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Is Autophagy in Response to Ischemia and Reperfusion Protective or Detrimental for the Heart?

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Abstract

Autophagy is a catabolic process which degrades long-lived proteins and damaged organelles through sequestration into double membrane structures termed autophagosomes and fusion with lysosomes. Autophagy is active in the heart at baseline and further stimulated under stress conditions, including starvation, ischemia/reperfusion and heart failure. Autophagy plays an adaptive role in the heart at baseline, thereby maintaining cardiac structure and function and inhibiting age-related cardiac abnormalities. Autophagy is activated by ischemia and nutrient starvation in the heart through Sirt1-FoxO and AMPK-dependent mechanisms, respectively. Activation of autophagy during ischemia is essential for cell survival and maintenance of cardiac function. Autophagy is strongly activated in the heart during reperfusion after ischemia. Activation of autophagy during reperfusion could be either protective or detrimental, depending upon the experimental model. However, strong induction of autophagy accompanied by robust upregulation of Beclin1 could cause autophagic cell death, thereby being detrimental. This review provides an overview regarding both protective and detrimental functions of autophagy in the heart and discusses possible applications of current knowledge to the treatment of heart disease.

Introduction

Autophagy is a catabolic process whereby long-lived proteins in the cytosol and organelles are sequestered into double membrane vesicles, termed autophagosomes, and transported to lysosomes for degradation (10, 14, 19). Autophagy occurs at basal conditions and mediates homeostatic functions in cells. However, autophagy is also induced by stress, such as nutrient starvation, hypoxia, endoplasmic reticulum (ER) stress, and oxidative stress (19). During nutrient starvation, autophagy allows cells to recycle extracted amino acids and fatty acids for ATP production via the TCA cycle. Autophagosomes sequester damaged organelles, such as mitochondria, ER and ribosomes, for degradation, thereby preventing release of reactive oxygen species and inducers of cell death (10, 14, 19). Autophagy can also induce cell death in some conditions. This form of cell death is referred to as type II programmed cell death, but may simply be caused by excessive activation of autophagy. For example, strong autophagy promotes depletion of essential proteins and organelles, and is therefore potentially harmful for cells (19).

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Autophagy is easily detected under basal conditions in the heart, but it is further enhanced during fasting and other pathological conditions, including cardiac remodeling, heart failure and ischemia/reperfusion (22, 32). The functional significance of autophagy in the heart during stress is complex: whether autophagy is salutary or detrimental is context-dependent. In general, a modest increase in autophagy under stress conditions appears to be protective, while massive activation may be detrimental in the heart. The nature of the stress triggering autophagy and the underlying signaling mechanism also appear to affect the functional significance of autophagy in the heart (17, 21, 22). This review discusses both protective and detrimental functions of autophagy in the heart at baseline and in response to myocardial ischemia and reperfusion.

Autophagy machinery and signaling

Autophagy occurs in at least three distinct forms: 1) microautophagy, in which lysosomes directly engulf cytosolic constituents through invagination of lysosomal membranes; 2) chaperone-mediated autophagy, in which specific proteins are delivered to lysosomes through their binding to the chaperone, heat shock cognate 70; and 3) macroautophagy, which is characterized by the formation of double membrane vesicles termed autophagosomes, which sequester macromolecules and finally fuse with lysosomes, where the contents are digested. In this review, we limit our discussion to macroautophagy (hereafter autophagy).

The autophagic process consists of four stages: induction, nucleation, expansion, and maturation/retrieval of autophagosomes (10, 14). Induction and nucleation include the formation of an isolation membrane, known as a phagophore, from a source site which could be the endoplasmic reticulum (ER), mitochondria or some other unknown origin (1, 10, 14). Phagophores localize to the phagophore assembly site (PAS), where constituents of the autophagy machinery are recruited, expand to engulf cytosolic constituents (cargo), and develop into autophagosomes. Autophagosomes are then transported through their binding to cytoskeletal constituents, such as microtubules, and, finally, are directed to lysosomes for fusion.

Autophagosome formation is regulated by the autophagy-related genes (ATG) (10, 14). Genetic screenings in yeast have identified more than thirty ATGs, and most of them have mammalian homologs. Phagophore induction and expansion are mainly induced by the class-III phosphoinositide 3-kinase, Vps34, which forms a multiprotein complex with Beclin1, Atg14 and Vps150, leading to the formation of phosphatidylinositol 3-phosphate (PtdIns3P). The latter is required for recruitment of other regulatory proteins at the phagophore assembly site, allowing expansion of the phagophore. Phagophore induction is also triggered by a macromolecular complex formed by Atg13, Unc-51-like kinase 1 (Ulk1, a homologue of the yeast Atg1) and Ulk2, which phosphorylates the focal adhesion kinase-family interacting protein 200 (FIP200), which promotes initiation of autophagy (10, 14). The elongation phase of the phagophore is regulated by two ubiquitination-like reactions. Firstly, Atg12 is conjugated to Atg5 in a reaction catalyzed by Atg7 and Atg10. The Atg12-Atg5 complex binds to the phagophore membrane and promotes its elongation. Subsequently, Atg7, Atg3 and the Atg12-Atg5 complex modulate the conjugation of the cytosolic form of LC3 (Atg8), known as LC3-I, to phosphatidylethanolamine, to form a membrane-associated form of LC3 known as LC3-II. LC3-II is crucial for autophagosome expansion, inducing membrane tethering and hemifusion, and controlling the size of the autophagosome by regulating the membrane curvature. Once autophagosome formation is completed, LC3-II is released to the cytosol after its Atg4-mediated cleavage from phosphatidylethanolamine (10, 14). The molecular mechanism and genes involved in the autophagosome fusion to lysosomes are still unclear in mammals, even though it is known

that such an event requires a lysosome membrane protein, LAMP-2, and a small GTPase, Rab7. SNARE proteins have been recognized to be crucial for autophagosome fusion to vacuoles in yeast (10, 14).

Autophagy in the heart under basal conditions and during aging

Autophagy is essential for cardiomyocytes (CMs) to maintain structure and function. Cardiac specific-deletion of *atg5* in adult mice rapidly induces cardiac hypertrophy, left ventricular dilatation and contractile dysfunction, which is accompanied by increases in protein ubiquitination/aggregation, ER stress, disorganized sarcomere structure, and mitochondrial misalignment (20). In humans, Danon's disease is caused by a deficiency of LAMP-2, where the loss of function in LAMP-2 causes impairment of autophagosome-lysosome fusion (25). As a consequence, the content of autophagosomes cannot be degraded properly. Danon's disease is characterized by mental retardation, myopathy, and a severe cardiomyopathy with LV dilation and dysfunction (22, 25). Autophagy is also required for maintaining cardiac function during aging. Even though cardiac autophagy is downregulated during aging, further deletion of *atg5* in the mouse heart during aging facilitates cardiac dysfunction and aging cardiomyopathy (30). Taken together, these results suggest that a certain level of autophagy is required for maintaining normal structure and function in the heart.

Autophagy during myocardial ischemia

Autophagy is stimulated during hypoxia and/or ischemia in the heart and the CMs therein (22). During hypoxia/ischemia, the energy reserve in the heart is rapidly depleted, together with decreases in the cellular ATP content. AMP-activated protein kinase (AMPK) acts as a sensor for energy deprivation, and activation of AMPK mediates metabolic adaptation during hypoxia/ischemia. Since inhibition of AMPK significantly suppresses autophagy during myocardial ischemia, activation of AMPK is required for induction of autophagy (16). Activation of AMPK not only inhibits mammalian target of rapamycin (mTOR) through phosphorylation of tuberous sclerosis complex 2 (TSC2), but also induces phosphorylation of eukaryotic elongation factor 2 (eEF2). Inhibition of mTOR and phosphorylation of eEF2 inhibit protein synthesis, an energy consuming process, and stimulate autophagy, both of which contribute to hypoxic adaptation (17). The relative importance of mTOR inhibition and eEF2 phosphorylation in mediating AMPK-induced autophagy is unknown. We have shown recently that mTOR is also inhibited during ischemia in the heart through dephosphorylation/activation of glycogen synthase kinase-3 β (GSK-3 β) (34). The molecular mechanism through which myocardial ischemia inhibits the activity of mTOR also requires further clarification. Besides these signaling mechanisms involving mTOR, stimulation of autophagy may be promoted by upregulation of hypoxia inducible factor-1 (HIF-1) during hypoxia/ischemia (2, 35). The relative contributions of mTOR inhibition and HIF-1 upregulation to induction of autophagy during myocardial hypoxia/ischemia are currently unknown.

Studies conducted thus far support the notion that autophagy is generally protective during myocardial ischemia. Autophagy may compensate for the loss of energy through regeneration of amino acids and fatty acids, which can be used for ATP synthesis through the TCA cycle. Obviously, however, ATP cannot be made through the TCA cycle when aerobic respiration is completely shut-down during severe and/or prolonged ischemia. In addition, since some processes in autophagy, such as autophagosome formation, are ATP-dependent, autophagy itself and the consequent production of ATP cannot take place under complete ischemia. Alternatively, autophagy removes damaged mitochondria, thereby reducing ROS and inhibiting the release of pro-apoptotic factors, such as cytochrome c.

Finally, autophagy may scavenge protein aggregates, which accumulate during ischemia (15, 17, 19).

Yan *et al.* demonstrated that autophagy is significantly upregulated during chronic ischemia (i.e. myocardial hibernation) in the pig heart (33). In this model, the level of autophagy inversely correlated with that of apoptosis in the ischemic area. The ischemic area was recovered with little cell death when the coronary flow was restored, suggesting that autophagy may protect myocardium from apoptosis during hibernation (33). Inhibition of endogenous AMPK suppressed autophagy during prolonged ischemia, which was accompanied by enlargement of the myocardial infarction (28). Since these studies demonstrate only correlation between autophagy and cardioprotection during ischemia, the causative role of autophagy in mediating protection against chronic/prolonged ischemia remains to be demonstrated. If stimulation of autophagy during ischemia is truly protective, interventions to enhance autophagy should be protective during myocardial ischemia. Currently, an intervention selectively inducing autophagy remains to be developed. Even if such an intervention were available, however, it would be difficult to treat the heart with the drug in the absence of perfusion. Recent evidence suggests, however, that autophagy is induced by ischemic preconditioning (6, 11). Thus, activation of autophagy through pharmacological preconditioning may reduce myocardial injury during prolonged ischemia. In addition, as discussed below, excessive activation of autophagy during reperfusion is potentially harmful. If this hypothesis is true, interventions to augment autophagy should be given only before or during ischemia but should not be extended to the reperfusion period.

Autophagy is progressively activated in mice after three days of fasting, which is paralleled by a progressive decline in the heart weight (12, 29). This is another example in which autophagy is activated by energy starvation in the heart. Although normal mice maintain cardiac function during fasting, inhibition of autophagy leads to the rapid development of cardiac dysfunction, suggesting that autophagy plays a critical role in maintaining cardiac function during nutrient starvation (9). Autophagy is critical for maintaining cardiac function immediately after birth when newborn mice suffer from nutrient starvation until they establish nutrition from breast feeding (13). We have shown recently that fasting activates FoxO through Sirt1-mediated deacetylation, which in turn induces expression of Rab7, thereby stimulating autophagosome-lysosome fusion. Downregulation of FoxO1 or suppression of FoxO1 deacetylation inhibits starvation-induced autophagy and promotes cardiac dysfunction during fasting (9). These results suggest that starvation-induced autophagy activated through the Sirt1-FoxO-dependent mechanism is adaptive for the heart and the CMs therein.

Autophagy during myocardial reperfusion

Although various interventions during the acute phase of myocardial infarction aim at restoring coronary flow and supply oxygen and nutrients to ischemic myocardium, reperfusion itself leads to further damage of the heart, termed reperfusion injury. Although one may speculate that activation of autophagy may be reverted as soon as ischemia is relieved, the level of autophagy in fact further increases during reperfusion (16). Mechanisms mediating autophagy during reperfusion appear different from those involved in autophagy during ischemia. Energy crisis, a major stimulus for autophagy, in the heart is at least partially resolved at the time of reperfusion. Instead, ROS appear to be a major promoter of autophagy during reperfusion. ROS induce mitochondrial damage/dysfunction, as evidenced by mitochondrial permeability transition pore (mPTP) opening and mitochondrial fragmentation, which in turn promotes autophagy and/or mitophagy, a specialized form of autophagy which removes mitochondria (4). ROS oxidize and inhibit the cysteine protease activity of Atg4, which results in LC3 lipidation and autophagy (27). We

have shown recently that ROS play an important role in mediating upregulation of Beclin1 and stimulation of autophagy in the mouse heart during ischemia/reperfusion (9). Since Beclin1 is strongly upregulated during the reperfusion phase, but not the ischemia phase, strong induction of Beclin1 by ROS may play an important role in mediating stimulation of autophagy during reperfusion. Angiotensin II (Ang II) receptor signaling may also play a role in stimulating autophagy in response to ischemia/reperfusion. In a recent study, Porrello et al. found that Ang II treatment in the presence of adenovirus-mediated Ang II type 1 receptor (AT1R) overexpression caused a marked increase in autophagy in cultured neonatal rat CMs. In contrast, Ang II type 2 receptor (AT2R) overexpression inhibited autophagy in an Ang II-independent manner. Neonatal CMs cultured from hypertrophic heart rats (HHRs) were more susceptible to AT1R-stimulated autophagy than CMs from normal heart rats (NHRs), and there was a significantly greater increase in autophagic marker upregulation in adult HHR hearts than in NHR hearts following *ex vivo* ischemia/reperfusion (24). Thus, Ang II/AT1R signaling may be involved in the stimulation of autophagy by ischemia/reperfusion.

Whether or not autophagy induced during reperfusion is beneficial or detrimental remains controversial. Hamacher-Brady *et al* (7) have shown that, although autophagic flux is inhibited during ischemia/reperfusion, enhancing autophagic flux during ischemia/reperfusion protects against ischemia/reperfusion injury in CMs *in vitro*. In contrast, Valentim *et al.* showed that inhibiting autophagy by treatment with 3-methyladenine or by Beclin1 knock down increases the survival of CMs after ischemia/reperfusion *in vitro* (31). Our group has shown that autophagosome formation and the size of myocardial infarction are significantly attenuated in *beclin1*^{+/-} mice subjected to ischemia/reperfusion, suggesting that upregulation of autophagy is detrimental during ischemia/reperfusion (16). Beclin1 regulates both autophagosome formation and autophagic flux by interacting with Rubicon, which inhibits autophagic flux (18, 36). Whether *beclin1*^{+/-} mice show decreases in autophagosomes during ischemia/reperfusion due to decreases in autophagy formation or increases in autophagic flux (aided by the absence of Rubicon breakdown) has been raised as a point of debate (5). Using tandem fluorescent LC3 indicator mice, we have shown recently that ischemia/reperfusion increases both autophagosome and autolysosome formation *in vivo*, indicating that autophagic flux is increased during ischemia/reperfusion (9). In this study, 2-mercaptopropionyl glycine, an anti-oxidant, protected against ischemia/reperfusion injury, which was accompanied by suppression of autophagic flux, suggesting that suppressing autophagic flux may be protective during ischemia/reperfusion (9). More recently, we found that suppression of autophagy by downregulation of Ulk1, another mediator of autophagy, inhibits the size of myocardial infarction in response to ischemia/reperfusion (not shown), consistent with the notion that the protective effect of Beclin1 and Ulk1 downregulation during ischemia/reperfusion is mediated through suppression of autophagy.

Molecular mechanisms mediating the detrimental effects of autophagy during reperfusion are not completely understood. Due to its relatively non-specific nature of degradation, autophagy may digest organelles and proteins that could protect CMs from cell death during reperfusion. For example, autophagy degrades catalase, a critical enzyme reducing H₂O₂ (26), which may in turn lead to increases in oxidative stress and apoptosis. It remains to be elucidated why autophagy can be protective during reperfusion under some experimental conditions. A direct correlation exists between the severity of ischemia and the extent of autophagy during the reperfusion phase (3). If ischemia is mild, activation of autophagy during reperfusion may be modest and, thus, it may not be harmful. In addition, inhibiting autophagy at the level of autophagosome formation and at the level of autophagosome-lysosome fusion may differentially affect ischemia/reperfusion injury.

Perspectives

Judging from the intimate involvement of autophagy in patho-physiology of the heart, modulation of autophagy could significantly affect the function of the heart in patients with heart disease. In order to translate our knowledge of autophagy into treatment of heart disease, it would be important to know more precisely when autophagy is protective and when it is detrimental for the heart (Figure). An important caveat in studies of autophagy with experimental animals is the fact that many interventions to inhibit autophagy affect autophagy-independent mechanisms as well. Thus, ideally, the function of autophagy during stress in the heart should be evaluated with multiple loss of function animal models.

Another important issue in the study of autophagy in the heart is to elucidate the upstream signaling mechanisms positively and negatively regulating autophagy in the heart. At present, the molecular mechanisms mediating adaptive and maladaptive autophagy are not well understood. We have proposed that autophagy mediated by either AMPK- (16) or Sirt1-FoxO-dependent signaling mechanisms (8) may be protective, whereas that caused by robust upregulation of Beclin1 due to oxidative stress could be detrimental for the heart (9, 16). Further studies are required to test whether this hypothesis can be extended to autophagy in the human heart in various clinical settings. Recent evidence suggests that autophagy could be mediated by both Atg5/Atg7-dependent and Atg5/Atg7-independent (Rab9-dependent) mechanisms (23). Thus, it is possible that distinct forms of autophagy may exist and that some forms of autophagy could be beneficial while other forms of autophagy are detrimental.

Finally, it would be important to develop small molecule compounds which can either stimulate or inhibit autophagy in the heart. Such compounds would allow adjustment of the level of autophagy in the heart to physiological levels during aging, as well as either stimulation or inhibition of autophagy during ischemia and reperfusion to maintain energy status, protein quality control and organelle function under better conditions. In addition, autophagy may be stimulated in order to compensate for the dysfunction of the ubiquitin proteasome, remove protein aggregates, and achieve better protein quality control in some conditions, such as aging and cardiomyopathy. Modulation of autophagy is expected to add unique benefits on top of conventional therapies for heart disease. Thus, further investigation into the role of autophagy in human heart disease should provide us with promising outcomes.

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Figure. The role of autophagy in the heart at baseline, and during aging and ischemia/reperfusion

The scheme describes our hypothesis as to how protective and detrimental autophagy is induced and how autophagy affects the function of the heart. m. means mitochondrial, alter. means alterations and oxid. means oxidation.