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Autophagy: Molecular mechanisms and roles in health and disease

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Abstract

Autophagy is the evolutionarily conserved process by which eukaryotic cells deliver cytoplasmic constituents to the lysosome for degradation and recycling. Once regarded as a nonselective response to starvation, it is now understood as a tightly regulated and frequently selective quality-control system that shapes proteostasis, organelle turnover, metabolism, immunity, and lifespan. This narrative review synthesises mechanistic and disease-oriented literature, emphasising work from 2019 onward while retaining the landmark genetic and cell-biological studies on which the field rests. We first dissect autophagosome biogenesis as a stepwise reaction—initiation by the ULK1 complex, nucleation by the class III phosphatidylinositol 3-kinase complex, membrane expansion driven by lipid transfer and ubiquitin-like conjugation of ATG8 proteins, closure, and fusion with the lysosome—because each step has become a candidate node for pharmacological intervention. We then examine cargo selectivity, the property that most clearly distinguishes contemporary understanding from the classical bulk-degradation model, and compare macroautophagy with microautophagy and chaperone-mediated autophagy. Against this mechanistic backdrop we appraise the evidence linking autophagy to neurodegeneration, cancer, infection and inflammation, metabolic and cardiovascular disease, and ageing. A central and recurring theme is context dependence: the same pathway that suppresses tumour initiation can sustain established tumours, and the therapeutic imperative is accordingly to induce autophagy in some settings and inhibit it in others. We critically compare discordant clinical results with autophagy inhibitors, notably the divergent outcomes of hydroxychloroquine trials in pancreatic cancer, and argue that much of this inconsistency reflects unresolved problems of drug specificity, flux measurement, and patient selection rather than a failure of the underlying biology. We conclude by identifying priority research gaps, foremost among them the need for selective, mechanism-based modulators and validated biomarkers of autophagic flux in humans.

Keywords: *Autophagy, Autophagosome, Lysosome, Mitophagy, Selective autophagy, Chaperone-mediated autophagy, Neurodegeneration, Hydroxychloroquine*

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1. Introduction

1.1. Background

The conceptual origins of autophagy lie in the discovery of the lysosome and the recognition that cells possess

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a compartment dedicated to intracellular digestion (de Duve and Wattiaux, 1966). For several decades the process remained morphologically defined and mechanistically opaque. This changed with the demonstration that autophagy could be observed genetically in *Saccharomyces cerevisiae* and, decisively, with the isolation of autophagy-defective yeast mutants that identified the genes now designated *ATG* (Takeshige *et al.*, 1992; Tsukada and Ohsumi, 1993; Klionsky *et al.*, 2003). The subsequent discovery that two ubiquitin-like conjugation systems—one producing the ATG12–ATG5 conjugate, the other lipidating ATG8/LC3 with phosphatidylethanolamine—are essential for autophagosome formation converted a descriptive phenomenon into a tractable biochemical pathway (Mizushima *et al.*, 1998; Ichimura *et al.*, 2000; Kabeya *et al.*, 2000). This genetic foundation remains the basis of nearly all subsequent work (Mizushima, 2018).

1.2. Importance of the topic

Autophagy matters because it is not merely a starvation response but a constitutive housekeeping process. Basal autophagy continuously removes damaged organelles, misfolded proteins, and protein aggregates that no other pathway can clear, since the proteasome cannot accommodate bulky substrates or entire organelles (Mizushima *et al.*, 2011; Dikic and Elazar, 2018). Its disruption therefore has consequences across essentially every organ system, and the causal genetic evidence in mice—where neural-specific deletion of core *Atg* genes alone suffices to cause neurodegeneration—places autophagy upstream of a broad class of pathologies rather than merely correlated with them (Hara *et al.*, 2006; Komatsu *et al.*, 2006).

1.3. Global relevance

The pathway's reach is global in both a biological and an epidemiological sense. Autophagy genes influence susceptibility to Crohn's disease, a condition of rising incidence worldwide, and contribute to host defence against *Mycobacterium tuberculosis*, which remains among the leading infectious causes of death (Hampe *et al.*, 2007; Gutierrez *et al.*, 2004). Autophagic decline is a proposed contributor to ageing and to the age-associated neurodegenerative diseases whose prevalence rises as populations age (Hansen *et al.*, 2018; Aman *et al.*, 2021). Because autophagy is nutrient-responsive, it also connects directly to metabolic disease and to dietary and exercise interventions of broad public-health relevance (Singh *et al.*, 2009; He *et al.*, 2012).

1.4. Current challenges

Three obstacles dominate. First, autophagy is a *flux*, yet most assays capture only a static snapshot; an accumulation of autophagosomes is equally consistent with increased formation or with blocked degradation, and this ambiguity has generated a substantial body of misinterpreted literature (Klionsky *et al.*, 2021; Mizushima *et al.*, 2010). Second, the pharmacological tools in clinical use are non-selective: chloroquine and hydroxychloroquine perturb lysosomal function broadly and exert well-documented autophagy-independent effects, so clinical outcomes are difficult to attribute to autophagy inhibition as such (Mauthe *et al.*, 2018; Galluzzi *et al.*, 2017). Third, the role of autophagy is context dependent to a degree that frustrates simple therapeutic logic, most conspicuously in cancer (Levy *et al.*, 2017).

1.5. Aim and objectives

This review aims to provide an integrative and critical account of autophagy for a biomedical readership. Our objectives are: (i) to explain the molecular machinery of autophagosome biogenesis and its regulation; (ii) to define cargo selectivity and compare the three principal autophagic routes; (iii) to appraise, rather than merely catalogue, the evidence implicating autophagy in major disease categories; (iv) to compare conflicting clinical findings and diagnose their causes; and (v) to delineate research gaps and realistic therapeutic directions.

2. Molecular Machinery of Autophagosome Biogenesis

2.1. Initiation: the ULK1 complex as a signalling hub

Autophagy is initiated by the ULK1/2 kinase complex, comprising ULK1 or ULK2 together with ATG13, FIP200 (RB1CC1), and ATG101. This complex is the principal node at which nutrient and energy status are transduced into membrane events. Under nutrient-replete conditions mTORC1 associates with the complex and inhibitorily phosphorylates ULK1 and ATG13; starvation dissociates mTORC1 and relieves this inhibition (Hosokawa *et al.*, 2009; Ganley *et al.*, 2009; Jung *et al.*, 2009). Conversely, energy stress activates AMPK, which

both inhibits mTORC1 and directly phosphorylates ULK1 at activating sites, producing a coherent bidirectional switch (Kim *et al.*, 2011; Egan *et al.*, 2011). That two opposing kinases converge on a single substrate is not redundancy but design: it allows autophagy to respond to the *ratio* of nutrient to energy availability rather than to either signal alone. Active ULK1 then phosphorylates downstream components, including Beclin-1, coupling initiation to nucleation (Russell *et al.*, 2013; Zachari and Ganley, 2017; Hurley and Young, 2017).

2.2. *Nucleation: PI3P generation and the omegasome*

The class III phosphatidylinositol 3-kinase complex I (PI3KC3-C1)—VPS34, VPS15, Beclin-1, and ATG14—generates phosphatidylinositol 3-phosphate (PI3P) at a specialised endoplasmic reticulum subdomain, producing the PI3P-enriched "omegasome" from which the phagophore emerges (Itakura *et al.*, 2008; Axe *et al.*, 2008). Complex composition dictates function: ATG14 directs the complex toward autophagosome formation, whereas UVRAG- and Rubicon-containing complexes act at later steps, Rubicon negatively (Zhong *et al.*, 2009; Matsunaga *et al.*, 2009). PI3P is not merely a lipid mark but a recruitment platform, binding WIPI2 and DFCP1 and thereby physically coupling nucleation to the downstream conjugation machinery (Polson *et al.*, 2010; Dooley *et al.*, 2014). Ultrastructural and imaging studies localise autophagosome formation to ER subdomains and to ER–mitochondria contact sites, although the relative contribution of different membrane sources remains genuinely unsettled (Hayashi-Nishino *et al.*, 2009; Hamasaki *et al.*, 2013).

2.3. *Expansion: Lipid transfer and ubiquitin-like conjugation*

Phagophore expansion requires both a lipid supply and a membrane identity. A major recent advance is the demonstration that ATG2 is a lipid-transfer protein bridging the ER and the growing phagophore, providing a direct, vesicle-independent conduit for phospholipids—an elegant resolution of the long-standing question of how the isolation membrane grows (Osawa *et al.*, 2019; Valverde *et al.*, 2019; Maeda *et al.*, 2019). Membrane

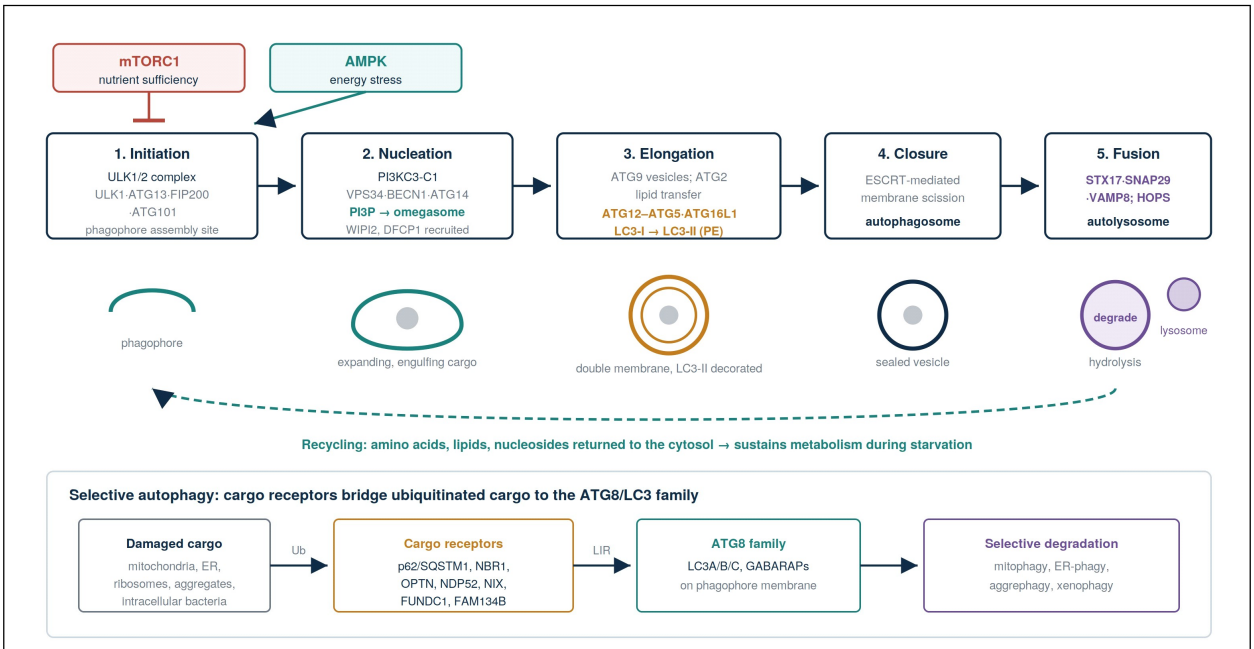

Figure 1: Molecular machinery of autophagosome biogenesis and selective cargo capture. Autophagy proceeds through five stages. Initiation by the ULK1 complex integrates opposing inputs from mTORC1 (inhibitory, signalling nutrient sufficiency) and AMPK (activating, signalling energy stress). Nucleation by PI3KC3-C1 generates PI3P and the ER-derived omegasome, recruiting WIPI2 and DFCP1. Elongation combines ATG2-mediated lipid transfer with ubiquitin-like conjugation, in which the ATG12–ATG5•ATG16L1 complex lipidates LC3-I to membrane-bound LC3-II. ESCRT-dependent closure yields the sealed autophagosome, which fuses with the lysosome via STX17•SNAP29•VAMP8 and the HOPS complex. Degradation returns amino acids and lipids to the cytosol. The lower panel depicts selective autophagy: cargo receptors bind ubiquitinated substrates and, through LIR motifs, the ATG8 family, conferring specificity for mitochondria, ER, ribosomes, aggregates, and intracellular bacteria

Table 1: Summary of selected landmark studies in autophagy research

Study / contribution	Year	Key advance	Significance
Tsukada and Ohsumi	1993	Autophagy-yeast mutants defective isolated	Founded the genetics of autophagy (*ATG* genes)
Liang *et al.*	1999	Beclin-1 identified as a tumour suppressor	First link between autophagy and cancer
Mizushima *et al.*	1998	ATG12–ATG5 conjugation system	First ubiquitin-like system in autophagy
Kabeya *et al.*	2000	LC3 as an autophagosome marker	Enabled routine detection; still the standard assay
Kuma *et al.*	2004	*Atg5*-null neonatal lethality	Proved physiological necessity in vivo
Hara *et al.*; Komatsu *et al.*	2006	CNS-specific *Atg* deletion causes neurodegeneration	Basal is intrinsically autophagy neuroprotective
Narendra *et al.*	2008	Parkin recruited to damaged mitochondria	Defined the mitophagy mechanism
Settembre *et al.*	2011	TFEB links autophagy to lysosomal biogenesis	Established transcriptional control of the system
Osawa *et al.*; Valverde *et al.*	2019	ATG2 is a lipid-transfer protein	Explained how the phagophore expands
Collier *et al.*	2021	Human *ATG7* deficiency characterised	Core autophagy defect causes human disease

identity is conferred by the two ubiquitin-like systems: the ATG12–ATG5•ATG16L1 complex acts as an E3-like ligase that conjugates ATG8-family proteins (LC3s and GABARAPs) to phosphatidylethanolamine, converting soluble LC3-I into membrane-bound LC3-II (Hanada *et al.*, 2007). This lipidation reaction underpins virtually every autophagy assay in use, which is precisely why its interpretation demands the care discussed in Section 9 (Melia *et al.*, 2020).

2.4. Closure and fusion with the lysosome

Sealing of the phagophore is topologically distinct from expansion and is mediated by ESCRT machinery. Fusion of the completed autophagosome with the lysosome then requires the autophagosomal SNARE syntaxin 17 (STX17), which partners with SNAP29 and the lysosomal VAMP8, assisted by tethering factors including the HOPS complex and ATG14 (Itakura *et al.*, 2012; Takats *et al.*, 2013; Diao *et al.*, 2015). Degradation is followed by autophagic lysosome reformation, an mTOR-dependent process that regenerates lysosomes and prevents their exhaustion during prolonged autophagy—an underappreciated step whose failure would be expected to limit sustained flux (Yu *et al.*, 2010). These stages are summarised in Figure 1.

2.5. Transcriptional control: The lysosome as a signalling platform

Autophagy is coordinated with lysosomal biogenesis through transcription factor EB (TFEB), which drives a coherent gene network encompassing both autophagic and lysosomal genes (Sardiello *et al.*, 2009; Settembre *et al.*, 2011). TFEB is itself governed at the lysosomal surface: mTORC1 phosphorylates and sequesters it in the cytosol, whereas lysosomal calcium release activates calcineurin, which dephosphorylates TFEB and permits nuclear translocation (Settembre *et al.*, 2012; Medina *et al.*, 2015). This architecture reframes the lysosome from a terminal degradative compartment into a nutrient-sensing signalling hub, and makes TFEB an attractive—if unavoidably pleiotropic—therapeutic target (Ballabio and Bonifacino, 2020).

3. Cargo selectivity and the forms of autophagy

3.1. Selective autophagy and cargo receptors

The most consequential conceptual shift of the past two decades is the recognition that autophagy is frequently

selective. Selectivity is achieved by cargo receptors—p62/SQSTM1, NBR1, optineurin, NDP52, and others—which simultaneously bind ubiquitinated cargo and, through LC3-interacting region (LIR) motifs, the ATG8-family proteins on the phagophore, physically tethering substrate to the nascent membrane (Bjorkoy *et al.*, 2005; Pankiv *et al.*, 2007; Johansen and Lamark, 2020). Distinct receptors confer distinct specificities, giving rise to mitophagy, ER-phagy, aggrephagy, ribophagy, and xenophagy (Gatica *et al.*, 2018; Kirkin and Rogov, 2019; Vargas *et al.*, 2023). Receptor-driven cargo recognition can itself nucleate autophagosome formation, inverting the classical sequence in which the membrane is thought to form first. Well-characterised examples include FAM134B and RTN3 in ER-phagy and NUFIP1 in ribophagy (Khaminets *et al.*, 2015; Grumati *et al.*, 2017; Wyant *et al.*, 2018).

3.2. Mitophagy as the paradigm of selective organelle turnover

Mitophagy is the best-understood selective pathway and is mechanistically instructive. On healthy mitochondria the kinase PINK1 is imported and degraded; upon depolarisation, import fails and PINK1 accumulates on the outer membrane, where it phosphorylates both ubiquitin and the E3 ligase Parkin (Narendra *et al.*, 2008; Narendra *et al.*, 2010; Matsuda *et al.*, 2010). Phospho-ubiquitin both activates Parkin allosterically and serves as its substrate, generating a feed-forward amplification loop that decorates the damaged organelle with ubiquitin chains (Kane *et al.*, 2014; Koyano *et al.*, 2014). These chains recruit optineurin and NDP52, which engage the autophagy machinery (Lazarou *et al.*, 2015). The elegance of this circuit—damage sensing encoded in the failure of protein import—explains its prominence as a model (Youle and Narendra, 2011; Pickles *et al.*, 2018). Receptor-mediated, Parkin-independent routes also operate, notably FUNDC1 under hypoxia (Liu *et al.*, 2012; Palikaras *et al.*, 2018). Critically, however, in vivo reporter studies indicate that substantial basal mitophagy proceeds independently of PINK1 in several high-demand tissues. This tempers extrapolation from depolarising agents in cultured cells to physiological turnover, and may partly explain the comparatively mild phenotypes of *Pink1*-null mice—a caution against assuming that a mechanism dominant in vitro is dominant in vivo (McWilliams *et al.*, 2018; Sun *et al.*, 2015).

3.3. Microautophagy and chaperone-mediated autophagy

Macroautophagy is not the only route to the lysosome. In microautophagy, the lysosomal or late endosomal

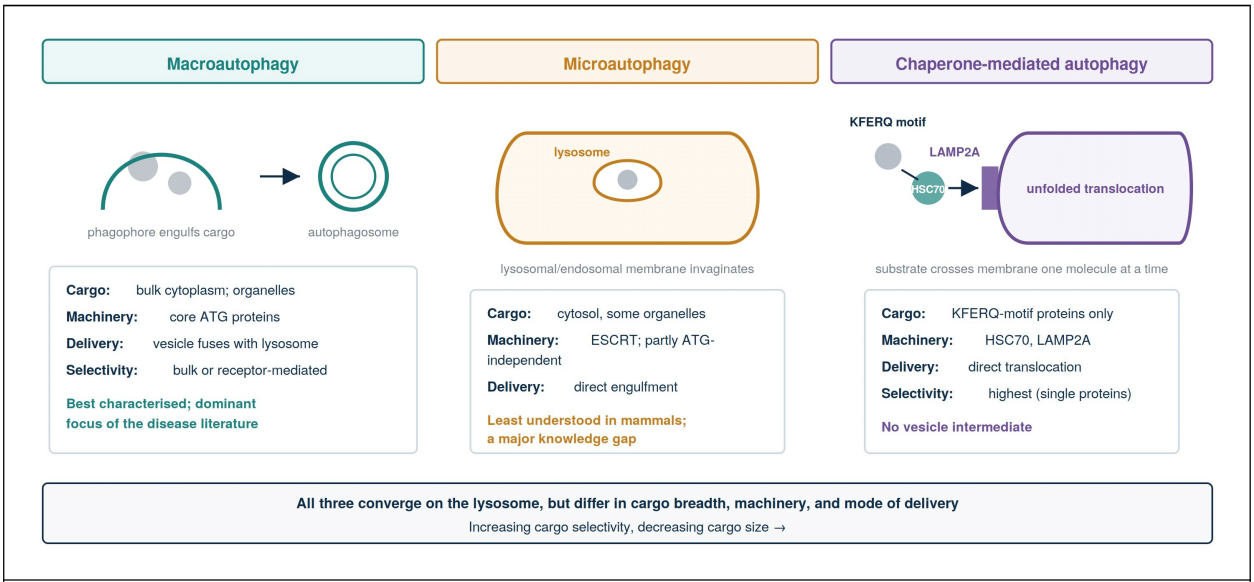

Figure 2: Comparative pathways of macroautophagy, microautophagy, and chaperone-mediated autophagy. All three converge on the lysosome but differ fundamentally in mechanism. Macroautophagy sequesters cargo within a double-membrane autophagosome that subsequently fuses with the lysosome, and may be bulk or receptor-mediated. Microautophagy proceeds by direct invagination of the lysosomal or endosomal membrane, is partly ESCRT-dependent, and remains the least characterised route in mammals. Chaperone-mediated autophagy requires no vesicle intermediate: HSC70 recognises a KFERQ-like motif and delivers the unfolded substrate through LAMP2A one molecule at a time, achieving the highest selectivity but restricted to soluble proteins

membrane directly invaginates to engulf cytosolic material, a process at least partly ESCRT-dependent and substantially less well characterised in mammals than in yeast (Sahu *et al.*, 2011; Schuck, 2020). Chaperone-Mediated Autophagy (CMA) is mechanistically distinct: substrates bearing a KFERQ-like pentapeptide motif are recognised by HSC70 and translocated one molecule at a time across the lysosomal membrane through LAMP2A, with no vesicular intermediate (Cuervo and Dice, 1996; Kaushik and Cuervo, 2018). CMA therefore attains the highest attainable selectivity—individual proteins—but cannot handle organelles. Its decline with age carries functional consequences: hepatic CMA loss causes metabolic dysregulation, and neuronal CMA failure destabilises the proteome in ways directly relevant to neurodegeneration (Schneider *et al.*, 2014; Bourdenx *et al.*, 2021). The three routes are compared in Figure 2 and Table 2.

Table 2: Comparison of the three principal autophagic pathways

Feature	Macroautophagy	Microautophagy	Chaperone-mediated autophagy
Cargo	Bulk cytoplasm, organelles, pathogens	Cytosolic components; some organelles	Soluble proteins with a KFERQ-like motif only
Vesicle intermediate	Yes (autophagosome)	No (direct invagination)	No (direct translocation)
Core machinery	ATG ATG8 proteins; lipidation (Nakatogawa, 2020)	ESCRT; ATG-partly independent (Schuck, 2020)	HSC70 and LAMP2A (Cuervo and Dice, 1996)
Selectivity	Bulk or receptor-mediated (Vargas *et al.*, 2023)	Bulk or selective	Highest (individual proteins) (Kaushik and Cuervo, 2018)
Principal disease relevance	Broadest; the dominates literature	Least in characterized mammals	Ageing, metabolic, Neurodegenerative (Schneider *et al.*, 2014; Bourdenx *et al.*, 2021)

4. Autophagy in health: Physiological functions

The physiological necessity of autophagy is demonstrated most clearly by genetics. Neonatal mice lacking *Atg5* survive birth but die during the early postnatal starvation period, establishing that autophagy-derived amino acids sustain metabolism in the interval before suckling is established (Kuma *et al.*, 2004). Beyond bulk nutrient supply, autophagy governs lipid mobilisation from lipid droplets, pancreatic beta-cell homeostasis, cardiomyocyte maintenance, and exercise-induced metabolic adaptation in skeletal muscle (Ebato *et al.*, 2008; Nakai *et al.*, 2007). In immunity it contributes both to the direct elimination of intracellular pathogens and to the restraint of inflammatory signalling—functions that are separable and occasionally opposed (Levine *et al.*, 2011; Deretic *et al.*, 2013). This functional breadth is the basis for regarding autophagy as a general homeostatic buffer, and it is also precisely why systemic pharmacological manipulation carries a broad liability for off-target physiological consequences.

5. Autophagy in Disease

5.1. Neurodegeneration: predominantly a loss-of-function relationship

Neurons are post-mitotic and cannot dilute damaged components by division, rendering them uniquely dependent on autophagic quality control. Conditional deletion of *Atg5* or *Atg7* in the mouse central nervous system causes progressive neurodegeneration with ubiquitinated inclusions even in the absence of any disease-associated aggregate-prone protein—evidence that basal autophagy is itself neuroprotective rather than merely a response to aggregates. Consistent with this, mTOR inhibition enhances clearance of mutant huntingtin and reduces toxicity in Huntington's disease models (Ravikumar *et al.*, 2004). In Alzheimer's disease the autophagic–lysosomal system is compromised at the level of lysosomal proteolysis, with presenilin-1 mutations impairing lysosomal acidification (Lee *et al.*, 2010; Nixon, 2013). The decisive human evidence, however, has come from genetics: biallelic *ATG7* variants cause a complex neurodevelopmental disorder with brain, muscle, and endocrine involvement, establishing that core autophagy deficiency produces disease in humans and not only in mice (Collier *et al.*, 2021). Other congenital disorders of autophagy—*EPG5* in Vici syndrome and *WDR45* in beta-propeller protein-associated neurodegeneration—reinforce the conclusion (Cullup *et al.*, 2013; Saitsu *et al.*, 2013; Ebrahimi-Fakhari *et al.*, 2016). Here the therapeutic logic is at least unambiguous in direction (induce autophagy), even if difficult in execution (Menzies *et al.*, 2017; Fleming *et al.*, 2022).

5.2. Cancer: A genuinely context-dependent relationship

Cancer is where simple narratives fail. Early evidence supported tumour suppression: *BECN1* is monoallelically deleted in a substantial fraction of human breast and ovarian cancers, *Becn1* heterozygous mice are tumour-prone, and autophagy-deficient mice develop benign liver tumours (Liang *et al.*, 1999; Qu *et al.*, 2003; Yue *et al.*, 2003; Takamura *et al.*, 2011). The mechanistic interpretation is that autophagy limits oxidative stress, genomic instability, and inflammation, thereby restraining tumour *initiation*. Yet an opposing body of work shows that established tumours—particularly those driven by RAS mutations—are autophagy-dependent, exploiting autophagic recycling to sustain mitochondrial metabolism under nutrient stress (Guo *et al.*, 2011; Yang *et al.*, 2011; Karsli-Uzunbas *et al.*, 2014). Autophagy further supports tumours non-cell-autonomously, with host autophagy supplying circulating arginine, and it promotes immune evasion by selectively degrading MHC-I in pancreatic cancer (Poillet-Perez *et al.*, 2018; Yamamoto *et al.*, 2020). These findings are not truly contradictory once disease stage is taken into account: autophagy suppresses initiation but supports maintenance (Kimmelman and White, 2017; Amaravadi *et al.*, 2019). The clinical corollary—that autophagy inhibition should benefit established tumours—is the hypothesis currently under test, and Section 7 argues that it has not yet been tested fairly.

5.3. Infection, inflammation, and immunity

Autophagy captures cytosolic bacteria (xenophagy), a defence demonstrated for *M. tuberculosis* and group A *Streptococcus*, and one that successful pathogens such as *Shigella* actively evade—the evasion itself being strong evidence of the pathway's selective pressure on microbial evolution (Nakagawa *et al.*, 2004; Ogawa *et al.*, 2005). Recognition is receptor-mediated, with NDP52 and optineurin—the latter activated by TBK1 phosphorylation—targeting ubiquitin-coated bacteria (Thurston *et al.*, 2009; Wild *et al.*, 2011). Separately, autophagy restrains inflammation: loss of ATG16L1 augments endotoxin-induced IL-1beta production and disrupts Paneth cell function, providing a mechanistic bridge to the *ATG16L1* risk variant identified in Crohn's disease by genome-wide association (Saitoh *et al.*, 2008; Cadwell *et al.*, 2008; Rioux *et al.*, 2007). That a common coding variant of modest effect converges on the same pathway as rare, severe monogenic disorders illustrates the allelic spectrum through which autophagy contributes to human disease (Deretic, 2021).

5.4. Metabolic, cardiovascular, and ageing-related disease

Autophagy declines with age across model organisms, and interventions that maintain it extend lifespan: *Atg5* overexpression, disruption of the inhibitory Beclin-1–BCL2 interaction, and administration of the polyamine spermidine each prolong murine lifespan or confer cardioprotection in an autophagy-dependent manner (Pyo *et al.*, 2013; Fernandez *et al.*, 2018; Eisenberg *et al.*, 2009; Eisenberg *et al.*, 2016; Madeo *et al.*, 2018). The dependence on autophagy in these experiments is what elevates the pathway from marker to mediator of longevity (Rubinsztein *et al.*, 2011; Lopez-Otin *et al.*, 2023). In the heart, however, the relationship is again conditional: basal autophagy is protective, whereas excessive autophagic activity in response to severe haemodynamic stress has been reported as maladaptive (Zhu *et al.*, 2007). These two findings are often presented as a contradiction; the more parsimonious reading is that they differ in the magnitude and duration of the stimulus, and that the dose–response relationship, rather than autophagy per se, determines the outcome. Such stimulus-dependent inversion recurs throughout the field and is a standing caution against treating "more autophagy" as uniformly beneficial (Levine and Kroemer, 2019).

6. Therapeutic modulation and clinical evidence

Therapeutic strategy divides cleanly by direction. *Induction* is sought in neurodegeneration and ageing, using mTOR inhibitors, spermidine, and TFEB activators; the evidence base here is largely preclinical, and the principal liabilities are the pleiotropy of mTOR inhibition and its attendant immunosuppression (Rubinsztein *et al.*, 2012). *Inhibition* is sought in established cancers, and here clinical data exist—but they are discordant, and the discordance is instructive rather than merely disappointing.

Chloroquine and hydroxychloroquine (HCQ), which impair lysosomal acidification and autophagosome–lysosome fusion, remain the only autophagy inhibitors in widespread clinical use. Early phase I combination trials established tolerability and hinted at activity (Rangwala *et al.*, 2014). The pivotal comparison, however, lies between two randomised phase 2 studies in pancreatic cancer that reached opposite conclusions. In

metastatic disease, adding HCQ to gemcitabine and nab-paclitaxel did *not* improve overall survival at 12 months, the primary endpoint, leading the investigators to advise against routine use in that setting (Karasic *et al.*, 2019). In the preoperative setting, by contrast, the same drug combination improved histopathologic response relative to chemotherapy alone, accompanied by pharmacodynamic evidence—accumulation of p62/SQSTM1—that autophagy had indeed been inhibited (Zeh *et al.*, 2020). Several explanations merit weighing rather than a premature verdict. The trials differed in disease stage, in endpoint (overall survival versus pathological response), and in the extent to which target engagement was verified; HCQ achieves incomplete and inter-individually variable lysosomal inhibition; and neither trial selected patients using any biomarker of autophagy dependence. The most defensible reading is therefore not that the biology is wrong, but that autophagy inhibition is pharmacodynamically achievable and biologically active, while HCQ is too blunt an instrument and unselected populations too heterogeneous for a survival benefit to emerge.

Preclinical work points toward a more rational path. Inhibition of the RAF–MEK–ERK axis induces a compensatory, protective autophagic response, and combining ERK or MEK inhibition with autophagy blockade produces synergistic tumour regression in RAS-driven models—a mechanism-based rationale for combination therapy and for selecting patients by driver genotype rather than by tumour histology alone (Kinsey *et al.*, 2019; Bryant *et al.*, 2019). Whether more potent and specific inhibitors, for example of ULK1 or VPS34, will widen the therapeutic index without unacceptable toxicity remains the central open question.

7. Recent innovations

Four developments stand out. First, the identification of ATG2 as a lipid-transfer protein resolved the mechanism of phagophore expansion and exposed a previously inaccessible druggable step. Second, the systematisation of selective autophagy—receptor–cargo pairings for mitochondria, ER, ribosomes, and pathogens—raises the realistic prospect of cargo-selective therapeutics that spare bulk autophagy and thereby avoid the toxicity inherent in global modulation. Third, in vivo flux reporters have begun to displace static, culture-based readouts and have already overturned assumptions, most notably by revealing PINK1-independent basal mitophagy in living tissue (Kimura *et al.*, 2007). Fourth, human genetics has arrived: the description of *ATG7* deficiency furnished the first proof that a core autophagy gene defect causes a defined human disease, converting inference from mouse models into direct human evidence (Mizushima and Levine, 2020).

8. Current challenges

The *measurement problem* is foundational. Because autophagy is a flux, LC3-II abundance alone is uninterpretable without lysosomal inhibitors or tandem reporters; the field's consensus guidelines exist precisely because this error remains so common. The *specificity problem* follows directly: CQ and HCQ act on the lysosome rather than on autophagy selectively, so clinical outcomes cannot be attributed cleanly to autophagy inhibition. The *context problem* is biological rather than technical: the direction of benefit depends on tumour stage, tissue, and stimulus intensity, so no single therapeutic posture can be correct everywhere. Finally, there is a *biomarker gap*: no validated, clinically deployable readout of autophagic flux in human

Table 3: Advantages versus limitations of the current evidence base

Dimension	Strengths	Limitations
Mechanistic understanding	Pathway steps defined biochemically and structurally	Membrane sources and closure incompletely resolved
Genetic evidence	Causal mouse models; now human *ATG7* disease	Congenital autophagy disorders remain rare and recently described
Measurement	Tandem reporters and in vivo flux tools now available	Static LC3-II assays still widely misinterpreted
Pharmacology	CQ/HCQ clinically available, inexpensive, tolerable	Non-selective; substantial autophagy-independent effects
Clinical trials	Target engagement demonstrable (p62 accumulation)	Discordant outcomes; no biomarker-based patient selection

Table 4: Current and investigational autophagy-directed therapeutic strategies

Strategy	Agent / target	Intended setting	Status of evidence
Lysosomal inhibition	Chloroquine/hydroxychloroquine (Mauthe *et al.*, 2018)	Established cancers	Randomised trials discordant (Karasic *et al.*, 2019; Zeh *et al.*, 2020)
Combination with MAPK blockade	ERK/MEK inhibitor plus autophagy inhibitor (Kinsey *et al.*, 2019; Bryant *et al.*, 2019)	RAS-driven tumours	Strong preclinical rationale; early clinical
Upstream induction	mTOR inhibitors (Ravikumar *et al.*, 2004)	Neurodegeneration	Preclinical; pleiotropy limits translation
Nutraceutical induction	Spermidine (Eisenberg *et al.*, 2016; Madeo *et al.*, 2018)	Ageing, cardioprotection	Preclinical and early clinical
Transcriptional enhancement	TFEB activation (Settembre *et al.*, 2011; Medina *et al.*, 2015)	Lysosomal storage, neurodegeneration	Preclinical; pleiotropy a safety concern
Selective node inhibition	ULK1, VPS34, ATG4 inhibitors (Amaravadi *et al.*, 2019)	Oncology	Investigational

tissue exists, which means trials cannot presently confirm target engagement or enrich for likely responders. This is arguably the single greatest impediment to translation, and it is a methodological rather than a conceptual failure (Klionsky *et al.*, 2021).

9. Future perspectives

Progress is likely to follow from selectivity and from measurement rather than from further pathway discovery. Cargo-selective modulators—agents that enhance mitophagy or aggrephagy without globally amplifying degradation—would decouple therapeutic benefit from the metabolic and immunological costs of broad induction. Potent, specific inhibitors of ULK1, VPS34, or ATG4 could replace HCQ and permit a genuine test of the autophagy-dependence hypothesis in cancer. Minimally invasive flux biomarkers would enable biomarker-guided patient selection and make negative trials interpretable. TFEB-directed therapy offers a route to coordinated enhancement of the entire autophagic–lysosomal system, though its pleiotropy demands careful safety evaluation. Finally, a systematic mechanistic characterisation of mammalian microautophagy is overdue and may reveal a therapeutically relevant pathway that is currently overlooked simply because it is hard to assay.

10. Clinical implications

Several implications follow for practising clinicians. Autophagy should be understood as a modifier of disease across specialties rather than the province of any one; congenital disorders of autophagy now belong in the differential diagnosis of complex neurodevelopmental phenotypes, and their recognition requires genomic rather than biochemical testing. In oncology, hydroxychloroquine should not be used routinely outside clinical trials, since the strongest randomised evidence in metastatic pancreatic cancer was negative for its primary survival endpoint. The widespread use of HCQ for rheumatological indications means that many patients are chronically exposed to a lysosomal inhibitor, a consideration of uncertain but non-trivial relevance to long-term autophagic function that has not been systematically studied. Conversely, lifestyle factors that induce autophagy, notably caloric restriction and exercise, have a plausible mechanistic basis for benefit; the inferential leap from mechanism to clinical recommendation should nonetheless be made cautiously, since the human outcome data remain thin.

11. Research gaps

Priority gaps include: validated human biomarkers of autophagic flux, without which trials remain fundamentally uninterpretable (Klionsky *et al.*, 2021); selective, potent pharmacological modulators to replace CQ and HCQ; a mechanistic account of mammalian microautophagy comparable to that available for macroautophagy; resolution of the extent to which PINK1–Parkin mitophagy operates physiologically as opposed to under artificial depolarisation; clarification of the membrane sources of the phagophore and their

relative contributions (Nakatogawa, 2020); and prospective, biomarker-stratified clinical trials capable of testing autophagy dependence rather than assuming it. A prioritised agenda appears in Table 5.

Table 5: Prioritised future research directions

Priority	Rationale	Illustrative goal
Human flux biomarkers	Trials cannot currently confirm target engagement (Klionsky *et al.*, 2021)	Minimally invasive, validated flux readout
Selective modulators	CQ/HCQ are too non-specific to test the hypothesis (Mauthe *et al.*, 2018)	Potent ULK1/VPS34 inhibitors with an acceptable index
Cargo-selective therapy	Avoids the costs of global modulation (Vargas *et al.*, 2023)	Enhance mitophagy without inducing bulk autophagy
Mammalian microautophagy	Mechanistically underdeveloped (Schuck, 2020)	Define the machinery and its physiological role
Biomarker-stratified trials	Unselected populations dilute any true effect (Levy *et al.*, 2017)	Enrich for autophagy-dependent tumours prospectively

12. Conclusion

Autophagy has advanced from a morphological curiosity to a molecularly defined, genetically tractable pathway with demonstrable causal roles in human disease. The machinery of autophagosome biogenesis is now understood as an ordered series of biochemical steps; cargo selectivity has displaced bulk degradation as the organising concept; and human genetics has supplied direct evidence that core autophagy failure causes disease. What remains unresolved is not principally mechanistic. The obstacles to clinical translation are the absence of selective pharmacological tools, the lack of validated flux biomarkers, and a context dependence that resists uniform therapeutic prescriptions—the same pathway must be enhanced in the degenerating neuron and suppressed in the established tumour. Progress will therefore depend less on discovering new components than on learning to measure autophagy reliably in patients and to modulate it selectively. Figure 3 situates these determinants within an integrative framework.

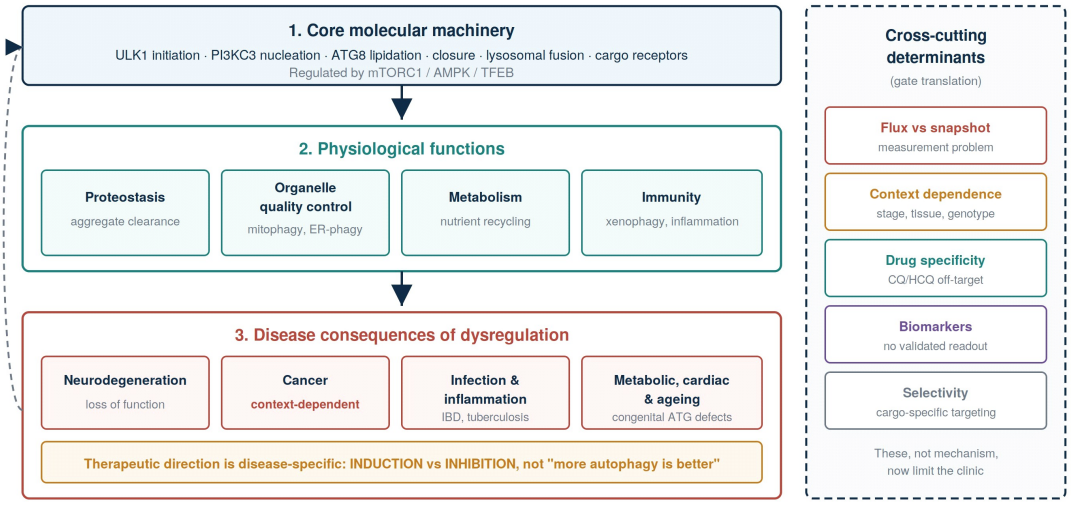

Figure 3: Conceptual framework linking autophagic machinery, physiology, and disease. The core machinery supports physiological functions spanning proteostasis, organelle quality control, metabolism, and immunity; their disruption produces disease phenotypes that differ in direction. Neurodegeneration reflects loss of function, whereas the relationship in cancer is stage-dependent, so the therapeutic imperative is induction in some settings and inhibition in others. Cross-cutting determinants—the flux-versus-snapshot measurement problem, context dependence, drug non-selectivity, absent biomarkers, and the challenge of cargo-selective targeting—now gate clinical translation more than mechanistic ignorance does. The dashed arrow denotes feedback from human disease genetics, which reveals non-redundant functions in vivo

13. Key takeaways

- Autophagy delivers cytoplasmic cargo to the lysosome through an ordered pathway: ULK1 initiation, PI3KC3 nucleation, ATG2-mediated lipid transfer with ATG8 lipidation, ESCRT-dependent closure, and STX17-mediated fusion.
- It is frequently *selective*, with cargo receptors bridging ubiquitinated substrates to ATG8 proteins; PINK1–Parkin mitophagy is the paradigm, though basal mitophagy in vivo can proceed without PINK1.
- Macroautophagy, microautophagy, and CMA differ in cargo breadth, machinery, and mode of delivery; CMA is the most selective and handles single proteins only.
- In neurodegeneration the relationship is loss-of-function; human *ATG7* deficiency provides direct causal evidence in patients.
- In cancer the relationship is stage-dependent: autophagy suppresses initiation but sustains established, RAS-driven tumours.
- Clinical evidence for autophagy inhibition is discordant—negative for survival in metastatic pancreatic cancer, positive for pathological response preoperatively—most plausibly reflecting HCQ's non-selectivity and unstratified populations rather than refuting the underlying biology.
- The rate-limiting needs are selective modulators and validated human flux biomarkers, not the discovery of further pathway components.

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