Autophagy: Molecular Mechanisms, Physiology & Pathology

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Introduction

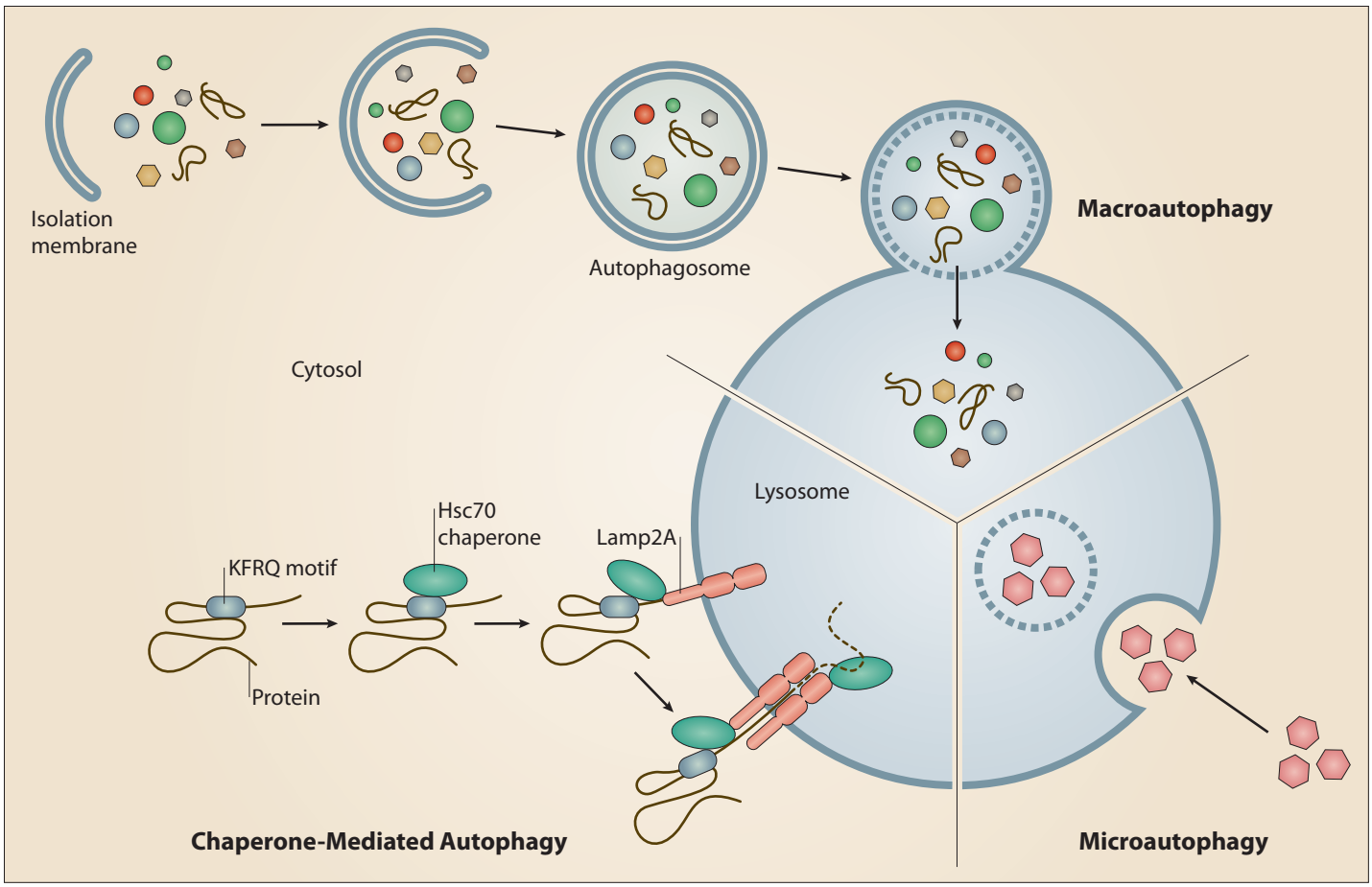
Cell homeostasis involves a fine balance between anabolism and catabolism. In cells, the main catabolic pathways are the ubiquitin-proteasome pathway and the autophagy-lysosomal pathway, through which degradation takes place inside lysosomes. Macroautophagy is a highly regulated cellular mechanism for the degradation and recycling of cytoplasmic contents, including proteins, lipids and whole organelles. This process begins with the formation of an autophagosome, a double membrane structure that engulfs parts of the cytoplasm and whole organelles, which ultimately fuses with a lysosome to enable degradation of the enclosed material. The final products – including amino acids, lipids and nucleotides – are released into the cytoplasm via permeases present in the lysosomal membrane, and can then participate in anabolic reactions required to maintain cellular functions. Autophagy is conserved from yeast to humans and is regulated by the Atg family of proteins.[1]

Types of Autophagy

In addition to macroautophagy there are two other forms of autophagy: chaperone-mediated autophagy (CMA) and microautophagy (Figure 1). CMA is a form of selective autophagy that, to date, has only been described in mammalian cells.[2] Proteins for CMA contain a KFERQ-related motif in their amino acid sequence. This motif is recognized by the cytosolic constitutive chaperone Hsc70 (heat shock cognate of the Hsp70 family), enabling lysosomal delivery of CMA substrates. The lysosomal membrane protein Lamp2A acts as a receptor mediating the translocation of unfolded polypeptides across the lysosomal membrane into the lysosome, where degradation then occurs.[2] Microautophagy involves the direct uptake of fractions of the cytoplasm by the lysosomal membrane. It is dependent on GTP hydrolysis and calcium, although the molecular basis of this process remains to be elucidated.[3]

Macroautophagy, hereafter referred to as autophagy, occurs occurs in all tissues and cell types. A basal level of autophagy is necessary to keep cells (particularly postmitotic cells, such as neurons) free of damaged proteins and organelles.[4,5] By contrast, induced autophagy is a stress response elicited in many conditions (such as nutrient starvation and metabolic stress), which recycles intracellular components to generate ATP and new 'building blocks', thus sustaining cell survival.[6] Since the discovery of the *Atg* genes a decade ago, the molecular

Figure 1 | Types of autophagy



Three main types of autophagy have been described in mammalian cells: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). All of these pathways converge in lysosomes to ensure intracellular degradation. Macroautophagy facilitates the recycling of cellular components, including organelles, through the formation of an autophagosome. During microautophagy, proteins translocate directly into the lysosomes. Chaperone-mediated autophagy enables the degradation of proteins which harbor a protein sequence that is recognized by chaperones (e.g. Hsc70). Recognition of the lysosomal membrane protein Lamp2A by Hsc70 facilitates the unfolding and translocation of this protein inside the lysosomal lumen, where degradation then occurs.

events that regulate this recycling process have been gradually unraveled. Atg proteins are involved in various stages of the process including induction, autophagosome formation, fusion and degradation (Figure 2). Two conjugation reactions, regulated by Atg7, are required for autophagosome biogenesis. First, the ubiquitin-activating enzyme E1-like protein, Atg7, induces the formation of the Atg5-Atg12 complex. LC3 is then conjugated to the lipid residue phosphatidylethanolamine (PE), facilitating its anchoring at the autophagosomal membrane (Figure 2). Later, this LC3-PE complex (also known as LC3-II) is deconjugated by the protease Atg4, enabling the release of LC3 from the membrane.[7]

Autophagy is generally considered a rather nonselective process for the bulk degradation of randomly enclosed cargo. However, recent evidence suggests a degree of selectivity, with many organelles and cytoplasmic components specifically targeted by autophagosomes.[6] These include mitochondria, ribosomes, peroxisomes, misfolded proteins and intracellular pathogens. In selective autophagy (Figure 4), ubiquitination of substrates permits their specific degradation by binding to autophagy receptors, such as p62, which act as bridges between the ubiquitinated cargo and the autophagosomal membrane.[8]

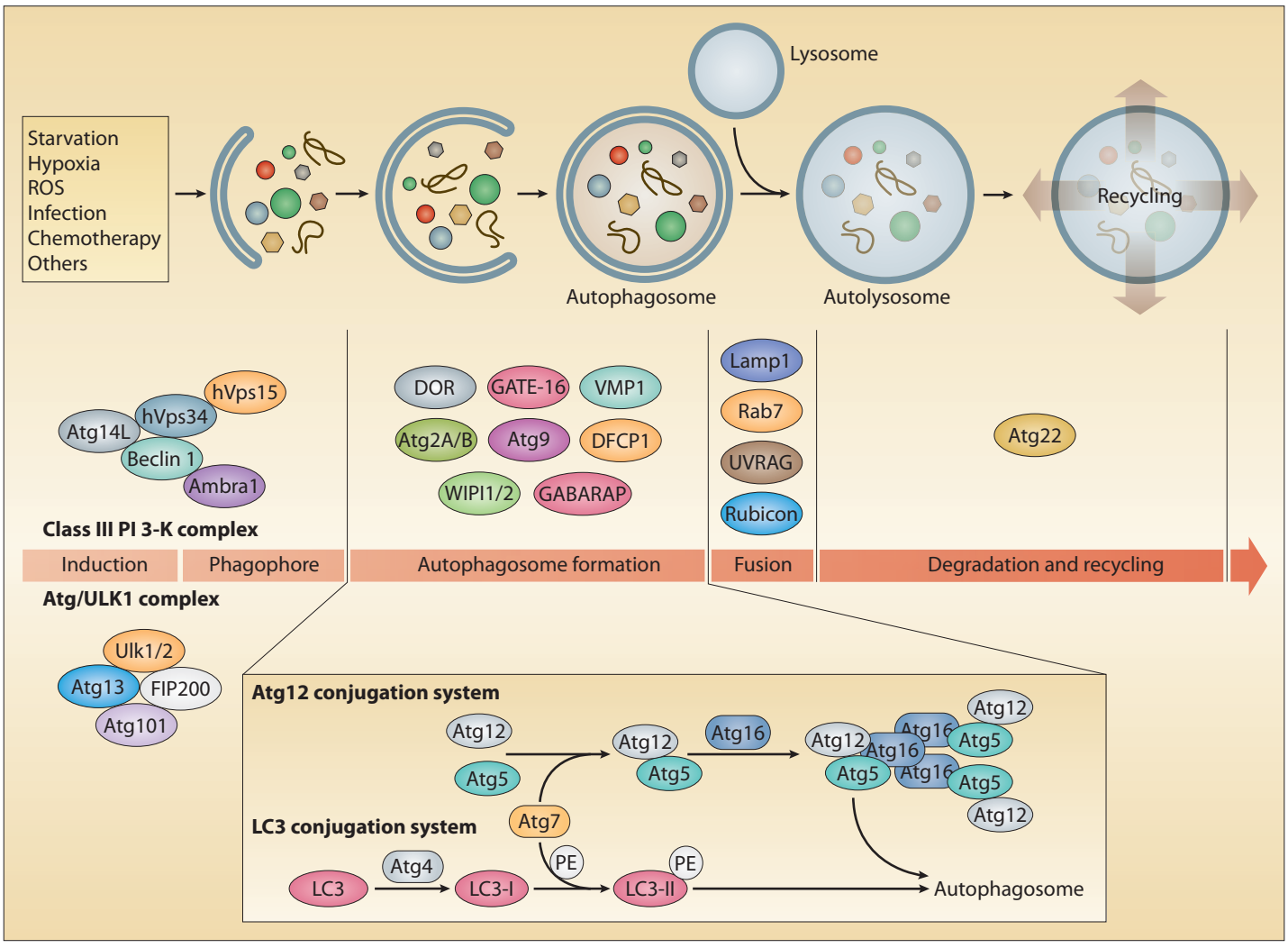
The Molecular Machinery of Autophagy

Autophagosome formation

Autophagy is initiated by the formation of a double membrane-bound vacuole known as the autophagosome, the size of which ranges from 300 to 900 nm. The origin of the preautophagosomal structure (PAS) and the molecular basis of autophagosome biogenesis represent long-standing challenges in autophagy research. The discovery of *Atg* genes in yeast was a major milestone in the understanding of autophagy.[9] Over 15 Atg proteins, as well as the class III PI 3-K hVps34, are involved in the construction of the autophagosome (Table 1; Figure 2). These Atg proteins are hierarchically recruited at the PAS.[9] Autophagosome formation is a multistep process that consists of biogenesis of the isolation membrane, followed by its elongation and closure. The process also requires the shuttling of Atg9, the only transmembrane Atg, between a peripheral site and the isolation membrane.[9]

Recently, several convincing arguments have been put forward regarding the role of the endoplasmic reticulum (ER) in the initiation of autophagy.[10-12] In response to nutrient starvation, two complexes congregate at the PAS to initiate autophagy. The PI 3-K complex is composed of Beclin 1, Atg14, hVps34, hVps15 and AMBA1. The ULK1 complex is composed of ULK1, FIP200, Atg13 and Atg101 (Figure 2). The mechanism mediating this co-regulation of the ULK1 and PI 3-K complexes remains to be elucidated. The production of phosphatidylinositol 3-phosphate (PtdIns3P) by hVps34 recruits WIPI1/2 and DFCP1, both of which are PtdIns3P binding proteins. DFCP1 is located at the Golgi in resting cells, but in response to autophagy stimulation it is recruited to an ER structure known as the omegasome.[10] The omegasome – serving as a PAS – accommodates the two ubiquitin-like conjugation systems (consisting of Atg12, Atg5, Atg16 and PE-conjugated LC3). These systems act sequentially to elongate the phagophore membrane, thus forming the autophagosome.[9] Recently, the mitochondrial outer membrane has been proposed as another source of the isolation membrane.[13] According to this scenario, the mitochondria-ER contact site provides the growing phagophore with lipids. Atg16L1-decorated vesicles derived from coated pits in the plasma also serve as a source of membrane for the phagophore.[14] Finally, the Golgi apparatus and post-Golgi compartments containing Atg9 also contribute to the formation of the autophagosome membrane.[15,16] Regardless of the origin of the membrane, Atg proteins are retrieved from the autophagosome membrane after closure, with the exception of a fraction of LC3-II, which is transported into the lysosomal compartment.[1,17] Following this discovery, several methods based on the analysis of the LC3 protein have been developed to monitor autophagy.[18]

Figure 2 | The molecular machinery implicated in macroautophagy



During the first stages of autophagy in mammals, two macromolecular complexes are formed: the Class III PI 3-K complex and the Atg1/ULK1 complex. Other regulators – including Atg2A/B, WIPI 1/2, DFCP1 and VMP1 – cooperate during autophagosome formation, together with two conjugation reactions catalyzed by Atg7. First, Atg5 and Atg12 are conjugated and bind to Atg16. Second, pre-LC3 is cleaved by the protease Atg4 and binds to the lipid phosphatidylethanolamine (PE), which facilitates its anchoring at the autophagosomal membrane. Once formed, the autophagosome then fuses with lysosomes or endosomes, a process that involves several lysosomal proteins such as Lamp1 and Rab7. After degradation by the action of lysosomal hydrolases, the final products – including amino acids, lipids and nucleotides – translocate to the cytoplasm via permeases, such as Atg22 (in yeast), present in the lysosomal membrane. They are then recycled for new anabolic reactions to sustain cell homeostasis.

Table 1 | Comparison of Atg and Atg-related proteins in yeast and mammals

Yeast Protein	Mammalian Protein(s)
Atg1	ULK1/2
Atg2	Atg2A/B
Atg3	Atg3
Atg4	Atg4A-D
Atg5	Atg5
Atg6/Vps30	Beclin1
Atg7	Atg7
Atg8	LC3A/B/C GABARAP GABARAPL1/2/3 (GABARAPL2 = GATE-16)
Atg9	Atg9L1
Atg10	Atg10
Atg11	N.d.
Atg12	Atg12
Atg13	Atg13
Atg14	Atg14L/Barkor
Atg16	Atg16
Atg17	FIP200*
Atg18	WIPI1/2/3/4
Atg29	N.d.
Atg31	N.d.
Vps34	Vps34

*Functional homolog to yeast Atg17, but no sequence similarity. N.d. = not determined.

The role of LC3 and of other members of the mammalian Atg8 family (GABARAPs) remains unclear. However, a recent study has demonstrated the participation of LC3s and GABARAPs at different stages of autophagosome biogenesis.[19] LC3s mediate the elongation of the autophagic membrane, while GABARAPs mediate a downstream event likely associated with the membrane closure. Atg8 proteins induce membrane fusion, which is involved in autophagy.[19] In both yeast and mammalian cells, autophagosome biogenesis also involves SNAREs.[20,21] Atg8 proteins can also serve as a scaffold for recruiting proteins that may regulate events upstream and downstream of autophagosome formation.[22-24]

After formation, autophagosomes can merge with endocytic compartments (such as early and late endosomes, multivesicular bodies) before fusing with the lysosomal compartment.[25-27] The term 'amphisome', from the Greek roots *amphi* (both) and *soma* (body), was coined by Per O. Seglen to describe the vacuole resulting from the fusion of the autophagosome with the endosome. The late stage of autophagy depends on molecules that regulate autophagosome maturation, including their fusion with endosomes and lysosomes, the acidification of the autophagic compartments, and the recycling of metabolites from the lysosomal compartment (Figure 2). These steps are fundamental for the movement of material through the autophagic pathway (defined here as spanning the cargo sequestration step through to lysosomal degradation). Any blockade of autophagosome maturation or fusion with the lysosomal compartment, or impairment of lysosomal function or biogenesis, results in an accumulation of autophagosomes. This inevitably slows down or interrupts the autophagic flux (Figure 3).[28,29]

Maturation and degradation of autophagosomes

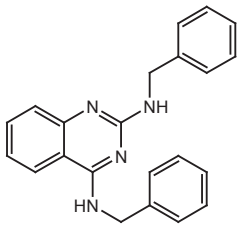
Rubicon and UVRAG

Rubicon and UVRAG (UV irradiation resistance-associated gene) are two Beclin 1-binding proteins that regulate autophagosome maturation and endocytic trafficking.[30-32] Moreover, the Beclin 1:hVps34:UVRAG:Rubicon complex appears to downregulate these trafficking events, whereas the Beclin 1:hVps34:UVRAG complex upregulates autophagosome maturation and endocytic trafficking.[31,32] Beclin 1 thus regulates both the formation of autophagosomes (via its interaction with Atg14L) and their maturation (via its interaction with UVRAG and Rubicon).

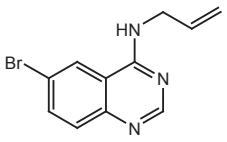
Rab proteins

Rab proteins belong to the Ras superfamily of G proteins. Some of these GTPases have been linked to autophagy. Colombo *et al.*[33] and Eskelinen *et al.*[34] have reported that Rab7 is required for autophagosome maturation. Autophagosome maturation is dependent on interactions with class C Vps proteins and UVRAG.[35] This function of UVRAG is independent of its interaction with Beclin 1, and stimulates Rab7 GTPase activity and the fusion of autophagosomes with late endosomes/lysosomes. Interestingly, Rab11 is required for the fusion of autophagosomes and multivesicular bodies (MVBs) during starvation-induced autophagy in the erythroleukemic cell line K562.[36] These findings suggest that specific membrane-bound compartment fusion processes during autophagosome maturation engage different sets of Rab proteins, and possibly

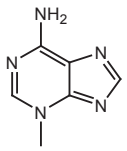
Figure 3 | Autophagosome synthesis, maturation and degradation



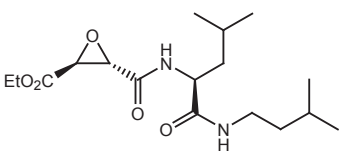
DBeQ (4417)
Selective p97 inhibitor; blocks autophagosome production



SMER 28 (4297)
Increases autophagosome synthesis

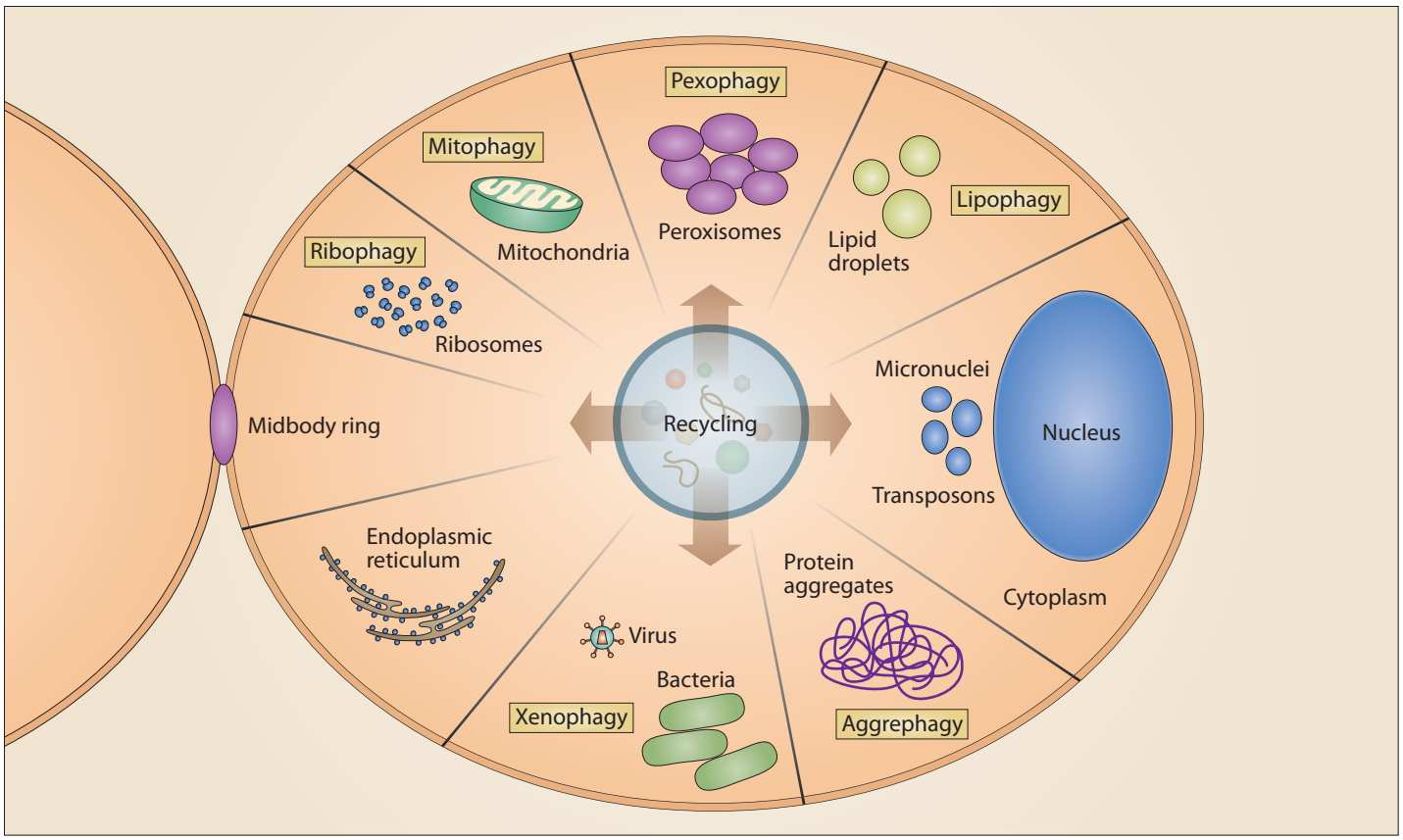


3-Methyladenine (3977)
Class III PI 3-kinase inhibitor; inhibits autophagic sequestration



E 64d (4545)
Inhibits lysosomal proteases and interferes with autolysosomal digestion

Figure 4 | Selective autophagy



Subcellular structures are specifically targeted for lysosomal degradation by autophagy. Depending on the cargo, the processes are named differently: mitophagy for the specific elimination of mitochondria; ribophagy for ribosomes; and lipophagy for the degradation of lipid droplets. Pexophagy degrades peroxisomes and aggrephagy degrades intracellular protein aggregates and misfolded proteins such as those observed in many neurodegenerative conditions. Xenophagy denotes the degradation of intracellular pathogens such as viruses and intracellular bacteria. Other cellular components – such as the endoplasmic reticulum (ER), micronuclei, glycogen and transposons – can also be specifically targeted by autophagosomes for degradation.

associated cohort proteins. Other Rab proteins, such as Rab22 and Rab24, have subcellular locations consistent with a role in autophagy.[37,38]

ESCRT and Hrs

The endosomal sorting complex required for transport (ESCRT) mediates the biogenesis of MVBs and the sorting of proteins in the endocytic pathway.[39] Recently, studies have also demonstrated the requirement of the multisubunit complex ESCRT III for autophagosome fusion with MVBs and lysosomes, which results in the generation of amphisomes and autolysosomes, respectively.[40-42] ESCRT III dysfunction associated with the autophagic pathway may have important implications in neurodegenerative diseases (such as frontotemporal dementia and amyotrophic lateral sclerosis).[40,41] The Hrs protein (hepatocyte growth factor-regulated tyrosine kinase substrate) plays a major role in endosomal sorting upstream of ESCRT complexes.[39] Hrs contains a FYVE domain that binds specifically to PtdIns3P, facilitating autophagosome maturation.[43] This raises the intriguing possibility that PtdIns3P may be required for both the formation and maturation of autophagosomes. However, the role of ESCRT proteins in autophagy remains to be elucidated. To date, the possibility that these proteins are involved in the closing of autophagosomes cannot be discounted (reviewed by Rusten *et al.*[44]).

SNAREs

Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are basic elements involved in intracellular membrane fusion.[45] In *S. cerevisiae* the vacuolar t-SNAREs Vam3 and Vti1 are required for complete fusion between the autophagosome and the vacuole (the name given to the lysosome in yeast).[46,47] Furthermore, Vti1b, the mammalian homolog of Vt1, may be involved in a late stage of autophagy, since maturation of autophagic vacuoles is delayed in hepatocytes isolated from mice in which Vti1b has been deleted.[48] More recently, Colombo and colleagues reported that the v-SNAREs VAMP3 and VAMP7 control autophagosome-MVB and amphisome-lysosome fusion, respectively.[49]

Endo/lysosomal membrane proteins

Lamps (Lysosomal associated membrane proteins) are a family of heavily-glycosylated, endo/lysosomal transmembrane proteins.[50] Autophagic degradation is impaired in hepatocytes isolated from Lamp2 knockout mice.[51] However, no defects in autophagy are observed in Lamp2 knockout mouse

fibroblasts.[52] A blockade in the later stage of autophagy occurs only in fibroblasts lacking both Lamp1 and Lamp2.[53] The differences in autophagic activity observed between hepatocytes and fibroblasts may be responsible for the cell type-specific effects of Lamp1 and Lamp2 depletion.[50]

DRAM

DRAM (damage-regulated autophagy modulator) encodes a 238-amino acid protein that is conserved through evolution, but has no ortholog in yeast.[54] DRAM, a direct target of p53, is a multispanning transmembrane protein found in the lysosome. The protein may regulate late stages of autophagy, but surprisingly also controls autophagosome formation,[54] suggesting the possibility of a new paradigm in which feedback signals from the lysosomes control early stages of autophagy.

Microtubules

The destabilization of microtubules by either vinblastine or nocodazole blocks autophagosome maturation, while their stabilization by taxol enhances fusion between autophagic vacuoles and lysosomes.[55] Recent findings have confirmed the role of microtubules in fusion with the acidic compartment.[56] Autophagosomes move bidirectionally along microtubules, and exhibit a centripetal movement dependent on the dynein motor.[57,58] Two types of fusion have been documented: complete fusion of the autophagosome with the lysosome; and transfer of material from the autophagosome to the lysosomal compartment via a kiss-and-run fusion process, in which two separate vesicles are maintained.[56] However, microtubule-independent autophagosome fusion with lysosomes can occur during starvation-induced autophagy, when autophagosomes are formed in the vicinity of lysosomes.[59]

Acidification and lysosomal degradation

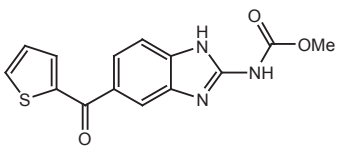
ATPases

Vacuolar ATPases (v-ATPases) are ubiquitous, multi-subunit proteins located in the acidic compartment. Inhibition of the activity of v-ATPase by bafilomycin A1 or concanamycin A blocks the lysosomal pumping of H^+ and consequently inhibits lysosomal enzymes, which are active at low pH (Figure 5). Bafilomycin A1 has been proposed to block the late stages of autophagy by affecting autophagosome fusion involving endosomes and lysosomes,[60] or by preventing the lysosomal degradation of sequestered material.[59] Overall, the effect of the v-ATPase inhibition is to interrupt the autophagic flux, as determined by lysosomal inhibition of autophagic cargo.

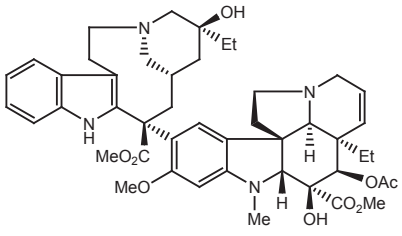
ATPases associated with various cellular activity proteins (AAA ATPases) form a family of proteins that are broadly involved in intracellular membrane fusion. N-ethylmaleimide sensitive factor (NSF) is an AAA ATPase that binds to SNARE complexes and disassembles them using ATP hydrolysis, thus facilitating SNARE recycling. In yeast mutants lacking sec18 (the yeast homolog of NSF), autophagosomes are formed, but fail to fuse with the vacuole.[47] It remains unknown whether the ATPase activity of NSF is involved in the later stages of autophagy in mammalian cells. Nonetheless, NSF activity is attenuated during starvation, providing a possible explanation for the slow fusion observed between autophagosomes and lysosomes when autophagy is induced by starvation.[59] Suppressor of K^+ transport growth defect 1 (SKD1-Vps4), another AAA ATPase protein, is required for autophagosome maturation in mammalian cells.[61] Vps4 controls the assembly of ESCRT complexes at the multivesicular membrane, and is involved in autophagosome maturation in *Drosophila*,[42] and autophagosome-vacuole fusion in yeast.[62]

Figure 5 | Microtubule agents and vacuolar ATPase inhibitors

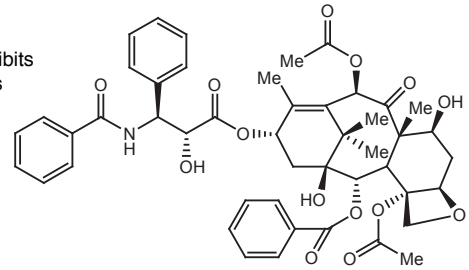
Nocodazole (1228)
Microtubule inhibitor



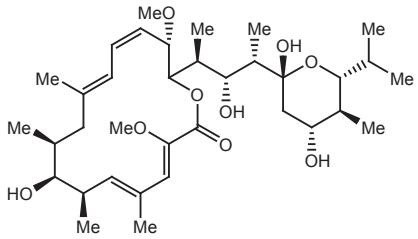
Vinblastine (1256)
Disrupts microtubules



Taxol (1097)
Promotes assembly and inhibits disassembly of microtubules



Bafilomycin A1 (1334)
Vacuolar H^+-ATPase inhibitor



Concanamycin A (2656)
Vacuolar H^+-ATPase inhibitor

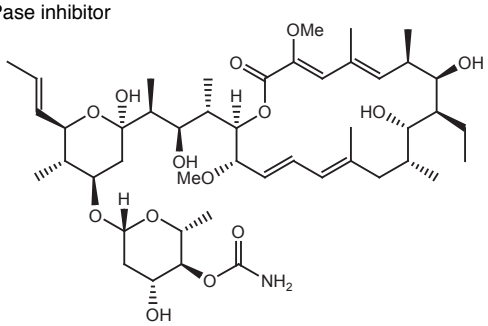
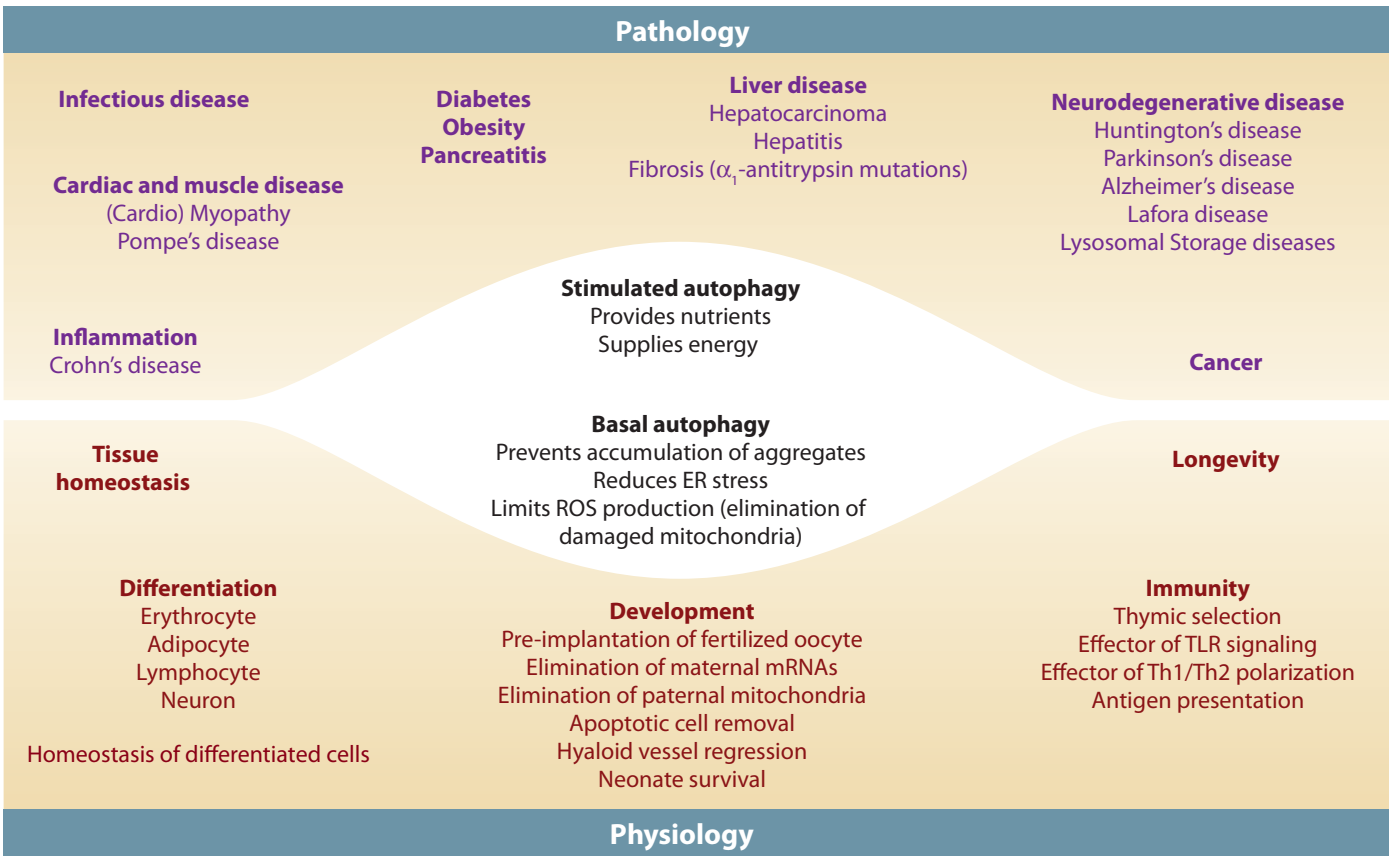


Figure 6 | Physiology and pathology



Autophagy has many essential functions in cells and tissues. Basal autophagy is essential to prevent the accumulation of damaged proteins and organelles; reduce ER stress; and limit the production of reactive oxygen species (ROS). On the other hand, induced autophagy is important to provide nutrients and building blocks during periods of starvation. Autophagy is essential during the development and differentiation of many cell types and in maintaining tissue homeostasis. Moreover, autophagy plays an essential role during immunity, participating in thymic selection and antigen presentation. Autophagy is also important in maintaining cellular homeostasis during aging. Given these essential physiological roles, it is unsurprising that dysregulation of autophagy has profound consequences, and is implicated in the pathology of many diseases. Defects in autophagy have been associated with numerous neurodegenerative diseases, including proteinopathies and lysosomal storage diseases, and have also been reported in liver and muscle diseases. Other pathological situations such as diabetes and obesity, as well as inflammatory pathologies such as Crohn's disease, have also been correlated with defective autophagy.

Degradation and lysosomal efflux

Autophagy contributes to the regulation of carbohydrate, lipid and protein metabolism via lysosomal degradation.[63] Similar to acidification defects in the endo/lysosomal compartment, defects in the transport or expression of lysosomal enzymes induce a blockade of autophagy, characterized by an accumulation of autophagic vacuoles.[64] The final stage of autophagy is the efflux of metabolites generated by the lysosomal degradation of macromolecules into the cytoplasm. Atg22 has recently been identified as a permease that recycles amino acids from the vacuole in *S. cerevisiae*.[65]

Autophagy and Cell Death

Programmed cell death is a process of controlled cellular autodestruction that allows an organism to control its morphogenesis and reformation, and to eliminate cells that jeopardize its survival. Cell death is of vital importance during both embryonic development and adult life, and plays a key role in pathological situations such as cancer, infection and neurodegenerative diseases.[66] Classically, three forms of cell death are defined, based on morphology: apoptosis, autophagy and necrosis.[67,68] At the cellular level, apoptosis is characterized by a reduction in cell size and chromatin condensation through the formation of blebs in the plasma membrane. The organelles remain intact, and in the final stage the cells are degraded through phagocytosis by neighboring cells, in a process activated by phosphatidylserine residues in the external part of the plasma membrane of the apoptotic cell. At the biochemical level, activation of caspases and proapoptotic proteins (such as cytochrome c) that leak out of the mitochondria provokes a cascade of degradation of cellular components.[69] Apoptosis is the best described form of programmed cellular cell death and plays a key role during the onset and progression of numerous pathologies. Autophagic cell death is morphologically characterized by the presence of numerous

autophagosomes in the cytoplasm prior to nuclear condensation.[67,68] This type of cell death has been observed *in vivo* in different animal species and different tissues, and is generally associated with situations of important tissue restructuring, such as that which occurs during insect metamorphosis, or during involution of the mammary gland after lactation.[67,68,70] However, this morphological definition must be complemented by biochemical and functional assessment. A recent study proposed that autophagic cell death should fulfill three specific criteria: (i) cell death occurs independent of apoptosis; (ii) there is an increase in autophagic flux, and not simply an increase in autophagy markers; and (iii) cell death can be prevented by suppression of autophagy by genetic means or by using chemical inhibitors (Figure 7).[71] Situations where these premises are fulfilled *in vivo* are relatively uncommon, and generally have only been observed in lower eukaryotes, for example, during developmental cell death in *Drosophila*, *Dictyostelium discoideum* and *Caenorhabditis elegans*.[70] In mammalian systems autophagic cell death is observed *in vitro* in some cancer cell lines.[70] However, in many instances, autophagy precedes apoptosis, as seen during hypoxic-ischemic injury in the brain,[72] in cancer cells and after *in vivo* chemotherapy.[73] In these settings, elimination of autophagy regulators reduces cell death. There is a high degree of cross-talk between autophagy and apoptosis, with several regulators, such as Beclin 1, Bcl-2, Atg5, Atg3, Atg12, caspases and p53, playing important roles in both pathways.[74-77] For example, the dissociation of Beclin 1 from Bcl-2 is essential for its autophagic activity, and Bcl-2 inhibits autophagy when it is present in the ER.[78]

Physiology and Pathology

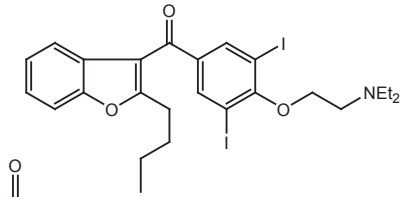
Autophagy is induced in cells after many stressful situations including starvation, hypoxia and infection (Figure 4). Its finely tuned regulation is essential to maintain cell and tissue homeostasis.[79] Stimulation of autophagy during periods of starvation is an evolutionarily conserved response to stress in eukaryotes.[1] Under starvation conditions, the degradation of proteins and lipids allows the cell to adapt its metabolism and meet its energy needs. The stimulation of autophagy plays a major role at birth in maintaining energy levels in various tissues after the maternal nutrient supply via the placenta ceases.[80] Furthermore, pharmacological and genetic down-regulation of autophagy induces rapid cell death after starvation in cells.[28]

Autophagy is also essential during development and differentiation (Figure 4). The preimplantation period after oocyte fertilization is dependent on autophagic degradation of components of the oocyte cytoplasm such as elimination of maternal mRNAs[81] and paternal mitochondria.[82,83] Autophagy is also implicated in the elimination of apoptotic bodies generated during naturally-occurring cell death associated with embryonic development.[84,85] Autophagy-mediated remodeling of the cytoplasm is involved in the differentiation of erythrocytes, lymphocytes and adipocytes,[86] and neural stem cells to neurons.[87] Moreover, autophagy is crucial for the homeostasis of immune cells and contributes to the regulation of self-tolerance.[88] Induction of autophagy during caloric restriction may contribute to the observed extension of lifespan in rats. Recent data have shown that the induction of autophagy increases longevity in a large variety of species.[89] This anti-aging effect likely depends, at least in part, on the quality control function of autophagy, which limits the accumulation of aggregation-prone proteins and damaged mitochondria.

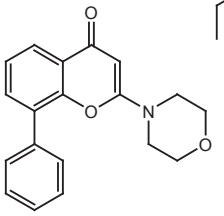
As described above, autophagy is essential to eliminate many harmful components in cells such as protein aggregates, damaged organelles and intracellular pathogens. It is thus unsurprising that dysregulation of this process has important consequences, and is implicated in many diseases. These include Huntington's, Alzheimer's and Parkinson's diseases, which are characterized by the accumulation of protein aggregates in the brain and in other tissues (such as muscle)[86], and

Figure 7 | Autophagy inhibitors and activators

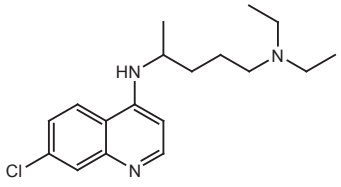
Amiodarone (4095)
Thought to stimulate autophagy by targeting upstream mTORC1 control pathways

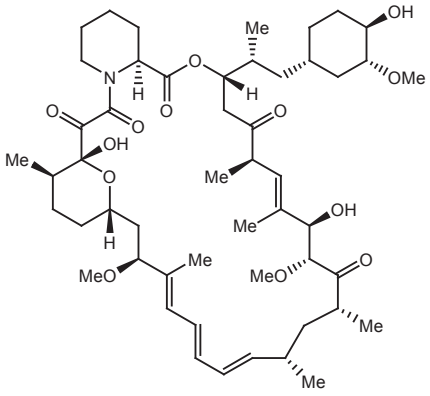


LY 294002 (1130)
Selective PI 3-kinase inhibitor; inhibits autophagic sequestration



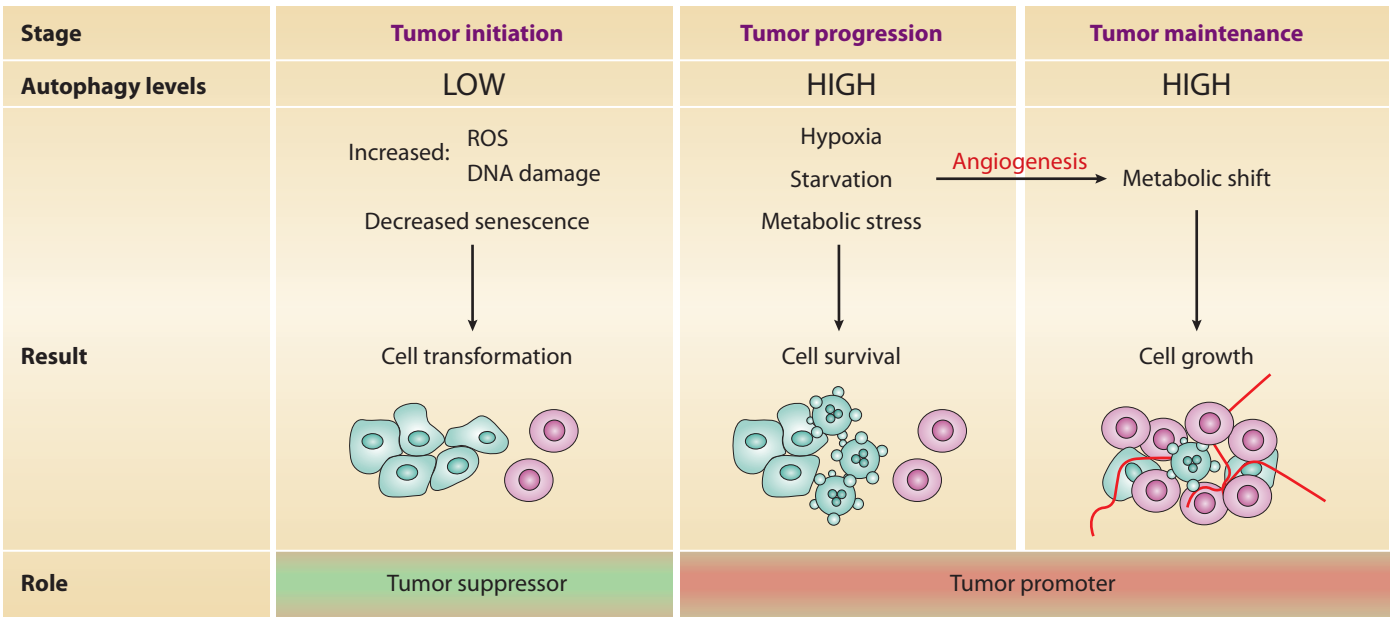
Chloroquine (4109)
Inhibits autophagy via a mechanism distinct from that of 3-methyladenine





Rapamycin (1292)
mTOR inhibitor; induces autophagy in yeast and mammalian cell lines

Figure 8 | Autophagy and cancer



The role of autophagy in cancer is complex and context-dependent. During tumor initiation, autophagy acts as a tumor suppressor, acting as a barrier to cell transformation by reducing cell proliferation and DNA damage. Autophagy also plays a role in preventing cell transformation through the induction of senescence. Later, during tumor progression, high levels of autophagy have been shown to increase cancer cell survival in conditions of starvation, hypoxia and metabolic stress – conditions often observed in primary tumors. Cancer cells subsequently become dependent on autophagy to sustain cell growth, since it provides the basic building blocks required for anabolic reactions. During these two later stages of tumor cell biology, autophagy thus acts as a tumor promoter mechanism.

in liver fibrosis.[90] In the heart, basal autophagy is necessary to maintain cellular homeostasis and is upregulated in response to stress in hypertensive heart disease, heart failure, cardiac hypertrophy, and ischemia-reperfusion injury.[91] In the pancreas, autophagy is required to maintain the architecture and function of pancreatic β-cells.[92] Defective hepatic autophagy likely contributes to insulin resistance and to a predisposition to Type 2 diabetes and obesity.[93] Given its role in the elimination of intracellular pathogens such as bacteria, viruses and parasites, autophagy also contributes to innate immunity.[94] Recently, polymorphisms of the genes that encode Atg16L1 and IRGM – two autophagy genes essential for the elimination of intracellular pathogens – have been associated with Crohn's disease, a chronic inflammatory bowel disease.[95]

Amino acids produced by autophagy in the muscles and liver can be used for gluconeogenesis in the liver,[96] and can contribute to the production of ATP by entering the tricarboxylic acid (TCA) cycle. Degradation of liver lipid droplets by autophagy, via lipophagy, contributes to the generation of free fatty acids that are oxidized in the mitochondria. Moreover, hepatocyte-specific Atg7 knockout mice exhibit elevated levels of hepatic lipids.[97]

Decreases in hepatic autophagy are observed in both genetic and dietary mouse models of obesity and insulin resistance.[93] This effect impacts ER function, including the response to stress. Restoration of Atg7 expression limits obesity-dependent ER stress, and rescues insulin resistance and glucose tolerance. However, autophagy plays an opposite role in white adipose tissue where its inhibition decreases white adipose mass and enhances insulin sensitivity.[97,98] The adipose tissue-specific deletion of Atg7 also favors the oxidation of free fatty acids by increasing the proportion of brown adipocytes, leading to a lean body mass.[97] The effects of pharmacological manipulation of autophagy in obese patients therefore remain uncertain, unless liver autophagy can be specifically targeted.[99] Autophagy is involved not only in the regulation of metabolism in the peripheral tissues, but also in regulating food intake via the brain, though its role in this process remains to be clearly demonstrated.[100,101]

While cancer is frequently associated with defects in autophagy, the role of autophagy is highly complex and dependent on cancer stage and context (Figure 8); autophagy has been shown to act as a tumor suppressing mechanism, but is also required in the later stages of tumor progression to enable tumor cells to cope with metabolic stress.[102] Several of the functions of autophagy – such as the elimination of defective organelles, which reduces oxidative stress and prevents DNA damage – also contribute to its tumor suppressive effects.[103] Remarkably, autophagy facilitates effective glucose uptake and glycolytic flux in Ras-transformed cells.[104] Moreover, the loss of autophagy in Ras-transformed cells is associated with reduced oxygen consumption and lower levels of the TCA intermediates citrate, aconitate, and isocitrate.[105] The high basal level of autophagy observed in tumors with Ras mutations is required for cancer cell survival.[106] In these tumors, autophagy constitutes an 'Achilles heel' that could prove useful

in the fight against cancer. Inhibiting autophagy is a challenging prospect, however, as in many tumors autophagy serves as a stress response to anticancer treatments.[107,108]

Future Prospects

Recent years have witnessed advances in our understanding of the origin of the membranes required to form autophagosomes. Pioneering work in the field of autophagy is notable for suggesting the implications of these recently-identified membrane origins (reviewed by Yang and Klionsky).[1] The coordinated recruitment of these different pools of membrane during the different stages of autophagosome formation has been elucidated.[7] However, whether the origin of the membrane involved in autophagosome formation varies depending on the stimulus that triggers autophagy remains an unanswered question. A recent proteomic approach has revealed hundreds of interactions between human proteins and the core autophagic machinery, suggesting some hitherto unknown aspects of autophagy regulation that could lead to a better understanding of its integration into cell function.[109] Knowing more about the structure of proteins belonging to the core machinery of autophagy could also accelerate the design of drugs to modulate the process.[110] Therefore, the targeting of autophagy as an adjuvant therapy represents a promising avenue of research in some major human diseases.

References

1. **Yang and Klionsky** (2010) *Curr.Opin.Cell.Biol.* **22** 124.
2. **Arias and Cuervo** (2011) *Curr.Opin.Cell.Biol.* **23** 184.
3. **Li** *et al* (2011) *Cell.Mol.Life.Sci.* **69** 1125.
4. **Hara** *et al* (2006) *Nature* **441** 885.
5. **Komatsu** *et al* (2006) *Nature* **441** 880.
6. **Mizushima and Komatsu** (2011) *Cell* **147** 728.
7. **Mizushima** *et al* (2011) *Annu.Rev.Cell.Dev.Biol.* **27** 107.
8. **Weidberg** *et al* (2011) *Annu.Rev.Biochem.* **80** 125.
9. **Nakatogawa** *et al* (2009) *Nat.Rev.Mol.Cell.Biol.* **10** 458.
10. **Axe** *et al* (2008) *J.Cell.Biol.* **182** 685.
11. **Yla-Anttila** *et al* (2009) *Autophagy* **5** 1180.
12. **Hayashi-Nishino** *et al* (2009) *Nat.Cell.Biol.* **11** 1433.
13. **Hailey** *et al* (2010) *Cell* **141** 656.
14. **Ravikumar** *et al* (2010) *Nat.Cell.Biol.* **12** 747.
15. **Mari** *et al* (2010) *J.Cell.Biol.* **190** 1005.
16. **Ohashi and Munro** (2010) *Mol.Biol.Cell* **21** 3998.
17. **Yang and Klionsky** (2010) *Nat.Cell.Biol.* **12** 814.
18. **Mizushima** *et al* (2010) *Cell* **140** 313.
19. **Weidberg** *et al* (2011) *Dev.Cell* **20** 444.
20. **Moreau** *et al* (2011) *Cell* **146** 303.
21. **Nair** *et al* (2011) *Cell* **146** 290.
22. **Garcia-Marcos** *et al* (2011) *Mol.Biol.Cell* **22** 673.
23. **Itoh** *et al* (2011) *J.Cell.Biol.* **192** 839.
24. **Mauvezin** *et al* (2010) *EMBO Rep.* **11** 37.
25. **Liou** *et al* (1997) *J.Cell.Biol.* **136** 61.
26. **Tooze and Razi** (2009) *Autophagy* **5** 874.
27. **Stromhaug and Seglen** (1993) *Biochem.J.* **291** (Pt 1) 115.
28. **Boya** *et al* (2005) *Mol.Cell.Biol.* **25** 1025.
29. **Rubinsztein** *et al* (2009) *Autophagy* **5** 585.
30. **Liang** *et al* (2006) *Nat.Cell.Biol.* **8** 688.
31. **Matsunaga** *et al* (2009) *Nat.Cell.Biol.* **11** 385.
32. **Zhong** *et al* (2009) *Nat.Cell.Biol.* **11** 468.
33. **Gutierrez** *et al* (2004) *J.Cell Sci.* **117** 2687.
34. **Jager** *et al* (2004) *J.Cell Sci.* **117** 4837.
35. **Liang** *et al* (2008) *Nat.Cell.Biol.* **10** 776.
36. **Fader** *et al* (2008) *Traffic* **9** 230.
37. **Mesa** *et al* (2001) *J.Cell.Sci.* **114** 4041.
38. **Egami** *et al* (2005) *Biochem.Biophys.Res.Commun.* **337** 1206.
39. **Raiborg and Stenmark** (2009) *Nature* **458** 445.
40. **Filimonenko** *et al* (2007) *J.Cell.Biol.* **179** 485.
41. **Lee** *et al* (2007) *Curr.Biol.* **17** 1561.
42. **Rusten** *et al* (2007) *Curr.Biol.* **17** 1817.
43. **Tamai** *et al* (2007) *Biochem.Biophys.Res.Commun.* **360** 721.
44. **Rusten and Stenmark** (2009) *J.Cell.Sci.* **122** 2179.
45. **Gurkan** *et al* (2007) *Adv.Exp.Med.Biol.* **607** 73.
46. **Darsow** *et al* (1997) *J.Cell.Biol.* **138** 517.
47. **Ishihara** *et al* (2001) *Mol.Biol.Cell* **12** 3690.
48. **Atlashkin** *et al* (2003) *Mol.Cell.Biol.* **23** 5198.
49. **Fader** *et al* (2009) *Biochim.Biophys.Acta* **1793** 1901.
50. **Eskelinen** (2005) *Autophagy* **1** 1.
51. **Tanaka** *et al* (2000) *Nature* **406** 902.
52. **Eskelinen** *et al* (2004) *Mol.Biol.Cell* **15** 3132.
53. **Gonzalez-Polo** *et al* (2005) *J.Cell Sci.* **118** 3091.
54. **Crighton** *et al* (2006) *Cell* **126** 121.
55. **Yu and Marzella** (1986) *Am.J.Pathol.* **122** 553.
56. **Jahreiss** *et al* (2008) *Traffic* **9** 574.
57. **Kimura** *et al* (2008) *Cell Struct.Funct.* **33** 109.
58. **Ravikumar** *et al* (2005) *Nat.Genet.* **37** 771.
59. **Fass** *et al* (2006) *J.Biol.Chem.* **281** 36303.
60. **Yamamoto** *et al* (1998) *Cell Struct.Funct.* **23** 33.
61. **Nara** *et al* (2002) *Cell Struct.Funct.* **27** 29.
62. **Shirahama** *et al* (1997) *Cell Struct.Funct.* **22** 501.
63. **Kotoulas** *et al* (2006) *Pathol.Res.Pract.* **202** 631.
64. **Koike** *et al* (2005) *Am.J.Pathol.* **167** 1713.
65. **Yang** *et al* (2006) *Mol.Biol.Cell* **17** 5094.
66. **Fuchs and Steller** (2011) *Cell* **147** 742.
67. **Schweichel and Merker** (1973) *Teratology* **7** 253.
68. **Clarke** *et al* (1990) *Anat.Embryol.(Berl)* **181** 195.
69. **Kroemer and Reed** (2000) *Nat.Med.* **6** 513.
70. **Denton** *et al* (2011) *Cell Death Differ.* **19** 87.
71. **Shen and Codogno** (2011) *Autophagy* **7** 457.
72. **Koike** *et al* (2008) *Am.J.Pathol.* **172** 454.
73. **Salazar** *et al* (2009) *J.Clin.Invest.* **119** 1359.
74. **Pattingre** *et al* (2005) *Cell* **122** 927.
75. **Tasdemir** *et al* (2008) *Nat.Cell Biol.* **10** 676.
76. **Radoshevich** *et al* (2010) *Cell* **142** 590.
77. **Rubinstein** *et al* (2011) *Mol.Cell* **44** 698.
78. **Boya and Kroemer** (2009) *Oncogene* **28** 2125.
79. **Kroemer** *et al* (2010) *Mol.Cell* **40** 280.
80. **Kuma** *et al* (2004) *Nature* **432** 1032.
81. **Tsukamoto** *et al* (2008) *Science* **321** 117.
82. **Sato and Sato** (2011) *Science* **334** 1141.
83. **Al Rawi** *et al* (2011) *Science* **334** 1144.
84. **Mellén** *et al* (2008) *Cell Death Differ.* **15** 1279.
85. **Mellén** *et al* (2009) *Autophagy* **5**.
86. **Ravikumar** *et al* (2010) *Physiol.Rev.* **90** 1383.
87. **Vazquez** *et al* (2012) *Autophagy* **8** 187.
88. **Nedjic** *et al* (2009) *Curr.Opin.Immunol.* **21** 92.
89. **Rubinsztein** *et al* (2011) *Cell* **146** 682.
90. **Hidvegi** *et al* (2010) *Science* **329** 229.
91. **Nakai** *et al* (2007) *Nat.Med.* **13** 619.
92. **Ebato** *et al* (2008) *Cell Metab.* **8** 325.
93. **Yang** *et al* (2010) *Cell Metab.* **11** 467.
94. **Deretic** (2011) *Curr.Opin.Immunol.* **24** 21.
95. **Virgin and Levine** (2009) *Nat.Immunol.* **10** 461.
96. **Rabinowitz and White** (2010) *Science* **330** 1344.
97. **Singh** *et al* (2009) *Nature* **458** 1131.
98. **Zhang** *et al* (2009) *Proc.Natl.Acad.Sci.USA* **106** 19860.
99. **Codogno and Meijer** (2010) *Cell Metab.* **11** 449.
100. **Kaushik** *et al* (2011) *Cell Metab.* **14** 173.
101. **Meng and Cai** (2011) *J.Biol.Chem.* **286** 32324.
102. **Kimmelman** (2010) *Genes Dev.* **25** 1999.
103. **Mathew and White** (2011) *Curr.Opin.Genet.Dev.* **21** 113.
104. **Lock** *et al* (2011) *Mol.Biol.Cell* **22** 165.
105. **Guo** *et al* (2011) *Genes Dev.* **25** 460.
106. **Yang** *et al* (2011) *Genes Dev.* **25** 717.
107. **Kondo** *et al* (2005) *Nat.Rev.Cancer* **5** 726.
108. **Janku** *et al* (2011) *Nat.Rev.Clin.Oncol.* **8** 528.
109. **Behrends** *et al* (2010) *Nature* **466** 68.
110. **Miller** *et al* (2010) *Science* **327** 1638.

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1231	Brefeldin A	Causes ER stress; induces autophagy in mammalian cells
4098	Carbamazepine	Reduces inositol levels; induces autophagy
1126	Dexamethasone	Anti-inflammatory glucocorticoid; also induces autophagy in ALL cell lines
3093	Dorsomorphin	Induces autophagy via an AMPK inhibition-independent mechanism
3993	EB 1089	Vitamin D receptor (VDR) agonist; induces autophagy in MCF-7 cells
0741	GF 109203X	Protein kinase C inhibitor
0681	L-690,330	Inositol monophosphatase inhibitor; induces autophagy independently of mTOR inhibition
1391	NF 449	Highly selective $P2X_1$ antagonist
4079	Niclosamide	STAT3 inhibitor; also inhibits mTORC1 signaling. Stimulates autophagy *in vitro*
0600	Nimodipine	Ca^{2+} channel blocker (L-type)
0601	Nitrendipine	Ca^{2+} channel blocker (L-type)
2930	PI 103	Inhibitor of PI 3-kinase, mTOR and DNA-PK
1267	Pifithrin-α hydrobromide	p53 inhibitor; aryl hydrocarbon receptor agonist
1292	Rapamycin	mTOR inhibitor; immunosuppressant
1610	Rottlerin	Reported PKCδ inhibitor; stimulates autophagy
4297	SMER 28	Positive regulator of autophagy
2706	Temozolomide	DNA-methylating antitumor agent; also induces autophagy
1138	Thapsigargin	Causes ER stress; induces autophagy in mammalian cells
4247	Torin 1	Potent and selective mTOR inhibitor
3516	Tunicamycin	Causes ER stress; induces autophagy in mammalian cells
2815	Valproic acid, sodium salt	Reduces inositol levels; induces autophagy
0654	Verapamil	Ca^{2+} channel blocker (L-type)
Autophagy Inhibitors		
1334	Bafilomycin A1	Vacuolar H^+-ATPase inhibitor; also inhibits autophagy
1544	(±)-Bay K 8644	L-type Ca^{2+} channel agonist; inhibits autophagy
4109	Chloroquine	Inhibits apoptosis and autophagy
2656	Concanamycin A	Vacuolar H^+-ATPase inhibitor
4417	DBeQ	Selective p97 inhibitor; blocks autophagosome maturation
4545	E 64d	Cathepsin inhibitor; interferes with lysosomal digestion
1130	LY 294002	Selective PI 3-kinase inhibitor; inhibits autophagic sequestration
3977	3-Methyladenine	Class III PI 3-kinase inhibitor; also inhibits autophagy
1228	Nocodazole	Microtubule inhibitor; inhibits autophagosome-lysosome fusion
1190	Pepstatin A	Protease inhibitor; interferes with lysosomal digestion
1097	Taxol	Promotes assembly and inhibits disassembly of microtubules
1256	Vinblastine	Disrupts microtubules; inhibits autophagosome maturation
1232	Wortmannin	Potent, irreversible inhibitor of PI 3-kinase; inhibits PLK1

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