CELL DEATH & AUTOPHAGY

Mechanism and medical implications of mammalian autophagy

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Abstract | Autophagy is a highly conserved catabolic process induced under various conditions of cellular stress, which prevents cell damage and promotes survival in the event of energy or nutrient shortage and responds to various cytotoxic insults. Thus, autophagy has primarily cytoprotective functions and needs to be tightly regulated to respond correctly to the different stimuli that cells experience, thereby conferring adaptation to the ever-changing environment. It is now apparent that autophagy is deregulated in the context of various human pathologies, including cancer and neurodegeneration, and its modulation has considerable potential as a therapeutic approach.

The discovery of lysosomes by Christian de Duve[1] more than 60 years ago marked the birth of a new research field and earned its trailblazer a Nobel Prize in Physiology or Medicine in 1974. The delivery of heterogenic intracellular material to lysosomal digestion was termed 'autophagy' (Greek for 'self-eating') by de Duve as early as 1963, but consequent research on autophagy did not receive much attention for more than 30 years. Major achievements at that period focused on the tight regulation of autophagy by nutrient availability[2], while the physiological relevance and manner of lysosomal delivery remained unknown. Then, Yoshinori Ohsumi's laboratory conducted a genetic screen to dissect the process in yeast[3], identifying 15 autophagy-related proteins (ATGs) essential for autophagic delivery of cargo to the vacuole (the counterpart of the lysosome in yeast) (FIG. 1; TABLE 1). From that point onwards, the field explosively increased in knowledge, and the fundamental physiological importance of autophagy for human health and disease was uncovered. In 2016, Ohsumi was awarded a Nobel Prize in Physiology or Medicine for his discovery of mechanisms of autophagy.

We now know that autophagy is an adaptive process that occurs in response to different forms of stress, including nutrient deprivation, growth factor depletion, infection and hypoxia. We also understand much better how the autophagic machinery is regulated and selects cargo and how its perturbation affects cellular and organismal function. The main function of autophagy is to provide nutrients for vital cellular functions during fasting and other forms of stress; thus, autophagy has long been considered a nonselective process. However, autophagy was more recently shown to selectively eliminate unwanted, potentially harmful cytosolic material, such as damaged mitochondria or protein aggregates (a process known as selective autophagy; see BOX 1), thereby acting as a major cytoprotective system. Intriguingly, autophagy is also used by cells to secrete cytoplasmic constituents. Accordingly, autophagic activity modulates many pathologies, including neurodegeneration, cancer and infectious diseases, thus also placing autophagy under the spotlight of pharmacologists and clinicians.

In this Review, we summarize the molecular mechanisms and regulation of mammalian autophagy and describe their involvement in several pathological conditions. We also discuss current strategies, limitations and challenges involved in targeting the pathway in cancer and neurodegenerative diseases where most knowledge has accumulated over the past years.

Mechanism of autophagy

Induction of autophagy results in recruitment of ATGs to a specific subcellular location termed the phagophore assembly site (PAS) and nucleation of an isolation membrane that forms a cup-shaped structure termed the phagophore (FIG. 1). Gradual elongation of the curved isolation membrane results in expansion of the phagophore into a sphere around a portion of the cytosol. The isolation membrane eventually seals into a double-membraned vesicle, termed the autophagosome, thereby trapping the engulfed cytosolic material as autophagic cargo. After clearance of most ATGs and delivery along microtubules to the lysosome, the outer membrane of the autophagosome fuses with the lysosomal membrane to form an autolysosome. This fusion results in the release of a single-membrane autophagic body into the lysosomal lumen, which is followed by the degradation of the autophagic body together with its cargo by the autolysosomal hydrolytic milieu[4–6].

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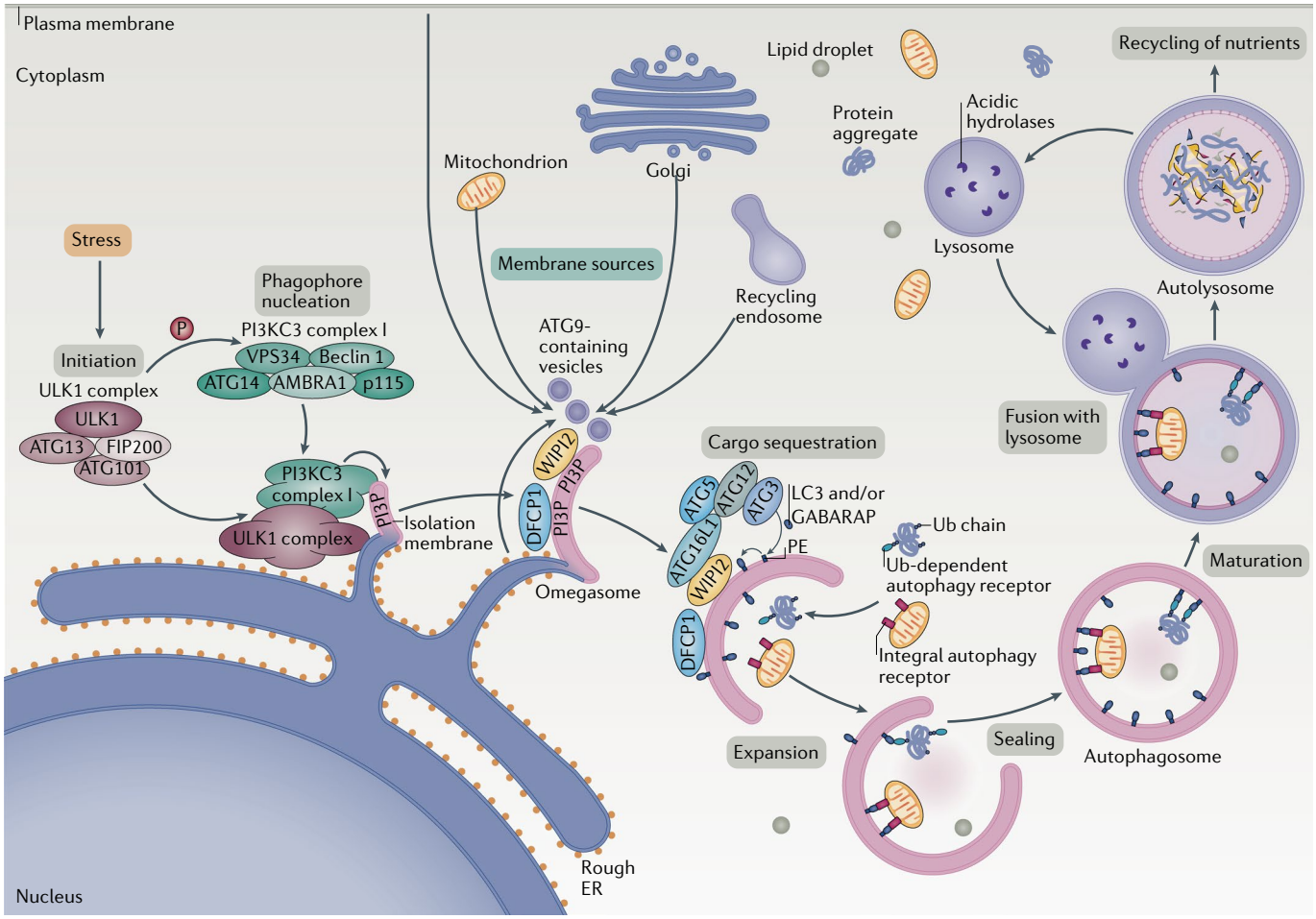


Fig. 1 | **Overview of the autophagy process.** Signals that activate the autophagic process (initiation) typically originate from various conditions of stress, such as starvation, hypoxia, oxidative stress, protein aggregation, endoplasmic reticulum (ER) stress and others. The common target of these signalling pathways is the Unc-51-like kinase 1 (ULK1) complex (consisting of ULK1, autophagy-related protein 13 (ATG13), RB1-inducible coiled-coil protein 1 (FIP200) and ATG101), which then triggers nucleation of the phagophore by phosphorylating components of the class III PI3K (PI3KC3) complex I (consisting of class III PI3K, vacuolar protein sorting 34 (VPS34), Beclin 1, ATG14, activating molecule in Beclin 1-regulated autophagy protein 1 (AMBRA1) and general vesicular transport factor (p115)), which in turn activates local phosphatidylinositol-3-phosphate (PI3P) production at a characteristic ER structure called the omegasome. PI3P then recruits the PI3P effector proteins WD repeat domain phosphoinositide-interacting proteins (WIPIs; here WIPI2) and zinc-finger FYVE domain-containing protein 1 (DFCP1) to the omegasome via interaction with their PI3P-binding domains. WIPI2 was recently shown to bind ATG16L1 directly, thus recruiting the ATG12~ATG5–ATG16L1 complex that enhances the ATG3-mediated conjugation of ATG8 family proteins (ATG8s), including microtubule-associated protein light chain 3 (LC3) proteins and γ-aminobutyric acid receptor-associated proteins (GABARAPs) to membrane-resident phosphatidylethanolamine (PE), thus forming the membrane-bound, lipidated forms; for example, in this conjugation reaction, LC3-I is converted into LC3-II — the characteristic signature of autophagic membranes. ATG8s not only further attract components of the autophagic machinery that contain an LC3-interacting region (LIR) but also are required for elongation and closure of the phagophore membrane. Moreover, in selective autophagy, LC3 is critically involved in the sequestration of specifically labelled cargo into autophagosomes via LIR-containing cargo receptors. Several cellular membranes, including the plasma membrane, mitochondria, recycling endosomes and Golgi complex, contribute to the elongation of the autophagosomal membrane by donating membrane material (part of these lipid bilayers is delivered by ATG9-containing vesicles, but the origin of the rest of the lipid bilayer is currently unknown). Sealing of the autophagosomal membrane gives rise to a double-layered vesicle called the autophagosome, which matures (including stripping of the ATG proteins) and finally fuses with the lysosome. Acidic hydrolases in the lysosome degrade the autophagic cargo, and salvaged nutrients are released back to the cytoplasm to be used again by the cell. Ub, ubiquitin.

The autophagic pathway and core autophagy proteins. The so-called core ATG proteins essential for autophagosome formation and lysosomal delivery of autophagic cargo are grouped by their functional and physical interactions into five complexes[7] (see also TABLE 1): (i) the ULK1 (Unc-51-like kinase 1) complex — the serine/threonine protein kinase ULK1, RB1-inducible coiled-coil protein 1 (FIP200; also known as RB1CC1), ATG13 and ATG101; (ii) ATG9 — the sole integral, transmembrane core ATG; (iii) the class III PI3K (PI3KC3) complex — the catalytic subunit vacuolar protein sorting 34 (VPS34) that converts PI into PI-3-phosphate (PI3P), Beclin 1 and general vesicular transport factor p115, joined by ATG14 in PI3KC3 complex I (PI3KC3–C1) or UV radiation resistance-associated gene protein (UVRAG) in complex II (PI3KC3–C2); (iv) WIPI (WD repeat domain phosphoinositide-interacting) proteins and their functional, optionally physical interaction partner ATG2;

Table 1 | **Key autophagic factors and their regulation**

Protein	Function	Mechanisms of regulation
Initiation and phagophore nucleation		
ULK1 and ATG1	Serine/threonine kinase; initiates autophagy by phosphorylating components of the autophagy machinery	Stress and nutrients (via mTORC1, AMPK and LKB1); TFEB and several miRNAs
FIP200	Component of ULK complex (possibly scaffolding function)	ULK1 and miRNAs
ATG13	Adaptor mediating the interaction between ULK1 and FIP200; enhances ULK1 kinase activity	ULK1, mTORC1 and AMPK
ATG101	Component of ULK complex; recruitment of downstream ATG proteins	ULK1
VPS34	Catalytic component of PI3KC3–C1; generates PI3P in the phagophore and stabilizes the ULK complex	AMPK, ULK1 and p300 (acetylation)
Beclin 1	Promotes formation of PI3KC3–C1 and regulates the lipid kinase VPS34	Activation: AMPK, ULK1, MAPKAPK2, MAPKAPK3, DAPK and UVRAG; inhibition: BCL-2, AKT and EGFR
ATG14	PI3KC3–C1 targeting to the PAS and expanding phagophore	PIPKIγI5 and mTORC1
ATG9	Delivery of membrane material to the phagophore	ULK1 complex
WIPI2	PI3P-binding protein that recruits ATG12~ATG5–ATG16L to the phagophore; retrieval of ATG9 from early autophagosomal membranes	TFEB (positive transcription regulator) and ZKSCAN3 (negative transcription regulator)
Phagophore expansion		
ATG4	Cysteine protease that processes pro-ATG8s; also, deconjugation of lipidated LC3 and ATG8s	ULK1 and ROS
ATG7	E1-like enzyme; activation of ATG8; conjugation of ATG12 to ATG5	miRNAs
ATG3	E2-like enzyme; conjugation of activated ATG8s to membranal PE	miRNAs
ATG10	E2-like enzyme that conjugates ATG12 to ATG5	miRNAs
ATG12~ATG5–ATG16L	E3-like complex that couples ATG8s to PE	CSNK2
PE-conjugated ATG8s	Scaffold for assembly of the ULK1 complex; supports membrane tethering and hemifusion events for phagophore expansion	ULK1, PKA, ATG4 and mTOR
ATG9	Delivery of membrane material to the phagophore	ULK1
Cargo sequestration		
Ubiquitin	Cargo labelling	PINK (phosphorylation)
Cardiolipin and ceramide	Cargo labelling	Phosphorylation
p62	Autophagy receptor	ULK1 and TBK1
OPTN	Autophagy receptor	TBK1
NBR1	Autophagy receptor	TBK1
NDP52	Autophagy receptor	TBK1
PE-conjugated LC3	Interaction with autophagy receptors; also phagophore expansion and sealing	ULK1, PKA, ATG4 and mTOR
Membrane sealing		
LC3s and GABARAPs	Unclear	Unclear; might involve phosphorylation and acetylation events
Autophagosome maturation		
ATG4	Removal of ATG8s from the surface of the autophagosome	Unknown
PE-conjugated LC3s and GABARAPs	Linking the autophagosome to microtubule-based kinesin motor	Unclear; might involve phosphorylation and acetylation events

Table 1 (cont.) | **Key autophagic factors and their regulation**

Protein	Function	Mechanisms of regulation
Fusion with the lysosome		
PE-conjugated LC3s and GABARAPs	Mediates autophagosome–lysosome fusion upon phosphorylation through PLEKHM1 and HOPS	STK3 and STK4
ATG14	Promotes SNARE-driven membrane fusion	Unknown
Rab GTPase RAB7	Unclear	Unknown

ATG, autophagy-related protein; AMPK, 5′ AMP-activated protein kinase; CSNK2, casein kinase 2; DAPK, death-associated protein kinase; EGFR, epidermal growth factor receptor; FIP200, RB1-inducible coiled-coil protein 1; GABARAP, γ-aminobutyric acid receptor-associated protein; HOPS, homotypic fusion and protein sorting; LC3, light chain 3; LKB1, liver kinase B1; MAPKAPK, MAPK-activated protein kinase; miRNA, microRNA; NBR1, neighbour of *BRCA1* gene; NDP52, nuclear dot protein 52; OPTN, optineurin; p62, also known as SQSTM1; p300, histone acetyltransferase 300; PAS, phagophore assembly site; PE, phosphatidylethanolamine; PI3P, phosphatidylinositol-3-phosphate; PINK, PTEN-induced putative kinase 1; PIPKIγi5, type Iγ PIP kinase isoform 5; PI3KC3, class III PI3K; PKA, protein kinase A; PLEKHM1, pleckstrin homology domain-containing protein family member 1; RAB, Ras-related protein; ROS, reactive oxygen species; STK, serine/threonine protein kinase; TBK1, TANK-binding kinase 1; TFEB, transcription factor EB; ULK1, Unc-51-like kinase 1; UVRAG, ultraviolent irradiation resistance-associated gene; VPS34, class III PI3K vacuolar protein sorting 34; WIPI2, WD repeat domain phosphoinositi de-interacting protein 2; ZKSCAN3, zinc-finger protein with KRAB and SCAN domains 3.

and (v) two ubiquitin (Ub)-like proteins and covalent conjugation targets (and their activation and conjugation machinery, see below): the Ub-like ATG12 conjugates with ATG5 (ATG12~ATG5), where ~ denotes conjugation, which further establishes a complex with ATG16L (ATG12~ATG5–ATG16L), and Ub-like ATG8 family proteins (ATG8s), which include the light chain 3 (LC3) subfamily (also known as microtubule-associated proteins 1 A/1B LC3, MAP1LC3): LC3A, LC3B, LC3C and the γ-aminobutyric acid receptor-associated protein (GABARAP) subfamily (GABARAP, GABARAPL1, GATE-16/GABARAPL2), which form conjugates with membrane-resident phosphatidylethanolamine (PE).

Induction and phagophore nucleation. Phagophores are nucleated at the PAS on endoplasmic reticulum (ER)-emanating membrane domains termed 'omegasomes' that are PI3P-rich and marked by the PI3P-binding protein zinc-finger FYVE domain-containing protein 1 (DFCP1; also known as ZFYVE1). However, ER-mitochondria and ER-plasma membrane contact sites[8,9] as well as other organelles, such as the Golgi complex, plasma membrane and recycling endosomes, were also recently implicated as PASs (see recent reviews[4]), possibly reflecting different experimental tools or the contribution of different intracellular membrane sources to autophagosome formation, which may be cell dependent and/or context dependent. Nucleation of the phagophore membrane is an intricate process, and its molecular details are still not completely understood. According to current understanding, phagophore formation involves the cooperative PAS formation, activation of the ULK1 complex and the PI3KC3–C1, possibly in concert with the activation of localized PI synthase. These events are accompanied by the recruitment of ATG9-containing vesicles generated by the secretory pathway to the PAS, which may deliver additional lipids and proteins contributing to membrane expansion[10–12]. Accordingly, activation of the ULK1 and PI3KC3–C1 are immediate responses to autophagy induction.

The most characterized trigger for induction of autophagy is deprivation of amino acids, which results in inhibition of the master cell growth regulator serine/threonine kinase mTOR[13,14]. mTOR is found in two distinct protein complexes, mTORC1 and mTORC2, but only mTORC1 directly regulates autophagy[15]. In high-nutrient conditions, ATG13 and ULK1 are both directly bound and phosphorylated by mTORC1 and remain inactive in this phosphorylated form[16]. Upon starvation, mTORC1 sites on ULK1 are dephosphorylated and ULK1 dissociates from mTORC1. Concomitantly, ULK1 undergoes autophosphorylation, followed by phosphorylation of ATG13 and FIP200. ULK1 is activated upon dissociation from mTOR after autophosphorylation, followed by phosphorylation of ATG13 and FIP200 by ULK1 (REFS[16,17]). Another key regulator of autophagy is TFEB (transcription factor EB), which is a master transcription factor controlling cellular clearance. Like ATG13 and ULK1, TFEB is negatively regulated by mTORC1 and released upon starvation to regulate the expression of genes involved in lysosomal biogenesis and lipid catabolism[18]. TFEB family members control autophagy by mTORC1 lysosomal recruitment and activity by directly regulating expression of the mTOR-activating Rag GTPase complex component Ras-related GTP-binding protein D (RagD)[19], thus providing a feedback circuit to balance the cellular metabolic state.

Autophagy may also be induced upon declining cellular energy levels, as in glucose starvation, sensed through the ATP:AMP ratio by cell homeostasis regulatory kinases 5′ AMP-activated protein kinase (AMPK) and serine/threonine-protein kinase STK11 (LKB1)[20]. LKB1 activates autophagy through AMPK by inhibition of mTORC1 indirectly via activation of the TSC2 (tuberous sclerosis 2) complex and possibly directly by phosphorylation of Raptor[21]. As TSC2 is regulated by interaction with WIPI3 and FIP200 (REF.[22]), involvement of the LKB1–AMPK–TSC2 axis in CREB-regulated transcription coactivator 1 (TORC1; also known as CRTC1) regulation provides a feedback control on autophagy induction. Because TSC2 is regulated by WIPI3 and FIP200, involvement of LKB1-AMPK-TSC2 axis in mTORC1 regulation allows coordination between autophagy induction and autophagosome formation. AMPK-mediated induction of autophagy can also bypass mTOR by directly inducing phosphorylation of ULK1, VPS34 and Beclin 1 (REF.[23]).

Contact sites
Interorganellar connections with distinct biochemical properties and a characteristic set of proteins that function as signalling hot spots.

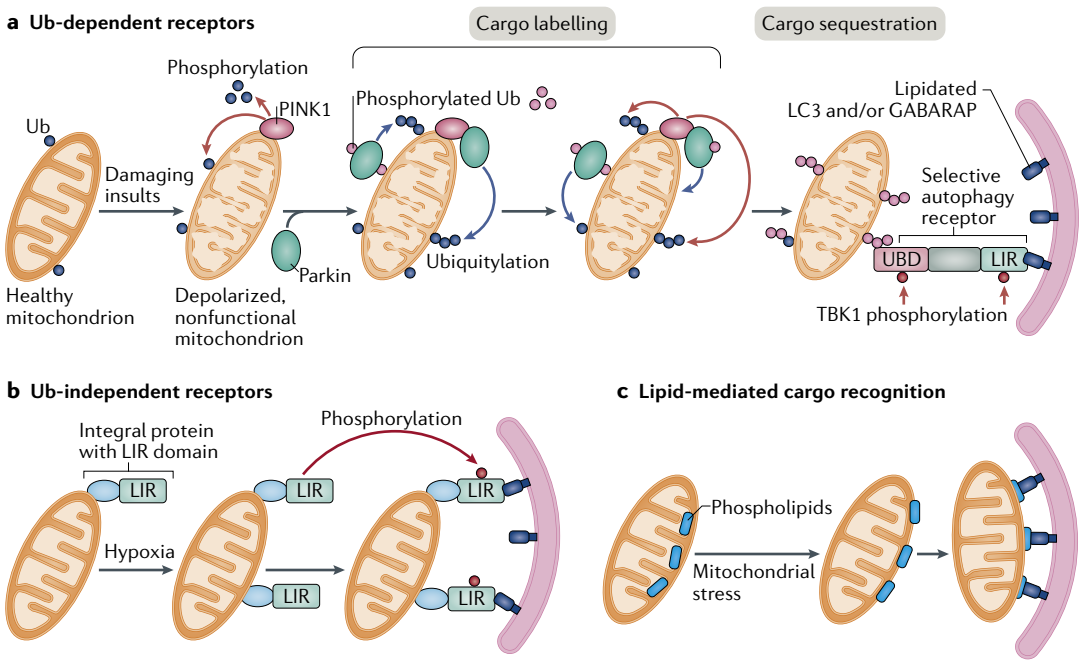
TSC2 (tuberous sclerosis 2) complex
Complex that is part of TSC that acts as a GTPase accelerating protein (GAP) for GTP-binding protein RHEB; because GDP-loaded RHEB is unable to activate mTORC1, TSC effectively shuts off mTORC1 signalling.

Raptor
Scaffold protein unique to mTORC1 (not present in mTORC2); binds substrates as well as regulators

Box 1 | **Cargo selection for selective autophagy**

Whereas starvation triggers bulk autophagy that nonspecifically engulfs any cytoplasmic material, certain signals or cellular events can evoke highly selective autophagic targeting of distinct cellular structures, such as damaged mitochondria (mitophagy), invading bacteria (xenophagy), aggregated proteins (aggrephagy) and others. Selective autophagy requires the labelling of cargo with 'eat-me' signals (most prominently ubiquitin (Ub) chains) recognized by autophagy receptors that link the cargo to the autophagic membrane via their light chain 3 (LC3)-interacting region (LIR). In selective autophagy, ULK1 is activated in an mTOR-independent manner that still awaits characterization. A recent report has implicated huntingtin (HTT), the protein product of the gene mutated in Huntington disease, as a possible molecular link between autophagic cargo and activation of ULK1[171]. In that study, HTT was shown to compete with mTOR complex 1 (mTORC1) for binding to ULK1, thus freeing ULK1 from mTORC1-mediated inhibition. HTT may also facilitate the interaction of the autophagy receptor sequestosome 1 (p62) with LC3 and K63-linked Ub chains, thereby coupling cargo recognition and activation of selective autophagy.

A well-studied targets of selective autophagy are mitochondria, which can be removed via different mechanisms depending on the physiological context. Upon damage or depolarization, the mitochondrial kinase PTEN-induced kinase 1 (PINK1) becomes stabilized and recruits the Ub E3 protein ligase Parkin (see figure part **a**). PINK1 and Parkin cooperate in a feedforward mechanism to assemble phosphorylated Ub (pUb) chains on several proteins of the outer mitochondrial membrane, which in turn recruit cargo receptors such as optineurin (OPTN), calcium-binding and coiled-coil domain-containing protein 2 (NDP52) and p62. In this process, PINK1 phosphorylates free Ub, polyUb attached by Parkin to the mitochondrial surface and the ubiquitin-like (UBl) domain of Parkin. These phosphorylation events enhance both the ubiquitin ligase activity of Parkin and its retention time on damaged mitochondria. Another player in mitophagy is TANK-binding kinase 1 (TBK1), which promotes coupling of the cargo to the phagophore by phosphorylating Ub-binding domains and LIRs of several cargo receptors, thereby increasing their affinity for pUb and LC3, respectively. Notably, mitophagy can also occur in a Ub-independent manner via mitochondrial proteins such as BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like (NIX), FUN14 domain-containing protein 1 (FUNDC1) and BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), which possess an LC3-interacting region (LIR) and therefore function as direct cargo receptors (see figure part **b**). They are typically regulated by stress-dependent phosphorylation. Finally, lipids, including phospholipids, such as cardiolipin[172] and ceramide[173], have been shown to mediate mitophagy (see figure part **c**). In neuronal cells, cardiolipin is located at the inner membrane of healthy mitochondria, but upon mitochondrial damage, it is externalized and presented on the mitochondrial surface, where it is recognized by LC3.



GABARAP, γ-aminobutyric acid receptor-associated protein; UBD, ubiquitin D.

Certain transcription regulators were implicated in the regulation of autophagy in different systems: the epigenetic reader bromodomain-containing protein 4 (BRD4) together with methyltransferase G9a were recently reported as repressors of a transcriptional programme of autophagic genes needed for autophagosome biogenesis[24], and the regulation of autophagy by FOXO (forkhead box O) proteins was demonstrated in cardiomyocytes[25,26].

FOXO (forkhead box O) proteins
Family of transcription factors activated in response to cell stress; they regulate genes involved in cellular energy production, oxidative stress resistance, cell viability and proliferation.

Several other regulators of autophagic proteins have been described in recent studies. Beclin 1 is inhibited by antiapoptotic molecule BCL-2 (REF.[27]) and is also the target of several kinases: phosphorylation by ULK1, MAPKAPK (mitogen-activated protein kinase–activated protein kinase) 2 and 3, AMPK and DAPK (death-associated protein kinase) promotes autophagy, whereas AKT and EGFR (epidermal growth factor receptor) inhibit autophagy through Beclin 1 inactivation. PI3KC3–C1 is further

regulated by interaction with AMBRA1 (activating molecule in BECN1-regulated autophagy protein 1), which promotes autophagy[28], whereby ULK1-phosphorylated AMBRA1 is released from microtubules to allow Beclin 1 binding and consequent PI3KC3–C1 activation[29]. In chondrocytes, fibroblast growth factor 18 (FGF18) and its receptor FGFR4 activate the VPS34–Beclin 1 complex in a JNK-dependent manner to initiate autophagy[30]. Progestin and adipoQ receptor family member 3 (PAQR3), a Golgi complex-localized multipass transmembrane protein, was found to shift the balance towards PI3KC3 association with ATG14 instead of with UVRAG upon glucose starvation and thereby increase autophagy[31]. Finally, it has been recently reported that autophagy in livers of fasting mice is regulated by acetylation of VPS34, which is mediated by the histone acetyltransferase p300 (REF.[32]).

The mechanistic determinant for recruitment of ULK1 to the PAS is largely unclear. In a recent study, the Golgi-localized WW domain-containing adaptor with coiled coil (WAC) was identified as a positive regulator of autophagy[33]. In a subsequent study, WAC was found to mediate translocation of GABARAP — a factor that mediates phagophore expansion — from the Golgi complex to the centrosome[34]. It was proposed that centrosomal GABARAP is then trafficked (possibly via microtubules) to the phagophore, where it recruits and activates the ULK1 complex. This centrosomal pool of GABARAP might support sustained activation of the ULK1 complex during autophagosome formation. PI3KC3–C1 is targeted to the PAS by ATG14 (REFS[35,36]) possibly through phosphorylation by ULK1 and consequent interaction with ATG13[37], while ATG14 is regulated by interaction with type Iγ PI-phosphate 5-kinase (PIPKIγi5), an enzyme that generates phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P2)[38]. Recruitment of ATG9 is regulated by the ULK1 complex[10,39] and by transport protein particle complex III (TRAPPIII), an activator of the ER-to-Golgi complex trafficking factor Ras-related protein RAB1 (also known as RAB1A)[40,41]. Moreover, guanine nucleotide exchange C9ORF72, a protein that is mutated in patients with amyotrophic lateral sclerosis (ALS) or with frontotemporal dementia, was recently shown to interact with ULK1 and RAB1 (REF.[42]), suggesting that RAB1 coordinates ATG9 recruitment with the activity of ULK1.

Of note, phagophore nucleation (and possibly expansion) probably also involves actin scaffolding, as autophagosome formation is promoted by F-actin-capping protein CapZ[43] and WASP homologue-associated protein with actin, membranes and microtubules (WHAMM) recruited to the PAS by PI3P and by actin nucleation-promoting factor junction-mediating and -regulatory protein (JMY), which is targeted to the phagophore via its LC3-interacting region (LIR; see also below)[44].

Phagophore expansion. The ATGs most prominently implicated in phagophore expansion are the Ub-like ATG8 family members[45]. Nascent pro-ATG8s are processed at their C-termini by the cysteine protease ATG4, exposing a glycine residue that is essential for their conjugation to PE[45]. The specificity of the four distinct ATG4 isoforms is not fully characterized, but ATG4B has been shown to recognize all ATG8s, whereas ATG4A is more specific to GABARAPs[46,47]. The processed ATG8s are activated by the E1-like enzyme ATG7 and conjugated to membrane-associated PE by the activity of ATG3, thereby converting it from a freely diffuse form (for LC3 this form is known as LC3-I)into a membrane-anchored, lipidated form (for LC3 referred to as LC3-II)[8]. For efficient PE conjugation in vivo, ATG3 requires stimulation by E3-like activity of the ATG12~ATG5 conjugate, formed by activation of ATG12 by ATG7 and conjugation to ATG5 by E2-like ATG10 (REF.[8]). The activity of ATG12~ATG5 is localized to the PAS by interaction with ATG16L in a dimeric ATG12~ATG5–ATG16L complex[48,49] that is recruited to the PAS through interaction of ATG16L1 with WIPI2 (REF.[50]). ATG16L1 can form homo-oligomers through a coiled-coil domain, which may allow ATG16L1 to crosslink multiple ATG12~ATG5 conjugates into a single large protein complex that possibly serves to scaffold the phagophore[51]. Conjugation of ATG8s to PE promotes phagophore expansion (and possibly also sealing)[52]. This conjugation event is suggested to occur on ER exit sites following starvation-induced and FIP200-mediated relocation of ER exit factor prolactin regulatory element-binding protein (SEC12) to the ER–Golgi intermediate compartment (ERGIC)[53,54] — in line with the observations that phagophores form in apposition to ER exit sites[55]. Notably, this view has been recently challenged by studies indicating that autophagosomes can form without the conjugation machinery[56] or even in the absence of all ATG8s[57]. Aside from their contribution to phagophore expansion, phagophore-anchored ATG8s also facilitate cargo recruitment in selective autophagy, as they interact with LIRs of cargo receptors (which themselves recognize the cargo through 'eat-me' signals, such as Ub or galectins (BOX 1)).

Apart from C-terminal processing of nascent ATG8s, ATG4 is also capable of deconjugating ATG8s from PE to release it from the membrane and limit phagophore expansion. Both activities of ATG4 are required for the normal progression of autophagy. As the autophagic activities of ATG8s are attributed to their conjugation to PE, it was originally postulated that ATG4 deconjugating activity must be tightly regulated both in time and in space[58]. Accordingly, in order to function properly on the autophagic membrane, lipidated ATG8s should be protected from ATG4 (REFS[58–60]). This may be regulated by mitochondria-generated reactive oxygen species (ROS)[58], in line with the suggestion that phagophores form preferentially at ER–mitochondria contact sites. Alternatively, ATG8s might be protected from deconjugation by inhibition of ATG4 through phosphorylation by ULK1 (REFS[59,60]).

Targeting of ATG8s to autophagic membranes can also be regulated by additional post-translational modifications. For example, phosphorylation of LC3 by protein kinase A (PKA) negatively regulates its autophagic activity[61]. Finally, autophagosomal size is also controlled by the AMPK-related kinases NUAK family SNF1-like kinase 2 (NUAK2) and

JNK
Member of the MAPK family activated by extracellular signals; associated with several pathological conditions, including neurodegenerative diseases, inflammation and cancer.

E1
Ubiquitin (Ub)-activating enzyme; first enzyme in the E1–E2–E3 ubiquitylation cascade that activates Ub in an ATP-dependent manner.

E3
Ubiquitin (Ub)-ligating enzyme; cooperates with E2 to attach Ub to a lysine residue in the target protein. Only component of the Ub machinery that interacts with the target, thus conferring substrate specificity to the reaction.

E2
Ubiquitin (Ub)-conjugating enzyme; takes over activated Ub from E1 and hands it over to E3. Plays a key role in defining the linkage type of Ub conjugation when chains of multiple Ub molecules are assembled.

ER exit sites
Areas of the endoplasmic reticulum (ER) where transport vesicles that contain lipids and proteins made in the ER detach from the ER and move to the Golgi complex.

Galectins
Carbohydrate-binding lectins that recognize intracellular bacteria-containing vesicles when their membrane integrity is compromised.

serine/threonine-protein kinase BRSK2 through ATG2 and WIPI4 (REF.[22]). Accordingly, WIPI molecules function as PtdIns3P effectors at the nascent autophagosome, acting as scaffold molecules with distinct interactions to different autophagic factors[50]. WIPI4 interacts with ATG2 to regulate autophagosome formation by an as yet unclear mechanism[22].

Autophagosome maturation. Following expansion and sealing of the phagophore, the autophagosome undergoes maturation, which involves gradual clearance of ATGs from the nascent autophagosome outer membrane and recruitment of machinery responsible for lysosomal delivery (microtubule-based kinesin motors) and machinery that mediates fusion with the lysosome, encompassing SNAREs: syntaxin 17 (STX17) and synaptosomal-associated protein 29 (SNAP29), on the autophagosome and vesicle-associated membrane protein 8 (VAMP8), on the lysosome[62,63], and the homotypic fusion and protein sorting (HOPS) complex, which mediates membrane tethering to support SNARE-mediated fusion. These processes all occur in a poorly characterized and probably coordinated manner that is slowly emerging[64] (see also REF.[65] for review).

ATG8s drive maturation by linking the autophagosome to kinesins through autophagy-specific kinesin adaptors such as FYCO1 (FYVE and coiled-coil domain-containing protein 1)[66]. ATG8s also recruit — via pleckstrin homology domain-containing family M member 1 (PLEKHM1) — the HOPS complex to the autophagosome[67]. Recruitment of the HOPS complex to the autophagosome was also proposed to be mediated by UVRAG, which is negatively regulated by mTORC1 (REF.[68]), thus potentially broadening the range of mTORC1 activities to late events along the autophagic process. However, a later study suggested an indirect role for UVRAG in autophagy that is secondary to its role in late stages of endocytic degradation[69].

There is now evidence that post-translational modifications of ATG8s further regulate autophagosome maturation, as the phosphorylation of LC3 on residue Thr50 by the Ste20 Hippo kinase orthologues serine/threonine-protein kinase 3 (STK3) and STK4 was recently found to be essential for autophagosome–lysosome fusion and for clearance of intracellular bacteria by autophagy[70]. Interestingly, the phagophore nucleation factor ATG14 was recently implicated in autophagosome maturation as well. ATG14 was shown to be recruited to the autophagosomal outer membrane by interaction with STX17 and to promote membrane tethering to enhance SNARE-mediated fusion[63].

Medical implications

Extensive research over the past two decades has not only established a central role for autophagy in cellular homeostasis but also unravelled molecular links to various disease conditions (TABLE 2). Chemical or genetic disturbance of autophagy and the age-dependent decline in autophagic activity have been implicated in the progression of cancer, neurodegeneration and immune diseases, as well as ageing [71].

Complex roles of autophagy in cancer development and progression. Autophagy is an important process during cancer progression, but the exact roles of autophagy in cancer cells are strongly context-dependent (FIG. 2a). Its cytoprotective function is believed to have tumour-suppressive potential before the onset of tumorigenesis, and loss of autophagy has been associated with increased risk of cancer[72]. However, autophagy has also been shown to allow premalignant cells to escape genotoxic stress and inflammation that promote tumorigenesis. There is good evidence, moreover, that autophagy provides cancer cells with metabolic plasticity, allowing them to thrive in suboptimal environments[73] and to exploit the prosurvival activity of autophagy to cope with therapy-induced stresses[74–76]. Accordingly, many types of advanced cancers exhibit high autophagic activity[77], and it was proposed that certain tumours, such as pancreatic cancer[78–80] or cancers with mutant RAS (rat sarcoma) genes[81], are highly dependent on autophagy. Interestingly, it has been revealed that autophagy induction is a side effect of many cancer therapies[82], and thus, pharmacological inhibition of autophagy has been proposed as a valid strategy to enhance the efficacy of therapies and to avoid resistance to treatment in certain cancers[81,83,84] (TABLE 2). Notably, some reports also highlight a beneficial role for autophagy activation in cancer therapies involving the induction of immunogenic cell death. In this context, autophagy-competent dying tumour cells actively release ATP[85–87] and the high-mobility group box 1 protein B1 (HMGB1)[88,89], which recruit immune effectors into the tumour bed to trigger a tumour-specific immune response. Thus, activation of autophagy rather than its inhibition could be considered as a strategy to boost the efficacy of cancer therapy[83]. In accordance with this notion, caloric restriction (which promotes autophagy by inactivation of mTORC1) was found to enhance tumour immunosurveillance but had this effect only in the case of autophagy-proficient tumours[90]. In order to therapeutically exploit these findings, it will be necessary to identify chemotherapy and/or radiotherapy regimens that trigger an optimal tumour-targeting immune response as well as to define the types of cancer that are sensitive to this treatment strategy. The genetic context was also shown to be important for determining the role of autophagy in cancer. For example, in a mouse model of pancreatic ductal adenocarcinoma, the loss of autophagy prevents the formation of high-grade pancreatic intraepithelial neoplasias in the presence of p53, whereas in the absence of p53, autophagy inhibition accelerates tumour growth[91]. Thus, autophagy seems to be a double-edged sword in the context of cancer therapies, and it remains to be established whether it can be successfully targeted — inhibited or induced — for therapeutic benefit.

The emerging notion that autophagy can shape the tumour microenvironment is further corroborated by the fact that autophagy can facilitate polarized sorting and unconventional secretion of certain cytosolic proteins[92,93]. Indeed, oncogenic RAS-driven invasion was shown to be dependent on autophagy-mediated secretion of multiple factors, including the pro-migratory cytokine interleukin 6 (IL-6) and WNT5a, which are

SNAREs
Proteins that mediate the fusion of vesicles with target membranes. SNARE proteins on the vesicle (v-SNAREs) and on the target membrane (t-SNAREs) combine to form a *trans*-SNARE complex that provides the force for membrane fusion.

Hippo kinase
A kinase that functions as a central node in the regulation of cell division and controls organ size in flies and mammals as well as the growth of cancer cells.

High-mobility group box 1 protein (HMGB1)
A protein that senses and coordinates the cellular stress response acting as a DNA chaperone, autophagy sustainer and protector from apoptotic cell death. Outside the cell, it functions as a prototypic damage associated molecular pattern molecule (DAMP).

Unconventional secretion
Comprises the translocation across the plasma membrane of cargo without a signal peptide or a transmembrane domain and cargos that reach the plasma membrane by bypassing the Golgi apparatus despite entering the endoplasmic reticulum (ER).

Table 2 | **Human diseases linked to autophagy and clinical translation**

Disease	Mechanism	Compounds
Autophagy activation in neurodegenerative diseases		
Alzheimer disease	mTOR inhibition (via 5-HT_6R activation)	AVN-211; Lu AE58054 (idalopirdine); SB-742457
	Inhibition of AKT–mTOR pathway	rAAV/Aβ vaccine
	ACAT1 inhibition	F12511
	mTOR inhibition	Rapamycin, latrepirdine and metformin
	AMPK activation	Resveratrol and resveratrol-like small molecules
	Lysosomal acidification	Nicotinamide
	GSK3β and IMPase inhibition	Lithium
	Unclear mechanism	Berberine
	MTMR14 (autophagy inhibitor) inhibition	AUTEN-67
Parkinson disease	NRF2 activation	DMF
	TFEB activation	Curcumin analogue
	Beclin 1 complex activation	*BECN1* gene transfer
	TFEB regulation	*TFEB* gene
	Beclin 1 activation	Dual GLP-1–GIP receptor agonists
ALS	Unclear mechanism	Berberine
Huntington disease	MTMR14 inhibition	AUTEN-67
	mTOR inhibition	Rapamycin
	Unclear mechanism	Berberine
	Calpain inhibition	Calpastatin
	Unknown	Rilmenidine
	Unknown	Trehalose
	mTOR activation	Constitutively active *RHEB* gene product
Interventions involving autophagy inhibition in cancer		
Breast cancer	Autophagy inhibition + microtubule inhibition	CQ + taxols
	Autophagy inhibition	CQ
Prostate cancer	Autophagy inhibition + BCL-2 inhibitor + antiandrogen	HCQ + ABT-263 + abiraterone
	Autophagy inhibition	HCQ
	Autophagy inhibition + androgen receptor inhibition	Metformin hydrochloride + enzalutamide
Pancreatic cancer	Autophagy inhibition + inhibition of DNA synthesis	CQ + gemcitabine
	Autophagy inhibition + inhibition of DNA synthesis + microtubule inhibition	HCQ + gemcitabine + abraxane
	Autophagy inhibition + inhibition of DNA synthesis	HCQ + gemcitabine
	Autophagy inhibition	CQ
Small-cell lung cancer	Autophagy inhibition	CQ
	Autophagy inhibition + DNA damage	CQ + radiotherapy
Non-small-cell lung cancer	Autophagy inhibition + microtubule inhibition + DNA damage + inhibition of angiogenesis	HCQ + paclitaxel + carboplatin + bevacizumab
Melanoma	Autophagy inhibition + DNA damage + DNA repair inhibitor	CQ + radiation + DT01
	Autophagy inhibition + MEK inhibition	HCQ + trametinib
	AKT–mTOR signalling	Curcumin
Colorectal cancer	Autophagy inhibition + alkylation + DNA damage + inhibition of angiogenesis	HCQ + oxaliplatin + 5-FU + bevacizumab
	Autophagy inhibition + inhibition of angiogenesis + alkylation and antimetabolite	HCQ + bevacizumab + XELOX
	Autophagy inhibition + HDAC inhibitor	HCQ + vorinostat
Renal cell carcinoma	Autophagy inhibition + mTOR inhibitor	HCQ + RAD001

Table 2 (cont.) | **Human diseases linked to autophagy and clinical translation**

Disease	Mechanism	Compounds
Interventions involving autophagy inhibition in cancer (cont.)		
Solid tumours	Autophagy inhibition + HDAC inhibitor	HCQ + vorinostat
	Autophagy inhibition + DNA damage	CQ + carboplatin and/or gemcitabine
Multiple myeloma	Autophagy inhibition + proteasome inhibition + alkylation	CQ + velcade + vyclophosphamide
Glioblastoma	Autophagy inhibition + DNA damage and/or alkylation	CQ + chemoradiation with temozolomide
Interventions involving autophagy activation in cancer and cancer-related phenotypes		
Adenocarcinoma bone metastasis	p53-dependent autophagy induction	Fluvastatin (HMG-CoA reductase inhibitor)
Hepatocellular carcinoma	AMPK activation	Palbociclib
Inclusion body myositis	mTOR inhibition	Rapamycin
Desmoid-type fibromatosis	mTOR inhibition	Rapamycin
Advanced cancers	mTOR inhibition + HDAC6 inhibition + autophagy inhibition	Rapamycin + vorinostat + HCQ
Other interventions		
Infection	AMPK-mediated autophagy activation	Ohmyungsamycins
	Autophagy activation by mTOR inhibition	Statin, gefitinib and carbamazepine
	Autophagy activation	TAT-Beclin1
Diabetes	SIRT1 upregulation	Resveratrol

For references, see Supplementary Table 1. 5-FU, 5-fluorouracil; 5-HT_6R, 5-hydroxytryptamine 6 receptor; ACAT1, acyl-CoA:cholesterol acyltransferase 1; ALS, amyotrophic lateral sclerosis; AMPK, 5′ AMP-activated protein kinase; BECN1, Beclin 1; CQ, chloroquine; DMF, dimethyl fumarate; DT01, DNA repair inhibitor; GLP-1/GIP, glucagon-like peptide 1/glucose-dependent insulinotropic polypeptide; GSK3β, glycogen synthase kinase-3β; HCQ, hydroxychloroquine; HDAC, histone deacetylase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IMPase, inositol monophosphatase; MTMR14, myotubularin-related protein 14; NRF2, nuclear factor erythroid 2-related factor 2; RAD001, 40-*O*-(2-hydroxyethyl) derivative of sirolimus; rAAV/Aβ, recombinant adeno-associated viral vector/amyloid-b; SIRT1, NAD-dependent protein deacetylase sirtuin-1; TAT, transactivator of transcription peptide derived from a region of Beclin 1, which binds HIV-1 Nef; TFEB, transcription factor EB.

normally secreted via the conventional pathway[94]. In addition, recent findings point to a close relationship between autophagy and the biogenesis and secretion of exosomes[95,96]. Exosomes transfer lipids, proteins, mRNAs, non-coding RNAs and even DNA out of cells and have been shown to promote tumour growth, alter the tumour microenvironment, facilitate cancer cell dissemination, modulate immune responses and mediate resistance to therapy[97].

To acquire metastatic potential, adherent cancer cells need to gain motility, which is achieved through epithelial–mesenchymal transition (EMT). Intriguingly, signals that trigger EMT, such as hypoxia or transforming growth factor-β (TGFβ), also activate autophagy pathways[98]. Yet, as activation of autophagy was shown to cause downregulation of major transcription factors of the EMT process, autophagy seems to inhibit rather than support EMT in most types of cancer[99–101]. Moreover, cadherin 6, which specifically marks cells undergoing EMT and actively drives the EMT process, downregulates autophagy by directly interacting with and blocking the functions of several autophagic proteins[102], suggesting that autophagy activation is not favoured during EMT itself.

A feature closely linked to EMT is anoikis, a specific form of apoptotic cell death that results from the prolonged detachment of cells from the extracellular matrix (ECM) and is mediated by BCL-2 protein family members, including Bcl-2 modifying factor (BMF) and Bcl-2-like protein 11 extra-long isoform (BIM-EL). Anoikis represents a critical challenge to metastasizing tumour cells. However, autophagy activated upon loss of ECM–integrin receptor engagement can promote resistance to anoikis in several tumour models, possibly by alleviating metabolic deficiencies in ECM-detached cells[98,103,104]. Intracellular signals governing this process remain poorly defined but might involve the integration of multiple pathways, including those that activate autophagy upon accumulation of ROS[105], glucose starvation, ER stress signalling via the PERK pathway[106,107] and activation of the IKK (IκB kinase) complex[108], which is a central activator of the NF-κB (nuclear factor-κB) pathway. Moreover, emerging evidence points to direct, negative regulation of the autophagy factor Beclin 1 by the proapoptotic factors BIM-EL and BMF: the interaction of BIM-EL with Beclin 1 was shown to inhibit autophagosome formation by sequestering Beclin 1 to microtubules, whereas BMF was reported to stabilize the inhibitory Beclin 1–BCL-2 protein complex[109–111]. However, the exact mechanisms and the pathophysiological consequences of this crosstalk between autophagy and anoikis are not yet fully understood.

Besides preventing anoikis, the ECM contact also has a major role in cancer cell migration. Cells bind to the ECM through large protein complexes called focal adhesions. In order for cells to migrate, their focal adhesions need to be taken apart and then reconstructed in a coordinated fashion. Autophagy contributes to focal adhesion remodelling by controlling the turnover of key components of focal adhesions, including paxillin, vinculin, zyxin and

Exosomes
Small extracellular vesicles that contain various molecular constituents and are released directly from the plasma membrane or when multivesicular bodies fuse with the plasma membrane.

NF-κB (nuclear factor-κB) pathway
A transcription factor that controls cytokine production and cell survival and plays a key role in the cellular response to infection. Disturbance of the pathway has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection and improper immune development.

a Autophagy and cancer

Cancer progression		Role of autophagy
Cancer initiation	Antitumoral	Protection against stress (metabolic, oxidative, inflammatory)
Growth of primary tumour	Protumoral	Protection against stress (metabolic, oxidative, inflammatory)
EMT	Antitumoral	Downregulation of EMT-promoting transcription factors
Anoikis resistance	Protumoral	Unclear mechanism, multiple pathways involved
Migration	Antitumoral	RHOA degradation
	Protumoral	Focal adhesion turnover

Cancer treatment		Role of autophagy
Treatment resistance	Protumoral	Cytoprotection
Immunogenic cell death	Antitumoral	Secretion of factors that trigger tumour-specific immune response

b Autophagy in cell migration

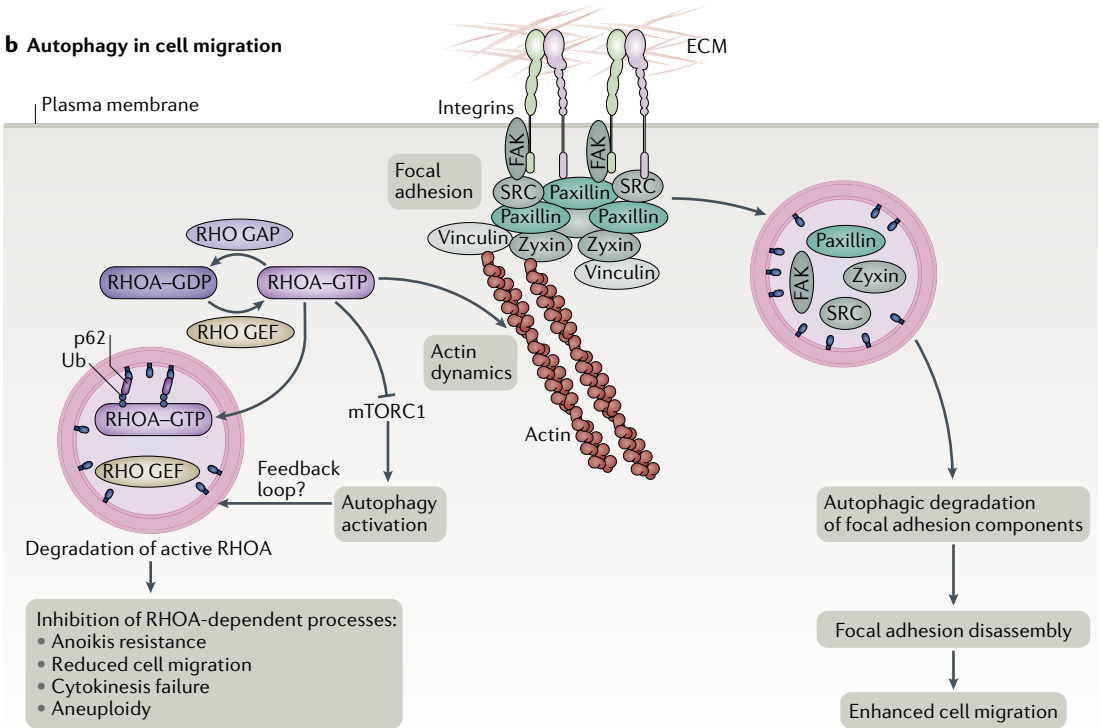


Fig. 2 | **Autophagy in cancer. a** | Autophagy impacts several aspects of cancer progression. High autophagic activity is believed to be cytoprotective and to suppress cancer initiation. However, in the primary tumours (after successful initiation), autophagy is often upregulated to overcome stresses resulting from fast growth (such as protein stress) as well as low nutrient availability (starvation) inside the tumour mass. Other cellular processes are associated with cancer progression and spreading crosstalk with the autophagy pathway. The upregulation of autophagy during epithelial–mesenchymal transition (EMT) appears to have an inhibitory effect on EMT, as several EMT-promoting transcription factors are downregulated in an autophagy-dependent manner. In contrast, another process that is required for cancer cells to gain migratory capacity and spread, anoikis resistance, is promoted by upregulation of autophagy in many cancers, yet the underlying mechanisms are unclear. Cell migration can be both promoted by autophagy (turnover of focal adhesions) and inhibited (autophagic degradation of actin dynamics regulator transforming protein RHOA); see also part **b**. On one hand, cancer therapies can induce autophagy, which contributes to the development of resistance. On the other hand, autophagy was reported to be required for immunogenic cancer cell death and was suggested to be antitumorigenic. **b** | Autophagy regulates cell migration in at least two opposing ways: on one hand, autophagy directly degrades active (GTP-bound) RHOA and the RHOA guanine nucleotide exchange factor (GEF) H1 in a ubiquitylation-dependent manner mediated by recognition through autophagy receptor sequestosome 1 (p62). This impacts actin dynamics, thus inhibiting cell migration and other RHOA-dependent cellular processes. Interestingly, autophagic RHOA degradation also contributes to anoikis resistance. Intriguingly, RHOA has been shown to inhibit signalling upstream of mTORC1, thus stimulating autophagy in a potential negative feedback loop. On the other hand, autophagy mediates the disassembly of focal adhesions by degrading several focal adhesion components, thus contributing to increased cell migration. ECM, extracellular matrix; FAK, focal adhesion kinase; mTORC1, mTOR complex 1; Ub, ubiquitin.

Leading edge
Front edge of a cell that is pushed forward by rapid actin polymerization.

focal adhesion kinase (FAK), and loss of autophagy inhibits migration and focal adhesion turnover at the leading edge[112,113] (FIG. 2b). Moreover, autophagy has been shown to directly target and degrade active transforming protein RHOA[114] as well as the RHOA–guanine nucleotide exchange factor (GEF) H1 (REF.[115]), which are crucial regulators of actin dynamics and cell migration. Remarkably, RHOA has also been implicated in mediating anoikis through cytoskeletal tension-dependent cell death in unattached cells[116]. Thus, RHOA degradation could be one way by which autophagy contributes to anoikis resistance. Importantly, in other contexts, autophagy-mediated RHOA degradation may inhibit cell migration as well as other RHOA-dependent events, including cell division, leading to cytokinesis failure or aneuploidy[114]. Intriguingly, RHOA can repress signalling through mTORC1, thus enhancing autophagy[117], further highlighting the complex interplay between cell–ECM attachment, cell migration and autophagy.

Taken together, autophagy can both suppress and promote cancer progression and metastasis at several stages. This complicates therapeutic intervention (BOX 2) and makes it necessary to evaluate the type of tumour cell, its genetic background, the stage of tumour progression and the tumour microenvironment in order to achieve the desired effect of autophagy modulation and avoid potential aggravation of the disease.

Autophagy against neurodegenerative diseases. Among the hallmarks of neurodegenerative diseases (including Alzheimer disease, Parkinson disease, Huntington disease, ALS, Vici[118], hereditary spastic paraplegia[119], static encephalopathy of childhood with neurodegeneration in adulthood (SENDA)[120] and others) are aggregates of misfolded or unfolded proteins that accumulate inside neuronal cells, eventually causing severe disturbances in their function and/or their death (FIG. 3).

In healthy cells, proteins that are not properly folded are tagged with Ub and degraded by the proteasome[121]. However, proteasomal activity is prone to impairment by various internal and external stresses and declines with age. If the degradative capacity of the proteasome is overloaded, the autophagy system becomes activated to remove accumulating aggregates as well as organelles that are irreparably damaged by aggregated and non-functional proteins[121]. Indeed, the most common cause of death of autophagy-deficient animals is neurodegeneration accompanied by the accumulation of ubiquitylated protein aggregates[122,123]. Moreover, numerous proteins that are mutated in neurodegenerative diseases have been implicated in autophagy[124,125] or in lysosomal function[126], and transcriptome studies using samples from patients revealed alterations in autophagy-related signalling[127–129]. The autophagic cargo receptor sequestosome 1 (p62), which binds Ub, has a key role in the clearance of protein aggregates, and post-mortem analysis of p62-positive inclusions is a defining diagnostic

Box 2 | **Autophagy as a pharmacological target**

The first US Food and Drug Administration (FDA)-approved agent capable of inhibiting autophagy was chloroquine, a drug previously used to treat malaria and arthritis, which also blocks autophagy by disrupting lysosome acidification[174]. Now, multiple targets within the pathway have been or are being evaluated for pharmacological intervention of autophagy (TABLE 2), including mTOR, serine/threonine protein kinases ULK1 and ULK2[175,176], vacuolar protein sorting 34 (VPS34)[177–179], and interactions within the Beclin 1 complex[180], the E1-like enzyme autophagy-related protein 7 (ATG7)[181] and ATG4B — the protease that processes pro-LC3 (light chain 3)[182].

The most thoroughly tested inducer of autophagy is rapamycin, which inhibits mTOR complex 1 (mTORC1) in mouse and fly models of various neurodegenerative diseases. However, considerable side effects on cellular pathways other than autophagy precluded its therapeutic use in humans[183]. Additionally, natural (often dietary) compounds, including resveratrol, polyphenols, berberine, artemisinin, sesamol, trehalose or spermidine, have moved into the focus of pharmacologists, yet knowledge of the mechanisms of action and potential side effects of these substances is currently incomplete[184–192]. Notably, in addition to pharmacological interventions, caloric restriction and exercise were also shown to induce autophagy and to contribute to protection against diabetes in mice[193], and the effect of alternate-day fasting on human metabolism and autophagy is currently being tested in a phase I clinical trial (NCT02673515).

In most cancer therapies, inhibition of autophagy is combined with other therapeutic interventions, including radiation, chemotherapies and targeted agents, including DNA-damaging agents, histone deacetylase (HDAC) inhibitors, proteasome inhibitors, mitotic inhibitors, antiandrogens and kinase inhibitors[194,195]. Unfortunately, although some reports indicated that autophagy inhibition might increase chemosensitization and may overcome acquired resistance to other anticancer agents, multiple clinical trials testing the efficacy of autophagy inhibition in cancer patients have been largely disappointing and have served to underline the vast complexity of networks in which autophagy is embedded. As the crosstalk between autophagy pathways and other cellular systems is usually reciprocal, modulation of autophagy activity not only affects the efficacy of protein aggregate clearance or the elimination of damaged organelles but also likely impacts the magnitude or duration of other fundamental cellular pathways, such as NF-kB signalling, cell migration or cell death programmes. Finally, autophagy regulation in the tumour stroma and in tumour cells may differ: whereas inhibition of autophagy in tumour cells might trigger cell death, it could at the same time promote the release of survival factors in the tumour stroma (particularly fibroblasts and tumour-infiltrating immune cells)[196], thereby precluding a positive therapeutic outcome. Thus, decisions as to whether autophagy activity in a certain disease condition, particularly in cancer, should be upregulated or downregulated are not trivial and require careful evaluation of tumour type, stage and microenvironment.

Whereas targeting of autophagy in cancer turns out to be a delicate task, the consensus with respect to neurodegeneration is that autophagy activation protects against several neurodegenerative disorders[197,198]. Nevertheless, also in the context of neurodegeneration, therapeutic targeting of autophagy is challenging, and optimal dosage of inhibitors and timing of inhibition are crucial parameters for maximal therapeutic efficiency[199]. Indeed, as with cancer therapies, the specific targeting of autophagy without affecting other cellular processes is currently one of the major challenges in the field.

Lastly, several FDA-approved drugs with proautophagy activity (see TABLE 2) have been shown to limit infection and inflammation in mouse models[200,201], and the peptide TAT-Beclin1 improved the outcome of chikungunya and West Nile virus infections in mice[180]. However, evaluations of human patients through clinical trials are still unavailable.

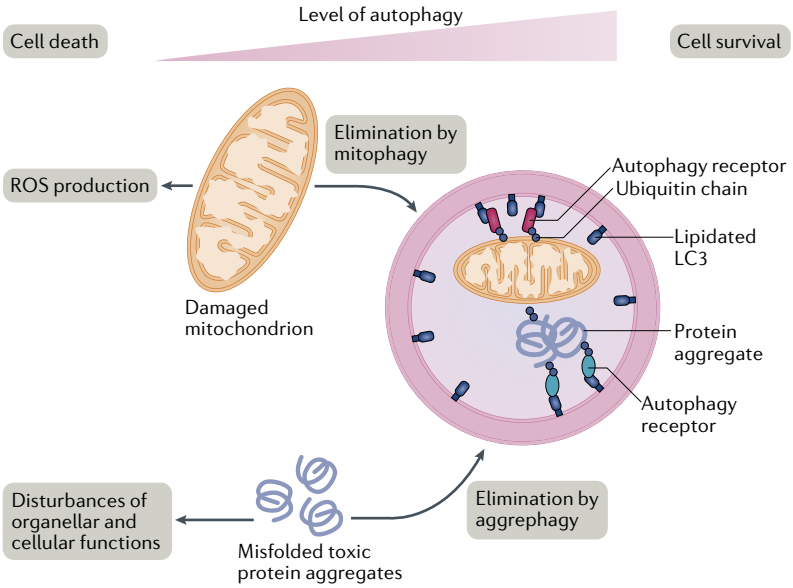


Fig. 3 | **Autophagy in neurodegeneration.** Autophagy protects against neurodegeneration by eliminating two hallmarks of neurodegenerative diseases: defective mitochondria and toxic protein aggregates. Damaged mitochondria produce high levels of reactive oxygen species (ROS) that pose a threat to many cellular components, including proteins, lipids and DNA. Protein aggregates, which are exacerbated by ROS-mediated oxidative damage, compromise the function of organelles and are considered particularly toxic for neurons. Reduced autophagy activity (age-related, pharmacologically or genetically caused) therefore increases the risk of neurodegenerative diseases. Accordingly, pharmacological stimulation of autophagy could be an effective therapeutic strategy against neurodegenerative diseases. LC3, light chain 3.

marker in several neurodegenerative diseases[130]. p62 participates in both aggregate formation by targeting misfolded aggregated proteins to the aggresome (a single intracellular location in which misfolded proteins are sequestered to minimize potential cytotoxic effects)[131] and the subsequent sequestration of aggresomes by the phagophore[132,133].

Besides potentially toxic protein aggregates, dysfunctional mitochondria have also been identified as a major cause of neurodegeneration. They pose a considerable threat to cells because they elevate cellular ROS levels that might in turn damage both the proteome and the genome (FIG. 3). Therefore, to maintain mitochondrial homeostasis, cells separate damaged mitochondria from the mitochondrial network and remove them by selective autophagy (termed mitophagy) (see BOX 1). Mitophagy is predominantly regulated by the PINK1 (PTEN-induced kinase 1)–Parkin pathway, which is activated upon depolarization of the mitochondrial membrane potential and involves a sophisticated interplay of PINK1-mediated phosphorylation and Parkin-mediated ubiquitylation events on the outer mitochondrial membrane, resulting in recruitment of autophagic machinery and the selective sequestration of ubiquitylated mitochondria within autophagosomes[134–136]. Mutations in Parkin and PINK1 are strongly associated with early-onset Parkinson disease[137]. In addition to the PINK1–Parkin pathway, NIP-like protein X (NIX; also known as BNIP3L) can serve as an alternative mediator of mitophagy in neurons. Recent evidence suggests that NIX overexpression restores mitophagy and mitochondrial function in Parkin-deficient or PINK1-deficient cell lines derived from patients with Parkinson disease[138].

A number of studies have also uncovered a link between TANK-binding kinase 1 (TBK1) and ALS[139–142] as well as Parkinson disease[143]. TBK1 belongs to the IKK family of kinases involved in innate immunity signalling pathways, but it also has a major role in autophagy and mitophagy. Through inducible phosphorylation of ubiquitylated cargo receptors (which, in addition to p62, include OPTN (optineurin) and NDP52 (calcium-binding and coiled-coil domain-containing protein 2; also known as CALCOCO2)), TBK1 enhances their affinity to Ub on the cargo, LC3 on the autophagosome or both, thereby contributing to efficient recruitment of ubiquitylated cargo to autophagosomes[136,144,145].

Taken together, in contrast to cancer where the function of autophagy is highly context dependent, activation of autophagy is clearly beneficial for counteracting the mechanisms involved in neurodegenerative diseases, and currently, autophagy induction is being explored as a strategy for neurodegenerative disease prevention as well as for the treatment of advanced-stage disease (BOX 2; TABLE 2).

Autophagy in infection, inflammation and immunity. Autophagy has been implicated in a variety of immune functions, such as removal of intracellular bacteria[146–148], inflammatory cytokine secretion[149], control of inflammation, antigen presentation[150,151] and lymphocyte development[152]. The importance of autophagy for these functions is highlighted by the susceptibility of autophagy-deficient animals to infection and the implication of autophagy defects in autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis, diabetes and multiple sclerosis[153,154]. Autophagy also acts within tumour cells to modulate recruitment of and interaction with components of both the adaptive and innate immune systems[155].

Mechanistically, autophagy extensively crosstalks with inflammatory signalling cascades, including multiple context-specific and bidirectional interactions with the IKK–NF-κB pathway[156,157]. NF-κB can induce autophagy by transactivating Beclin 1 (REF.[158]). Moreover, in the presence of various physiological and pharmacological stress signals, the IKK complex can induce autophagy[156]. Yet, the NF-κB pathway may also inhibit autophagy, for example, in the context of tumour necrosis factor-α (TNFα)-induced cell death[159] and in macrophages infected by *Escherichia coli*[160]. Reciprocally, in several cell lines, TNFα-driven NF-κB activation requires a functional autophagy pathway[161]. Autophagy can also suppress NF-kB signalling by the autophagic degradation of active IKKβ, mediated either by KEAP1 (Kelch-like ECH-associated protein 1)[162] or by the E3 ubiquitin-protein ligase RO52 (also known as TRIM21)[163].

In a process called xenophagy, autophagy also directly targets and eliminates invading bacteria such

as *Mycobacteria*[164], *Listeria*, *Salmonella*, *Legionella*, *Shigella*, *Listeria* and group A streptococcus (see REF.[147] for review). As soon as these bacteria enter the cytosol, they are labelled with various types of Ub chain and galectin and sequestered by autophagic membranes involving the same autophagic receptors (p62, NDP52 and others) that also engage endogenous selective autophagy substrates. The various types of Ub modification to the bacterial coat transform bacterial surfaces into signalling platforms. For example, linear Ub chains not only attract the autophagic machinery but also locally activate NF-kB signalling for a maximal antibacterial response[165,166]. Notably, many pathogens have evolved strategies to escape the autophagic machinery by secreting factors that interfere with autophagosome maturation[167], blocking fusion of the autophagosome with the lysosome[168] and competing with host autophagy receptors for binding to LC3 (REF.[169]) and so on. Some bacteria even manipulate autophagy for their own benefit and are able to replicate effectively within autophagosome-like vesicles[170]. Nevertheless, autophagy activation is considered a valid therapeutic strategy to combat bacterial infections (BOX 2; TABLE 2).

Conclusions and perspectives

Autophagy currently enjoys star status in cell biology. Initially described as a nonselective mechanism for intracellular garbage disposal and recycling, autophagy has emerged as a highly selective and powerful programme that is critically implicated in various fundamental cellular processes. The cytoprotective properties of autophagy have raised the particular interest of scientists and clinicians. However, initial excitement about therapeutic targeting of autophagy in cancer and other diseases has given way to a sober, more realistic view of autophagy as a druggable process (BOX 2). The uncovering of diverse and sometimes unexpected challenges demands an unbiased re-evaluation of therapeutic strategies. The major task seems to be mapping the context-dependent functional networks in which autophagy is embedded. The challenge is therefore to modulate autophagy without adversely affecting other cellular processes. The fact that autophagy crosstalks with virtually every other cellular system may give some idea of the vast scope of this task, yet it also indicates the enormous potential for beneficial modulation that we can expect to find while exploring this fundamental pathway.

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