**Review**

**Autophagy in cardiac myocyte homeostasis, aging, and pathology**

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**Abstract**

Autophagy, an intralysosomal degradation of cells’ own constituents that includes macro-, micro-, and chaperone-mediated autophagy, plays an important role in the renewal of cardiac myocytes. This cell type is represented by long-lived postmitotic cells with very poor (if any) replacement through differentiation of stem cells. Macroautophagy, the most universal form of autophagy, is responsible for the degradation of various macromolecules and organelles including mitochondria and is activated in response to stress, promoting cell survival. This process is also involved in programmed cell death when injury is irreversible. Even under normal conditions, autophagy is somewhat imperfect, underlying gradual accumulation of defective mitochondria and lipofuscin granules within aging cardiac myocytes. Autophagy is involved in the most important cardiac pathologies including myocardial hypertrophy, cardiomyopathies, and ischemic heart disease, a fact that has led to increased attention to this process.

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1. **Introduction**

Life is associated with a continuous renewal of biological structures ensuring that worn-out and damaged components of the organisms are removed and replaced by newly synthesized ones. Cellular components are turned over by several complementary degradation systems. Many cytoplasmic and nuclear proteins, mainly short-lived ones, are cleaved by calcium-dependent neutral proteases, calpains [1], and by multicatalytic proteinase complexes, proteasomes [2]. Mitochondria possess their own proteolytic systems represented by matrix Lon [3] and membrane-bound AAA [4] proteases, allowing them to autonomously replace certain defective proteins. Most long-lived proteins, many other macromolecules, biological membranes and whole organelles, including mitochondria, ribosomes, endoplasmic reticulum and peroxisomes, are turned over through autophagy (also called autophagocytosis), that is, an intralysosomal degradation of the cells’ own constituents [5–7]. Irreversibly damaged, diseased and redundant cells can initiate a self-killing program in the form of apoptotic or autophagic cell death, followed by phagocytosis of cellular remains by neighboring similar cells, macrophages or other professional scavengers [8–10].

Cardiac myocytes, as well as neurons, have long been considered terminally differentiated cells that cannot be replaced, unlike intestinal epithelium, epidermal keratinocytes or mature blood cells, which are constantly renewed owing to division and differentiation of stem cells. However, recent evidences showing that both adult heart muscle and brain contain multipotent stem cells that can differentiate into mature myocytes and neurons, especially in response to injury [11], seem to partially invalidate this dogma. Nevertheless, the replacement of cardiac myocytes and neurons under normal conditions is an imperfect and slow process and, thus, they can be called long-lived postmitotic cells to distinguish them from mature intestinal, epidermal and blood cells, which are also postmitotic, although short-lived.
The slow and incomplete replacement of cardiac myocytes through stem cell differentiation makes intracellular renewal of particular importance for the maintenance of heart function. Here we review the role of autophagy, the most universal mechanism involved in the renewal of cellular constituents, in cardiac myocyte homeostasis, response to stress, programmed cell death, aging, and disease.

2. General characteristics of autophagy

Autophagy is an evolutionary conserved pathway of self-digestion that occurs in various eukaryotic organisms from yeast to mammals [5]. Depending on how intracellular material is delivered to lysosomes, three basic types of autophagy are recognized in mammalian cells: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA).

In macroautophagy, portions of cytoplasm are first isolated (sequestered) within a double membrane enclosed vacuole called autophagosome. In studies on yeast, the isolation membrane has been shown to develop from a small vesicle that later transforms into a cup-like structure surrounding the material to be degraded [5,6,12]. The formation of an autophagosome (sequestration) is complete when the edges of the “cup” merge. It has been found that the proteins forming the isolation membrane in yeast are unique, different from those in other cellular compartments, which suggests a de novo formation of this membrane [13]. It is still discussed whether the isolation membrane also forms de novo in higher organisms, including mammals, or if it originates from another organelle, such as endoplasmic reticulum, lysosome or Golgi complex [12]. The sequestration membrane that later gives rise to the autophagosome has been termed phagophore [12], or pre-autophagosome [14].

The autophagocytosed material undergoes degradation by a large spectrum of lysosomal enzymes (acid hydrolases), which the autophagosomes receive when they fuse with lysosomes or late endosomes [12]. Consequently, the autophagic and endocytic pathways merge and combine. Late endosomes, in turn, develop from early endosomes following their acidification and enrichment with newly synthesized lysosomal enzymes, which are transported to mannose-6-phosphate receptors (MPR) within small vesicles (occasionally called “primary lysosomes”) that pinch off from the trans-Golgi network (TGN) [12,15]. The acidic pH of late endosomes, created by the activity of vacuolar ATPase, triggers the release of lysosomal enzymes into the lumen, while MPR are recycled back to TGN. Mature lysosomes differ from late endosomes by an even lower pH and the absence of MPR [12,15]. It has been suggested that autophagosomes, like late endosomes, also may receive acid hydrolases directly from TGN through vesicular transport [16]. The implication is that intralysosomal material is propagated through the vacuolar compartment, which may be considered a box, although in any moment divided up in a number of vacuoles that receive hydrolytic enzymes from TGN and materials to be degraded from the outside (endocytosis) or inside (autophagy) of the cell. Vacuoles that form as a result of fusion between autophagosomes and late endosomes are called “amphisomes” [12], while the structures developing from autophagosomes fusing with lysosomes are termed “autophagolysosomes” [17]. All vacuoles that contain autophagocytosed material including autophagosomes and autophagolysosomes are commonly called “autophagic vacuoles”. The term “secondary lysosome” refers to any lysosome containing material under degradation, either endocytosed, or autophagocytosed [17].

Sequestration is not required for microautophagy, in which small components of cytoplasm enter lysosomes through invaginations of the limiting membrane, often forming multiple intralysosomal vesicles [6]. The cargo for microautophagy may include various macromolecules or small organelles [6].

The study of molecular mechanisms involved in macro- and microautophagy dramatically benefited from the analysis of yeast mutants. This approach appeared particularly fruitful for molecular dissection of macroautophagy, allowing identification of several dozens of proteins responsible for different steps of the process including induction, cargo packaging, pre-autophagosome nucleation, expansion and completion (resulting in autophagosome formation), retrieval of proteins, targeting and docking of the autophagosome and its fusion with the vacuole (yeast lysosome homolog), and the breakdown of the inner autophagosome membrane in the vacuole (extensively reviewed in [5]). According to a new nomenclature, proteins involved in autophagic function are designated as Atg proteins, such as Atg1, Atg8, etc. [5]. Orthologs of many autophagy-related proteins and corresponding genes (ATG genes) have been determined in mammals, as well as in fruit flies and worms [5].

Two ubiquitin-like systems, which include Atg12 and Atg8 ubiquitin-like proteins, respectively, have been found to play a key role in autophagosome formation [18]. Atg7, a homolog of the E1 enzyme, activates both Atg12 and Atg8 (the latter is first cleaved by a cysteine protease Atg4). Atg12 conjugates with Atg5, and the conjugate binds to Atg16 resulting in the formation of a complex needed for conjugation of activated Atg8 with phosphatidylethanolamine. Atg10 and Atg3, homologs of the E2 enzyme, trigger conjugation events (extensively reviewed in [5,18]). LC3, a mammalian homolog of yeast Atg8, localizes to mammalian autophagosomes after processing to LC3-II [19]. Along with quantitative electron microscopy, visualization of LC3-II (using immunocytochemistry or green fluorescent protein labeling) is a helpful tool for macroautophagy evaluation [19].

In CMA, particular cytosolic proteins are selectively transported to lysosomes by molecular chaperones [6]. All
proteins intended for CMA contain a specific sequence of five amino acids, designated as KFERQ (a single letter amino acid abbreviation for lysine–phenylalanine–glutamate–arginine–glutamine). A heat-shock type chaperone protein of 73 kDa recognizes this sequence; the substrate-chaperone complex binds to the lysosomal-associated membrane protein type 2a (LAMP-2a) and then enters the lysosomal lumen [6]. The principal pathways of autophagic degradation in mammalian cells are schematically presented in Fig. 1.

Previously, it was suggested that proteins associated with different cellular compartments have equal chances to be sequestered for macroautophagy, indicating a random process [20]. Later evidences suggest, however, that not only CMA, but also macro- and microautophagy can be selective. For example, peroxisomes can undergo non-random macro- and micropexophagy [6], while selective mitochondrial degradation, or mitophagy [21], occurs in cells undergoing programmed autophagic death (see below). Recently, a mitochondrial outer membrane protein, Uth1p, has been found essential for autophagy of these organelles in yeast [22]. Some studies suggest that mitochondria can be tagged by ubiquitin for degradation, such as those in degenerating insect flight muscles and sperm mitochondria in fertilized oocytes (reviewed in [23]). It is not known whether ubiquitination plays any role in other cases of selective organelle degradation.

3. Regulation of autophagy and adaptive response to stress

Autophagy is a nonstop life-sustaining renewal process, which is active under normal conditions and can be further stimulated by different stressors. Such stressors are usually associated either with increased damage to cellular components, requiring repair (reparative autophagy), or with a request for essential end products of lysosomal degradation, as it occurs under starvation, when cells partially digest themselves to generate energy and provide their anabolic machinery with new building blocks.

Regulation of macroautophagy (later referred to as “autophagy” if not otherwise stated) has been more extensively studied and is better understood than that of microautophagy and CMA. A protein kinase called Tor (for the “target of rapamycin”, an antibiotic isolated from Streptomyces hygroscopicus) is believed to play a central role in the intracellular control of the autophagic pathway [7]. Tor orthologs occur in all eukaryotes; the mammalian Tor being designated as mTor. Activation of Tor, for example by nutrients, induces phosphorylation of Atg1 and Atg13 (proteins involved in the induction step of autophagy), resulting in their disassembling and suppression of autophagy, while nutrient deprivation and rapamycin down-regulate Tor and therefore induce autophagy [5–7]. In addition, Tor is involved in the

Fig. 1. Macroautophagy, microautophagy and chaperone-mediated autophagy are the principal lysosomal degradation pathways in mammalian cells. Black arrows show evolution of structures, while gray double-ended arrows indicate fusion. Lysosomes differ from late endosomes by the absence of mannose phosphate receptors. Autophagosomes form autophagolysosomes by fusion either with late endosomes or lysosomes. Detailed explanations and references are given in Section 2.
regulation of transcription of certain autophagy-related proteins [5–7]. Some cellular regulatory factors seem to work as “buffers”, preventing excessive activation or suppression of autophagy through the Tor pathway. For instance, Tor stimulation, known to inhibit autophagy, has been found to up-regulate p70S6 and eIF2α kinases, as well as Gen2 (the yeast homolog of mammalian eIF2α) kinase, all of which, in turn, induce autophagy [5,6]. A number of additional proteins, such as Snf1, proteinkinases A and B, GTPases, Notch and Ras have been also shown to act as regulators of autophagy, although their relation to the TOR pathway is not well understood (reviewed in [5–7]). Induction of autophagy requires activation of the enzyme class III phosphatidylinositol-3-kinase (PI3K), which allows the use of inhibitors of this enzyme, such as 3-methyladenine (3MA), wortmannin and LY294002, for suppression of autophagic sequestration [24].

Some hormones, first of all insulin and glucagon, are important players in nutrient-related regulation of autophagy in vivo. In nutrient-rich conditions, the activation of insulin receptor turns on the enzyme class I PI3K (different from class III PI3K, involved in sequestration), which modifies the lipid phosphatidylinositol in the plasma membrane and, consequently, through the activation of several intermediate enzymes, results in up-regulation of Tor and inhibition of autophagy (reviewed in [5–7]). Glucagon, an antagonist of insulin, which activates autophagy in hepatocytes, represses Tor signaling through a protein kinase A-related mechanism [25].

Autophagic response of cells to nutrients may also be unrelated to hormones. For example, stimulation of autophagy by amino acid deprivation may occur independently of insulin and, in particular, of the Tor pathway [26]. Consistent with activation of autophagy by withdrawal of glucose, the latter has been found to stimulate Tor in vitro [27], suggesting that glucose regulation of autophagy may not depend on insulin either. Furthermore, Tor is reported to act as an ATP sensor [28], which may also explain why glucose starvation induces autophagy, considering that the level of glucose is an important determinant of cellular energy status. However, due to the fact that the autophagic pathway is energy dependent, excessive ATP depletion by itself would suppress autophagy [20]. In cultured cells, autophagy is usually activated by growth factor withdrawal, which, as was recently reported, occurs due to the fact that growth factors are needed for normal uptake of nutrients [29].

Reparative autophagy is activated by various types of injury including those induced by oxidative stress, ionizing radiation, bacterial toxins, or chemical agents [21,30,31]. The molecular mechanisms involved in the activation of autophagic response to injury still remain to be investigated. Recent evidences, however, suggest that the effects of many damaging agents on autophagy may be mediated by ceramide that is released as a result of membrane damage [32]. Ceramide is cleaved by ceramidases to sphingosine, which is a lysosomotropic detergent that may cause lysosomal rupture and apoptosis [33].

Hormones other than insulin and glucagon are also known to influence autophagy. Insulin-like growth factor 1 (IGF-1) acts similarly to insulin, while growth hormone may inhibit autophagy by stimulating secretion of IGF-1 [34]. Autophagy has also been found induced by hydrocortisone [35] and corticotropin [36] and suppressed by epinephrine and other adrenergic agonists [20]. Many hormonal effects on autophagy are poorly understood and may often represent secondary lysosomal responses to initial metabolic changes induced by hormones.

The presence of mTor, p70S6, eIF2α and other autophagy-related proteins in cardiac myocytes [37] might suggest that autophagic processes in the heart apparently share key regulatory mechanisms with other mammalian tissues. Variations, however, exist, first of all, in autophagic response to hormones that may have different modes of action in different tissues. For example, glucagon and beta-adrenergic agents, in contrast to their effects on hepatocytes, inhibit autophagy in myocardial and skeletal muscle cells, while insulin hinders autophagic degradation in all these tissues [38]. Thyroid hormones usually suppress degradation in cardiac myocytes, inducing myocardial hypertrophy, but increase catabolism in skeletal muscles [39]. These results point to the need of further studies on the regulation of autophagy specifically in cardiac myocytes.

Microautophagy in hepatocytes is a constitutive degradation pathway that, unlike macroautophagy, has been found tolerant to nutrient deprivation. Its activity, however, decreased under prolonged starvation in parallel with an overall decline of basal protein turnover [38]. In cardiac myocytes, microautophagy (assessed using electron microscopy) was not apparent under normal conditions and short-term starvation, but was activated after prolonged starvation [40].

Starvation also stimulates CMA that shows only minor activity under normal conditions but becomes obvious a few hours following withdrawal of nutrients, substituting declining macroautophagy as a source of amino acids [6]. Starvation induces CMA mainly in the liver, heart, kidneys and spleen, but not in skeletal muscles [6]. Recently, oxidative stress has been demonstrated to activate CMA in rat hepatocytes and mouse fibroblasts [41]. The increase of CMA is accompanied by up-regulation of LAMP-2a, which binds the substrate-chaperone complex [6,41].

4. Autophagy and cell death

Autophagy is not only important for repair of cellular damages induced by various stressors or genetic defects, but is also involved in the execution of self-killing program of irreversibly injured cells. Programmed cell death (PCD) is essential for elimination of damaged and diseased cells,
including those about to undergo malignant transformation, as well as for tissue remodeling during development, requiring periodical removal of certain groups of cells [8–10,42]. In PCD, cellular components undergo a controlled enzymatic digestion, allowing surrounding tissue to escape excessive damage and consequent inflammation due to release of toxic substances from cells that die in an uncontrolled manner, i.e., through necrosis. Necrotic death, associated with cell swelling and ensuing plasma membrane rupture, occurs when the degree of damage is too high and/or PCD is somehow disturbed. In contrast, cells undergoing PCD preserve their plasma membrane, and cell remains are usually phagocytosed by macrophages [8–10]. When phagocytes are suppressed or not present, as is common in cell culture conditions, cell destruction at end stages of PCD becomes uncontrolled and is called “post-apoptotic necrosis” [43]. In some cases, the progress of PCD can be delayed soon after its induction, for example, when viruses that infect cells encode anti-apoptotic proteins. Such cells develop necrosis-like death, occasionally called “programmed necrosis” [8].

PCD is classified into typical apoptosis (PCD-1) and autophagic cell death (PCD-2), differing by the predominance of caspase activation or autophagy, respectively [8,9,42]. Both types of PCD are described in cardiac myocytes [44–48]. Morphologically, PCD-1 is characterized by chromatin condensation and fragmentation of nucleus and cytoplasm into apoptotic bodies, while PCD-2 is associated with cell shrinkage and formation of multiple autophagic vacuoles. The distinction between PCD-1 and PCD-2 is, however, opaque, since the processes involved in the two types of cell death are often combined and even work in tandem [9,49]. Moreover, even in pure PCD-1 (if this is at all possible) lysosomal enzymes importantly contribute to cell death (see below), suggesting indirect involvement of autophagy in the process.

Caspases, the main determinants of PCD-1, is a family of cytosolic cysteine endopeptidases that are activated either following stimulation of death receptors on the plasma membrane, such as tumor necrosis factor receptors, or in response to internal cell injury, mediated by mitochondrial depolarization and release of cytochrome c, Smac/DIABLO, apoptosis-inducing factor (AIF) and some other regulatory proteins. Signaling from death receptors leads to caspase-8 activation, while the mitochondrial pathway results in the activation of caspase-9. Caspases 8 and 9, called initiation caspases, activate execution caspases such as 3 and 7, which are responsible for the final cleavage of cellular constituents (extensively reviewed in [8–10]). Both receptor-mediated and mitochondrial pathways may be interdependent and converge at the execution stage [8,10]. When caspases are inhibited, injured cells are still able to undergo PCD due to mitochondria-to-nucleus translocation of AIF, causing DNA fragmentation and chromatin condensation [50].

Lysosomal membrane rupture, resulting in the release of acid hydrolases into the cytosol, was observed in various cell types including cardiac myocytes at early stage of PCD-1, often before any mitochondrial alterations [43]. Apparently, lysosomal enzymes, such as cathepsins B and D, induce mitochondrial permeability transition either directly, or by activating phospholipase A2, promoting cytoplasmic translocation of cytochrome c, AIF and other pro-apoptotic agents [51]. Recent studies suggest that lysosomal enzymes can induce mitochondrial permeability transition more specifically, through the cleavage of Bid (cathepsins B, H, L, S and K) or activation of Bax (cathepsin D); in addition, cathepsin B has been reported to directly activate caspases [52,53].

The reason for considering PCD-2 as a distinct form of cell death is that inhibitors of autophagy suppress it, and that neither caspases nor AIF are required for PCD-2 to occur [9]. PCD-2 is usually involved in removal of long-lived postmitotic cells with abundant cytoplasm, such as cardiac myocytes, as well as of large variety of cells during developmental remodeling [42]. In addition, along with PCD-1, it can be responsible for elimination of malignant cells [54,55]. Although it is clear that lysosomal enzymes and not caspases are the main executors of cell digestion during PCD-2, the molecular machinery remains largely unknown. Recently, however, a novel group of regulatory proteins, called death associated protein kinases (DRPk), have been implicated in death receptor-activated autophagic death [56].

Cell death also develops due to prolonged inhibition of autophagy, leading to the impairment of homeostasis associated with excessive accumulation of damaged, non-recycled cellular constituents [45,57]. Cells exposed to inhibitors of autophagy usually die through PCD-1, since the PCD-2 (autophagic) pathway is disabled [45,57]. These in vitro observations underline the role of autophagy in life maintenance and are important for understanding cellular aging (see below).

It should be mentioned that in some cases autophagy contributes to cell death by promoting cell injury, for example, induced by iron-catalyzed oxidative reactions. Cells contain small amounts of “loose” redox-active iron, which can catalyze the formation of the highly reactive hydroxyl radical (HO\(^·\)), or similarly reactive iron-centered (ferryl and perferryl) radicals. These radicals can attack almost all cellular constituents and initiate chain reactions that lead to cell death. Although neither hydrogen peroxide (H\(_2\)O\(_2\)) nor superoxide (O\(_2\)\(^·⁻\)) are particularly reactive per se, in the presence of redox-active iron even low concentrations of these reactive oxygen species (ROS) are highly cytotoxic [23].

It has recently been recognized that the lysosomal compartment, especially in long-lived postmitotic cells such as cardiac myocytes and neurons with a limited uptake of iron from the outside, is the main forming place for redox-active, low mass iron. The reason why low mass iron accumulates intralysosomally is the normal autophagic degradation of many iron-rich particulates, such as ferritin,
and mitochondria [58]. In the case of oxidative stress, with enhanced cellular formation of hydrogen peroxide, high intralysosomal concentration of redox-active iron predisposes lysosomes to oxidant-induced damage and rupture followed by reparative autophagy, PCD, or necrosis, depending on the magnitude of lysosomal damage [43,58].

5. Autophagy and myocardial aging

Although the slow replacement of cardiac myocytes might be advantageous for myocardial function, facilitating sychronic work of many cells composing the heart and the preservation of adaptive changes in the form of hypertrophy, it also makes these cells vulnerable to aging and complicates repair after injury. Even under favorable conditions, degenerative changes gradually advance in the aging heart, suggesting that renewal mechanisms, first of all autophagy, are inherently imperfect. There are strong evidences that age-related changes largely represent the accumulation of macromolecular damage, which is a side-effect of normal mitochondrial respiration, associated with inevitable oxidative stress (reviewed in [23]). The mechanisms involved in age-related accumulation of oxidative macromolecular damage, which is a side-effect of normal mitochondrial respiration, associated with inevitable electron leak [23,59]. Autophagy appears unable to completely remove all damaged structures (so-called biological "garbage" or "waste"), which accumulate extralysosomally and intralysosomally, suggesting insufficiency of autophagic sequestration and degradation, respectively.

Defective mitochondria represent the bulk of extralysosomal "waste" in senescent myocardium. Senescent mitochondria are usually enlarged, often excessively ("giant" mitochondria), showing structural deterioration such as loss of cristae and swelling and being deficient in ATP production (reviewed in [23]). The mechanisms involved in age-related accumulation of defective mitochondria are still somewhat disputed. Initial mitochondrial changes, which appear in early life, may arise from oxidative damage combined with imperfect autophagy, inadequate function of Lon and AAT proteases or insufficient mitochondrial DNA (mtDNA) repair. Later, additional factors can contribute to the progress of mitochondrial damage. It has been repeatedly demonstrated that aging cardiac myocytes can accumulate homoplasmic mtDNA point mutations or deletions, finally resulting in complete substitution of normal mitochondria with mutated ones, apparently because some mitochondrial DNA mutations may facilitate mitochondrial fission [60]. Such a possibility is supported by the facts that even actively proliferating tumor cells, which efficiently dilute damaged structures, accumulate homoplasmic mtDNA mutations [61]. Not excluding enhanced replication of defective mitochondria, it has also been hypothesized that, because of reduced respiration, mitochondria with mutated DNA experience less membrane damage and, therefore, are less targeted to autophagy compared to normal mitochondria [62]. However, because mtDNA mutations occur only in a relatively small proportion of cardiac myocytes (only in one out of seven cells in the aged human myocardium [60]), it is likely that many age-related mitochondrial changes develop independently of any mutations [63].

One possible factor responsible for poor autophagic removal of damaged mitochondria is their enlargement. In a recent observation, a prolonged (12 days) 3MA-induced inhibition of autophagy in cultured neonatal rat cardiac myocytes resulted in a dramatic accumulation of apparently normal small mitochondria, but only in a moderate increase in the number of large, senescent-like mitochondria [45]. These results suggest that small mitochondria are normally turned over with a higher rate than large ones and, therefore, preferentially accumulate following a blockade of autophagy. Under normal conditions, when autophagy is not inhibited but still somewhat imperfect, large mitochondria predominately accumulate with age, apparently because they are less easily autophagocytosed than small mitochondria (we suppose that the formation of a large autophagic vacuole is more energy consuming than that of a small one, or that autophagosomes by some other reason cannot exceed a certain size). Mitochondrial enlargement may result from impaired fission (for example, due to initial oxidative damage to mitochondrial components), as supported by decreased DNA synthesis in large mitochondria [45]. A tentative mechanism behind age-related accumulation of large ("giant") mitochondria is presented in our "bottleneck" model, symbolizing a non-random turnover of mitochondria of different sizes (Fig. 2).

Finally, decreased mitochondrial autophagy may result from age-related disturbance of lysosomal function secondary to the accumulation of intralysosomal "waste" material, lipofuscin, or age pigment [64]. We have shown that the normal presence of redox-active iron within lysosomes, which catalyzes formation of hydroxyl radicals from hydrogen peroxide (see above) that diffuses into the lysosomes or is produced there by autophagocytosed mitochondria, is the driving force behind lipofuscin formation [17]. This non-degradable material forms due to intralysosomal peroxidation of lipid and protein residues and progressively accumulates in cardiac myocytes and other long-lived postmitotic cells [65]. By time lipofuscin accumulates and forms large aggregates that sometimes almost completely fill many lysosomes. Such structures were formerly often called "residual bodies" and considered indolent structures. It has been shown, however, that lipofuscin granules are integrated parts of the lysosomal compartment that fuse with late endosomes and thus receive newly produced acid hydrolases [66]. In agreement of this, lysosomal enzymes within lipofuscin granules have been demonstrated histochemically [67]. Because cross-linked polymeric lipofuscin cannot be degraded by lysosomal hydrolases, the delivery of enzymes to lipofuscin-loaded lysosomes is a complete waste. When lipofuscin-loaded lysosomes within aged postmitotic cells become abundant, these cells end up in a situation when most lytic enzymes are directed to lipofuscin-loaded lysosomes (making up the majority of lysosomes in senescent cells) leaving too little for useful purposes, such as autophagy.
Harmful effects of lipofuscin accumulation are not limited to its inhibitory effect on autophagy. Being rich in heavy metals, particularly in iron, lipofuscin seems to sensitize aged cells to oxidative stress by causing enhanced lysosomal rupture [68]. Mitochondrial damage leads to increased ROS generation and enhanced lipofuscinogenesis, while lipofuscin accumulation promotes accretion of damaged mitochondria by compromising their autophagic turnover. Consequently, postmitotic cells undergo progressive deleterious changes, finally resulting in PCD, as is extensively described in the “mitochondrial–lysosomal axis” theory of aging [64]. In support of this theory, lipofuscin accumulation in cultured cardiac myocytes showed a strong positive correlation with both oxidative
stress and mitochondrial damage [69]. In agreement with the “mitochondrial–lysosomal axis” theory, other components of cardiac myocytes appear much less affected by age. For example, senescent myocytes with numerous defective mitochondria and lipofuscin deposits usually contain apparently intact myofibrils [45]. Clearly, myofibrils experience less pronounced ROS attack than actively respiring mitochondria and besides, not lysosomes but calpains are responsible for their turnover [70]. The role of autophagic malfunction in aging is schematically presented in Fig. 3.

Considering the role of autophagic insufficiency in aging, stimulation of autophagy might become a prospective anti-aging strategy. In favor of this view, caloric restriction, to date the only effective life-extending intervention in mammals, is associated with up-regulation of autophagy [34]. In addition to life extension, caloric restriction has been shown to decrease production of ROS and associated damage in the heart [71].

6. Autophagy and heart disease

Alterations of autophagy occur in various cardiac pathologies, reflecting either compensatory responses of heart muscle cells, or autophagic involvement in PCD. Sometimes, as in lysosomal storage diseases (LSD), cellular damage develops as a result of initial autophagic dysfunction, while in other cases, such as in hemochromatosis, normal autophagic activity is involved in the induction of cell injury (see below).

In cardiac hypertrophy, which develops in hypertension or various heart defects, autophagy decreases, promoting an enhancement of myocardial mass [72]. During regression of hypertrophy (for example, as a result of therapy), autophagy is activated, reflecting increased catabolism [73], while the development of dilated cardiomyopathy and consequent heart failure is associated with enhanced PCD, including both classical apoptotic (PCD-1) and autophagic (PCD-2) cell death [44,74]. Myocardial damage due to intoxication, infection or autoimmune disease (often combined with myocarditis) results in the activation of reparative autophagy, which is followed by PCD if the injury is advanced [46,75]. Autophagy plays an important role in the ischemic injury of the heart, either as a repair process, or as a component of PCD, depending on the degree of myocardial damage [47,76–78].

Cardiomyopathy is a common manifestation of LSD, such as the Farby, Pompe and Danon diseases [79–81]. Cells in LSD are overloaded with lysosomes containing indigested material, which accumulates due to genetic defects of specific lysosomal enzymes or other proteins involved in lysosomal degradation. In comparison with age-related accumulation of lipofuscin, the increase of intralysosomal waste in LSD is more pronounced, resulting in early myocardial degeneration. Disturbed autophagic turnover in LSD is likely to result in the accumulation of defective mitochondria within cardiac myocytes according to the scenario that is described above for senescent lipofuscin-loaded cells. Although a possible involvement of mitochondria has not been looked for in the myocardium of LSD patients, the findings of senescence-like mitochondrial abnormalities in LSD-affected skeletal muscles and brains [82,83] support such a possibility.

Another large group of hereditary cardiomyopathies develop as a result of mutations in mtDNA [84]. In these diseases, often called ‘mitochondrial cardiomyopathies’, heart muscle cells usually contain increased numbers of enlarged and poorly functioning mitochondria [84]. Apparently, the accumulation of large mitochondria may reflect their poor autophagy, as described above for aging cells, although this possibility has never been studied.

Hemochromatosis is a disorder, which is characterized by iron deposition within different tissues, including the myocardium and often resulting in heart failure [85]. Primary hemochromatosis is a hereditary disease associated with increased gastrointestinal absorption of iron, while secondary hemochromatosis is a consequence of dietary iron intoxication, hemolytic anemia, blood transfusions and other conditions leading to iron overload [86]. The cytosolic iron-binding protein, ferritin, normally performs a dual function of iron detoxication and iron reserve. Autophagic degradation of ferritin is believed to play an important role in intracellular iron recycling [87]. In hemochromatosis, the excess of iron is mainly localized to the lysosomal compartment, both in the form of ferritin (which is often fully iron-saturated) and in the form of hemosiderin, an insoluble substance produced due to intralysosomal oxidation of ferritin and other iron-rich proteins [88]. Such iron-loaded lysosomes show increased fragility when exposed to oxidants, for example, to hydrogen peroxide or redox-cycling cytostatics such as doxorubicin, when signs of oxidative stress in the form of reparative autophagy and enhanced intracellular concentrations of toxic aldehydes are found [58,89]. A reasonable explanation for increased sensitivity of hemochromatosis-affected cells to oxidants is that lysosomal degradation of ferritin results in the liberation of substantial amounts of low mass redox-active iron. As was pointed out in Section 4 of this review, the amount of intralysosomal redox-active iron determines lysosomal sensitivity to oxidative stress. Therefore, the increase of intralysosomal low mass iron within cardiac myocytes may induce oxidative stress even at a normal production of hydrogen peroxide, resulting in lysosomal rupture and ensuing cell injury followed by reparative autophagy, PCD or necrosis.

The often rather sudden appearance of heart failure in hemochromatosis [90] may be explained by an increasing degree of ferritin iron-saturation in affected cells. As long as the uptake of iron into myocardial cells is balanced by storing in non-saturated ferritin, the normal autophagy of ferritin [87] results in a temporary reduction of intralysosomal low mass iron by its binding to engulfed non-
saturated ferritin. Of course this buffering effect may disappear when ferritin is degraded, but autophagy of additional non-saturated ferritin will create a steady-state condition. However, if autophagocytosed ferritin is already iron-saturated, as in pronounced iron overload, the situation will change drastically. Then ferritin will no longer be able to bind intralysosomal low mass iron, resulting in the increase of its intralysosomal concentration, creating a situation when lysosomal fragility and sensitivity to oxidative stress may suddenly increase dramatically.

7. Conclusion

For heart muscle cells, which are poorly replaced and experience increased ROS attack as a result of high oxygen consumption, autophagy (including macro-, micro- and chaperone-mediated autophagy) is a critically important life-sustaining mechanism. Marcautophagy (invoking autophagic sequestration and subsequent intralysosomal degradation of sequestered material) is the most universal type of autophagy, providing for turnover of various oxidatively or otherwise damaged macromolecules and organelles, including mitochondria, being the main site of ROS production and the main target of ROS attack. Normal myocardial aging is associated with gradual accumulation of “waste” material such as defective mitochondria and lipofuscin, an intralysosomal indigestible substance, suggesting that both autophagic sequestration and degradation are imperfect to a certain extent. In response to stress, autophagy is compulsorily activated either to meet the increased requirements for repair and detoxication as a result of exposure to various damaging factors, or to produce energy and deliver building blocks for anabolic processes, as it occurs under starvation. Irreversible damage to cardiac myocytes results in the initiation of PCD in the form of classical apoptosis (PCD-1) or autophagic cell death (PCD-2), in which caspases and lysosomal enzymes, respectively, play the main role in cells’ self-digestion. Both types of PCD are often combined and, moreover, autophagy can be indirectly involved in PCD-1, as lysosomal enzymes can activate caspases following destabilization of autophagolysosomes. The involvement of autophagy in myocardial aging and in a wide spectrum of cardiac pathologies including myocardial hypertrophy, cardiomyopathies, ischemic heart disease and hemochromatosis attracts attention to this important process opening new strategies for the management of cardiovascular diseases and development of cardiotoxic drugs.

References

van Empel VP, De Windt LJ. Myocyte hypertrophy and apoptosis: a
Terman A, Dalen H, Eaton JW, Brunk UT. Radiation-induced cell
dermal-mediated macroautophagy involves inhibition of
protein kinase B and up-regulation of beclin 1. J Biol Chem
Kagedal K, Zhao M, Svensson I, Brunk UT. Sphingosine-induced
apoptosis is dependent on lysosomal proteases. Biochem J 2001;359:
335–43.

Bergamini E, Cavallini G, Donati A, Gori Z. The anti-ageing effects of
caloric restriction may involve stimulation of macroautophagy and
lysosomal degradation, and can be intensified pharmacologically.

Kalamidas SA, Kotoulas OB. Studies on the breakdown of glycogen
in the lysosomes: the effects of hydrocortisone. Histol Histopathol

de Waal EJ, Vreeling-Sindelarova H, Schellens JP, James J. Starvation-
induced microautophagic vacuoles in rat myocardial cells. Cell

Kiffin R, Christian C, Knecht E, Cuervo AM. Activation of
chaperone-mediated autophagy during lumen formation in vitro. Proc Natl

Dennis PB, Jaeschke A, Saitoh M, Fowler B, Kozma SC, Thomas G.
Mammalian TOR: a homoeostatic ATP sensor. Science 2001;294:
1102–5.

Growth factor regulation of autophagy and cell survival in the absence

Sandvig K, van Deurs B. Toxin-induced cell lysis: protection by 3-

Persson LH, Kurz T, Eaton JW, Brunk UT. Radiation-induced cell
Scarlattii F, Bauvy C, Venturii A, Sala G, Cluezaud F, Vandewalle A,
et al. Ceramide-mediated macroautophagy involves inhibition of
protein kinase B and up-regulation of beclin 1. J Biol Chem


Brunk UT, Terman A. Lipofuscin: mechanisms of age-related
lysosomal degradation, and can be intensified pharmacologically.

Kalamidas SA, Kotoulas OB. Studies on the breakdown of glycogen
in the lysosomes: the effects of hydrocortisone. Histol Histopathol

Bergamini E, Cavallini G, Donati A, Gori Z. The anti-ageing effects of
caloric restriction may involve stimulation of macroautophagy and
lysosomal degradation, and can be intensified pharmacologically.

Kalamidas SA, Kotoulas OB. Studies on the breakdown of glycogen
in the lysosomes: the effects of hydrocortisone. Histol Histopathol

Bergamini E, Cavallini G, Donati A, Gori Z. The anti-ageing effects of
caloric restriction may involve stimulation of macroautophagy and
lysosomal degradation, and can be intensified pharmacologically.

Kalamidas SA, Kotoulas OB. Studies on the breakdown of glycogen
in the lysosomes: the effects of hydrocortisone. Histol Histopathol

Bergamini E, Cavallini G, Donati A, Gori Z. The anti-ageing effects of
caloric restriction may involve stimulation of macroautophagy and
lysosomal degradation, and can be intensified pharmacologically.

Kalamidas SA, Kotoulas OB. Studies on the breakdown of glycogen
in the lysosomes: the effects of hydrocortisone. Histol Histopathol

Bergamini E, Cavallini G, Donati A, Gori Z. The anti-ageing effects of
caloric restriction may involve stimulation of macroautophagy and
lysosomal degradation, and can be intensified pharmacologically.


