Chapter 11

Autophagy in Model Organisms: Insights into Cancer

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Additional information is available at the end of the chapter

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

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 stomatisis* 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 αsynuclein 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 pseudoceolom (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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