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# Breakthroughs and bottlenecks in autophagy research

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**Abstract**

The study of autophagy has grown exponentially over the past two decades, and significant progress has been made in our understanding of its mechanisms and physiological significance. However, its application to human diseases remains limited. Here, we summarize the current status of autophagy research, with a particular focus on human diseases.

## Basic mechanisms and physiology

Autophagy is the process by which intracellular material is degraded in the lysosome in animals and in the vacuole in yeasts and plants [1,2]. There are several types of autophagy: macroautophagy (mediated by the autophagosome [**Figure 1**]), microautophagy (mediated by invagination of the lysosomal membrane), and chaperone-mediated autophagy. Here, we focus on macroautophagy, which will hereafter be referred to simply as autophagy. One of the most important breakthroughs in the history of autophagy research was the isolation of yeast autophagy mutants (**Figure 2**) [3]. Subsequently, many autophagy genes were identified, and these were unified as *ATG* (autophagy-related) genes in 2003 [3]. By characterizing *ATG* gene products, the mechanisms of autophagosome formation and maturation have been rapidly elucidated over the past two decades.

Of the more than 40 *ATG* genes identified in yeasts to date, approximately half are conserved in many eukaryotes (including mammals), allowing us to carry out reverse genetic studies through the generation of "knockout" organisms. These studies have revealed the physiological roles of autophagy, including metabolic adaptation (particularly during starvation), organismal development, cell differentiation, quality control of intracellular proteins and organelles, regulation of immune and stress responses, and anti-aging [1]. Although the degradation of cytoplasmic contents in autophagy is seemingly random (particularly during starvation), autophagy can also recognize substrates in a selective manner (**Figure 1**). In fact, "selective autophagy" was characterized by early yeast studies in which selective cargos such as vacuolar enzymes and peroxisome were identified. Later, additional substrates such as damaged organelles, protein aggregates, and liquid droplets as well as cargo receptors (or adaptors) such as Atg32, p62/SQSTM1, and optineurin were identified in yeasts and other organisms, providing more information about the roles of autophagy [1].

It has also been revealed that many of these *ATG* genes have non-autophagic functions, including unconventional secretion as well as LC3-associated phagocytosis and endocytosis [4]. Therefore, not all of the phenotypes observed in *ATG* gene-

deficient organisms are caused by autophagy defects. The distinction between autophagy-dependent and -independent functions is often difficult to make, and thus these autophagy-independent functions of *ATG* genes should be taken into account when investigating the roles of autophagy and developing autophagy-modulating therapeutics.

**Monitoring and Biomarkers**

Steady-state levels of autophagy-related proteins or structures (i.e., autophagosomes) do not always serve as autophagy indicators. Instead, autophagic flux (degradation of autophagy substrates) should be monitored by biochemical methods (e.g., lysosomal turnover of autophagosome-associating ATG8 family proteins) or fluorescence microscopy (e.g., lysosomal delivery of RFP-GFP-ATG8s) [5]. However, precise measurement of autophagic flux in vivo (e.g., in model animals) remains a challenge [5]. Furthermore, methods for evaluating autophagy in humans and tissue therefrom remain limited, and this has created a major bottleneck for autophagy research in humans. Autophagic activity can be measured in blood cells *ex vivo*, but culture procedures might affect the results [5]. Ideal indicators might be autophagy-related biomarkers in blood and urine [5]. Alternatively, autophagic activity might be predicted by using nuclear imaging such as positron emission tomography to detect autophagy substrates in vivo. Therefore, the development of non-invasive methods to measure autophagic activity is critical.

**Autophagy in neurodegenerative diseases**

A major breakthrough was the discovery that autophagy was neuroprotective after it was found to degrade aggregate-prone cytoplasmic proteins that cause neurodegenerative diseases, like Huntington's disease, certain dementias and Parkinson's disease **(Figure 2)** [6]. Indeed, subsequent work demonstrated that autophagy also has broader proteostatic properties and buffers the aggregation of wild-type endogenous proteins. The beneficial roles of autophagy in the brain may be broader, as it also protects against certain forms of cell death and likely buffers certain neuroinflammatory processes. Unsurprisingly, since postmitotic cells like neurons cannot dilute proteins by cell division, the brain is perhaps the most vulnerable organ in mammals after global autophagy inhibition.

The importance of autophagy in the brain is highlighted by the discoveries that it is compromised in certain Mendelian neurodegenerative disease genes as well as risk loci for complex diseases, including forms of Parkinson's disease and dementia,

Huntington's disease and forms of motor neuron disease [6]. Such disease-associated genes do not only affect bulk autophagy but may also specifically impact selective autophagy, where additional machinery enables preferential sequestration of different types of substrates, including mitochondria (mitophagy), endoplasmic reticulum (ER-phagy), aggregate-prone proteins (aggrephagy). Indeed, studies of PINK1 and Parkin, two genes mutated in autosomal recessive Parkinsonism, have enabled detailed molecular elucidation of an important form of mitophagy.

Strategies that enhance autophagic flux may also be beneficial in various neurodegenerative diseases, for example, by lowering the levels of toxic disease-causing proteins. Support for this approach is provided by both small molecule and genetic approaches in animal models of diseases like Huntington's disease, Alzheimer's disease, and Parkinson's disease [6, 7].

**Autophagy in metabolism and cancer**

Autophagy is active at a low-level in normal cells and tissues but is robustly induced by and required for survival to starvation [8]. By degrading intracellular cargoes in lysosomes, autophagy provides breakdown products to sustain cellular and organismal metabolism during interruptions in nutrient availability. In contrast, a major breakthrough was the discovery that tumor cells commonly have high levels of autophagy even in the presence of nutrients and depend on it for sustaining metabolism and survival **(Figure 2)** [8]. Elevated metabolic demand imposed by cell growth may be responsible, as autophagy loss in tumor cells causes an energy crisis and sensitivity to starvation, with death due to depletion of nucleotide pools. Genetic ablation of essential autophagy genes specifically in tumor cells in a variety of mouse models for cancer impairs tumor growth, survival, and malignancy, demonstrating the functional dependency on autophagy in vivo. Moreover, induction of systemic loss of autophagy in mice with established cancer has potent anti-tumor activity prior to damage to normal tissues, revealing the selective dependency of tumors on autophagy and the utility of targeting autophagy for cancer therapy.

Whereas tumor-cell intrinsic autophagy promotes tumorigenesis, host autophagy also promotes tumor growth. Autophagy-deficient mice or tumor stroma commonly lack the ability to support tumor growth [9]. Loss of autophagy in mice causes the release of arginase 1 from hepatocytes that degrades circulating arginine, an essential tumor nutrient, which limits tumor growth. Loss of autophagy in tumor stroma prevents alanine secretion required for tumor metabolism and survival. Thus, autophagy in both tumor cells and host tissues provides metabolic support that drives

tumorigenesis [9].

In addition to its important metabolic role, autophagy also suppresses inflammation by eliminating damaged intracellular material and extracellular dead cells (via LAP) that would otherwise activate the innate immune response. A major breakthrough was the discovery that autophagy deficiency and immune activation limits tumor growth **(Figure 2)** [9]. Autophagy suppresses activation of type I and II interferon signaling and thereby tumor rejection by T cells enabling tumors to evade immune surveillance [10]. Autophagy prevents cell surface expression of MHC-I by promoting its degradation in lysosomes, thereby preventing tumor recognition and killing by T cells [11]. Importantly, autophagy inhibition not only promotes tumor rejection by the immune system but also enhances response to immunotherapy, potential expanding the patient population that will benefit from this significant improvement in cancer treatment [9].

## Autophagy-modulating drugs

The realization that autophagy stimulation (e.g., to mitigate neurodegeneration) or inhibition (e.g., to eliminate cancer) may have clinical benefit depending on the target disease has led to efforts in autophagy drug development. These have included chemical screens of drug libraries, repurposing studies, as well as focused efforts to develop drugs targeting specific components of the autophagy machinery (e.g., ATG4 or ULK1 inhibitors) and lysosome function (e.g., chloroquine analogs) [9]. Recent approaches have revealed the potential for targeting specific substrates for selective autophagy [12]. These include autophagosome-tethering compounds (ATTEC), which aim to link targets like mutant huntingtin to LC3 and facilitate autophagic capture of the substrate. The autophagy-targeting chimera (AUTAC) approach modifies specific substrates with *S*-guanylation, which serves as a signal enabling selective autophagy by inducing K63 polyubiquitination. Bottlenecks in the therapeutic domain include the availability of a repertoire of specific chemical inhibitors and activators with drug-like properties to be used both as high fidelity tools for modulating autophagy in the lab in cell and animal models to provide proof-of-concept studies as a prelude for further therapeutic development for patients, improved autophagy biomarkers and assays to enable accurate quantitative assessments of autophagy in patients, especially in the brain and tumors.

## Concluding remarks

Autophagy is a cellular process important not only for normal physiological functions

but also for disease development, launching initiatives to develop autophagy modulators providing new, exciting approaches for human disease treatment. Whereas autophagy is normally protective and its deficiency contributes to a variety of diseases, cancer hijacks the protective functions of autophagy to promote tumorigenesis, necessitating development of both positive and negative regulators for treating human diseases.

**References**


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**Figure legends**

**Figure 1. Macroautophagy pathway**

A small membrane cisterna elongates and encloses part of the cytoplasm to become the autophagosome. The endoplasmic reticulum (ER) serves as a scaffold for autophagosome formation. Both bulk cytosol and selective cargos such as mitochondria and the ER can be engulfed. After closure, the autophagosome fuses with lysosomes to degrade the inner autophagosomal membrane and the enclosed contents. Autophagosome formation is triggered by stress signals (e.g., starvation) or selective cargos (e.g., damaged mitochondria).

**Figure 2. Breakthroughs in the field of macroautophagy research**

Major breakthroughs in basic and disease-related fields are shown. ATTEC, autophagosome-tethering compounds; AUTAC, autophagy-targeting chimera; BECN1, Beclin 1; CQ, chloroquine; ESCRT, endosomal sorting complexes required for transport; HCQ, hydroxychloroquine; LAP, LC3-associated phagocytosis; LIR, LC3-interacting region; SNARE, soluble N-ethylmaleimide-sensitive fusion attachment protein receptor; SNP, single nucleotide polymorphism; TFEB, transcription factor EB.

**Figure 1**

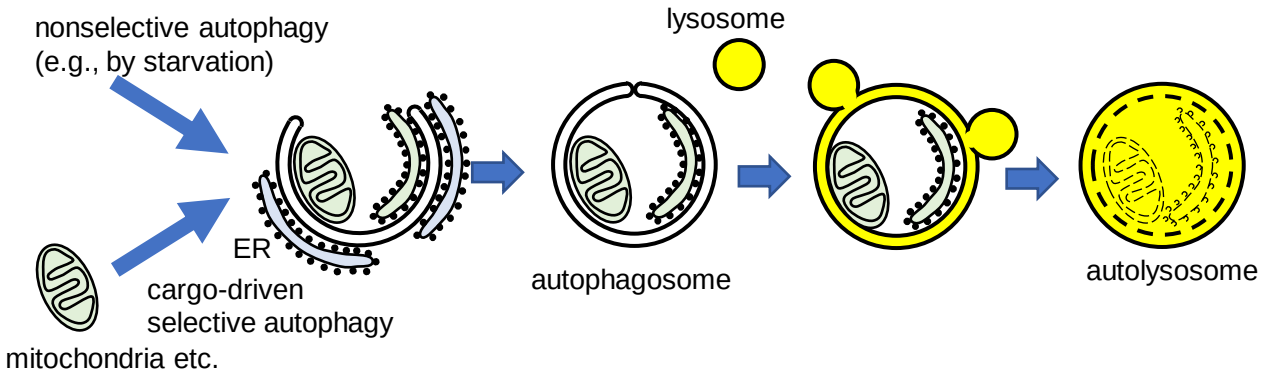
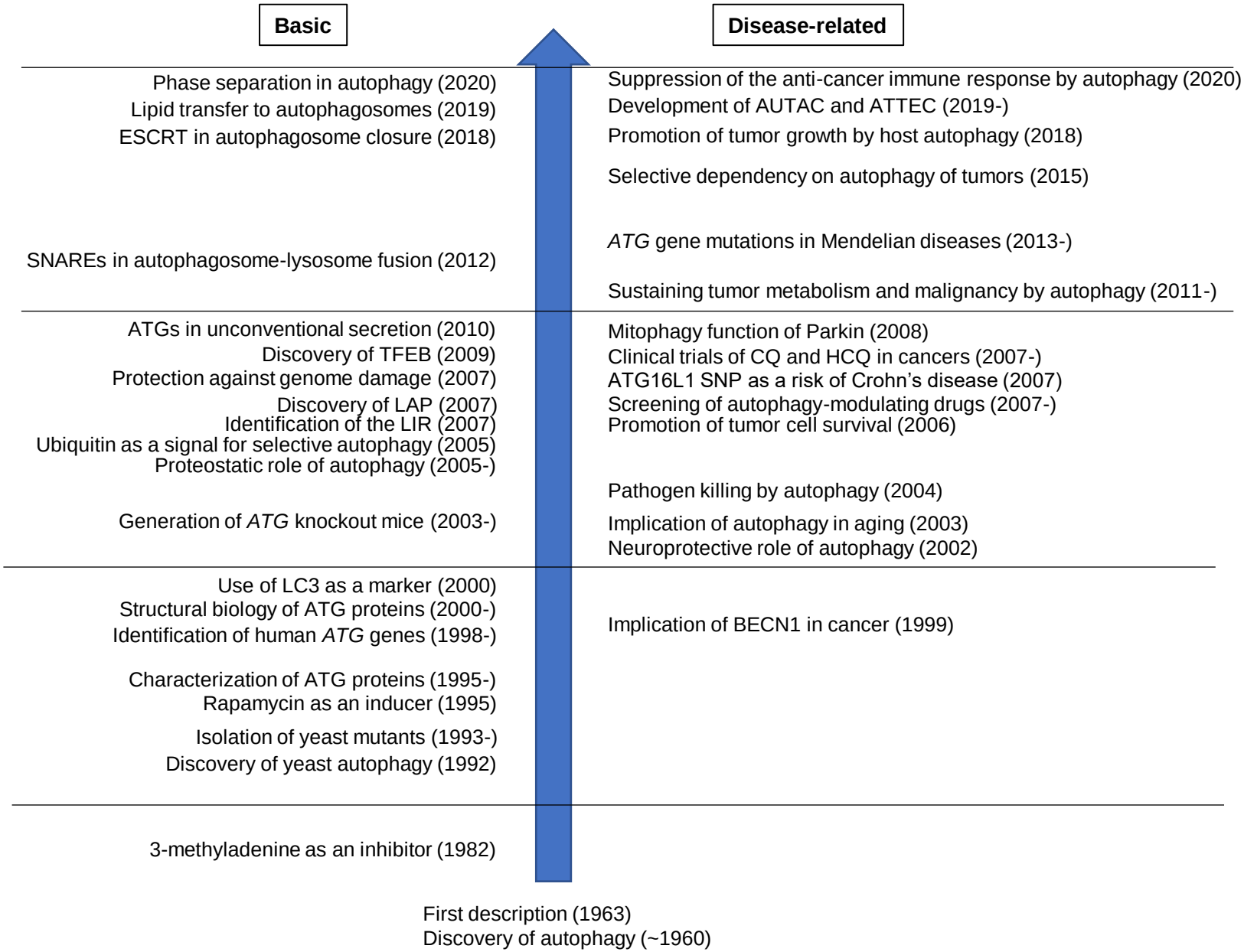

nonselective autophagy
(e.g., by starvation)
lysosome
ER
cargo-driven
selective autophagy
mitochondria etc.
autophagosome
autolysosome

**Figure 2**


Basic
Disease-related
Phase separation in autophagy (2020)
Lipid transfer to autophagosomes (2019)
ESCRT in autophagosome closure (2018)
SNAREs in autophagosome-lysosome fusion (2012)
Suppression of the anti-cancer immune response by autophagy (2020)
Development of AUTAC and ATTEC (2019-)
Promotion of tumor growth by host autophagy (2018)
Selective dependency on autophagy of tumors (2015)
*ATG* gene mutations in Mendelian diseases (2013-)
Sustaining tumor metabolism and malignancy by autophagy (2011-)
ATGs in unconventional secretion (2010)
Discovery of TFEB (2009)
Protection against genome damage (2007)
Discovery of LAP (2007)
Identification of the LIR (2007)
Ubiquitin as a signal for selective autophagy (2005)
Proteostatic role of autophagy (2005-)
Generation of *ATG* knockout mice (2003-)
Mitophagy function of Parkin (2008)
Clinical trials of CQ and HCQ in cancers (2007-)
ATG16L1 SNP as a risk of Crohn's disease (2007)
Screening of autophagy-modulating drugs (2007-)
Promotion of tumor cell survival (2006)
Pathogen killing by autophagy (2004)
Implication of autophagy in aging (2003)
Neuroprotective role of autophagy (2002)
Use of LC3 as a marker (2000)
Structural biology of ATG proteins (2000-)
Identification of human *ATG* genes (1998-)
Characterization of ATG proteins (1995-)
Rapamycin as an inducer (1995)
Isolation of yeast mutants (1993-)
Discovery of yeast autophagy (1992)
Implication of BECN1 in cancer (1999)
3-methyladenine as an inhibitor (1982)
First description (1963)
Discovery of autophagy (~1960)