REVIEW

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Autophagy in aging and disease

MĂDĂLINA FÎLFAN[1,2)], RALUCA ELENA SANDU[2)], ALEXANDRA-DANIELA ZĂVĂLEANU[2)], ANDREI GREŞIŢĂ[2)], DANIELA-GABRIELA GLĂVAN[1)], DENISSA-GRETA OLARU[3)], AUREL POPA-WAGNER[4)]

[1)] *2nd Psychiatry Clinical Hospital, University of Medicine and Pharmacy of Craiova, Romania*

[2)] *Department of Functional Sciences, Center of Clinical and Experimental Medicine, University of Medicine and Pharmacy of Craiova, Romania*

[3)] *Department of Ophthalmology, MedLife Clinic, Craiova, Romania*

[4)] *Chair of Vascular Neurology and Dementia, University of Medicine Essen, Germany*

Abstract

Autophagy is a catabolic degradation system used to destroy and recycle the unnecessary or damaged components of a cell. Autophagy is present at a basal level in all mammals and is regulated by some conditions, such as oxidative stress, starvation or hypoxia. In aged tissues, increased but also decreased expression of autophagy-specific proteins, Beclin 1, LC3, Atg5 and Atg7 has been reported. Likewise, it could be shown that the lifespan of yeast, nematodes and flies is prolonged by pharmacologically stimulated autophagy using exogenous administered spermidine. Autophagy is potentially implicated in acute lung injury and sepsis, two main causes of morbidity and mortality worldwide. Finally, a quite recent study supports the hypothesis that autophagy might be useful in vascular disease prevention by stimulating cholesterol efflux, which leads to inhibition of necrotic core formation and lipid accumulation. Since autophagy is also implicated in neuro-protection, in Alzheimer's and Huntington's disease animal models and many others normal and pathological states, including immunity, diabetes mellitus, different kind of tumors, colorectal cancer, different inflammations, lung diseases, neurodegenerative diseases, autophagy is of interest to many biomedical researchers.

Keywords: autophagy, aging, polyamines, neurodegenerative diseases, cancer.

Introduction

Autophagy, a protein degradation system, is a catabolic process as a stress-response or a natural way to destroy and recycle the unnecessary or damaged components. The word provenience is the Greek "self-eating" and the process is evolutionarily conserved in all eukaryotic cells. The process was first seen in rat liver cells after receiving glucagon, by K. Porter and T. Ashford, in 1962. The name was given soon after that by a Belgian biochemist Christian de Duve (1963) [1].

There are three types of autophagy: microautophagy, macroautophagy (referred to as autophagy) and chaperone-mediated autophagy. Of these, macroautophagy is the prevalent form and is also the subject of most studies [2]. During autophagy, cell components are enclosed in double membrane vesicles, named autophagosomes and presented to lysosomes, in order to be degraded. In this way, self-digestion of the cell is achieved as part of a "rejuvenating" process or removal of intracellular pathogens.

In multicellular organisms, many cells die by apoptosis, from the Greek word "falling off" – a programmed cell death. In adult tissues, where cell number is constant, apoptosis should match cell division, so diminished (reduced) or enhanced apoptosis is the cause of many diseases (cancer, stroke, neurodegenerative diseases) [3].

Apoptosis and autophagy are interconnected. In dying cells, if stress persists and autophagy is not able to support cell survival, apoptosis will be activated to avoid local inflammation and induce an efficient elimination of cell debris [4].

Autophagy is present at a basal level in all mammals and is regulated by some conditions, such as oxidative stress, starvation or hypoxia [5–7]. Being related to many normal and pathological states, including immunity, diabetes mellitus, different kind of tumors, colorectal cancer, different inflammations, lung diseases, neuro-degenerative diseases, autophagy is of interest to many biomedical researchers.

Autophagy and aging

There are multiple theories of aging, including the accumulation of oxygen reactive species, which cause cumulative cell damage and senescence [8]. Also, the mitochondrial and DNA damages could be involved in aging [9].

Regarding autophagy in aged tissues, increased but also decreased expression of Beclin1, LC3, Atg5 and Atg7 have been reported [10–12]. Several studies have shown that the 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) and sirtuin 1 (SIRT1) activity are decreased with age [13, 14]. AMPK, that plays a role in cellular energy homeostasis, is expressed in several tissues and consists of three subunits. Sirtuin 1, also known as nicotinamide adenine dinucleotide (NAD)-dependent deacetylase sirtuin 1, is an enzyme that deacetylates proteins that contribute to cellular regulation in response to stressors or longevity.

Eisenberg *et al.* noted that in aging also the polyamine named spermidine is decreased [15]. Polyamines are considered important for survival, being involved in multiple pathways and biological processes, while abnormal changes in polyamine levels are associated with diseases and aging [16]. Thus, an analysis of polyamine levels in female mice aged 3, 10 and 26 weeks-old, revealed that spermidine concentration decreased with age in 11 from 14 tissues, spermine decreased only in the skin, muscles and heart, while putrescine was decreased in all the 14 tissues and ages [17].

Autophagy and polyamines in aging and disease

In 2009, it could be shown that the lifespan of yeast, nematodes and flies is prolonged by pharmacologically stimulated autophagy using exogenous spermidine. Resveratrol gave similar results in nematodes [18]. However, the mechanism of action is not the same for both substances: while resveratrol action is caused by the activation of the deacetylase activity of sirtuin 1, spermidine inhibits the general histone acetylase activity in yeast.

The most important polyamines are putrescine, spermidine and spermine. In mice, rich polyamine food and rapamycin increase lifespan [19, 20]. In the same year, Eisenberg *et al.* used spermidine to stimulate autophagy and extend lifespan in yeast, flies and worms [15].

Spermidine had a positive effect over nematodes, reducing α-synuclein-induced loss of dopaminergic neurons. In the same study, exogenous administration of spermidine to flies inhibited early death over heterologous expression of human α-synuclein [21].

In a recent study, spermidine is considered a novel therapeutic agent for airway diseases (asthma and chronic obstructive pulmonary disease – COPD), based on the agonism of T-cell protein tyrosine phosphatase (TCPTP) [22].

A quite recent study supports the idea that autophagy might be useful in vascular disease prevention by stimulating cholesterol efflux, which leads to inhibition of necrotic core formation and lipid accumulation [23].

Several authors consider that autophagy is potentially implicated in acute lung injury and sepsis, two main causes of morbidity and mortality worldwide [24]. Acute lung injury might be produced in case of hyperoxia treatments or mechanical ventilation. Hyperoxia causes reactive oxygen species (ROS) production, a condition that can be replicated in a mice acute lung injury model, with long exposure to >95% oxygen [25]. Tanaka *et al.* reported that exposure to hyperoxia will cause, *in vivo*, high values of biochemical and histological markers of autophagy, which will lead to LC3B-II accumulation and will ignite the autophagy pathway [26]. The same authors have shown that *in vitro* cultured pulmonary epithelial cells, the main target in acute lung injury, are affected by hyperoxia, followed by LC3B activation [26].

In another study, Lee *et al.* (2014) found out that LC3 expression is increased and autophagy is stimulated in lung tissue of a mouse animal model for sepsis achieved by cecal ligation and puncture. In this study, carbon monoxide, a candidate for anti-inflammatory therapy, was used as a therapeutic agent. Treatment effectiveness was assessed by monitoring Beclin 1 expression. The authors concluded that Beclin 1 contributes to sepsis survival and bacterial clearance [27].

Autophagy influences innate and adaptive immunity functions, helps host defense against bacteria, viruses parasites and has more demonstrated anti-pathogenic functions [28–31].

Genetic deficiencies in autophagy molecular pathway are implicated in several diseases: Atg9 in the case of *Legionella pneumophila* and Atg7 in the case of *Klebsiella pneumoniae* [32, 33].

COPD is a pulmonary disease with clinical phenotypes of emphysema and bronchitis. The pathogenesis is not completely elucidated, but smoking is considered an important risk factor [34, 35].

Chen *et al.* investigated autophagy markers in human lung tissue of patients suffering from COPD. They discovered that there is an elevation of autophagy markers, including LC3-II and other autophagy-related (Atg) proteins and also there is an increased density of autophagosomes *in situ*. Also, *in vitro* epithelial cells responded with LC3B-II elevation and increased autophagosomes formation to cigarette smoke [36].

Several authors claim that there is a connection between loss of Atg5 and the presence of benign adenomas in liver, but the presence of Atg5 has not been confirmed in other tissues, yet [37]. The authors suggest that a cause for this benign tumor to progress to cancer would be a defect in autophagy, which is necessary for tumor progression. There are studies which confirm that both mammary and pancreatic cancer are dependent on autophagy for tumorigenesis [38].

Menzies *et al.* (2011) suggested that autophagy is implicated in neuroprotection, in Alzheimer's and Huntington's disease animal models [39]. Parkinson's disease pathogenesis is connected with mitochondrial autophagy in other studies [40, 41].

A neurodegenerative disease, in which iron is accumulated in basal ganglia, SENDA (static encephalopathy of childhood with neurodegeneration in adulthood) could be related to autophagy blocking leading to ferritin accumulation at the autophagosome formation site [42, 43]. A defect in the autophagy gene WDR45/WIPI4 is considered the cause of this disease [44–46]. De Domenico *et al.* have suggested that an iron deficit could stimulate autophagy [47] (Figure 1).

Also, hepatic C virus (HCV) replication is conditioned by autophagy. Therefore, inhibition of autophagy may lead to a suppression of HCV replication and could be a novel therapeutic target [48–52]. Autophagy has a negative effect on the antiviral immunity in case of HCV infection. A study confirms that Atg5 was present in the HCV-induced membranous web, so autophagy is considered to have a proviral contribution in HCV replication complex formation. Another study confirmed that autophagic membranes might be used as viral RNA replication site as viral RNA was present in the same place with the autophagosome. Autophagy mechanism, with a modified expression of a protease inactive mutant ATG4BC47A, also contributes to the survival of the HCV-infected cells, although cellular stress induced by viral replication also

exists [53–55]. Vescovo *et al.* (2012) obtained biopsies from HCV-infected patients and discovered that there is an inverse correlation between autophagy activation and liver steatosis level, and concluded that redundant lipids could be eliminated by autophagic mechanisms [52, 56].

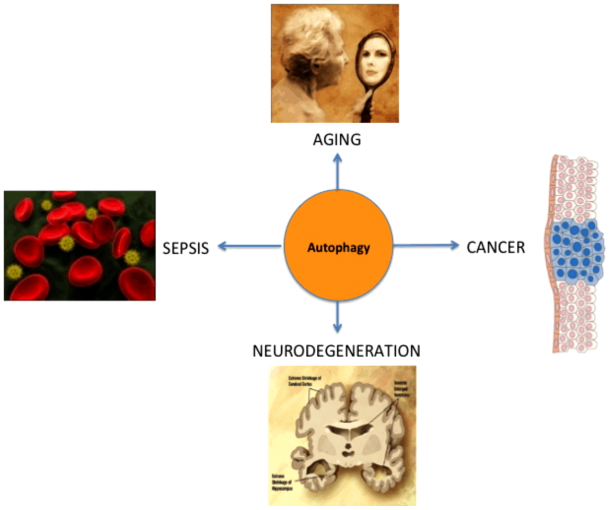

Figure 1 – ***Autophagy has been implicated in many others normal and pathological states, including immunity, diabetes mellitus, different kind of tumors, colorectal cancer, different inflammations, lung diseases, neurodegenerative diseases.***

Autophagy and ubiquitin-proteasome systems activation contribute to muscle loss, but at the same time, myofibers are protected by autophagosomes, which eliminate the damaged organelles or proteins sequestered in the vesicles [57].

Knocked-out mice for autophagy genes Atg5 or Atg7 suffer of muscular atrophy [58, 59]. Nair & Klionsky, in their study, concluded that during physically exercise autophagy activity is necessary to eliminate affected mitochondria and degenerated muscle fibers [59].

Molecular mechanisms underlying regulation of autophagy

Autophagy is negatively regulated by the mTOR (mechanistic target of rapamycin) pathway and cellular functions such as translation and cell growth are mediated by the serine/threonine protein kinase mTOR. The pathway consists of two functional complexes: mTORC1 and mTORC2, each one of them including several subunits [60].

In some studies, a reduced mTORC1 activity and a diminished autophagy led to a delayed onset of aging. A study done on human diploid fibroblasts suggests that some autophagy related genes are upregulated in sense-cence, especially ULK3 [61]. Likewise, other studies using human and rodent cell lines emphasized that rapamycin, one of the mTORC1 inhibitors, also delayed the onset of aging. The authors suggest that during the cell cycle arrest, irreversible arrest was transformed into a reversible condition by rapamycin, a condition that allows for pharmacological modulation of senescence [62].

The autophagic mechanism consists of several steps: the first step is the formation of the isolation membrane named phagophore at the preautophagosomal site (PAS). In the second step, the phagophore is elongated and the third step includes autophagosome maturation and confi-nement of the cytosolic content. Lysosome fusion with a mature autophagosome forms a single membrane compart-ment named autolysosome. The last step is digestion and is performed by lysosomal proteases and other degradative enzymes [24, 63–67].

Atg proteins, that are well described in yeast, are responsible for autophagy regulation. In mammals, their homologues are the Atg-related proteins, which are responsible for autophagy regulation [68, 69].

Conclusions and future perspectives

Autophagy is present at a basal level in all mammals and is regulated by some conditions, such as oxidative stress, starvation or hypoxia. Studies done on human diploid fibroblasts suggest that autophagy related genes are upregulated in senescence. Likewise, it has been shown that the lifespan of yeast, nematodes and flies is prolonged by pharmacologically stimulated autophagy using exogenous spermidine. Since autophagy is also implicated in neuro-protection in Alzheimer's and Huntington's disease animal models and many others normal and pathological states, including immunity, diabetes mellitus, different kind of tumors, colorectal cancer, different inflammations, lung diseases, neurodegenerative diseases, autophagy is of interest to many biomedical researchers.

Conflict of interests

The authors declare that they have no conflict of interests.

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Corresponding author
Aurel Popa-Wagner, Professor, PhD, Chair of Vascular Neurology and Dementia, University of Medicine Essen, Hufelandstraße 55, 45147 Essen, Germany; Phone 0201–723–2180, e-mail: aurel.popa@uk-essen.de

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