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Unleashing The Power Within: The Marvels of Autophagy in Cellular Health

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Abstract: Autophagy is a critical cellular process essential for maintaining cellular health and functionality. It involves the regulated degradation and recycling of damaged organelles and cellular components, promoting cellular homeostasis and preventing the accumulation of harmful substances. This self-cleaning mechanism is fundamental to various physiological processes and is implicated in numerous diseases, making it an area of intense research

Key words: Autophagy, Apoptosis, homeostasis

Introduction

Autophagy stands as a fundamental cellular process, an intricate mechanism that holds profound significance in upholding cellular health and equilibrium. The term "autophagy" originates from the Greek words "auto" (self) and "phagy" (to eat), epitomizing the concept of cellular self-cleansing. Through a meticulously orchestrated sequence of events, cells undergo a process of targeted disintegration and recycling, specifically targeting damaged organelles, proteins, and other cellular components. This systematic breakdown serves the purpose of preserving proper cellular functionality and preventing the accumulation of detrimental substances. By engaging in this process, cells not only sustain their vitality but also exert influence over an array of physiological and pathological conditions. Given its intricate nature and broad implications, autophagy continues to captivate the curiosity of scientific exploration[1].

Cell death

Cellular decisions, driven by intricate communication processes, form the cornerstone of maintaining organismic homeostasis. Among the various types of cell death, three primary classifications emerge: type 1 cell death through apoptosis, type 2 involving autophagy, and type 3 marked by necrosis.

As elucidated by Wen and Klionsky (2016), autophagy, rooted in the Greek terms "auto" (self) and "phagein" (to eat), represents a meticulously regulated process of cellular degradation and recycling. This process has been conserved across organisms, extending from yeast to more complex eukaryotes. Autophagy stands as a form of programmed non-apoptotic cell death and is crucial for intracellular homeostasis. It operates through the disintegration of waste components from the cytoplasm within acidic lysosomal compartments, thus averting cell demise.

Autophagy holds considerable significance in targeting numerous pathogens, particularly intracellular bacteria. Changes in the autophagy machinery can give rise to various pathological conditions, encompassing cancer, aging, and neurodegeneration. This process involves the envelopment of cytoplasmic matrix fragments and damaged organelles by cell membranes, culminating in the formation of autophagosomes. These autophagosomes subsequently merge with lysosomes. A similar process, known as heterophagy, is witnessed when phagocytic white cells engulf dying or deceased cells.

Autophagy serves as a common response in sublethally injured cells, epithelial cells like those found in the endometrium undergoing cyclic physiologic regression, as well as in instances of atrophy due to diverse causes[2].

In tissues such as the liver and kidneys, the presence of autophagic vacuoles is frequent. These vacuoles can be observed under a light microscope as eosinophilic inclusions. While some vacuoles are extricated from the cell through exocytosis, others persist as residual bodies. The contents of these residual bodies ultimately contribute to the formation of lipofuscin, often referred to as the "wear-and-tear" pigment.

Morphological features of autophagy

Plasma membrane blebbing, the absence of chromatin condensation, extensive cytoplasmic vacuolization, minimal or no phagocytic cell uptake, and the in-vivo accumulation of autophagic vacuoles constitute noteworthy characteristics. These processes involve the conveyance of cargo to lysosomes or vacuoles through an autophagosome—a double-membrane vesicle or structure.

Phagophore

The initiation of autophagosome formation within the cytoplasm is marked by the generation of a cup-shaped membrane structure known as the Phagophore or isolation membrane. This process is most effectively initiated at the preautophagosomal structure or site (PAS).

How Autophagy is activated? Extracellular and Intracellular factors, Growth factors deprivation, Nutrient deprivation/ under starvation, Stress-Oxidative stress, ER stress,during pathogen invasion, protein aggregation/ organelle aggregation, Ageing, Exercise

Physiological functions

Assisting in catabolism's nutrient provision, generating ATP under stress or starvation, signaling for the removal of apoptotic cells through heterophagy, degrading misfolded proteins, eliminating damaged or excess organelles, ensuring genomic stability (which aids in tumor suppression), and offering defense against metabolic stress are all functions attributed to autophagy.

Autophagy also plays a defensive role against microbes, engaging in the targeted transport of microorganisms to degradative lysosomes in a process termed Xenophagy. Furthermore, autophagy is activated by detachment of the extracellular matrix, safeguarding cancer cells from anoikis-induced cell death. In the context of metastasis, autophagy has dual pro-metastatic roles: facilitating the adaptation of pre-metastatic cells in the face of metabolic stress or challenging environments and being vital for the maintenance of cancer stem cells and drug resistance.

Conversely, autophagy exhibits anti-metastatic qualities, inducing apoptotic cell death in cancer cells in specific cases. This capacity may impede cancer metastasis and counteract oncogene-induced cell senescence. Autophagy also governs the release of HMGB1 from cancer cells, activating dendritic cells and thereby inciting an immune response against cancer.

Classes of Autophagy:

Variants of autophagy are classified based on their modes of transport to the lysosome or vacuole: these include macroautophagy (often referred to simply as autophagy), microautophagy, and chaperone-mediated autophagy (CMA).

Macroautophagy

A universal intracellular catabolic process, autophagy facilitates the degradation of cytoplasmic components such as macromolecules and organelles, directing them to vacuoles or lysosomes. Particularly during periods of starvation, autophagy serves as a significant source of amino acids and other vital elemental components like lipids, sugars, and nucleotides.

In macroautophagy, segments of cytosol and organelles are encapsulated within a double-membrane autophagosome. This autophagosome later merges with a lysosome, resulting in the formation of a single-membrane autophagolysosome. This sequence of events characterizing macroautophagy involves the following steps:

Initiation

At the initiation of the autophagy signaling pathway, the ULK1 complex emerges, composed of ULK1 (akin to UNC-51 kinase), FIP200 (a protein that interacts with kinases), along with the autophagy-related gene components ATG13 and ATG101. This complex plays a pivotal role. In parallel, the ULK1 complex actively contributes to the generation of the isolation membrane, also recognized as the PAS (Pre-autophagosomal structure), as depicted in Figure 1.

Nucleation

Activated by the ULK1 complex, the PI3K (Phosphatidylinositol-3 kinase) complex comes into play. This complex consists of Beclin 1, VPS34, VPS15, and ATG15. While other cell membranes might have a role, this complex predominantly triggers nucleation at the juncture of mitochondria and the endoplasmic reticulum

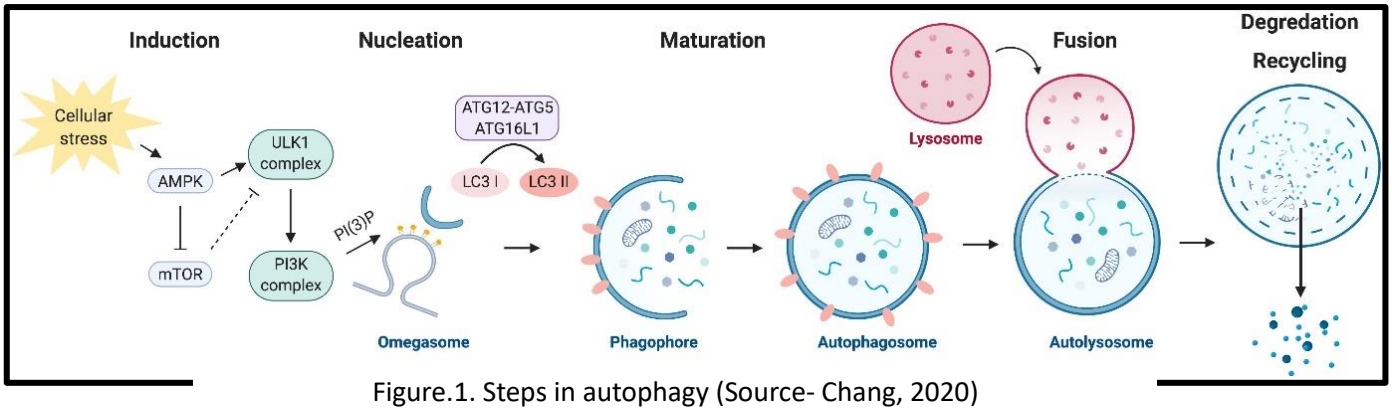

Figure.1. Steps in autophagy (Source- Chang, 2020)

(ER). This process culminates in the formation of a cup-shaped membrane termed a phagophore.

Lipids are attracted to the isolation membrane through the involvement of transmembrane proteins ATG9 and VMP1, as illustrated in Figure 1.

Elongation and maturation

Two ubiquitin-like (UBL) protein conjugation systems facilitate the attachment of ATG proteins: the ATG12-UBL system and the LC3-UBL system involving the protein Light Chain (LC3). The ubiquitin-like conjugation mechanism links lipid, specifically phosphatidylethanolamine (PE), covalently to microtubule-associated Light chain 3 (LC3). This process triggers the elongation of the phagophore, followed by its fusion to generate an autophagosome.

Throughout autophagosome formation, neighbouring intracellular contents are ensnared within the autophagosome. This loading of cargo is not a haphazard capture; rather, it's a selectively orchestrated process, as depicted in Figure 1.

Fusion of lysosome to autophagosome

In the concluding stages, the docking and fusion of lysosomes with autophagosomes are facilitated by proteins akin to soluble NSF attachment protein receptors (SNAREs). This intricate process brings about the creation of a singularly membrane-bound structure called an Autophagolysosome. Within this, a segment of cytosol containing malfunctioning organelles is contained. This encapsulation culminates in the degradation of these dysfunctional organelles, after which the constituent components are transported back into the cytoplasm through lysosomal permeases. Here, they serve the cell's needs, contributing to biosynthetic processes or energy generation, as portrayed in Figure 1.

Regulation of Autophagy:

MTORC1 orchestrates the activation of diverse biosynthetic pathways, including the synthesis of proteins, lipids, and nucleotides. This, in turn, hinders the process of autophagy while promoting cellular growth. Through a comprehensive RNAi protein screening, it was revealed that glucosyl ceramidase beta (GBA) acts as a positive regulator of autophagy-dependent cell death within human cells. GBA plays a role in converting glucosylceramide into ceramide and glucose. In a specific type of autophagy-dependent cell death termed autosis, the activity of the plasma membrane Na+/K+ ATPase holds significance.

Notably, the inhibition of Na+/K+ ATPase, achieved by compounds like cardiac glycosides, imparts neuroprotection. This was demonstrated in a rat model of neonatal hypoxia and ischemia, indicating its potential therapeutic impact [3].

Chaperone mediated autophagy (CMA)

The process I'm discussing is specific to mammalian cells. This pathway, known as Chaperone-Mediated Autophagy (CMA), is responsible for breaking down a diverse array of

substrate proteins. These include glycolytic enzymes, transcription factors, their inhibitors, proteins binding calcium and lipids, proteasome subunits, and proteins involved in vesicular trafficking.

In CMA, the recognition of the KFERQ motif is executed by the heat shock 70 kDa protein 8 (HSPA8/HSC70), alongside other cochaperones. HSPA8 then transports the substrate to the lysosomal membrane, where it likely assists in unfolding the substrate. At the lysosomal membrane, the substrate binds to monomers of lysosomal-associated membrane protein 2A (LAMP2A), the receptor for CMA substrates. This interaction leads to the multimerization of LAMP2A.

HSP90 plays a role in stabilizing the subunits of the complex formed on the lumenal side of the lysosomal membrane, as the multimeric translocation complex forms. After the substrate has been successfully moved into the lysosomal lumen, cytosolic HSPA8 actively dismantles the translocation complex. Subsequently, LAMP2A reverts to its monomeric state, ready to bind new substrate and initiate a fresh round of translocation. Lumenal HSPA8 plays a role in this process. The mechanism of translocation is regulated, particularly at the point of substrate binding to LAMP2A, which is the rate-limiting step in CMA. Conditions like mild oxidative stress, exposure to protein-damaging toxins, and extended periods of nutrient deprivation all lead to an upregulation of CMA, highlighting its responsiveness to various stimuli.

Role of CMA

Quality control of oxidised proteins, Aggregate prone proteins, metabolism of proteins and energetics, regulatory functions-Transcriptional regulation cell cycle, proliferation and growth.T- cell activation and immune functions.

Activity of CMA

CMA exhibits an upregulation after a period of 10 hours or more of nutrient deprivation. Interestingly, this heightened activity of CMA persists even beyond three days of starvation, contributing as a source of recycled amino acids for the synthesis of proteins. The orchestration of this process involves two key proteins, namely GFAP (glial fibrillary acidic protein) and EFL alpha (Elongation factor L alpha). These proteins play a role in governing the stability of the multimerized LAMP2A complex, and this regulation occurs in a GTP-dependent manner.

When the need arises for activating CMA, the GTPase Rac1 steps in, recruiting the phosphatase PHLPP (Pleckstrin homology domain and leucine-rich repeat protein phosphatase) to the lysosome. PHLPP is responsible for the dephosphorylation of AKt1. This intricate mechanism ensures the proper activation of CMA.

Notably, the mTOR pathway, along with its effector kinase Akt, negatively regulates the assembly of LAMP2A into the CMA translocation complex. As part of the process, the lysosome or vacuole engulfs small cytoplasmic components through an inward invagination of the lysosomal membrane.

Microautophagy

Micro-autophagy operates in a synchronized manner alongside macroautophagy and CMA, collectively contributing to cellular resilience. This coordinated effort aids cells in enduring prolonged periods of starvation by perpetually recycling nutrients and energy resources.

A specific form of micro-autophagy, known as endosomal micro-autophagy, plays a significant role. It enables the selective uptake and subsequent degradation of cytosolic proteins within late endosomes and multivesicular bodies (LE/MVB). Unlike other forms of autophagy, proteins carrying KFERQ-like motifs are not a prerequisite for their association with late endosomes. In this context,

Hsc70 directly binds to phosphatidylserine on the LE membrane.

In the scenario of Endosomal Micro-autophagy (EMI), Hsc70 experiences internalization and subsequent degradation, along with the cargo protein, marking a key mechanism through which this process operates.

Microlipophagy is triggered by Atg14 and 5AMP-activated protein kinase.

Classes of microautophagy

Non- selective: Cytoplasmic material is trapped in the lysosome/ vacuole by the random process of membrane invagination.

Selective

Micropexophagy- Cluster of damaged and / or superfluous peroxisomes, **Micromitophagy-** Mitochondria, **Pexophagy**- Peroxisomes, **Ribophagy**- Ribosomes, **Xenophagy**- Virus, bacteria, **Lipophagy**- Lipid droplets

Techniques to study Autophagy

Transmission electron microscopy: Transmission electron microscopy remains the golden standard.

Molecular markers

Transmission electron microscopy remains the gold standard in microscopy. Molecular markers like ULK1, WIPI1, WIPI2, ATG5, and microtubule-associated Light chain 3 (LC3) play pivotal roles in autophagy studies. The redistribution of the GFP-LC3 fusion protein into vesicular structures like autophagosomes or autolysosomes offers valuable insights.

Autophagic activity can be assessed directly through methods such as measuring LDH (lactate dehydrogenase) and monitoring protein turnover from long-lived proteins. Indirect evaluation is possible using autophagy-specific antibodies in techniques like Western blot assays, flow cytometry, and fluorescence microscopy-based assays. These methodologies contribute to unravelling the intricacies of autophagy processes.

Conclusion

Autophagy stands as a captivating cellular phenomenon that has engrossed the scientific community, offering substantial potential for enhancing our comprehension of cellular biology and enhancing human well-being. The ongoing investigation into the mechanisms and therapeutic prospects of autophagy holds the potential to unveil innovative treatments and interventions down the line. This collective effort could ultimately usher in a healthier and more robust society.

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