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Autophagy in Model Organisms: Insights into Cancer

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Abstract

Autophagy is an evolutionarily conserved process utilized by most organisms to clear cellular damage and recycle building blocks for energy production. In this chapter, we emphasize the importance of genetic model organisms, including yeast, nematodes, flies, and mammals in the discovery and understanding of the autophagy process. We highlight the important roles of autophagy in aging, stress tolerance, neuronal health, organismal development, and pathogen resistance in invertebrate and vertebrate model organisms. We provide examples on how the same autophagy-related pathways that increase stress response and longevity in lower organisms could be utilized by cancer cells to survive harsh microenvironments, proliferate, and metastasize, with emphasis on the dual role of autophagy in cancer: an antitumorigenic or a protumorigenic process.

Keywords: autophagy, model organisms, stress tolerance, aging, organismal development, cancer

1. Introduction to autophagy

Autophagy is an evolutionarily conserved “self-degradation” process through which cytosolic compartments and organelles are delivered to the lysosome for degradation [1]. Autophagy exists in three forms: microautophagy where cytosolic components are directly engulfed in lysosomes, chaperone-mediated autophagy through which designated proteins are selectively targeted to the lysosomes, and macroautophagy (noted herein as autophagy) where cytosolic material is enclosed in a double-membrane autophagosomal structure that is delivered to lysosomes for degradation by acidic hydrolases [1]. Autophagy is selectively activated to remove cellular damage or is non-selectively activated under stress situations to supply energy and sustain cellular/organismal viability.

The autophagy machinery components and the physiology of this process are highly conserved across evolution from yeast to mammals. The autophagy-related genes (ATGs) have been initially identified in yeast *Saccharomyces cerevisiae* by pioneering genetic screens [2–7]. Later, their orthologues in other organisms have been determined, which led to the assessment of the functional roles of autophagy. ATG proteins form distinct autophagic complexes that function upon phagophore biogenesis, autophagosome formation, and maturation. The autophagy process comprises several steps. First, it starts with the nucleation and formation of the phagophore, which elongates and closes to form the double-membrane autophagosome, engulfing material to be recycled. Then, the autophagosome fuses with the lysosome to form the autolysosome where the material is digested by hydrolases [8–12]. The autophagy proteins are classified into six functional groups: the Atg1 autophagy initiation complex, the autophagy-specific phosphatidylinositol PI 3-kinase complex, the Atg12 the Atg2-Atg18 complex, the Atg9 transmembrane protein, the Atg12 autophagy conjugation system, and the Atg8 lipid conjugation system [8, 9]. The autophagic components of every group, their functions, and homologues in yeast, *Drosophila*, and the nematode *Caenorhabditis elegans* are described in **Table 1**.

	Yeasts	<i>Caenorhabditis elegans</i>	<i>Drosophila melanogaster</i>	Mammals
Regulation of induction	yTOR	<i>let-363</i>	dTOR	<i>mTOR</i>
	Snf1	<i>aak-1, aak-2</i>	AMPK	AMPK
Atg1/ULK autophagy initiation complex	Atg1	<i>unc-51</i>	Atg1	<i>ULK1, ULK2</i>
	Atg13	<i>atg-13</i>	Atg13	<i>ATG13</i>
	Fip200	<i>atg-11</i>	Fip200	<i>ATG17</i>
	Atg101	<i>epg-9</i>	Atg101	<i>ATG101</i>
Class III PI3K complex	Vps34	<i>vps-34</i>	Vps34	<i>VPS34</i>
	Vps15	<i>vps-15</i>	Vps15	<i>VPS15</i>
	Atg6	<i>Bec-1</i>	Atg6	<i>ATG6</i>
	Atg14	<i>epg-8</i>	Atg14	<i>ATG14L</i>
Atg2-Atg18 conjugation complex	Atg2	<i>atg-2</i>	Atg2	<i>ATG2</i>
	Atg18	<i>atg-18, epg-6</i>	Atg18a, Atg18b	<i>WIPI1, WIPI2, WIPI3, WIPI4</i>
Atg 9 transmembrane	Atg9	<i>atg-9</i>	Atg9	<i>ATG9A, ATG9B</i>
Atg12 conjugation system	Atg12	<i>lgg-3</i>	Atg12	<i>ATG12</i>
	Atg5	<i>atg-5</i>	Atg5	<i>ATG5</i>
	Atg10	<i>atg-10</i>	Atg10	<i>ATG10</i>
	Atg16	<i>atg-16.1, atg-16.2</i>	Atg16	<i>ATG16L1, ATG16L2</i>
	Atg7	<i>atg-7</i>	Atg7	<i>ATG7</i>
Atg8 conjugation system	Atg8	<i>lgg-1, lgg-2</i>	Atg8a, Atg8b	<i>GABARAP, LC3, GABARAPL1, GABARAPL2</i>
	Atg3	<i>atg-3</i>	Atg3	<i>ATG3</i>
	Atg4	<i>atg-4.1, atg-4.2</i>	Atg4a, Atg4b	<i>ATG4A, ATG4B, ATG4C, ATG4D</i>
	Atg7	<i>atg-7</i>	Atg7	<i>ATG7</i>

Table 1. Conserved autophagy genes in yeast, nematodes, flies, and mammals.

This review focuses on the multifaceted roles of autophagy in model organisms and how these conserved pathways could be adopted by cancer cells to suppress or promote tumorigenesis.

2. The importance of invertebrate model organisms

Although mammalian model organisms such as mice and rats are highly advantageous to study disease-related biological processes in humans due to the close anatomical and physiological similarities between systems, they have disadvantages including space, cost, and time-consuming transgenic technologies. Yeast models including budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) and fission yeast *Schizosaccharomyces pombe* (*S. pombe*), the fruit fly *Drosophila melanogaster* (*D. melanogaster*), the nematode *Caenorhabditis elegans* (*C. elegans*), and other invertebrate models have emerged as excellent model organisms to study conserved signaling pathways. Many biological processes including autophagy are highly evolutionary conserved such that findings in these models are often applicable to humans.

First, yeasts, flies, and nematodes are characterized by their short lifespans and rapid reproductive lifecycles. Second, their genomes are fully sequenced [13–15] and well annotated, and a large number of tools and resources are available in accessible bioinformatics databases specific to every model (Yeast: www.yeastgenome.org; *Drosophila*: www.flybase.org; *C. elegans*: www.wormbase.org). Third, a high percentage of genes in invertebrate model organisms is homologous to disease-associated genes in humans. Fourth, several tools have been invented and developed in these systems including microscopy, transgenic techniques, biochemical methods, and others, rendering them attractive models to study genetically signaling pathways linked to diseases in humans including autophagy.

Although autophagy has been first observed by electron microscopy in mammalian cells in the 1950s [16], more than 30 autophagy genes have been discovered using genetic screens in yeast, and many of them have homologues in humans [2–7]. The rapid reproductive life cycles and short lifespans, the massive generation of tools to study autophagy, and the ease with which researchers pursue genetics work *in vivo* emphasize the importance of these models to study not only the molecular basics of the autophagic process but also the multifaceted roles of autophagy in organismal aging, stress tolerance, neuronal health, metabolism, pathogen infection, and others.

Despite the large advantages of invertebrate model organisms, they also have many limitations. The anatomy and physiology of the organismal systems, including immune, circulatory, respiratory, and nervous systems, largely differ from that of humans. Therefore, the importance of mammalian *in vitro* and *in vivo* models in studying autophagy is also unquestionable.

3. Methods to monitor autophagy in model organisms

Similar methods to study autophagy have been used in invertebrate model organisms and mammalian systems with the employment of the benefits of every system. These methods are

recently reviewed in detail for yeast [17–20], *C. elegans* [21–25], flies [26–30], and mammalian systems [31–36].

Despite its complexity and difficulty to pursue, electron microscopy is one of the most reliable methods to visualize autophagic structures and has been used to monitor autophagy in many model organisms. However, since it requires a substantial specialized expertise, most researchers currently rely on light microscopic and biochemical methods, which are more accessible and easier to perform in most organisms. The fluorescent image analysis of autophagic components using reporters of tagged autophagic proteins has been widely used. LC3/ATG8 exists in two forms: LC3-I is cytosolic and soluble, and LC3-II is conjugated with phosphatidylethanolamine and is bound to the autophagosomal membranes. When autophagy is induced, the conjugation reaction can be monitored using the LC3:GFP reporter and the change between the diffuse localization of LC3 into autophagosomal puncta structures reflects the autophagic activity. This reporter is one of the most popular with its orthologues in *C. elegans* (LGG1:GFP) [23, 24] and in *Drosophila* and yeast (ATG8:GFP) [18, 29, 30]. The autophagic activity has been also assessed using Western blotting of the LC3:GFP protein extracts with or without inhibitors to determine the conversion of LC3-I to LC3-II. Moreover, previous studies in yeast, *C. elegans*, and mammalian cells have demonstrated that LC3-II is degraded inside the autolysosomes and that the GFP fragment is resistant to degradation and accumulates when autophagy is induced [37–40]. Therefore, researchers have used Western blot analysis on protein extracts to assess the levels of GFP and cleavage of GFP-LC3-I.

Since autophagic proteins also accumulate upon defective autophagy, researchers have monitored the degradation of cargo proteins such as p62 in most model organisms as well [24, 25, 28, 41, 42]. Furthermore, autophagy inhibitors have been used to determine whether the accumulation of autophagosomes is due to impaired autophagy or to a heightened autophagic flux. The most recent studies employ the mRFP-GFP-LC3, which enables the distinction between heightened autophagic flux and impaired autophagy. In this method, mCherry and GFP have been used as red and green fluorescent protein markers, respectively, to trace the autophagic protein LC3. Upon physiological pH in newly formed autophagosomes or when autophagy is impaired, both GFP and mCherry colocalize in puncta leading to yellow puncta structures, whereas upon lysosomal fusion and acidification, the GFP signal is lost and only mCherry is detected.

High-resolution live-cell imaging to visualize the dynamics of autophagy has been also employed and reviewed in detail [36].

4. Autophagy-related biological roles in model organisms

Despite the anatomical, morphological, and physiological differences between model organisms, autophagy appears to play similar important roles across evolution. In this section, we review the major autophagy-associated roles at the cellular and organismal levels in invertebrate and mammalian model systems.

4.1. Stress tolerance

In most organisms, autophagy is activated by different stresses including nutrient deprivation, oxidative stress, hypoxia, temperature shifts, and others, to eliminate damaged macromolecules and to produce energy

In yeast, mutation of *Atg1*, *Atg2*, *Atg4*, *Atg7*, or *Atg8* genes increases sensitivity to the oxidative stressor paraquat [43]. In *C. elegans*, starvation, oxidative stress, and hypoxia stresses induce autophagy in multiple tissues of the animal as monitored by the number of positive GFP::LGG-1 puncta [44–47]. The increased autophagy levels induced by stress are essential for organismal survival to stressful conditions. In addition, the inhibition of autophagy genes causes defects in the formation of the *C. elegans* dauer animals, a static larval stage adapted to survive prolonged starvation [45]. Furthermore, autophagy is required for the survival of *C. elegans* nematodes to starvation [47, 48], hypoxic environments [44], oxidative stress [46], and hyperosmotic stress [49].

In *Drosophila*, *Atg7* mutant flies are hypersensitive to complete starvation, sugar-only diets, and oxidative stress [50, 51]. Moreover, JNK signaling induces the transcription of autophagy genes to help protect flies from oxidative stress [52]. Specifically, mutation of *Atg1* and *Atg6* in young adult flies overexpressing JNK signaling suppressed their increased resistance to the oxidative stressor paraquat [52]. Consistently, the spermidine-induced autophagy is required for the resistance of *Drosophila* animals to paraquat [53].

The role of autophagy in stress resistance has been demonstrated not only in invertebrate models but also with mammalian cell culture and *in vivo* models. For example, in mice, ATG5 overexpression induces autophagy, increases oxidative stress resistance, and extends lifespan [54]. Additionally, autophagy is significantly induced following the early starvation-associated postnatal period in mouse neonates and is required for their survival until supply with milk nutrients [55]. Several studies also reported that following ischemic injuries, autophagy is activated and contributes to neuroprotection by delaying neuronal cell death in rats [56–58]. Collectively, these studies demonstrate an evolutionarily conserved role of autophagy in stress tolerance. However, how autophagy mediates stress tolerance is still unclear. While many studies highlight the important role of autophagy in the clearance of stress-induced damaged organelles, others claim that the stress resistance is due to the role of autophagy in sustaining energy levels and providing building blocks for mitochondrial energy production.

4.2. Extension of lifespan

Accumulating evidence demonstrates that longevity pathways converge on autophagic processes in many organisms to regulate diverse cellular functions including the clearance of damaged proteins and organelles and the remodeling of cellular metabolism. In *C. elegans*, multiple genetic or pharmacological manipulations extend lifespan [59]. For instance, mutations of genes in the insulin-signaling pathway, including *daf-2* and *age-1*, which are orthologues of the insulin signaling receptor and PI3K, respectively, deficiency in target of rapamycin (TOR) signaling, overexpression of activated protein kinase (AMPK) signaling, mutation of mitochondrial genes, dietary restriction through mutation of *eat-2*, mutation in

sitruin-1, are all genetic alterations that extend lifespan in *C. elegans* [59]. Pharmacological alterations, such as spermidine, resveratrol, and w-6 polyunsaturated fatty acids treatment also prolong lifespan in *C. elegans* [60, 61]. Importantly, autophagy is induced in most of the above-mentioned longevity pathways and contributes to the lifespan extension phenotypes in *C. elegans*. For example, the inhibition of the autophagy gene *bec-1* suppresses the increased lifespan mediated by caloric restriction in *eat-2* mutant animals or by TOR inhibition [62]. Furthermore, the inhibition of *bec-1* in *daf-2* long-lived *C. elegans* mutants severely reduces their lifespan [45]. In addition, autophagy is highly induced in calcineurin *C. elegans* mutant animals and its inhibition by RNAi feeding against *bec-1* or *atg-7* abolishes the increased longevity phenotype [63]. Moreover, the mutation of *cep-1*, the worm orthologue of P53 promotes an autophagy-dependent lifespan extension [64]. Additionally, both spermidine and resveratrol extend *C. elegans* lifespan by inducing autophagy [60, 65]. Mitophagy also contributes to the extension of lifespan upon low insulin signaling and mitochondrial mutations [66].

HLH-30 is the worm homologue of transcription factor EB (TFEB), a master transcriptional regulator of lysosomal and autophagic pathways [67, 68]. The overexpression of HLH-30 increases lifespan in *C. elegans* [67]. Furthermore, the impairment of the production of the yolk lipoprotein vitellogenin extends lifespan in *C. elegans* [69]. Importantly, autophagy and HLH-30 are both induced by the reduction in vitellogenesis and contribute to the extension of lifespan in vitellogenesis-defective *vit* mutant animals [69].

In *Drosophila*, mutations in *Atg7* and *Atg8* genes shorten lifespan [50, 51]. In addition, mutation of the autophagic protein FIP200, a component of the Atg1 autophagy initiation complex, leads to neuronal degeneration and shortens lifespan [70]. The administration of phosphatidylethanolamine enhances autophagic flux and increases lifespan in yeast, *Drosophila*, and mammalian cells in culture [71].

In yeast, the role of autophagy in aging seems to be context-dependent. Autophagy has been shown to be required for the extension of chronological lifespan by low doses of the mammalian target of rapamycin (mTOR) inhibitor rapamycin [72, 73], methionine limitation [74], and calorie restriction [75]. In contrast, Tang et al., 2008 claim that autophagy genes may be required or not for the lifespan extension by calorie restriction depending on their role in the autophagy process. Specifically, they show that the deletion of genes involved in autophagosome formation including *Atg1*, *Atg6*, *Atg7*, and *Atg8* did not affect lifespan of budding yeast upon calorie restriction [76]. However, the deletion of *Atg15*, *Atg17*, or other genes involved in vacuole-vacuole fusion reduced the lifespan extension promoted by calorie restriction [76].

In mammals, the link between autophagy and the organismal extension of lifespan has not been clearly established. A few studies support the role of autophagy in promoting longevity in mammals. For instance, ATG5 overexpression has been shown to extend lifespan by 17.2% in mice [54]. Interestingly, rapamycin feeding of mice at their old age extends their lifespan, which could be due to autophagy activation [77]. While rapamycin is a strong mTOR inhibitor and autophagy inducer, the link between rapamycin feeding and increased autophagy has not been made, and therefore, the extension of lifespan by administration of rapamycin in mice may not be due to autophagy activation per se but to other mechanisms [77].

Although the role of autophagy in mammalian organismal lifespan is still not clearly elucidated, many studies demonstrate an important role for autophagy in delaying the acquisition of aging features of multiple cells and tissues. Numerous studies also claim a decline in the autophagic activity in many mammalian organs upon aging [78–83]. For example, autophagy genes *Atg5*, *Becn1*, and *Atg7* are significantly downregulated in the human aging brain [84]. Cardiac-specific *Atg5* deficiency in mice leads to cardiac abnormalities after 6 months of age and early death [85]. Consistently, cardiac-specific overexpression of *Atg7* increased autophagic flux and improved cardiac function in desmin-related cardiomyopathies in mice [86]. Furthermore, the hyperactivation of chaperone-mediated autophagy in aging livers maintains hepatic function in old mice to a level comparable to that reported in young mice [87]. Recently, autophagy inhibition has been shown to increase aging features in macrophages including the reduction in phagocytosis and nitrite burst and increased inflammatory response [78]. Numerous studies have also linked autophagy to improved neuronal health in mice and protection from age-associated neurological disorders [58, 81, 88–93]. This is further detailed in the neuronal health section of this chapter. Moreover, the role of autophagy in suppressing tumor initiation is well described at the end of this chapter. Therefore, although it is not clear whether autophagy extends organismal lifespan in mammals, collective evidence supports its implication in the extension of healthy living or health span and the delay of the appearance of age-associated diseases.

4.3. Resistance to pathogen infection

The induction of autophagy has been widely shown to contribute to the organismal survival to infection with pathogens. In *C. elegans*, autophagy genes are required for survival to infection with pathogens, including *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and other pathogens [68, 94, 95]. Using the GFP::LGG1 reporter, autophagy has been shown to be induced in the hypodermal seam cells and intestinal cells of wild-type animals following infection with *Pseudomonas aeruginosa* [95] and *Staphylococcus aureus* [68]. Importantly, inhibition of autophagy genes suppresses not only the resistance of wild-type animals but also the resistance of highly stress-resistant strains including *daf-2* mutant animals and *daf-16* overexpressing animals to infection with *Salmonella typhimurium* [94].

In *Drosophila*, IRD1 is the fly homologue of mammalian VPS15, an important autophagic serine/threonine kinase implicated in phagosome maturation. IRD1 plays an important role in antibacterial immune responses in *Drosophila* [96]. *Ird1* mutant flies are incapable of expressing antimicrobial peptide genes upon infection [96]. In addition, the conditional inactivation of autophagy genes *Atg5*, *Atg7*, *Atg12* in *Drosophila* reduces survival of the animals upon infection with *Escherichia Coli* [97]. Moreover, autophagy genes *Atg5*, *Atg8a*, and *Atg18* are also required to limit the infection of *Drosophila* cells with the *Vesicular stomatitis virus* [98]. Furthermore, the inhibition of *Atg5* using RNAi in flies increased the susceptibility of the animals to infection with *Listeria monocytogenes* [99].

The transcriptional upregulation of autophagy genes by TFEB has been also associated with increased resistance to pathogens. Upon infection with *Staphylococcus aureus*, HLH-30 rapidly translocates to the nucleus and activates the expression of autophagy genes, lysosomal genes,

and antimicrobial peptide genes in both *C. elegans* [68]. In murine cell lines, TFEB translocates to the nucleus following infection and induces the transcription of chemokines and cytokines [68]. Mitophagy is also another mechanism of defense against invasion with *P. aeruginosa* [100].

How autophagy mediates resistance to pathogens is still not clear. Xenophagy (eating the pathogen) is a cellular defense mechanism through which cells direct autophagy to degrade the invading pathogens. Autophagy genes restrict *Salmonella* bacterial replication in both hosts, the unicellular organism *Dictyostelium discoideum* and in *C. elegans* [94]. However, autophagy only increased resistance of *C. elegans* to *Pseudomonas aeruginosa* and to *Staphylococcus aureus* without decreasing bacterial load suggesting that xenophagy is not the only defense mechanism attributed to autophagy [68, 95].

In mammalian cells, autophagy also plays an essential role in the protection against invading pathogens, including *Streptococcus*, *Shigella flexneri*, *Mycobacterium tuberculosis*, and *Toxoplasma gondii* [12, 101–103]. Autophagy has also been shown to protect against toxins released by bacterial pathogens [37]. In mice, recent work demonstrates the involvement of autophagy in the clearance of pathogens, including *Listeria monocytogenes*, and moreover, IRF8 directs stress-induced autophagy in macrophages and promotes clearance of *L. monocytogenes* [104] and *Staphylococcus aureus* [105, 106] and *Mycobacterium tuberculosis* [107, 108]. However, recent work demonstrates a unique role of ATG5 in the resistance of mice to *Mycobacterium tuberculosis* infection distinct from autophagy in contrast to previous reports. ATG5 prevents polymorphonuclear cell-mediated immunopathology enhancing resistance to *Mycobacterium tuberculosis* infection [109].

4.4. Organismal development

Accumulating evidence highlights an important role for autophagy during organismal development. Deletion of autophagy genes leads to severe defects and causes early lethality in many organisms. For example, *bec-1* mutation leads to severe defects during embryogenesis in *C. elegans* and mutant animals display a highly penetrant lethal phenotype where only few animals are capable of reaching adulthood [110]. The *unc-51/atg-1* *C. elegans* mutant animals exhibit an uncoordinated movement and paralysis. Moreover, autophagy is highly induced at several stages during *C. elegans* development and a genome-wide genetic screen has identified signaling pathways that regulate this process in *C. elegans* [111]. In *Drosophila*, mutations in *Atg1* are pupal lethal [112] and strong hypomorphic mutations in *Atg8* lead to a semi-lethality phenotype [50, 51]. Autophagy is also induced during the development of Zebrafish larvae and the knockdown of autophagy genes *Atg5*, *Beclin1*, and *Atg7* results in aberrant cardiac morphogenesis and reduced survival in Zebrafish [113]. ATG5 deficiency in Zebrafish impairs nervous system development, specifically brain morphogenesis [114]. Additionally, AMBRA1 (autophagy/Beclin 1 regulator 1) is an evolutionary conserved positive regulator of BECN1 and is essential for proper autophagic activity. The inhibition of AMBRA1 in Zebrafish leads to incomplete organogenesis and defects in skeletal muscle development [115, 116].

In mice, *Becn1* homozygous deletion leads to embryonic lethality [117], while *Atg7* and *Atg5* null mice are born alive but die soon after birth. Similarly to what has been reported in

Zebrafish, *Atg5* is required for the proper cardiac development [113] and cortical astrocyte differentiation [118] during embryogenesis in mice. Autophagy is also involved in chondrocyte differentiation and bone formation through fibroblast growth factor (FGF) signaling in mice [119]. FIP200 is an important autophagic protein that interacts with ULK1 in the autophagy initiation complex. Homozygous deletion of FIP200 in mice leads to embryonic lethality due to heart failure and severe hepatic defects [120]. Other than its important role in the heart and liver, FIP200 plays a central role in the differentiation of neural stem cells and is essential for maintenance and function of fetal hematopoietic stem cells [121]. Supporting the role of autophagy in stem cell differentiation during development, a recent study reports a retardation in stem cell differentiation during the embryonic development of mice hypomorphic for *Atg16l1* [122].

The discovery that autophagy is involved in the degradation of the paternal mitochondria is another important aspect during development. In most eukaryotes, the maternal mitochondrial genome is believed to be the one inherited and thus the degradation of the sperm-inherited mitochondrial genome is essential. In *C. elegans*, autophagosomes engulf the paternal mitochondria and target them to the lysosomes for degradation during embryonic development [123]. Similarly, paternal mitochondria are also destroyed by endocytic and autophagic pathways in *Drosophila* [124]. However, in mammalian zygotes, the degradation of the paternally inherited mitochondria requires the ubiquitin proteasome system rather than autophagy [125]. Therefore, autophagy plays central role(s) in organismal development across evolution, which includes key checkpoints during embryogenesis, cellular differentiation, and tissue organization.

4.5. Neuronal health

The accumulation of autophagosomes has been observed in the neurons of individuals affected with neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis. Autophagy improves neuronal health by degrading damaged proteins, specifically mutant proteins associated with neurological disorders and toxic aggregation-prone proteins [88, 91, 126–128]. Non-mammalian model systems are excellent to study protein homeostasis in regard to fatal neurological disorders. In addition, *C. elegans* [129–133] and flies [134–139] researchers have generated transgenic animals that express polyglutamine repeats, beta-amyloid peptides, and the α synuclein protein, to mimic the pathologies of Huntington's disease, Alzheimer's disease, and Parkinson's disease, respectively. Using electron microscopy and the LGG-1:GFP reporter, the expression of human beta-amyloid (1–42) in *C. elegans* muscles resulted in the accumulation of autophagic vacuoles. Autophagy contributes to the degradation of the Beta-amyloid peptide in *daf-2* mutant nematodes [129]. In *Drosophila*, inhibition of autophagy genes increases neuronal toxicity of amyloid beta 1–42 peptides [140]. In *C. elegans*, inactivation of autophagy genes *atg-18* and *atg-7* accelerates the accumulation of polyQ40:YFP protein aggregates in the body wall muscles of the animals over time [133]. In *C. elegans*, the *unc-51 (atg-1)* gene is essential for normal axonal elongation and structure [141].

In *Drosophila*, mutation of *Atg7* or *Atg8* genes enhanced the accumulation of insoluble poly-ubiquitinated proteins with age as determined by Western blot analysis using fly head extracts [50, 51]. Consistently, the overexpression of *Atg8* in the central nervous system of adult flies reduced the accumulation of insoluble ubiquitinated proteins [51]. The *Drosophila* homologue of P62, refractory to sigma P (Ref(2)P), a scaffold protein with diverse biological roles, marks ubiquitinated protein aggregates for degradation [142, 143]. Ref(2)P acts as a receptor for selective autophagic degradation. In flies, p62-tagged ubiquitinated protein aggregates accumulate in the brains of older animals as compared to young animals [143]. The accumulation of protein aggregates correlates not only with poor autophagic functions with age but also with a decline in fly behaviors and aging [144]. While the accumulation of Ref(2)P-linked protein aggregates is enhanced in *Drosophila Atg8* [143] and *Vps15* [145] mutant flies, Ref(2)P is also required to form these aggregates [143]. In *Drosophila*, *Atg17/FIP200* localizes to Ref(2)P protein aggregates proximate to the lysosomes and interacts with the autophagy-activating protein ATG1 to promote autophagy [146]. By sustaining autophagy, the N-ethyl-maleimide-sensitive fusion protein (NSF1) protects dopaminergic neurons from degeneration and promotes longevity in *Drosophila* [147]. Also, the inhibition of the ectopic P-granules autophagy protein 5 (*Epg5*) in the retina of adult *Drosophila* animals leads to the degeneration of photoreceptor neurons and loss of the retina [148] mirroring the genetic neurological disorders of EPG5-related Vici Syndrome in humans. Mutation of the autophagy gene-related proteinase ATG4D in dogs has been recently associated with a novel neurodegenerative disorder in the Lagotto Romagnolo dog breed [149]. Accordingly, knockdown of *Atg4D* in Zebrafish also leads to neurodegeneration of the central nervous system [149].

Consistently with what has been observed in *C. elegans* and *Drosophila*, the induction of autophagy by starvation [150] or by rapamycin [151] reduced the amount of poly-ubiquitinated proteins [150] or α -synuclein [151] protein aggregates in yeast. However, yeast *Atg8* mutants displayed an accumulation of ubiquitinated aggregate-prone proteins upon starvation and high temperature stresses [150]. Moreover, the mutation of *Atg1* or *Atg7* delayed the clearance of α -synuclein aggregates in yeast [152, 153].

Numerous studies highlight an important role for autophagy in mammalian neurogenesis and neuronal “maintenance.” Several neurological disorders in humans are associated with impaired autophagy and defects in the clearance of damaged organelles and proteins [154, 155]. Among several examples, mutations in *WDR45*, one of the mammalian homologues of yeast *Atg18*, cause encephalopathy in children and neurodegeneration in adults [156]. Importantly, V471A polymorphism in the *Atg7* gene in human patients, mostly of Italian origin, has been strongly correlated with an earlier onset of Huntington’s disease [157, 158]. In mice, lack of autophagy genes *Atg7* and *Atg5* in the neurons promotes the accumulation of poly-ubiquitinated aggregation-prone proteins leading to neuronal degeneration [92, 93]. The specific knockout of *Atg7* in the Purkinje cells of mice leads to neurodegeneration and destabilization of axonal homeostasis [159]. Moreover, the induction of autophagy in neuronal of amyotrophic lateral sclerosis models decreases proteotoxicity by enhancing TDP43 turnover and neuronal survival [89]. An increasing number of studies support the correlation between

autophagy deficiency and neurodegeneration in mammals. Here, we only listed few examples to support this idea. For detailed reviews, please see [154, 155].

4.6. Autophagic cell death and clearance of cellular corpses

Apoptosis or programmed cell death is a fundamental component in the development of *C. elegans* nematodes [160]. Pioneering studies in *C. elegans* led to the discovery of evolutionarily conserved key players implicated in this important biological process. There are two types of programmed cell death in *C. elegans*: “developmental cell death,” which occurs in the somatic tissues throughout worm development, and “germ cell death,” which takes place in the gonads of adult hermaphrodites [160–165]. During the embryonic and postembryonic stages of *C. elegans* development, only 131 cells of 1090 cells undergo apoptosis to form the adult hermaphrodite [160–165]. The morphological changes in apoptotic *C. elegans* cells are similar to those of mammalian cells and include DNA fragmentation, chromatin condensation, and changes in mitochondrial and plasma membrane potentials [160]. Autophagy plays a major role in the clearance of apoptotic corpses generated during both the developmental cell death and germ cell death [166–169]. Specifically, the number of embryonic apoptotic corpses is significantly increased in nine *C. elegans* strains harboring mutations in essential genes of the autophagic pathway [167]. Autophagy proteins LGG-1, ATG-18, and EPG-5 are recruited to engulfed apoptotic corpses and are essential for the degradation inside the phagocyte [169].

In *Drosophila*, several studies have reported the requirement of autophagy in the death and clearance of specific cells throughout the fly development. In contrast to the role of autophagy in mediating cellular survival, autophagy contributes to fly development by killing particular cells in specific tissues. For instance, autophagy genes are required for the killing and clearance of cells in the salivary glands, ovary, intestine, and embryonic serosa membranes [170–175]. Autophagy also occurs in dying midgut cells and is essential for the clearance of this tissue.

In mice, autophagy contributes to the programmed cell death-mediated clearance of apoptotic cell corpses. Lack of *Atg5* leads to defective apoptotic corpses engulfment in the developing mice embryos [176]. Autophagy is also required for the clearance of cell corpses in the retinal neuroepithelium of developing chick embryos [177]. Therefore, the role of autophagy in the clearance of corpses is evolutionarily conserved and essential for the proper organogenesis and development in most animals.

4.7. Metabolism

In invertebrates, the storage and biosynthesis of energy reserves, including yolk particles, lipids, and glycogen, play a crucial role in development during early embryogenesis and later during adulthood [178]. In *C. elegans*, the yolk particles accumulate with age and are synthesized in the intestine and transported later to the pseudocoelom (body cavity) of *C. elegans* animals. These granules are essential to survival upon starvation during L1 diapause [178, 179]. In *Drosophila*, the yolk particles are also important for embryonic development. Importantly, ATG1 is required for the catabolism of yolk particles in *Drosophila* [180].

The role of autophagy in lipid metabolism has been reported in many organisms. In *C. elegans*, inhibition of autophagy genes leads to a decline in organismal lipid content supporting an essential role for autophagy in lipid metabolism [181]. Moreover, autophagy and lipolysis work inter-dependently to promote longevity in germline-less *C. elegans* strains [181, 182]. The role of autophagy in the degradation of lipid droplets has not been clearly elucidated in *C. elegans*. The fact that autophagy mutants display reduced lipid contents in *C. elegans* could be due to the role of autophagy in the restoration of energy levels and storage in the form of yolk, glycogen, and fat. To determine whether autophagy plays a role in lipid degradation in nematodes, both wild-type and autophagy mutant *C. elegans* strains should be subjected to an energy depletion stress that induces lipid degradation and the difference in the efficiency of degradation should be investigated. A similar experiment has been conducted upon loss of HLH-30, the TFEB homologue in *C. elegans*. In this case, *hlh-30* mutant animals displayed a less efficient degradation of lipid content upon starvation in comparison with the wild-type animals supporting a potential role of autophagy in the mobilization of lipids upon stress in *C. elegans* [183]. This role of HLH-30 is evolutionarily conserved. In fact, TFEB has been also shown to prevent diet-induced obesity in mice [183].

Following stress and energy depletion, the mobilization of “energy-rich” intracellular contents is essential. The autophagic degradation of lipids has been reported throughout evolution. In contrast to what has been observed in *C. elegans*, where the inhibition of autophagy leads to a decrease in lipid content, autophagic pathways are important for targeting lipid droplets for lysosomal degradation in yeast [184, 185]. In mammalian systems, autophagy has been linked to lipid metabolism but with opposite effects depending on the context. In hepatocytes, the pharmacological or genetic inhibition of autophagy increases triglyceride content supporting an important role of autophagy in lipid breakdown. Consistently, lipid content is significantly increased in *Atg7* liver-specific knockout mice as compared to the controls [186]. However, knockdown of *Atg7*, *Atg5*, or the pharmacological inhibition of autophagy in 3T3-L1 pre-adipocytes reduced lipid accumulation [187]. This is in accordance with the observation that the mass of white adipose tissue decreased significantly in *Atg7* adipocyte-specific knockout mice in comparison with the control [187]. The connection between autophagy and lipid metabolism is reviewed in detail in Ref. [188].

In accordance with the role of autophagy in lipid metabolism, autophagy also plays an important role in glycogen metabolism. In *Drosophila*, the inhibition of autophagy in the fly skeletal muscles using chloroquine reduced the efficiency of glycogen degradation [189]. Using electron microscopy, the same group has revealed glycogen as electron dense material inside the double membrane structures of the autophagosomes [189]. Importantly, *Vps15* deficiency led to the accumulation of glycogen in murine skeletal muscles, whereas the overexpression of *Vps34/Vps15* in myoblasts from Danon autophagic vacuolar myopathy patients decreased glycogen storage [190]. In humans, the impairment of lysosomal and autophagic functions is associated with glycogen storage diseases and is linked to muscle atrophy and neurodegeneration [191–194]. Altogether, accumulating evidence supports the role of autophagy in the degradation of lipids and glycogen across evolution.

5. From model organisms to cancer in humans

Genetic pathways that alter autophagy in model organisms are often linked to cancer in humans. For instance, AMPK, TOR, Insulin, SKN-1/NRF2, CEP-1/p53, FLCN-1, and other signaling pathways modulate autophagy in model organisms and are associated with cancer initiation and progression in humans. Two major kinases are important in stress sensing and autophagy regulation: the mammalian target of rapamycin (mTOR) and the 5' AMP-activated protein kinase (AMPK). TOR is a serine/threonine kinase that is activated during nutrient-rich conditions and is inhibited by starvation. In *S. cerevisiae*, *D. melanogaster*, and mammalian systems, TOR has been linked to autophagy through the regulation of the autophagy initiation complex ULK1/ATG1 [112, 195–199]. AMPK is activated upon starvation and drives autophagy in mammalian cells and in invertebrate model organisms. In yeast, ATG1 and ATG13 have been found as potential genetic interactors and downstream effectors of *SNF1*, the yeast AMPK homologue [200]. In mammals, two groups reported the ability of AMPK to induce autophagy through ULK1/ATG1 activation [195, 201]. In this section of this chapter, we will emphasize the dual role of autophagy in cancer.

Autophagy deregulation has been widely reported in human cancers. This is reviewed in detail in Refs. [202, 203]. Whether autophagy plays a tumor-suppressing role or a tumor-promoting role is still controversial since both cases have been reported. Although autophagy protects against tumorigenesis since it plays a central role in the clearance of damaged cellular macromolecules and organelles, increasing evidence suggests that autophagy could also acquire tumor-promoting functions. By supplying cancer cells with energy, autophagy may promote their survival because they are often exposed to nutrient deprivation and hypoxia due to lack of blood vessels.

5.1. Autophagy as a tumor-suppressing mechanism

The observation that autophagy gene ATG6/BECN1 is monoallelically lost in a large number of prostate, breast, and ovarian cancers supported the tumor suppression role of autophagy at first [117, 204–206]. Consistently, autophagy genes are frequently downregulated in tumors. In mice, homozygous deletion of *Becn1* leads to embryonic lethality. However, *Becn1* heterozygous mice exhibit a high frequency of spontaneous tumors that still express the wild-type *Becn1* mRNA and protein supporting a role of *Becn1* as a haploinsufficient tumor suppressor gene [117, 206]. Moreover, BIF-1 and UVRAC, which are essential components of the Beclin1/class III PI3K complex, also contribute to the control of proliferation and suppression of tumor growth [207]. Furthermore, the deficiency in autophagy genes *Atg5*, *Atg7*, and *Becn1* in mice leads to benign hepatic tumors [208].

How autophagy acts as a tumor suppressor is not clear yet. A plausible explanation could be that loss of autophagy increases oxidative stress, which leads to the accumulation of damaged macromolecular cellular components [209, 210]. This is supported by the fact that impaired autophagy increases genomic instability presumably through lack of degradation of damaged mitochondria and an intracellular increase in the levels of reactive oxygen species (ROS) [211, 212]. The selective degradation of damaged mitochondria by autophagy has been shown to

protect against oxidative stress and mitochondrial dysfunction [213]. Autophagy deficiency has been shown to contribute to the tumorigenesis induced by oncogene activation or by chemical carcinogens. Deletion of *Atg7* in mice drives early tumorigenesis induced by BRAF^{V600E} activation [214], supporting the tumor suppression function of autophagy in the initiation of tumorigenesis. However, *Atg7* deletion also abrogated the ability of the BRAF^{V600E}-driven tumors to progress into a more malignant phenotype [214]. Also, *Atg4C*/autophagin3 knock-out mice exhibited an increased susceptibility to develop fibrosarcomas induced by chemical carcinogens [215].

Autophagy has been recently shown to mediate cellular senescence through the degradation of nuclear lamina upon oncogenic events, suggesting that this guardian role of autophagy might prevent tumorigenesis [216].

5.2. Autophagy as a tumor-promoting mechanism

The balance between autophagy and apoptosis is a key factor in the cellular decision between life and death. These two pathways are connected, and deregulation in this balance is a main factor in carcinogenesis. Upon cellular exposure to stress, when the damage cannot be repaired, cells normally undergo programmed cell death to eliminate them. When cells escape these control mechanisms and are unable to die, resistant clones emerge which could lead to cancer. Therefore, mechanisms of resistance to stress are often utilized by cancer cells to survive and proliferate. Autophagy is induced in hypoxic and highly nutrient-stressed tumor microenvironments [211, 212]. Autophagy is also required to promote tumorigenesis by activating mutations of multiple oncogenes, including *Kras*^{G12D} [217–219] and *Braf*^{V600E} [214]. In fact, *Atg7* deletion in mice extends the lifespan of mice carrying an activating mutation in *Braf*^{V600E} that drives lung tumor growth and impairs mitochondrial metabolism and survival to starvation [214]. Similarly, the inhibition of autophagy using the autophagy inhibitor chloroquine abrogates the growth of lymphoma tumors induced by Myc activation. Additionally, deletion of the autophagic component FIP200 in mammary epithelial cells in mice suppressed mammary tumor growth in the MMTV-PyMT mouse model of human breast cancer [220].

The role of P62/SQSTM1 in tumorigenesis is controversial and context-dependent. While autophagy suppresses tumorigenesis by eliminating P62, recent findings demonstrate that P62 synergizes with autophagy to promote tumor growth *in vivo* [221].

Several tumor suppressor genes are associated with aberrant autophagic flux. Mutation in the tumor suppressor gene *Flcn* in humans, responsible for the Birt-Hogg-Dubé neoplastic syndrome, increases the predisposition to renal cysts and tumors [222, 223]. Importantly, autophagy is required for survival to oxidative and nutrient deprivation stresses of FLCN-deficient cells and for the FLCN-driven tumorigenesis [46, 224]. A similar role for VHL, another renal tumor suppressor, in the regulation of autophagic events in renal cell carcinomas has also been described [225]. Autophagy inhibition by MiR-204 suppressed the tumor growth in VHL-deficient cells and the inhibition of LC3B/ATG5 suppressed the development of VHL-deficient renal cell carcinomas in nude mice [225]. Autophagy also contributes to the tumorigenesis induced by loss of the tumor suppressor tuberous sclerosis complex TSC2 [226].

Recently, ATG7 has been shown to cooperate with loss of PTEN to drive tumorigenesis in prostate cancer [227].

Autophagy also plays a critical role in sustaining cancer cell viability and promoting tumor growth in pancreatic ductal adenocarcinoma [228]. MiT/TFE-dependent transcriptional activation of the lysosomal-autophagic pathway is essential for metabolic reprogramming in pancreatic ductal adenocarcinomas and drives aggressive malignancies [229].

6. Conclusion and perspectives

The autophagy-associated pathways that alter lifespan, stress tolerance, neuronal health, resistance to pathogens, and metabolism in lower organisms are highly evolutionarily conserved and are associated with tumorigenesis in mammals. Although the autophagic process does not change between cells/tissues/organisms, its roles are diverse and depend on the context. The important role of autophagy as a “guardian” of cellular integrity by clearing damaged components helps protect organisms against many diseases, including neurological disorders and cancer. Moreover, the important role of autophagy in energy supply and survival to harsh environmental conditions could be employed by cancer cells to survive hypoxic tumor microenvironments. Due to the fact that the molecular and functional basis of autophagic processes are highly conserved between organisms, it is of great interest to use these organisms to link autophagy to important disease-associated signaling pathways. Finding pathways that alter autophagic activities is essential and could help the development of cures for multiple diseases with the common denominator: autophagy. Performing such assays in invertebrate models is an advantageous fast, inexpensive, and a reliable method that has great potential and value for the understanding and treatment of human diseases linked to autophagy including cancer.

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References

- [1] Kaur J, Debnath J. Autophagy at the crossroads of catabolism and anabolism. *Nat Rev Mol Cell Biol.* 2015;16(8):461–72.

- [2] Tsukada M, Ohsumi Y. Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. FEBS Lett. 1993;333(1–2):169–74.
- [3] Thumm M, Egner R, Koch B, Schlumpberger M, Straub M, Veenhuis M, et al. Isolation of autophagocytosis mutants of *Saccharomyces cerevisiae*. FEBS Lett. 1994;349(2):275–80.
- [4] Harding TM, Morano KA, Scott SV, Klionsky DJ. Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. J Cell Biol. 1995;131(3):591–602.
- [5] Hutchins MU, Klionsky DJ. Vacuolar localization of oligomeric alpha-mannosidase requires the cytoplasm to vacuole targeting and autophagy pathway components in *Saccharomyces cerevisiae*. J Biol Chem. 2001;276(23):20491–8.
- [6] Klionsky DJ, Cregg JM, Dunn WA, Jr., Emr SD, Sakai Y, Sandoval IV, et al. A unified nomenclature for yeast autophagy-related genes. Dev Cell. 2003;5(4):539–45.
- [7] Wang CW, Klionsky DJ. The molecular mechanism of autophagy. Mol Med. 2003;9(3–4):65–76.
- [8] Bento CF, Renna M, Ghislat G, Puri C, Ashkenazi A, Vicinanza M, et al. Mammalian autophagy: how does it work? Annu Rev Biochem. 2016.
- [9] Noda NN, Inagaki F. Mechanisms of autophagy. Annu Rev Biophys. 2015;44:101–22.
- [10] Mizushima N. Autophagy: process and function. Genes Dev. 2007;21(22):2861–73.
- [11] Xie Z, Klionsky DJ. Autophagosome formation: core machinery and adaptations. Nat Cell Biol. 2007;9(10):1102–9.
- [12] Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y. Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol. 2009;10(7):458–67.
- [13] Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, et al. Life with 6000 genes. Science. 1996;274(5287):546, 63–7.
- [14] Wood V, Gwilliam R, Rajandream MA, Lyne M, Lyne R, Stewart A, et al. The genome sequence of *Schizosaccharomyces pombe*. Nature. 2002;415(6874):871–80.
- [15] Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, et al. The genome sequence of *Drosophila melanogaster*. Science. 2000;287(5461):2185–95.
- [16] Klionsky DJ. Autophagy: from phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol. 2007;8(11):931–7.
- [17] Guimaraes RS, Delorme-Axford E, Klionsky DJ, Reggiori F. Assays for the biochemical and ultrastructural measurement of selective and nonselective types of autophagy in the yeast *Saccharomyces cerevisiae*. Methods. 2015;75:141–50.

- [18] Delorme-Axford E, Guimaraes RS, Reggiori F, Klionsky DJ. The yeast *Saccharomyces cerevisiae*: an overview of methods to study autophagy progression. *Methods*. 2015;75:3–12.
- [19] Klionsky DJ, Abdalla FC, Abeliovich H, Abraham RT, Acevedo-Arozena A, Adeli K, et al. Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy*. 2012;8(4):445–544.
- [20] Nakatogawa H, Ohsumi Y. SDS-PAGE techniques to study ubiquitin-like conjugation systems in yeast autophagy. *Methods Mol Biol*. 2012;832:519–29.
- [21] Palmisano NJ, Melendez A. Detection of autophagy in *Caenorhabditis elegans*. *Cold Spring Harb Protoc*. 2016;2016(2):pdb top070466.
- [22] Palmisano NJ, Melendez A. Detection of autophagy in *Caenorhabditis elegans* by western blotting analysis of LGG-1. *Cold Spring Harb Protoc*. 2016;2016(2):pdb prot086512.
- [23] Palmisano NJ, Melendez A. Detection of autophagy in *Caenorhabditis elegans* using GFP::LGG-1 as an autophagy marker. *Cold Spring Harb Protoc*. 2016;2016(1):pdb prot086496.
- [24] Zhang H, Chang JT, Guo B, Hansen M, Jia K, Kovacs AL, et al. Guidelines for monitoring autophagy in *Caenorhabditis elegans*. *Autophagy*. 2015;11(1):9–27.
- [25] Jenzer C, Simionato E, Legouis R. Tools and methods to analyze autophagy in *C. elegans*. *Methods*. 2015;75:162–71.
- [26] DeVorkin L, Gorski SM. Genetic manipulation of autophagy in the *Drosophila* ovary. *Cold Spring Harb Protoc*. 2014;2014(9):973–9.
- [27] DeVorkin L, Gorski SM. LysoTracker staining to aid in monitoring autophagy in *Drosophila*. *Cold Spring Harb Protoc*. 2014;2014(9):951–8.
- [28] DeVorkin L, Gorski SM. Monitoring autophagic flux using Ref(2)P, the *Drosophila* p62 ortholog. *Cold Spring Harb Protoc*. 2014;2014(9):959–66.
- [29] DeVorkin L, Gorski SM. Monitoring autophagy in *Drosophila* using fluorescent reporters in the UAS-GAL4 system. *Cold Spring Harb Protoc*. 2014;2014(9):967–72.
- [30] Mauvezin C, Ayala C, Braden CR, Kim J, Neufeld TP. Assays to monitor autophagy in *Drosophila*. *Methods*. 2014;68(1):134–9.
- [31] Ligeon LA, Barois N, Werkmeister E, Bongiovanni A, Lafont F. Structured illumination microscopy and correlative microscopy to study autophagy. *Methods*. 2015;75:61–8.
- [32] Tooze SA, Dooley HC, Jefferies HB, Joachim J, Judith D, Lamb CA, et al. Assessing mammalian autophagy. *Methods Mol Biol*. 2015;1270:155–65.
- [33] Joachim J, Jiang M, McKnight NC, Howell M, Tooze SA. High-throughput screening approaches to identify regulators of mammalian autophagy. *Methods*. 2015;75:96–104.

- [34] Esteban-Martinez L, Boya P. Autophagic flux determination *in vivo* and *ex vivo*. *Methods*. 2015;75:79–86.
- [35] Seglen PO, Luhr M, Mills IG, Saetre F, Szalai P, Engedal N. Macroautophagic cargo sequestration assays. *Methods*. 2015;75:25–36.
- [36] Karanasios E, Ktistakis NT. Live-cell imaging for the assessment of the dynamics of autophagosome formation: focus on early steps. *Methods*. 2015;75:54–60.
- [37] Gutierrez MG, Saka HA, Chinen I, Zoppino FC, Yoshimori T, Bocco JL, et al. Protective role of autophagy against *Vibrio cholerae* cytolysin, a pore-forming toxin from *V. cholerae*. *Proc Natl Acad Sci USA*. 2007;104(6):1829–34.
- [38] Hosokawa N, Hara Y, Mizushima N. Generation of cell lines with tetracycline-regulated autophagy and a role for autophagy in controlling cell size. *FEBS Lett*. 2006;580(11):2623–9.
- [39] Shintani T, Klionsky DJ. Cargo proteins facilitate the formation of transport vesicles in the cytoplasm to vacuole targeting pathway. *J Biol Chem*. 2004;279(29):29889–94.
- [40] Djeddi A, Michelet X, Culetto E, Alberti A, Barois N, Legouis R. Induction of autophagy in ESCRT mutants is an adaptive response for cell survival in *C. elegans*. *J Cell Sci*. 2012;125(Pt 3):685–94.
- [41] Jiang P, Mizushima N. LC3- and p62-based biochemical methods for the analysis of autophagy progression in mammalian cells. *Methods*. 2015;75:13–8.
- [42] Bjorkoy G, Lamark T, Pankiv S, Overvatn A, Brech A, Johansen T. Monitoring autophagic degradation of p62/SQSTM1. *Methods Enzymol*. 2009;452:181–97.
- [43] Mikawa T, Kanoh J, Ishikawa F. Fission yeast Vps1 and Atg8 contribute to oxidative stress resistance. *Genes Cells*. 2010;15(3):229–42.
- [44] Samokhvalov V, Scott BA, Crowder CM. Autophagy protects against hypoxic injury in *C. elegans*. *Autophagy*. 2008;4(8):1034–41.
- [45] Melendez A, Tallozy Z, Seaman M, Eskelinen EL, Hall DH, Levine B. Autophagy genes are essential for dauer development and life-span extension in *C. elegans*. *Science*. 2003;301(5638):1387–91.
- [46] Possik E, Jalali Z, Nouet Y, Yan M, Gingras MC, Schmeisser K, et al. Folliculin regulates ampk-dependent autophagy and metabolic stress survival. *PLoS Genet*. 2014;10(4):e1004273.
- [47] Kang C, You YJ, Avery L. Dual roles of autophagy in the survival of *Caenorhabditis elegans* during starvation. *Genes Dev*. 2007;21(17):2161–71.
- [48] Gomez TA, Banfield KL, Trogler DM, Clarke SG. The L-isoaspartyl-O-methyltransferase in *Caenorhabditis elegans* larval longevity and autophagy. *Dev Biol*. 2007;303(2):493–500.

- [49] Possik E, Ajisebutu A, Manteghi S, Gingras MC, Vijayaraghavan T, Flamand M, et al. FLCN and AMPK confer resistance to hyperosmotic stress via remodeling of glycogen stores. *PLoS Genet.* 2015;11(10):e1005520.
- [50] Juhasz G, Erdi B, Sass M, Neufeld TP. Atg7-dependent autophagy promotes neuronal health, stress tolerance, and longevity but is dispensable for metamorphosis in *Drosophila*. *Genes Dev.* 2007;21(23):3061–6.
- [51] Simonsen A, Cumming RC, Brech A, Isakson P, Schubert DR, Finley KD. Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult *Drosophila*. *Autophagy.* 2008;4(2):176–84.
- [52] Wu H, Wang MC, Bohmann D. JNK protects *Drosophila* from oxidative stress by transcriptionally activating autophagy. *Mech Dev.* 2009;126(8–9):624–37.
- [53] Minois N, Carmona-Gutierrez D, Bauer MA, Rockenfeller P, Eisenberg T, Brandhorst S, et al. Spermidine promotes stress resistance in *Drosophila melanogaster* through autophagy-dependent and -independent pathways. *Cell Death Dis.* 2012;3:e401.
- [54] Pyo JO, Yoo SM, Ahn HH, Nah J, Hong SH, Kam TI, et al. Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nat Commun.* 2013;4:2300.
- [55] Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, et al. The role of autophagy during the early neonatal starvation period. *Nature.* 2004;432(7020):1032–6.
- [56] Ginet V, Puyal J, Clarke PG, Truttmann AC. Enhancement of autophagic flux after neonatal cerebral hypoxia-ischemia and its region-specific relationship to apoptotic mechanisms. *Am J Pathol.* 2009;175(5):1962–74.
- [57] Yan W, Zhang H, Bai X, Lu Y, Dong H, Xiong L. Autophagy activation is involved in neuroprotection induced by hyperbaric oxygen preconditioning against focal cerebral ischemia in rats. *Brain Res.* 2011;1402:109–21.
- [58] Sheng R, Zhang LS, Han R, Liu XQ, Gao B, Qin ZH. Autophagy activation is associated with neuroprotection in a rat model of focal cerebral ischemic preconditioning. *Autophagy.* 2010;6(4):482–94.
- [59] Lapierre LR, Hansen M. Lessons from *C. elegans*: signaling pathways for longevity. *Trends Endocrinol Metab.* 2012.Dec; 23(12): 637–644.
- [60] Morselli E, Galluzzi L, Kepp O, Criollo A, Maiuri MC, Tavernarakis N, et al. Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol. *Aging (Albany NY).* 2009;1(12):961–70.
- [61] O'Rourke EJ, Kuballa P, Xavier R, Ruvkun G. omega-6 Polyunsaturated fatty acids extend life span through the activation of autophagy. *Genes Dev.* 2013;27(4):429–40.
- [62] Hansen M, Chandra A, Mitic LL, Onken B, Driscoll M, Kenyon C. A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. *PLoS Genet.* 2008;4(2):e24.

- [63] Dwivedi M, Song HO, Ahnn J. Autophagy genes mediate the effect of calcineurin on life span in *C. elegans*. *Autophagy*. 2009;5(5):604–7.
- [64] Tavernarakis N, Pasparaki A, Tasdemir E, Maiuri MC, Kroemer G. The effects of p53 on whole organism longevity are mediated by autophagy. *Autophagy*. 2008;4(7):870–3.
- [65] Morselli E, Maiuri MC, Markaki M, Megalou E, Pasparaki A, Palikaras K, et al. Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction of autophagy. *Cell Death Dis*. 2010;1:e10.
- [66] Palikaras K, Lionaki E, Tavernarakis N. Coordination of mitophagy and mitochondrial biogenesis during ageing in *C. elegans*. *Nature*. 2015;521(7553):525–8.
- [67] Lapierre LR, De Magalhaes Filho CD, McQuary PR, Chu CC, Visvikis O, Chang JT, et al. The TFEB orthologue HLH-30 regulates autophagy and modulates longevity in *Caenorhabditis elegans*. *Nat Commun*. 2013;4:2267.
- [68] Visvikis O, Ihuegbu N, Labed SA, Luhachack LG, Alves AM, Wollenberg AC, et al. Innate host defense requires TFEB-mediated transcription of cytoprotective and antimicrobial genes. *Immunity*. 2014;40(6):896–909.
- [69] Seah NE, de Magalhaes Filho CD, Petrashen AP, Henderson HR, Laguer J, Gonzalez J, et al. Autophagy-mediated longevity is modulated by lipoprotein biogenesis. *Autophagy*. 2016;12(2):261–72.
- [70] Kim M, Park HL, Park HW, Ro SH, Nam SG, Reed JM, et al. *Drosophila* Fip200 is an essential regulator of autophagy that attenuates both growth and aging. *Autophagy*. 2013;9(8):1201–13.
- [71] Rockenfeller P, Koska M, Pietrocola F, Minois N, Knittelfelder O, Sica V, et al. Phosphatidylethanolamine positively regulates autophagy and longevity. *Cell Death Differ*. 2015;22(3):499–508.
- [72] Alvers AL, Fishwick LK, Wood MS, Hu D, Chung HS, Dunn WA, Jr., et al. Autophagy and amino acid homeostasis are required for chronological longevity in *Saccharomyces cerevisiae*. *Aging Cell*. 2009;8(4):353–69.
- [73] Alvers AL, Wood MS, Hu D, Kaywell AC, Dunn WA, Jr., Aris JP. Autophagy is required for extension of yeast chronological life span by rapamycin. *Autophagy*. 2009;5(6):847–9.
- [74] Ruckenstuhl C, Netzberger C, Entfellner I, Carmona-Gutierrez D, Kickenweiz T, Stekovic S, et al. Lifespan extension by methionine restriction requires autophagy-dependent vacuolar acidification. *PLoS Genet*. 2014;10(5):e1004347.
- [75] Aris JP, Alvers AL, Ferraiuolo RA, Fishwick LK, Hanvivatpong A, Hu D, et al. Autophagy and leucine promote chronological longevity and respiration proficiency during calorie restriction in yeast. *Exp Gerontol*. 2013;48(10):1107–19.

- [76] Tang F, Watkins JW, Bermudez M, Gray R, Gaban A, Portie K, et al. A life-span extending form of autophagy employs the vacuole-vacuole fusion machinery. *Autophagy*. 2008;4(7):874–86.
- [77] Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature*. 2009;460(7253):392–5.
- [78] Stranks AJ, Hansen AL, Panse I, Mortensen M, Ferguson DJ, Puleston DJ, et al. Autophagy controls acquisition of aging features in macrophages. *J Innate Immun*. 2015;7(4):375–91.
- [79] Yang F, Chu X, Yin M, Liu X, Yuan H, Niu Y, et al. mTOR and autophagy in normal brain aging and caloric restriction ameliorating age-related cognition deficits. *Behav Brain Res*. 2014;264:82–90.
- [80] Zhao S, Lin L, Kan G, Xu C, Tang Q, Yu C, et al. High autophagy in the naked mole rat may play a significant role in maintaining good health. *Cell Physiol Biochem*. 2014;33(2):321–32.
- [81] Kaushik S, Arias E, Kwon H, Lopez NM, Athonvarangkul D, Sahu S, et al. Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. *EMBO Rep*. 2012;13(3):258–65.
- [82] Vittorini S, Paradiso C, Donati A, Cavallini G, Masini M, Gori Z, et al. The age-related accumulation of protein carbonyl in rat liver correlates with the age-related decline in liver proteolytic activities. *J Gerontol A Biol Sci Med Sci*. 1999;54(8):B318–23.
- [83] Cuervo AM, Dice JF. Age-related decline in chaperone-mediated autophagy. *J Biol Chem*. 2000;275(40):31505–13.
- [84] Lipinski MM, Zheng B, Lu T, Yan Z, Py BF, Ng A, et al. Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer’s disease. *Proc Natl Acad Sci USA*. 2010;107(32):14164–9.
- [85] Taneike M, Yamaguchi O, Nakai A, Hikoso S, Takeda T, Mizote I, et al. Inhibition of autophagy in the heart induces age-related cardiomyopathy. *Autophagy*. 2010;6(5):600–6.
- [86] Bhuiyan MS, Pattison JS, Osinska H, James J, Gulick J, McLendon PM, et al. Enhanced autophagy ameliorates cardiac proteinopathy. *J Clin Invest*. 2013;123(12):5284–97.
- [87] Zhang C, Cuervo AM. Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. *Nat Med*. 2008;14(9):959–65.
- [88] Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. Alpha-Synuclein is degraded by both autophagy and the proteasome. *J Biol Chem*. 2003;278(27):25009–13.

- [89] Barmada SJ, Serio A, Arjun A, Bilican B, Daub A, Ando DM, et al. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. *Nat Chem Biol.* 2014;10(8):677–85.
- [90] Park HK, Chu K, Jung KH, Lee ST, Bahn JJ, Kim M, et al. Autophagy is involved in the ischemic preconditioning. *Neurosci Lett.* 2009;451(1):16–9.
- [91] Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science.* 2004;305(5688):1292–5.
- [92] Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature.* 2006;441(7095):880–4.
- [93] Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature.* 2006;441(7095):885–9.
- [94] Jia K, Thomas C, Akbar M, Sun Q, Adams-Huet B, Gilpin C, et al. Autophagy genes protect against *Salmonella typhimurium* infection and mediate insulin signaling-regulated pathogen resistance. *Proc Natl Acad Sci USA.* 2009;106(34):14564–9.
- [95] Zou CG, Ma YC, Dai LL, Zhang KQ. Autophagy protects *C. elegans* against necrosis during *Pseudomonas aeruginosa* infection. *Proc Natl Acad Sci USA.* 2014;111(34):12480–5.
- [96] Wu J, Randle KE, Wu LP. ird1 is a Vps15 homologue important for antibacterial immune responses in *Drosophila*. *Cell Microbiol.* 2007;9(4):1073–85.
- [97] Ren C, Finkel SE, Tower J. Conditional inhibition of autophagy genes in adult *Drosophila* impairs immunity without compromising longevity. *Exp Gerontol.* 2009;44(3):228–35.
- [98] Shelly S, Lukinova N, Bambina S, Berman A, Cherry S. Autophagy is an essential component of *Drosophila* immunity against vesicular stomatitis virus. *Immunity.* 2009;30(4):588–98.
- [99] Yano T, Mita S, Ohmori H, Oshima Y, Fujimoto Y, Ueda R, et al. Autophagic control of listeria through intracellular innate immune recognition in *Drosophila*. *Nat Immunol.* 2008;9(8):908–16.
- [100] Kirienko NV, Ausubel FM, Ruvkun G. Mitophagy confers resistance to siderophore-mediated killing by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA.* 2015;112(6):1821–6.
- [101] Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. Escape of intracellular *Shigella* from autophagy. *Science.* 2005;307(5710):727–31.

- [102] Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell*. 2004;119(6):753–66.
- [103] Ling YM, Shaw MH, Ayala C, Coppens I, Taylor GA, Ferguson DJ, et al. Vacuolar and plasma membrane stripping and autophagic elimination of *Toxoplasma gondii* in primed effector macrophages. *J Exp Med*. 2006;203(9):2063–71.
- [104] Gupta M, Shin DM, Ramakrishna L, Goussetis DJ, Platanias LC, Xiong H, et al. IRF8 directs stress-induced autophagy in macrophages and promotes clearance of *Listeria monocytogenes*. *Nat Commun*. 2015;6:6379.
- [105] Maurer K, Torres VJ, Cadwell K. Autophagy is a key tolerance mechanism during *Staphylococcus aureus* infection. *Autophagy*. 2015;11(7):1184–6.
- [106] Maurer K, Reyes-Robles T, Alonzo F, 3rd, Durbin J, Torres VJ, Cadwell K. Autophagy mediates tolerance to *Staphylococcus aureus* alpha-toxin. *Cell Host Microbe*. 2015;17(4):429–40.
- [107] Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science*. 2006;313(5792):1438–41.
- [108] Vergne I, Singh S, Roberts E, Kyei G, Master S, Harris J, et al. Autophagy in immune defense against *Mycobacterium tuberculosis*. *Autophagy*. 2006;2(3):175–8.
- [109] Kimmey JM, Huynh JP, Weiss LA, Park S, Kambal A, Debnath J, et al. Unique role for ATG5 in neutrophil-mediated immunopathology during *M. tuberculosis* infection. *Nature*. 2015;528(7583):565–9.
- [110] Takacs-Vellai K, Vellai T, Puoti A, Passannante M, Wicky C, Streit A, et al. Inactivation of the autophagy gene bec-1 triggers apoptotic cell death in *C. elegans*. *Curr Biol*. 2005;15(16):1513–7.
- [111] Guo B, Huang X, Zhang P, Qi L, Liang Q, Zhang X, et al. Genome-wide screen identifies signaling pathways that regulate autophagy during *Caenorhabditis elegans* development. *EMBO Rep*. 2014;15(6):705–13.
- [112] Scott RC, Schuldiner O, Neufeld TP. Role and regulation of starvation-induced autophagy in the *Drosophila* fat body. *Dev Cell*. 2004;7(2):167–78.
- [113] Lee E, Koo Y, Ng A, Wei Y, Luby-Phelps K, Juraszek A, et al. Autophagy is essential for cardiac morphogenesis during vertebrate development. *Autophagy*. 2014;10(4):572–87.
- [114] Hu Z, Zhang J, Zhang Q. Expression pattern and functions of autophagy-related gene atg5 in zebrafish organogenesis. *Autophagy*. 2011;7(12):1514–27.
- [115] Benato F, Skobo T, Gioacchini G, Moro I, Ciccocanti F, Piacentini M, et al. Ambra1 knockdown in zebrafish leads to incomplete development due to severe defects in organogenesis. *Autophagy*. 2013;9(4):476–95.

- [116] Skobo T, Benato F, Grumati P, Meneghetti G, Cianfanelli V, Castagnaro S, et al. Zebrafish *ambra1a* and *ambra1b* knockdown impairs skeletal muscle development. *PLoS One*. 2014;9(6):e99210.
- [117] Yue Z, Jin S, Yang C, Levine AJ, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. *Proc Natl Acad Sci USA*. 2003;100(25):15077–82.
- [118] Wang S, Li B, Qiao H, Lv X, Liang Q, Shi Z, et al. Autophagy-related gene Atg5 is essential for astrocyte differentiation in the developing mouse cortex. *EMBO Rep*. 2014;15(10):1053–61.
- [119] Cinque L, Forrester A, Bartolomeo R, Svelto M, Venditti R, Montefusco S, et al. FGF signalling regulates bone growth through autophagy. *Nature*. 2015;528(7581):272–5.
- [120] Gan B, Peng X, Nagy T, Alcaraz A, Gu H, Guan JL. Role of FIP200 in cardiac and liver development and its regulation of TNF α and TSC-mTOR signaling pathways. *J Cell Biol*. 2006;175(1):121–33.
- [121] Liu F, Guan JL. FIP200, an essential component of mammalian autophagy is indispensable for fetal hematopoiesis. *Autophagy*. 2011;7(2):229–30.
- [122] Wu X, Fleming A, Ricketts T, Pavel M, Virgin H, Menzies FM, et al. Autophagy regulates Notch degradation and modulates stem cell development and neurogenesis. *Nat Commun*. 2016;7:10533.
- [123] Sato M, Sato K. Degradation of paternal mitochondria by fertilization-triggered autophagy in *C. elegans* embryos. *Science*. 2011;334(6059):1141–4.
- [124] Politi Y, Gal L, Kalifa Y, Ravid L, Elazar Z, Arama E. Paternal mitochondrial destruction after fertilization is mediated by a common endocytic and autophagic pathway in *Drosophila*. *Dev Cell*. 2014;29(3):305–20.
- [125] Song WH, Ballard JW, Yi YJ, Sutovsky P. Regulation of mitochondrial genome inheritance by autophagy and ubiquitin-proteasome system: implications for health, fitness, and fertility. *Biomed Res Int*. 2014;2014:981867.
- [126] Liberski PP, Sikorska B, Bratosiewicz-Wasik J, Gajdusek DC, Brown P. Neuronal cell death in transmissible spongiform encephalopathies (prion diseases) revisited: from apoptosis to autophagy. *Int J Biochem Cell Biol*. 2004;36(12):2473–90.
- [127] Ravikumar B, Duden R, Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum Mol Genet*. 2002;11(9):1107–17.
- [128] Shibata M, Lu T, Furuya T, Degterev A, Mizushima N, Yoshimori T, et al. Regulation of intracellular accumulation of mutant Huntingtin by Beclin 1. *J Biol Chem*. 2006;281(20):14474–85.

- [129] Florez-McClure ML, Hohsfield LA, Fonte G, Bealor MT, Link CD. Decreased insulin-receptor signaling promotes the autophagic degradation of beta-amyloid peptide in *C. elegans*. *Autophagy*. 2007;3(6):569–80.
- [130] Lakso M, Vartiainen S, Moilanen AM, Sirvio J, Thomas JH, Nass R, et al. Dopaminergic neuronal loss and motor deficits in *Caenorhabditis elegans* overexpressing human alpha-synuclein. *J Neurochem*. 2003;86(1):165–72.
- [131] Cao S, Gelwix CC, Caldwell KA, Caldwell GA. Torsin-mediated protection from cellular stress in the dopaminergic neurons of *Caenorhabditis elegans*. *J Neurosci*. 2005;25(15):3801–12.
- [132] Karpinar DP, Balija MB, Kugler S, Opazo F, Rezaei-Ghaleh N, Wender N, et al. Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson's disease models. *EMBO J*. 2009;28(20):3256–68.
- [133] Jia K, Hart AC, Levine B. Autophagy genes protect against disease caused by polyglutamine expansion proteins in *Caenorhabditis elegans*. *Autophagy*. 2007;3(1):21–5.
- [134] Ye Y, Fortini ME. Apoptotic activities of wild-type and Alzheimer's disease-related mutant presenilins in *Drosophila melanogaster*. *J Cell Biol*. 1999;146(6):1351–64.
- [135] Wittmann CW, Wszolek MF, Shulman JM, Salvaterra PM, Lewis J, Hutton M, et al. Tauopathy in *Drosophila*: neurodegeneration without neurofibrillary tangles. *Science*. 2001;293(5530):711–4.
- [136] Feany MB, Bender WW. A *Drosophila* model of Parkinson's disease. *Nature*. 2000;404(6776):394–8.
- [137] Auluck PK, Chan HY, Trojanowski JQ, Lee VM, Bonini NM. Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease. *Science*. 2002;295(5556):865–8.
- [138] Jackson GR, Salecker I, Dong X, Yao X, Arnheim N, Faber PW, et al. Polyglutamine-expanded human huntingtin transgenes induce degeneration of *Drosophila* photoreceptor neurons. *Neuron*. 1998;21(3):633–42.
- [139] Warrick JM, Paulson HL, Gray-Board GL, Bui QT, Fischbeck KH, Pittman RN, et al. Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in *Drosophila*. *Cell*. 1998;93(6):939–49.
- [140] Omata Y, Lim YM, Akao Y, Tsuda L. Age-induced reduction of autophagy-related gene expression is associated with onset of Alzheimer's disease. *Am J Neurodegener Dis*. 2014;3(3):134–42.
- [141] Ogura K, Wicky C, Magnenat L, Tobler H, Mori I, Muller F, et al. *Caenorhabditis elegans* unc-51 gene required for axonal elongation encodes a novel serine/threonine kinase. *Genes Dev*. 1994;8(20):2389–400.

- [142] Saitoh Y, Fujikake N, Okamoto Y, Popiel HA, Hatanaka Y, Ueyama M, et al. p62 plays a protective role in the autophagic degradation of polyglutamine protein oligomers in polyglutamine disease model flies. *J Biol Chem*. 2015;290(3):1442–53.
- [143] Nezis IP, Simonsen A, Sagona AP, Finley K, Gaumer S, Contamine D, et al. Ref(2)P, the *Drosophila melanogaster* homologue of mammalian p62, is required for the formation of protein aggregates in adult brain. *J Cell Biol*. 2008;180(6):1065–71.
- [144] Ratliff EP, Mauntz RE, Kotzebue RW, Gonzalez A, Achal M, Barekat A, et al. Aging and autophagic function influences the progressive decline of adult *Drosophila* behaviors. *PLoS One*. 2015;10(7):e0132768.
- [145] Lindmo K, Brech A, Finley KD, Gaumer S, Contamine D, Rusten TE, et al. The PI 3-kinase regulator Vps15 is required for autophagic clearance of protein aggregates. *Autophagy*. 2008;4(4):500–6.
- [146] Nagy P, Karpati M, Varga A, Piracs K, Venkei Z, Takats S, et al. Atg17/FIP200 localizes to perilyosomal Ref(2)P aggregates and promotes autophagy by activation of Atg1 in *Drosophila*. *Autophagy*. 2014;10(3):453–67.
- [147] Babcock DT, Shen W, Ganetzky B. A neuroprotective function of NSF1 sustains autophagy and lysosomal trafficking in *Drosophila*. *Genetics*. 2015;199(2):511–22.
- [148] Byrne S, Jansen L, JM UK-I, Siddiqui A, Lidov HG, Bodi I, et al. EPG5-related Vici syndrome: a paradigm of neurodevelopmental disorders with defective autophagy. *Brain*. 2016;139(Pt 3):765–81.
- [149] Kyostila K, Syrja P, Jagannathan V, Chandrasekar G, Jokinen TS, Seppala EH, et al. A missense change in the ATG4D gene links aberrant autophagy to a neurodegenerative vacuolar storage disease. *PLoS Genet*. 2015;11(4):e1005169.
- [150] Lu K, Psakhye I, Jentsch S. Autophagic clearance of polyQ proteins mediated by ubiquitin-Atg8 adaptors of the conserved CUET protein family. *Cell*. 2014;158(3):549–63.
- [151] Zabrocki P, Pellens K, Vanhelmont T, Vandebroek T, Griffioen G, Wera S, et al. Characterization of alpha-synuclein aggregation and synergistic toxicity with protein tau in yeast. *FEBS J*. 2005;272(6):1386–400.
- [152] Petroi D, Popova B, Taheri-Talesh N, Irniger S, Shahpasandzadeh H, Zweckstetter M, et al. Aggregate clearance of alpha-synuclein in *Saccharomyces cerevisiae* depends more on autophagosome and vacuole function than on the proteasome. *J Biol Chem*. 2012;287(33):27567–79.
- [153] Tenreiro S, Reimao-Pinto MM, Antas P, Rino J, Wawrzycka D, Macedo D, et al. Phosphorylation modulates clearance of alpha-synuclein inclusions in a yeast model of Parkinson's disease. *PLoS Genet*. 2014;10(5):e1004302.

- [154] Nixon RA. The role of autophagy in neurodegenerative disease. *Nat Med.* 2013;19(8):983–97.
- [155] Menzies FM, Fleming A, Rubinsztein DC. Compromised autophagy and neurodegenerative diseases. *Nat Rev Neurosci.* 2015;16(6):345–57.
- [156] Saitsu H, Nishimura T, Muramatsu K, Kodera H, Kumada S, Sugai K, et al. De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. *Nat Genet.* 2013;45(4):445–9, 9e1.
- [157] Metzger S, Saukko M, Van Che H, Tong L, Puder Y, Riess O, et al. Age at onset in Huntington's disease is modified by the autophagy pathway: implication of the V471A polymorphism in Atg7. *Hum Genet.* 2010;128(4):453–9.
- [158] Metzger S, Walter C, Riess O, Roos RA, Nielsen JE, Craufurd D, et al. The V471A polymorphism in autophagy-related gene ATG7 modifies age at onset specifically in Italian Huntington disease patients. *PLoS One.* 2013;8(7):e68951.
- [159] Komatsu M, Wang QJ, Holstein GR, Friedrich VL, Jr., Iwata J, Kominami E, et al. Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. *Proc Natl Acad Sci USA.* 2007;104(36):14489–94.
- [160] Lettre G, Hengartner MO. Developmental apoptosis in *C. elegans*: a complex CEDnario. *Nat Rev Mol Cell Biol.* 2006;7(2):97–108.
- [161] Sulston JE, Brenner S. The DNA of *Caenorhabditis elegans*. *Genetics.* 1974;77(1):95–104.
- [162] Sulston J, Dew M, Brenner S. Dopaminergic neurons in the nematode *Caenorhabditis elegans*. *J Comp Neurol.* 1975;163(2):215–26.
- [163] Sulston JE, Schierenberg E, White JG, Thomson JN. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev Biol.* 1983;100(1):64–119.
- [164] Sulston JE, Horvitz HR. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev Biol.* 1977;56(1):110–56.
- [165] Kimble J, Hirsh D. The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. *Dev Biol.* 1979;70(2):396–417.
- [166] Huang S, Jia K, Wang Y, Zhou Z, Levine B. Autophagy genes function in apoptotic cell corpse clearance during *C. elegans* embryonic development. *Autophagy.* 2013;9(2):138–49.
- [167] Cheng S, Wu Y, Lu Q, Yan J, Zhang H, Wang X. Autophagy genes coordinate with the class II PI/PtdIns 3-kinase PIK1-1 to regulate apoptotic cell clearance in *C. elegans*. *Autophagy.* 2013;9(12):2022–32.
- [168] Zou W, Wang X, Vale RD, Ou G. Autophagy genes promote apoptotic cell corpse clearance. *Autophagy.* 2012;8(8):1267–68.

- [169] Li W, Zou W, Yang Y, Chai Y, Chen B, Cheng S, et al. Autophagy genes function sequentially to promote apoptotic cell corpse degradation in the engulfing cell. *J Cell Biol.* 2012;197(1):27–35.
- [170] Berry DL, Baehrecke EH. Growth arrest and autophagy are required for salivary gland cell degradation in *Drosophila*. *Cell.* 2007;131(6):1137–48.
- [171] Nezis IP, Shrivastava BV, Sagona AP, Lamark T, Bjorkoy G, Johansen T, et al. Autophagic degradation of dBruce controls DNA fragmentation in nurse cells during late *Drosophila melanogaster* oogenesis. *J Cell Biol.* 2010;190(4):523–31.
- [172] Nezis IP, Lamark T, Velentzas AD, Rusten TE, Bjorkoy G, Johansen T, et al. Cell death during *Drosophila melanogaster* early oogenesis is mediated through autophagy. *Autophagy.* 2009;5(3):298–302.
- [173] Mohseni N, McMillan SC, Chaudhary R, Mok J, Reed BH. Autophagy promotes caspase-dependent cell death during *Drosophila* development. *Autophagy.* 2009;5(3):329–38.
- [174] Lee CY, Cooksey BA, Baehrecke EH. Steroid regulation of midgut cell death during *Drosophila* development. *Dev Biol.* 2002;250(1):101–11.
- [175] Denton D, Shrivastava B, Simin R, Mills K, Berry DL, Baehrecke EH, et al. Autophagy, not apoptosis, is essential for midgut cell death in *Drosophila*. *Curr Biol.* 2009;19(20):1741–46.
- [176] Qu X, Zou Z, Sun Q, Luby-Phelps K, Cheng P, Hogan RN, et al. Autophagy gene-dependent clearance of apoptotic cells during embryonic development. *Cell.* 2007;128(5):931–46.
- [177] Mellen MA, de la Rosa EJ, Boya P. The autophagic machinery is necessary for removal of cell corpses from the developing retinal neuroepithelium. *Cell Death Differ.* 2008;15(8):1279–90.
- [178] Braeckman BP. Intermediary metabolism. In: Koen Houthoofd JRV, editor. 16 February 2009 ed. wormbook2009. http://www.wormbook.org/chapters/www_intermetabolism/intermetabolism.html
- [179] Chotard L, Skorobogata O, Sylvain MA, Shrivastava S, Rocheleau CE. TBC-2 is required for embryonic yolk protein storage and larval survival during L1 diapause in *Caenorhabditis elegans*. *PLoS One.* 2010;5(12):e15662.
- [180] Kuhn H, Sopko R, Coughlin M, Perrimon N, Mitchison T. The Atg1-Tor pathway regulates yolk catabolism in *Drosophila* embryos. *Development.* 2015;142(22):3869–78.
- [181] Lapierre LR, Silvestrini MJ, Nunez L, Ames K, Wong S, Le TT, et al. Autophagy genes are required for normal lipid levels in *C. elegans*. *Autophagy.* 2013;9(3):278–86.
- [182] Lapierre LR, Melendez A, Hansen M. Autophagy links lipid metabolism to longevity in *C. elegans*. *Autophagy.* 2012;8(1):144–6.

- [183] Settembre C, De Cegli R, Mansueto G, Saha PK, Vetrini F, Visvikis O, et al. TFEB controls cellular lipid metabolism through a starvation-induced autoregulatory loop. *Nat Cell Biol.* 2013;15(8):1016.
- [184] Toulmay A, Prinz WA. Direct imaging reveals stable, micrometer-scale lipid domains that segregate proteins in live cells. *J Cell Biol.* 2013;202(1):35–44.
- [185] van Zutphen T, Todde V, de Boer R, Kreim M, Hofbauer HF, Wolinski H, et al. Lipid droplet autophagy in the yeast *Saccharomyces cerevisiae*. *Mol Biol Cell.* 2014;25(2):290–301.
- [186] Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, et al. Autophagy regulates lipid metabolism. *Nature.* 2009;458(7242):1131–5.
- [187] Singh R, Xiang Y, Wang Y, Baikati K, Cuervo AM, Luu YK, et al. Autophagy regulates adipose mass and differentiation in mice. *J Clin Invest.* 2009;119(11):3329–39.
- [188] Singh R, Cuervo AM. Lipophagy: connecting autophagy and lipid metabolism. *Int J Cell Biol.* 2012;2012:282041.
- [189] Zirin J, Nieuwenhuis J, Perrimon N. Role of autophagy in glycogen breakdown and its relevance to chloroquine myopathy. *PLoS Biol.* 2013;11(11):e1001708.
- [190] Nemazanyy I, Blaauw B, Paolini C, Caillaud C, Protasi F, Mueller A, et al. Defects of Vps15 in skeletal muscles lead to autophagic vacuolar myopathy and lysosomal disease. *EMBO Mol Med.* 2013;5(6):870–90.
- [191] Duran J, Gruart A, Garcia-Rocha M, Delgado-Garcia JM, Guinovart JJ. Glycogen accumulation underlies neurodegeneration and autophagy impairment in Lafora disease. *Hum Mol Genet.* 2014;23(12):3147–56.
- [192] Criado O, Aguado C, Gayarre J, Duran-Trio L, Garcia-Cabrero AM, Vernia S, et al. Lafora bodies and neurological defects in malin-deficient mice correlate with impaired autophagy. *Hum Mol Genet.* 2012;21(7):1521–33.
- [193] Nascimbeni AC, Fanin M, Masiero E, Angelini C, Sandri M. Impaired autophagy contributes to muscle atrophy in glycogen storage disease type II patients. *Autophagy.* 2012;8(11):1697–700.
- [194] Nascimbeni AC, Fanin M, Masiero E, Angelini C, Sandri M. The role of autophagy in the pathogenesis of glycogen storage disease type II (GSDII). *Cell Death Differ.* 2012;19(10):1698–708.
- [195] Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol.* 2011;13(2):132–41.
- [196] Kamada Y, Yoshino K, Kondo C, Kawamata T, Oshiro N, Yonezawa K, et al. Tor directly controls the Atg1 kinase complex to regulate autophagy. *Mol Cell Biol.* 2010;30(4):1049–58.

- [197] Nazio F, Strappazzon F, Antonioli M, Bielli P, Cianfanelli V, Bordi M, et al. mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat Cell Biol.* 2013;15(4):406–16.
- [198] Alers S, Loffler AS, Wesselborg S, Stork B. Role of AMPK-mTOR-Ulk1/2 in the regulation of autophagy: cross talk, shortcuts, and feedbacks. *Mol Cell Biol.* 2012;32(1):2–11.
- [199] Scott RC, Juhasz G, Neufeld TP. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. *Curr Biol.* 2007;17(1):1–11.
- [200] Wang Z, Wilson WA, Fujino MA, Roach PJ. Antagonistic controls of autophagy and glycogen accumulation by Snf1p, the yeast homolog of AMP-activated protein kinase, and the cyclin-dependent kinase Pho85p. *Mol Cell Biol.* 2001;21(17):5742–52.
- [201] Egan D, Kim J, Shaw RJ, Guan KL. The autophagy initiating kinase ULK1 is regulated via opposing phosphorylation by AMPK and mTOR. *Autophagy.* 2011;7(6):643–4.
- [202] Wu WK, Coffelt SB, Cho CH, Wang XJ, Lee CW, Chan FK, et al. The autophagic paradox in cancer therapy. *Oncogene.* 2012;31(8):939–53.
- [203] White E. The role for autophagy in cancer. *J Clin Invest.* 2015;125(1):42–6.
- [204] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature.* 1999;402(6762):672–6.
- [205] Aita VM, Liang XH, Murty VV, Pincus DL, Yu W, Cayanis E, et al. Cloning and genomic organization of beclin 1, a candidate tumor suppressor gene on chromosome 17q21. *Genomics.* 1999;59(1):59–65.
- [206] Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *J Clin Invest.* 2003;112(12):1809–20.
- [207] Takahashi Y, Coppola D, Matsushita N, Cuaing HD, Sun M, Sato Y, et al. Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat Cell Biol.* 2007;9(10):1142–51.
- [208] Takamura A, Komatsu M, Hara T, Sakamoto A, Kishi C, Waguri S, et al. Autophagy-deficient mice develop multiple liver tumors. *Genes Dev.* 2011;25(8):795–800.
- [209] Abedin MJ, Wang D, McDonnell MA, Lehmann U, Kelekar A. Autophagy delays apoptotic death in breast cancer cells following DNA damage. *Cell Death Differ.* 2007;14(3):500–10.
- [210] Katayama M, Kawaguchi T, Berger MS, Pieper RO. DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. *Cell Death Differ.* 2007;14(3):548–58.

- [211] Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell*. 2006;10(1):51–64.
- [212] Karantza-Wadsworth V, Patel S, Kravchuk O, Chen G, Mathew R, Jin S, et al. Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis. *Genes Dev*. 2007;21(13):1621–35.
- [213] Lemasters JJ. Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Res*. 2005;8(1):3–5.
- [214] Strohecker AM, Guo JY, Karsli-Uzunbas G, Price SM, Chen GJ, Mathew R, et al. Autophagy sustains mitochondrial glutamine metabolism and growth of BrafV600E-driven lung tumors. *Cancer Discov*. 2013;3(11):1272–85.
- [215] Marino G, Salvador-Montoliu N, Fueyo A, Knecht E, Mizushima N, Lopez-Otin C. Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3. *J Biol Chem*. 2007;282(25):18573–83.
- [216] Dou Z, Xu C, Donahue G, Shimi T, Pan JA, Zhu J, et al. Autophagy mediates degradation of nuclear lamina. *Nature*. 2015;527(7576):105–9.
- [217] Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, et al. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev*. 2011;25(5):460–70.
- [218] Lock R, Roy S, Kenific CM, Su JS, Salas E, Ronen SM, et al. Autophagy facilitates glycolysis during Ras-mediated oncogenic transformation. *Mol Biol Cell*. 2011;22(2):165–78.
- [219] Guo JY, Karsli-Uzunbas G, Mathew R, Aisner SC, Kamphorst JJ, Strohecker AM, et al. Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis. *Genes Dev*. 2013;27(13):1447–61.
- [220] Wei H, Wei S, Gan B, Peng X, Zou W, Guan JL. Suppression of autophagy by FIP200 deletion inhibits mammary tumorigenesis. *Genes Dev*. 2011;25(14):1510–27.
- [221] Wei H, Wang C, Croce CM, Guan JL. p62/SQSTM1 synergizes with autophagy for tumor growth *in vivo*. *Genes Dev*. 2014;28(11):1204–16.
- [222] Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: a metabolic disease. *Nature Reviews Urology*. 2010;7(5):277–85.
- [223] Tee AR, Pause A. Birt-Hogg-Dube: tumour suppressor function and signalling dynamics central to folliculin. *Fam Cancer*. 2013;12(3):367–72.
- [224] Yan M, Gingras MC, Dunlop EA, Nouet Y, Dupuy F, Jalali Z, et al. The tumor suppressor folliculin regulates AMPK-dependent metabolic transformation. *J Clin Invest*. 2014;126(6):2640–2650.

- [225] Mikhaylova O, Stratton Y, Hall D, Kellner E, Ehmer B, Drew AF, et al. VHL-regulated MiR-204 suppresses tumor growth through inhibition of LC3B-mediated autophagy in renal clear cell carcinoma. *Cancer Cell*. 2012;21(4):532–46.
- [226] Parkhitko A, Myachina F, Morrison TA, Hindi KM, Auricchio N, Karbowniczek M, et al. Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent. *Proc Natl Acad Sci USA*. 2011;108(30):12455–60.
- [227] Santanam U, Banach-Petrosky W, Abate-Shen C, Shen MM, White E, DiPaola RS. Atg7 cooperates with Pten loss to drive prostate cancer tumor growth. *Genes Dev*. 2016;30(4):399–407.
- [228] Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, et al. Pancreatic cancers require autophagy for tumor growth. *Genes Dev*. 2011;25(7):717–29.
- [229] Perera RM, Stoykova S, Nicolay BN, Ross KN, Fitamant J, Boukhali M, et al. Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. *Nature*. 2015;524(7565):361–5.