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Review

Autophagy: A cell repair mechanism that retards ageing and age-associated diseases and can be intensified pharmacologically

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Abstract

The process of ageing denotes a post-maturational deterioration of cells and organisms with the passage of time, an increased vulnerability to challenges and prevalence of age-associated diseases, and a decreased ability to survive. Causes may be found in an enhanced production of reactive oxygen species (ROS) and oxidative damage and not completed housekeeping, with an accumulation of altered ROS-hypergenerating organelles in older cells. It has been shown that autophagy is the only tier of defence against the accumulation of effete mitochondria and peroxisomes; that functioning of autophagy declines with increasing age and determinates cell and individual lifespan; that autophagy can be intensified by drugs; and that the pharmacological intensification of autophagy may be a big step towards retardation of ageing and prevention and therapy of age-associated diseases including neurodegeneration.

Keywords: Ageing; Anti-ageing therapy; Autophagy; Cancer; Neurodegenerative diseases; Oxidative stress; ROS

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Contents

1. Introduction and overview

Process of ageing denotes a post-maturational deterioration of cells and organisms with the passage of time, that underlies an increased vulnerability to challenges and a decreased ability to survive (Masoro, 1999). The issue wether biological ageing is distinct from age-associated diseases is still a matter of debate (Holliday, 2004; Hayflick, 2004). The author's opinion is that recent advancements in Biogerontology may support the hypothesis that all existing living beings are affected by ageing, a fatal inherited disease (''senectus ipsa morbus est'') whose incubation period is long enough to enable patients to reproduce successfully. In this comprehensive view, ageing is a disease, not a risk factor for disability and illness, and age-associated diseases like atherosclerosis, tumours and neurodegeneration should rather be seen like signs or complications of the underlying fundamental disease, and may share its mechanism(s) at least in part.

There is a general consensus on the fundamental mechanisms of ageing: cell molecules and substructures are subject to a variety of destructive forces that originate in both the internal and external environments of an organism, and lesions may accumulate in cells and tissues. At the molecular level, causes may be found in oxidative damage and not completed housekeeping. Both factors are responsible for the progressive senescence-associated accumulation of deleterious alterations of macromolecules and organelles, responsible for cell misfunctioning, starting from young–adult age (Weindruch and Sohal, 1997).

In regard to oxidative damage, the hypothesis is that as much as 1–2% of the used oxygen molecules might generate reactive oxygen species (ROS), endogenously produced by mitochondria and peroxisomes, which may hit and alter DNA, protein, cell membranes and organelles as well as extracellular components (Weindruch and Sohal, 1997). At an older age, accumulation of altered mitochondria and peroxisomes may boost the yield of ROS per unit of produced energy (see Bergamini et al., 2004). The effects of ROS are not counteracted fully by the antioxidant defenses in the hydrophylic and in the lipophylic compartments of the cell. Level of oxidative stress cannot be modulated at will by the use of antioxidants because signal is used to monitor the rate of cell metabolism, in order to adjust cell functions and blood supply. Thus, a long-term administration of excess antioxidants may have severe unwanted effects (Yu et al., 1998).

Failure in housekeeping (inadequate ability to repair or degrade altered macromolecules, cell membranes and organelles and replace them with new) appears to

be the concause of ageing, and the stimulation of turnover of altered proteins, membranes and organelles (e.g., by caloric restriction (Yu, 1995) and physical exercise (Holloszy and Kohrt, 1995) or drugs (Bergamini et al., 2003; Berger et al., 2006)) may be a remedy and a part in the mechanism of antiageing interventions. Table 1 shows mechanisms responsible for cell maintenance. In summary, repair at the molecular level can treat any type of DNA, protein and lipid damage; autophagy and lysosomal degradation regulate the turnover rate of macromolecules and subcellular structures, and also help maintenance of cell in case of failure of molecules repair; in mammals, targeting to lysosomal degradation provides an additional degradative pattern of altered proteins and aggregates, possibly endowed with anti-ageing effects, whose function may decline with ageing (Kaushik and Cuervo, 2006); macroautophagy may also recognize and dispose altered organelles quite selectively (Donati et al., 2006); failure of repair at the molecular and subcellular level may trigger apoptosis (Boya et al., 2005), the last tier of defence against the accumulation of irreversibly altered cells in the body. All cells in the body must die, including cardiac and muscle cells and neurons; time-lag before cell death may be influenced by several factors and eventually might be determinated by the time of autophagy failure (Table 2). Enhanced apoptosis may precipitate cardiac, muscle and neurodegenerative age-associated diseases.

In this issue, attention will be focused on macroautophagy, the physiological mechanism that controls cell nutrition and volume and adapts cell composition to a changing environment (Stevens and Lowe, 2000); mechanism also protects cells from the age-related accumulation of altered proteins, membranes and organelles (Stevens and Lowe, 2000; see also Terman et al., 2006). Macroautophagy was said to be the putative mechanism of the anti-ageing action of caloric restriction (Bergamini and Gori, 1995; see also Donati, 2006). Macroautophagy is a highly conserved degradation/recycling system ubiquitous in eukaryotic cells, in which the cytoplasm, including excess or aberrant organelles, is sequestered into double-membrane vesicles and delivered to the degradative organelle, the lysosome/vacuole, for breakdown and

Table 1
Anti-ageing cell repair mechanisms

Mechanism	Decline with ageing	Prevention by antiageing intervention
Molecular level		
DNA repair	Not proved	Not proved
Protein repair		
- Stress proteins	Yes	Yes
- Proteasomal proteolysis	Yes	Yes
- Lysosomal proteolysis	Yes	Not proved
Membrane lipid repair	Not proved	PUFA supplementation?
Subcellular level		
Autophagy	Yes	Yes (caloric restriction) Intensified by antilipolytic drugs and rapamycin
Cell and tissue level		
Apoptosis	Yes	Yes (same as autophagy)

Table 2
Factors that may affect time of neurodegeneration and neuron loss in Alzheimer Disease (AD)

I. *Amyloidogenesis*
1. Intracellular production of amyloidogenic peptides by selective (γ-secretase-catalyzed) cleavage of altered APP molecules
2. Secretion of amyloidogenic peptides
3. Degradation of Aβ through regulation of matrix metalloproteases 2 and 3 (MMP-2 and MMP-3)
4. Metal (Cu, Zn, Fe) concentration and formation of amyloid fibrils

II. *Inflammation*
5. Production of ROS by amyloid–metal complexes, which may start inflammation
6. Stimulation of microglia may enhance production of ROS. Polymorphism of cytokines may modulate process

III. *Cell injury*
7. Increase in oxidative stress, which leads to protein, lipid and membrane alteration
8. Early tau lesions in the somatodendritic compartment

IV. *Failure of quality control*
9. Levels of molecular chaperones and ubiquitin E3 ligase
10. Proteasome function
11. Functioning of macroautophagic proteolysis
12. Apoptosis of irreversibly injured neurons

Notes: Many AD researchers embraced the amyloid-cascade hypothesis, which states that beta-amyloid (Aβ) is the trigger for all cases of AD and that the tau pathology and other degenerative changes are a downstream consequence of the Aβ pathology (Hardy and Selkoe, 2002). Based on this hypothesis, many different factors may affect time of neurodegeneration and development of clinical symptoms. Membrane alteration (e.g., an alteration in the antioxidant machinery of membranes – see Bergamini et al., 2004) or mutation of APP gene may increase the frequency of post-translational modification of APP at some site and increase yield of Aβ1–42 by APP cleavage and accelerate neuropathology. (Dolichol and ubiquinone are part in the antioxidant machinery and involvement might perhaps account for the beneficial effects of statins). Emerging evidence indicates that intraneuronal Aβ also plays an early pathophysiological role in AD (Billings et al., 2005), and serves as a source for some of the extracellular amyloid deposits (Oddo et al., 2006). The secreted Aβ may be rapidly degraded or not by matrix MMP-2 and MMP-3 (White et al., 2006). Neocortical Aβ binds Cu^{2+} with very high affinity, forming a redox-active complex that catalyzes H_2O_2 production from O_2 (Puglielli et al., 2005). The generation of the Aβ toxic species is modulated by the Cu^{2+} concentration and the ability to form an intermolecular His bridge, and toxicity correlates with lipid peroxidation and dityrosine formation.(Smith et al., 2006). Inflammation is a critical component of the pathogenesis of AD. Although not an initiator of this disorder, inflammation nonetheless plays a pivotal role as a driving force that can modulate the neuropathology (Kitazawa et al., 2005). Accumulation and aggregation of Aβ peptide in the brain may activate glial cells which, in turn, initiate neuroinflammatory responses that involve reactive oxygen intermediates and release of inflammatory cytokines (Cheng et al., in press). Sustained brain inflammation might have deleterious effect through the activation of microglia, secretion of many pro-inflammatory cytokines, and generation of oxidative products and can be an essential cofactor in AD and other neurodegenerative disorders such as Parkinson disease, dementia with Lewy bodies, Huntington's and prion diseases. (Bonifati and Kishore, in press). The quality-control machinery in neuron might play an important role in retarding the pathogenesis of tauopathy and neuron death. For instance, an increase in heat-shock cognate (Hsc)70-interacting protein may protect against NFT formation in the early stages of AD (Sahara et al., 2005); several studies implicate the proteasome as a major factor in the clearance of early tau lesions (Oddo et al., 2004); stimulation of macroautophagy may help to clear accumulated aggresomes and retard apoptosis (Ravikumar and Rubinsztein, 2006).

eventual recycling (Wang and Klionsky, 2003). Process generates nutrients during fasting under the control of amino acids and pancreatic hormones (Kanazawa et al., 2004; Miotto and Kadowaki, this issue) and gives place to resynthesis, turnover and rejuvenation of cellular components (long-lived proteins, cytomembranes and organelles) (Bergamini et al., 2004; see also Donati, 2006). This process has an important role in various biological events such as adaptation to changing environmental conditions and cellular remodeling during development and differentiation (Yorimitsu and Klionsky, 2005). Autophagic degradation has an important role in physiology of the nervous system, heart and muscle (Eskelinen, 2006). Autophagy also samples proteins in cell (viral, tumour and autoantigens) and generates peptides which are then presented on major histocompatibility complex (MHC) class II (Munz, 2006; Schmid et al., 2006). Thus autophagy represents a previously unrecognized immune mechanism that may act against cells with intracellular microbes (Deretic, 2005) and perhaps against cells (e.g., cancer cells) exhibiting abnormal protein synthesis and composition.

It has been known for decades that autophagy regulates mitochondria (Pfeifer, 1978) and peroxisome turnover (Veenhuis et al., 1983; Locci Cubeddu et al., 1985; for recent advancements, see Monastyrska and Klionsky, 2006). Very recent evidence shows that macroautophagy recognizes and eliminates older or altered ROS-hypergenerating organelles quite selectively (Gu et al., 2004; Donati et al., 2006). The molecular identity of the "opsonizing" mechanism is not known yet (Klionsky, 2005) but perhaps ARF might be in the game (Reef et al., 2006). The accumulation of altered protein (Yamamoto et al., 2006) and any type of cell injury, including ROS (Moore, 2004), ischemic (Hamacher-Brady et al., in press) and mechanic (Diskin et al., 2005) injuries can ignite a nutrition-independent m-TOR-independent macroautophagy (see Meijer and Codogno, 2006). These two types (nutrition- and injury-dependent) of activation might interact each-other to potentiate protection.

Signalling and autophagy regulation have been investigated and clarified (Meijer and Codogno, 2006) and may undergo progressive deterioration with increasing age, both in vitro (Cavallini et al., 2001) and in vivo (Del Roso et al., 2003). The age-related decline in functioning of autophagy might account for the age-dependency of many age-associated diseases in ad libitum-fed animals, including neurodegeneration (Boland and Nixon, 2006), and for retardation by caloric restriction (Donati, 2006) and by the pharmacological intensification of autophagic degradation (Donati et al., 2004; Ravikumar and Rubinsztein, 2006).

2. Conclusions

There may be support to the hypothesis that during evolution, in order to promote rapid growth and successful reproduction, Nature tuned down autophagic degradation too much and function of cell maintenance suffered and determinated lifespan. A dramatically increasing number of scientific contributions is being published showing that autophagy is primarily a pro-survival mechanism (Levine and

Yuan, 2005). Currently available data show that the pharmacological intensification of the process of autophagic degradation may be a big step towards retardation of ageing and prevention and therapy of neurodegenerative diseases.

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