H, Heimbucher T, Qi W, et al. Mitochondrial perturbations couple mTORC2 to autophagy in *C. elegans*. *Cell Rep*. 2019;29(6):1399-1409.e5. [CrossRef]
12. Chiang CK, Wang CC, Lu TF, et al. Involvement of endoplasmic reticulum stress, autophagy and apoptosis in advanced glycation end products-induced glomerular mesangial cell injury. *Sci Rep*. 2016;6(1):34167. [CrossRef]
13. Baan CC, Kannegieter NM, Felipe CR, Tedesco Silva Jr HT. Targeting JAK/STAT signaling to prevent rejection after kidney transplantation: a reappraisal. *Transplantation*. 2016;100(9):1833-1839. [CrossRef]
14. Hu P, Lai D, Lu P, Gao J, He H. ERK and Akt signaling pathways are involved in advanced glycation end product-induced autophagy in rat vascular smooth muscle cells. *Int J Mol Med*. 2012;29(4):613-618. [CrossRef]
15. Shi M, Yang S, Zhu X, et al. The RAGE/STAT5/autophagy axis regulates senescence in mesangial cells. *Cell Signal*. 2019;62:109334. [CrossRef]
16. Wharram BL, Goyal M, Wiggins JE, et al. Podocyte depletion causes glomerulosclerosis: diphtheria toxin–induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *J Am Soc Nephrol*. 2005;16(10):2941-2952. [CrossRef]
17. Cinà DP, Onay T, Paltoo A, et al. Inhibition of MTOR disrupts autophagic flux in podocytes. *J Am Soc Nephrol*. 2012;23(3):412-420. [CrossRef]
18. Li L, Wang ZV, Hill JA, Lin F. New autophagy reporter mice reveal dynamics of proximal tubular autophagy. *J Am Soc Nephrol*. 2014;25(2):305-315. [CrossRef]
19. Shintani T, Klionsky DJ. Autophagy in health and disease: a double-edged sword. *Science*. 2004;306(5698):990-995. [CrossRef]
20. Kimura T, Takabatake Y, Takahashi A, et al. Autophagy protects the proximal tubule from degeneration and acute ischemic injury. *J Am Soc Nephrol*. 2011;22(5):902-913. [CrossRef]
21. Guan X, Qian Y, Shen Y, et al. Autophagy protects renal tubular cells against ischemia/reperfusion injury in a time-dependent manner. *Cell Physiol Biochem*. 2015;36(1):285-298. [CrossRef]
22. Peintner L, Venkatraman A, Waeldin A, et al. Loss of PKD1/polycystin-1 impairs lysosomal activity in a CAPN (calpain)-dependent manner. *Autophagy*. 2021;17(9):2384-2400. [CrossRef]
23. Ramírez-Sagredo A, Quiroga C, Garrido-Moreno V, et al. Polycystin-1 regulates cardiomyocyte mitophagy. *FASEB J*. 2021;35(8):e21796. [CrossRef]
24. Lu J, Boheler KR, Jiang L, et al. Polycystin-2 plays an essential role in glucose starvation-induced autophagy in human embryonic stem cell-derived cardiomyocytes. *Stem Cells*. 2018;36(4):501-513. [CrossRef]
25. Peña-Oyarzun D, Rodriguez-Peña M, Burgos-Bravo F, et al. PKD2/polycystin-2 induces autophagy by forming a complex with BECN1. *Autophagy*. 2021;17(7):1714-1728. [CrossRef]
26. Criollo A, Altamirano F, Pedrozo Z, et al. Polycystin-2-dependent control of cardiomyocyte autophagy. *J Mol Cell Cardiol*. 2018;118:110-121. [CrossRef]
27. Cebotaru V, Cebotaru L, Kim H, et al. Polycystin-1 negatively regulates Polycystin-2 expression via the aggresome/autophagosome pathway. *J Biol Chem*. 2014;289(10):6404-6414. [CrossRef]
28. Belibi F, Zafar I, Ravichandran K, et al. Hypoxia-inducible factor-1α (HIF-1α) and autophagy in polycystic kidney disease (PKD). *Am J Physiol Ren Physiol*. 2011;300(5):F1235-F1243. [CrossRef]
29. Zhu P, Sieben CJ, Xu X, Harris PC, Lin X. Autophagy activators suppress cystogenesis in an autosomal dominant polycystic kidney disease model. *Hum Mol Genet*. 2017;26(1):158-172. [CrossRef]

30. Rowe I, Chiaravalli M, Mannella V, et al. Defective glucose metabolism in polycystic kidney disease identifies a new therapeutic strategy. *Nat Med*. 2013;19(4):488-493. [CrossRef]
31. Masyuk AI, Masyuk TV, Lorenzo Pisarello MJ, et al. Cholangiocyte autophagy contributes to hepatic cystogenesis in polycystic liver disease and represents a potential therapeutic target. *Hepatology*. 2018;67(3):1088-1108. [CrossRef]
32. Chou LF, Cheng YL, Hsieh CY, et al. Effect of trehalose supplementation on autophagy and cystogenesis in a mouse model of polycystic kidney disease. *Nutrients*. 2018;11(1):42. [CrossRef]
33. Ravichandran K, Zafar I, He Z, et al. An mTOR anti-sense oligonucleotide decreases polycystic kidney disease in mice with a targeted mutation in Pkd2. *Hum Mol Genet*. 2014;23(18):4919-4931. [CrossRef]
34. Holditch SJ, Brown CN, Atwood DJ, et al. The consequences of increased 4E-BP1 in polycystic kidney disease. *Hum Mol Genet*. 2019;28(24):4132-4147. [CrossRef]
35. Zafar I, Belibi FA, He Z, Edelstein CL. Long-term rapamycin therapy in the Han:SPRD rat model of polycystic kidney disease (PKD). *Nephrol Dial Transplant*. 2009;24(8):2349-2353. [CrossRef]
36. Becker JU, Opazo Saez A, Zerres K, et al. The mTOR pathway is activated in human autosomal-recessive polycystic kidney disease. *Kidney Blood Press Res*. 2010;33(2):129-138. [CrossRef]
37. Fischer DC, Jacoby U, Pape L, et al. Activation of the AKT/mTOR pathway in autosomal recessive polycystic kidney disease (ARPKD). *Nephrol Dial Transplant*. 2009;24(6):1819-1827. [CrossRef]
38. Yim NH, Jung YP, Kim A, Ma CJ, Cho WK, Ma JY. Oyaksungisan, a traditional herbal formula, inhibits cell proliferation by induction of autophagy via JNK activation in human colon cancer cells. *Evid Based Complement Alternat Med*. 2013;2013:231874. [CrossRef]
39. Giansanti V, Torriglia A, Scovassi AI. Conversation between apoptosis and autophagy: "Is it your turn or mine?" *Apoptosis*. 2011;16(4):321-333. [CrossRef]
40. Kaushal GP, Kaushal V, Herzog C, Yang C. Autophagy delays apoptosis in renal tubular epithelial cells in cisplatin cytotoxicity. *Autophagy*. 2008;4(5):710-712. [CrossRef]
41. Huber TB, Walz G, Kuehn EW. mTOR and rapamycin in the kidney: signaling and therapeutic implications beyond immunosuppression. *Kidney Int*. 2011;79(5):502-511. [CrossRef]
42. Zafar I, Ravichandran K, Belibi FA, Doctor RB, Edelstein CL. Sirolimus attenuates disease progression in an orthologous mouse model of human autosomal dominant polycystic kidney disease. *Kidney Int*. 2010;78(8):754-761. [CrossRef]
43. Shillingford JM, Piontek KB, Germino GG, Weimbs T. Rapamycin ameliorates PKD resulting from conditional inactivation of Pkd1. *J Am Soc Nephrol*. 2010;21(3):489-497. [CrossRef]
44. Tao Y, Kim J, Schrier RW, Edelstein CL. Rapamycin markedly slows disease progression in a rat model of polycystic kidney disease. *J Am Soc Nephrol*. 2005;16(1):46-51. [CrossRef]
45. Atwood DJ, Brown CN, Holditch SJ, et al. The effect of trehalose on autophagy-related proteins and cyst growth in a hypomorphic Pkd1 mouse model of autosomal dominant polycystic kidney disease. *Cell Signal*. 2020;75:109760. [CrossRef]
46. Boehlke C, Kotsis F, Patel V, et al. Primary cilia regulate mTORC1 activity and cell size through LKB1. *Nat Cell Biol*. 2010;12(11):1115-1122. [CrossRef]
47. Bell PD, Fitzgibbon W, Sas K, et al. Loss of primary cilia upregulates renal hypertrophic signaling and promotes cystogenesis. *J Am Soc Nephrol*. 2011;22(5):839-848. [CrossRef]
48. Wang S, Livingston MJ, Su Y, Dong Z. Reciprocal regulation of cilia and autophagy via the MTOR and proteasome pathways. *Autophagy*. 2015;11(4):607-616. [CrossRef]
49. Lenoir O, Tharaux PL, Huber TB. Autophagy in kidney disease and aging: lessons from rodent models. *Kidney Int*. 2016;90(5):950-964. [CrossRef]
50. Tang Z, Lin MG, Stowe TR, et al. Autophagy promotes primary ciliogenesis by removing OFD1 from centriolar satellites. *Nature*. 2013;502(7470):254-257. [CrossRef]
51. Pampliega O, Orhon I, Patel B, et al. Functional interaction between autophagy and ciliogenesis. *Nature*. 2013;502(7470):194-200. [CrossRef]
52. Dong K, Zhang C, Tian X, et al. Renal plasticity revealed through reversal of polycystic kidney disease in mice. *Nat Genet*. 2021;53(12):1649-1663. [CrossRef]
53. Shi W, Xu D, Gu J, et al. Saikosaponin-d inhibits proliferation by up-regulating autophagy via the CaMKKβ-AMPK-mTOR pathway in ADPKD cells. *Mol Cell Biochem*. 2018;449(1-2):219-226. [CrossRef]
54. Sun L, Hu C, Zhang X. Histone deacetylase inhibitors reduce cysts by activating autophagy in polycystic kidney disease. *Kidney Dis (Basel)*. 2019;5(3):163-172. [CrossRef]
55. Liu G, Kang X, Guo P, et al. miR-25-3p promotes proliferation and inhibits autophagy of renal cells in polycystic kidney mice by regulating ATG14-Beclin 1. *Ren Fail*. 2020;42(1):333-342. [CrossRef]
56. Chang MY, Ma TL, Hung CC, et al. Metformin inhibits cyst formation in a zebrafish model of Polycystin-2 deficiency. *Sci Rep*. 2017;7(1):7161. [CrossRef]
57. Lee EJ, Ko JY, Oh S, et al. Autophagy induction promotes renal cyst growth in polycystic kidney disease. *EBiomedicine*. 2020;60:102986. [CrossRef]