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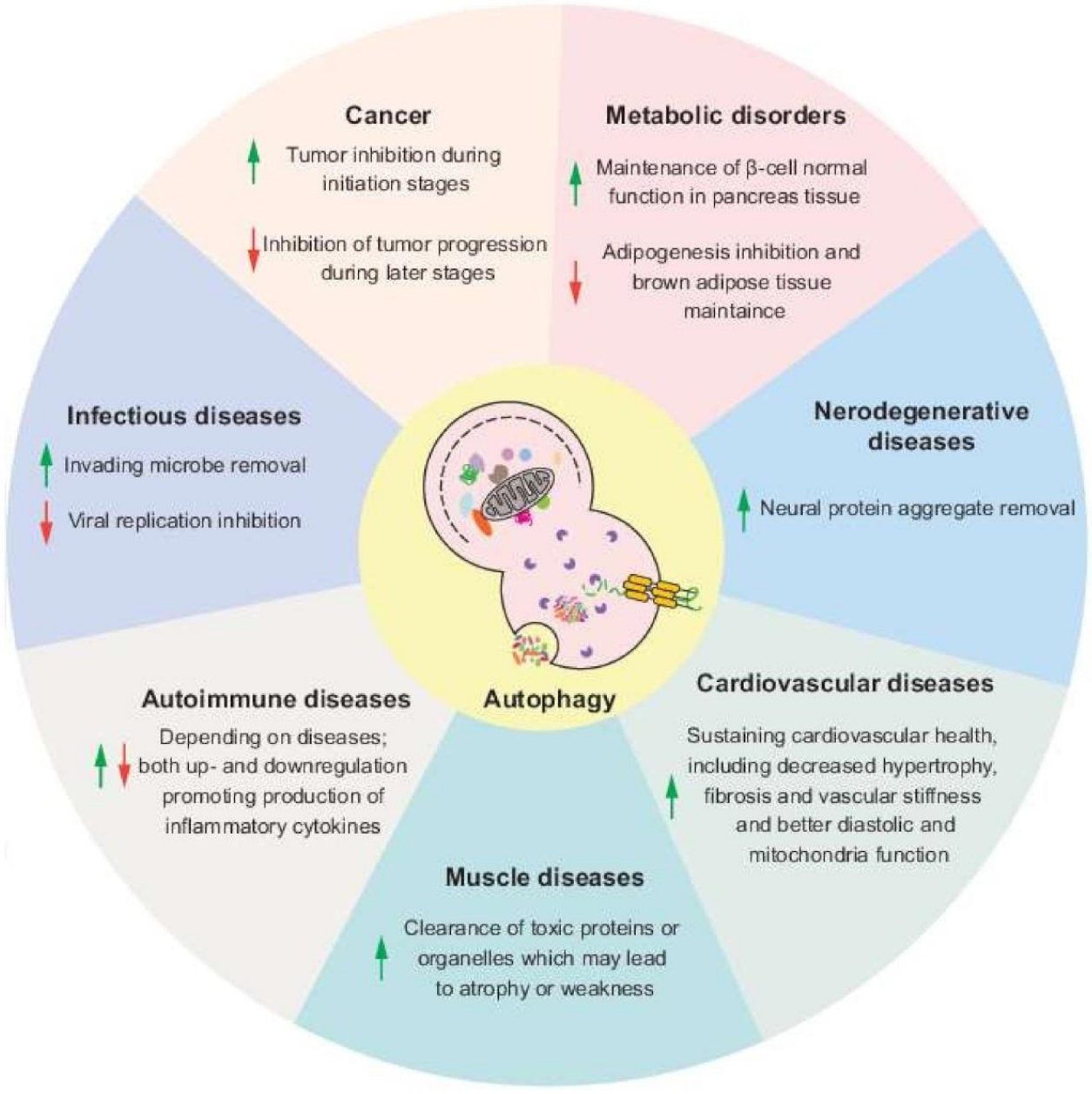


Autophagy & human diseases

Half century ago, the Belgian Biochemist Christian de Duve first coined the term "Autophagy" to describe a process where the cell digests its cytoplasmic materials within the lysosomes. The term comes from the Greek literature which means "self-eating".

Targeted interplay between bacterial pathogen and host autophagy:

Autophagy targets genus-specific proteins, so orthologous proteins which share sequence homology with each other are recognized as substrates by a particular autophagy targeting protein. There are complementary autophagy targeting proteins which potentially increase infection risk upon mutation. autophagy can target different sets of bacterial proteins from a same pathogen. the complementarity in the specific bacterial proteins can make the host more susceptible to chronic disorders and infections if the gene encoding one of the autophagy targeting proteins becomes mutated, and the autophagy system is overloaded or suffers other malfunctions. Moreover, autophagy targets virulence factors which are responsible for nutrient acquisition and motility recognized by multiple autophagy targeting proteins. The specialized virulence factors such as autolysins, and iron sequestering proteins are potentially recognized uniquely by a single autophagy targeting protein. The autophagy proteins CALCOCO2/NDP52 and MAP1LC3/LC3 may have evolved specifically to target pathogens or pathogenic proteins for autophagic degradation. While SQSTM1/p62 targets more generic bacterial proteins containing a target motif but not related to virulence.

The bacterial proteins from various pathogenic genera are also able to modulate autophagy. There are genus-specific patterns in the phases of autophagy that are potentially regulated by a given pathogen group. Some autophagy phases can only be modulated by particular pathogens, while some phases are modulated by multiple pathogen genera. Some of the interplay-related bacterial proteins have proteolytic and post-translational activity such as phosphorylation and ubiquitination and can interfere with the activity of autophagy proteins

Molecular basis:

Autophagy is executed by autophagy-related (Atg) genes. Prior to 2003, ten or more names were used, but after this point a unified nomenclature was devised by fungal autophagy researchers. Atg or ATG stands for autophagy related. It does not specify gene or a protein.

The first autophagy genes were identified by genetic screens conducted in Saccharomyces cerevisiae. Following their identification those genes were functionally characterized and their orthologs in a variety of different organisms were identified and studied. Today, thirty-six Atg proteins have been classified as especially important for autophagy, of which belong to the core machinery

In recent years, genetic deletion of autophagy related gene (Atg) in mammals reveals that autophagy plays a critical role in adaptive responses to starvation & other forms of stress, homeostasis, cellular differentiation & development. Analysis of tissue specific deletion of Atg genes has revealed the connection between dysregulation autophagy and various kinds of disease like phenotypes including

cancer, neurogenerative diseases, infectious diseases and metabolic diseases.

Types of autophagy:

At least three major types of autophagy have been identified.

Macroautophagy is characterized by formation of a unique doubled membraned organelle (autophagosome).

Microautophagy characterized by engulfment of cytoplasmic materials by inward invagination of lysosomal membrane. **Chaperone mediated autophagy** is the third type of autophagy which is mediated by hsc70, co-chaperones and lysosomal-associated membrane protein type 2A

Crinophagy is a poorly understood type in which the unnecessary secretory granules are degraded and recycled.

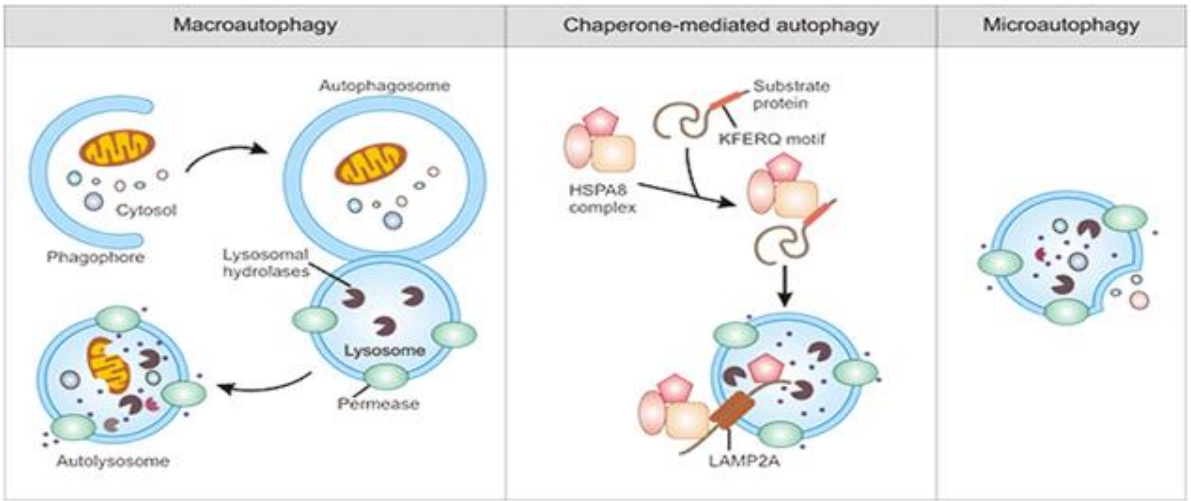
Mitophagy is the selective degradation of mitochondria by autophagy. It often occurs to defective mitochondria following damage or stress. It promotes turnover of mitochondria and prevents the accumulation of dysfunctional mitochondria which can lead to cellular degradation.

Lipophagy is the degradation of lipids by autophagy. In lipophagy, the target are lipid structures called lipid droplets (LDs), which a spherical organelle with a core mainly made up of triglycerides and a unilayer made up of phospholipids and proteins. In animal cells main lipophagic pathway is via engulfment of LDs via phagophore.

Functions:

Nutrient starvation

Autophagy has roles in various cellular functions. One particular example is in yeasts, where the nutrient starvation induces a high level of autophagy. This allows the unneeded proteins to be degraded and the amino acids recycled for the synthesis of proteins that are essential for survival. In higher eukaryotes, autophagy is induced in response to the nutrient depletion that occurs in animals at birth after severing of the trans-placental food supply, as well as that of nutrient starved cultured cells and tissues. Mutant yeast cells that have a reduced autophagic capability rapidly perish in nutrition-deficient conditions. Studies on the *apg* mutants suggest that autophagy via autophagic bodies is indispensable for



protein degradation in the vacuoles under starvation conditions, and that at least 15 APG genes are involved in autophagy in yeast.

Infection

Vesicular stomatitis virus is believed to be taken up by the autophagosome from the cytosol and translocated to the endosomes where detection takes place by a pattern recognition receptor called toll-like receptor 7, detecting single stranded RNA. Following activation of the toll-like receptor, intracellular signaling cascades are initiated, leading to induction of interferon and other antiviral cytokines. A subset of viruses and bacteria subvert the autophagic pathway to promote their own replication.

Repair mechanism

Autophagy degrades damaged organelles, cell membranes and proteins, and insufficient autophagy is thought to be one of the main reasons for the accumulation of damaged cells and aging. Autophagy and autophagy regulators are involved in response to lysosomal damage, often directed by galectins such as galectin-3 and galectin-8.

Programmed cell death

One of the mechanisms of programmed cell death (PCD) is associated with the appearance of autophagosomes and depends on autophagy proteins. This form of cell death most likely corresponds to a process that has been morphologically defined as autophagic PCD. Though any morphological and histochemical studies have not so far proved a causative relationship between the autophagic process and cell death.

Role in human diseases:

With the completion of the Human Genome Project in 2003 and the International HapMap project in 2005, the powerful set of research tools, including the high-speed DNA sequencing technology made it possible to identify the genetic contributions to the specific diseases, even if they are rare.

Table 1 summarizes the association between genetic variants of autophagy related genes and selected human diseases:

Genes	Functions in autophagy	Associated human diseases
ATG5	Autophagosome formation	Genetic polymorphisms are associated with asthma and enhanced risk of SLE
ATG16L1	Autophagosome formation	T300A mutation is associated with increased risk of Crohn's disease
EI24	Autophagosome formation and degradation	Mutations and deletions are associated with human early onset breast cancers
IRGM	Phagosome degradation	SNPs and deletion mutation are associated with

		enhanced risk oh Crohn's disease
PARK2/Parkin	Mitophagy induction	Mutations are associated with autosomal recessive or sporadic early onset Parkinson's disease
SMURF1	Selective autophagy	SNP associated with enhanced risk of ulcerative colitis
UVRAG	Autophagosome degradation	Deletion mutation is associated with human colorectal cancer

Autophagy & Parkinson's disease:

Parkinson's disease is the most common form of a group of progressive neurogenerative disorders characterized clinically by bradykinesia, rest tremor, muscle rigidity, shuffling gait & flexed posture. Many genes, mutations and polymorphisms have been implicated pathogenesis of the disease. Among them PARK2/Parkin and PARK6/PINK1 are commonly involved in autosomal recessive or sporadic juvenile onset Parkinson's disease.

PTEN-induced putative kinase protein 1 (PINK1, encoded by PARK6/PINK1) is a mitochondria associated protein kinase that acts upstream of Parkin (encoded by PARK2/Parkin), an E3 ubiquitin ligase implicated in selective degeneration of damaged mitochondria by autophagy. When the mitochondria are damaged, and loose their membrane potential, mitochondrial PINK1 is stabilized and recruits Prakin, which ubiquitinates mitochondrial membrane proteins, resulting in selective mitophagy. Excessive mitochondrial damage is associated with Parkinson's disease.

Lysosomal storage disease:

These diseases are characterized by progressive accumulation of undigested macromolecules within the cell. They are caused by inherited gene mutations that perturb lysosomal homeostasis. As lysosomes play an important role in autophagy pathway by fusing autophagosome & degrading autophagic cargo, lysosomal dysfunction in LSDs impacts the autophagy pathway. In most LSDs, the lysosomal dysfunction is accompanied by impaired autophagic flux, resulting in defective autophagosome lysosome fusion and secondary accumulation of autophagy substrates such as SQSTM1/p62, polyubiquitinated proteins, and damaged mitochondria. So, in some sense, LSDs can be considered as "Autophagy disorders".

Cancer:

The role of autophagy likely differs in different stages of cancer cells could utilize autophagy for their own cytoprotection.

Monoallelic deletion of BECN1 has been detected in human breast, ovarian and prostate specimens. In particular, the aberrant expression of Beclin 1 (encoded by the human BECN1 gene) in many kinds of tumor tissues correlates with poor prognosis. Beclin 1 plays an essential role

in autophagy. It interacts with the class III PtdIns 3 kinase, Vps34 to form to form the Beclin 1-Atg14-Vsp34-Vsp15 complex, which is important for localization of downstream autophagic proteins to the autophagosome formation site to induce autophagy.

Crohn's disease:

Genome wide association studies of non-synonymous SNPs have linked ATG16L1 variants with susceptibility to Crohn's disease. Atg16L1, a core component of autophagy machinery, forms a complex with Atg12-Atg15 to induce LC3 lipidation and is essential for autophagosome formation. The Atg16L1 protein has a C terminal WD repeat domain, the Crohn's disease-associated mutation is within or immediately upstream of this domain. Investigations of mice carrying two distinct mutations that reduces or eliminate the expression of Atg16L1 have suggested potential links between Atg16L1 and Crohn's disease. It was shown that Atg16L1 deficient macrophages produce more inflammatory cytokines IL-1β and IL-18 upon stimulation with lipopolysaccharides. On the other hand, the Atg16L1 hypomorph mice exhibited aberrant granule formation in Paneth cell, which play an important role in the innate immune response of the intestine.

Autophagy modulating compounds in clinical trials:

The development of pharmacological agents that modulate autophagy in thiese pathological conditions is critical. Pharmacological approaches to activate or inhibit autophagy are also required because autophagy can play either a protective or destructive role in different diseases, even in different stages of the same disease

Drugs	Autophagy target	Diseases
Chloroquine	Lysosomal inhibitor	Small cell lung cancer
Hydroxychloroquine	Lysosomal inhibitor	Breast cancer
Carbamazepine	Autophagy inducer	Alpha1antitrypsin deficiency liver cirrhosis
Lithium carbonate	Autophagy inducer	Amyotrophiclateral sclerosis
Trehalose	Autophagy inducer	Vascular aging

It is the most challenging issue to monitor autophagic activities in humans, in tissue samples and more preferably in blood samples. It is more important to measure autophagic flux than autophagosome number. To date, however, measurement of autophagic flux in paraffin embeded tissue samples has been successful and simple detection of endogenous LC3-II, a commonly used biomarker for autophagosomes is also done. But the appearance of LC3 positive autophagosomes do not necessarily indicate the higher autophagic activity in the tissue. Autophagosomes can accumulate due to the induction of autophagy or due to blocking of a late step of the autophagic pathway including impaired autophagosome-lysosome fusion and compromised lysosomal activity. this is a frequent occurrence in human diseases and even during the normal aging process. to overcome these problems, it may be beneficial to combine immunohistochemical assays of other autophagy related marker proteins such as ATG5 and Beclin 1 to detect autophagy in clinical tissue samples.

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