Molecular mechanisms of autophagy in cardiac ischemia/reperfusion injury (Review)

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Abstract. Autophagy is a maintenance process for recycling long-lived proteins and cytoplasmic organelles. The level of this process is enhanced during ischemia/reperfusion (I/R) injury. Autophagy can trigger survival signaling in myocardial ischemia, whereas defective autophagy during reperfusion is detrimental. Autophagy can be regulated through multiple signaling pathways in I/R, including Beclin-1/class III phosphatidylinositol-3 kinase (PI-3K), adenosine monophosphate activated protein kinase/mammalian target of rapamycin (mTOR), and PI-3K/protein kinase B/mTOR pathways, which consequently lead to different functions. Thus, autophagy has both protective and detrimental functions, which are determined by different signaling pathways and conditions. Targeting the activation of autophagy can be a promising new therapeutic strategy for treating cardiovascular disease.

Contents

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1. Introduction

Myocardial ischemia was one of the main causes of sudden cardiac death in the past decades (1). Acute myocardial infarction is a leading cause of death worldwide (2). Strategies for reducing ischemia/reperfusion (I/R)-induced injury in cardiomyocytes are receiving considerable attention due to the failure of cardiomyocytes to regenerate (1). Coronary reperfusion is the most effective treatment for ischemic diseases. However, it may initially aggravate cellular damage during the ischemic period (3).

Cardiac ischemic preconditioning (IPC), which is achieved through repeated brief I/R periods, is one of the well-known protective strategies of the myocardium against I/R injury (4). Recently, autophagy has been linked to IPC-mediated cardioprotection (5). In addition, a number of pharmaceutical therapies targeting I/R injury have been developed to orchestrate multiple protein complexes and signaling pathways in autophagy (6,7). In this review, we aim to draw attention to the role of autophagy in cardioprotection.

2. Autophagy

Autophagy is a self-protective mechanism of living cells under various stress conditions (8). During autophagy, cellular cytoplasm constituents are delivered to lysosomes for degradation and recycling (9). Autophagy limits the production of reactive oxygen species and excessive protein aggregation to maintain intracellular or extracellular homeostasis (10). Autophagy has emerged as a potential drug target for numerous diseases including cancer, neurodegenerative diseases, and cardiovascular disease (11,12).

Autophagy plays multifaceted roles in heart and diseases (13). Under basal conditions, autophagy is a maintenance process for recycling long-lived proteins and cytoplasmic organelles in the heart (14). Furthermore, autophagy plays significant roles in starvation, aging, inflammation, and reverse cardiac remodeling by maintaining cellular homeostasis (15). Autophagy can be regarded as an end effector in hypoxic and ischemic conditions to eliminate superfluous, damaged, or aged cells or organelles (16-18). However, this process will

cause detrimental autophagic cell death when triggered by severe ischemia or in cardiovascular diseases (19).

3. Autophagy and cardioprotection

Myocardial I/R injury is a complex process that destroys proteins, DNA, and plasma membrane, thereby resulting in cell death and decreased cardiac output (20,21). Many studies have reported an increase in the number of autophagosomes in the heart during I/R in animal models (5,22). Autophagy induced by ischemia was subsequently enhanced by reperfusion in isolated rabbit hearts (23) and in mouse hearts (24). The activation of autophagy is reflected in the abundance of autophagy-related protein pathways, such as light chain 3 (LC3), Beclin-1, autophagy-related gene (ATG) 5-12 complex, and p62 (25-27). Hu *et al* reported that approximately 20 min of aortic clamping with hyperkalemic cold blood cardioplegia to achieve total autophagy, which in accordance with previous evidence (28). The abundance of autophagic proteins will actually decrease with the progress of autophagy because of self-degradation (25,26). In particular, in biopsies from the right atrial appendage of patients undergoing valve surgery or coronary artery bypass grafting, the expression of autophagy-related proteins, including LC3-I, LC3-II, ATG5-12, Beclin-1, and p62, is reduced during reperfusion (26).

Until recently, the debate continues whether autophagy plays a protective or deleterious role in the I/R injury process. On the one hand, modest levels of autophagy triggered by mild to moderate hypoxia/ischemia are protective and seem to prevent the activation of apoptosis (23,29). On the other hand, high levels of autophagy induced by severe hypoxia or I/R may cause self-digestion and eventual cell death (30). Therefore, autophagic flux induced by ischemia during the early stage of I/R has been speculated to be beneficial; however, it is harmful during reperfusion at the later stage of I/R (15,19).

Autophagy may play an alternative role in I/R, which determines cell fate. The extent of autophagy in response to ischemia is considered based on the severity and duration of ischemic insults (31). Nutrient and oxygen deprivation in the heart threatens cellular survival during I/R, and increased autophagy may provide at least a temporary reprieve for a threatened myocardium by serving as a source of intracellular nutrients (32). Oxidative stress, calcium overload, endoplasmic reticulum (ER) stress, and mitochondrial dysfunction maintain a high level of autophagy during reperfusion (33). However, high levels or long-term upregulation of autophagy can lead to excessive degradation of essential proteins and organelles (34). If intracellular energy sources become inadequate, then autophagic processes will be a particular form of cell death, called type II or autophagic cell death (35). In fact, aware that necrosis and apoptosis are not the only mechanisms of cell death is increasing (36). Autophagic cell death has been identified as a cell death phenotype via electron microscope observations; it has a morphological term characterized by abundant autophagic vacuoles in the cytoplasm (37,38).

Moreover, increased autophagy after I/R is not due to increased autophagosome formation, but instead, to impaired clearance of autophagosomes (39); this assumption is derived from the concept of autophagic flux (40). Furthermore, a rapid decline induced by reperfusion in LAMP2, which is a critical protein for autophagosome-lysosome fusion, can impair autophagosome processing and mitochondrial permeabilization, thereby increasing ROS generation and triggering cardiomyocyte death (41). In addition, when the engulfed targets or autophagosomes cannot fuse with lysosomes and digest their contents, a cell may eject the autophagosomes as a response, which induces an acute and significant inflammatory response (42).

4. Mechanism of autophagy in cardioprotection

Autophagy is a complex and dynamic multi-step process that depends on strict regulation and coordination through multiple signaling pathways (43). To date, several cellular signaling pathways are considered to trigger autophagy in I/R. In addition, autophagy has been shown to be regulated by several signaling pathways (44), including Beclin-1/class III phosphatidylinositol-3 kinase (PI-3K), AMPK/mammalian target of rapamycin (mTOR), and PI-3K/Akt/mTOR pathways.

Beclin-1/class III PI-3K pathway. Beclin-1, which is a phylogenetically conserved protein, the mammalian homologue of the yeast Atg6, and the interacting protein of the anti-apoptotic protein Bcl-2, is a key molecule involved in mediating autophagy (45,46). It plays a crucial role in engaging class III PI-3K to positively modulate autophagy in mammalian cells (47,48). Autophagy in mammalian cells is reported to be activated by the class III PI-3K complex, which contains Vps34 and Beclin-1 (29,49). Moreover, a coiled-coil domain (aa 140-268) is present in this 450 amino acid-long protein in Beclin-1; this domain can mediate binding to class III PI-3K Vps34 by interacting with an evolutionarily conserved domain (ECD; aa 244-337) (50). RNA interference of Beclin-1, which inhibits autophagy, will subsequently enhance cardiac cell survival (51).

Autophagy is involved in delayed cardioprotection induced by sevoflurane preconditioning (52). Sevoflurane preconditioning reduces the autophagy induced by H/R by decreasing the Beclin-1 expression (52). Accordingly, IPC protects the rat heart against MI/R injury by inhibiting Bcl-2 dissociation from Beclin-1 during the reperfusion phase *in vivo*, although IPC-induced autophagy reflects a compensatory pro-survival response to I/R injury (53). Bcl-2 is the prototype of a protein family, which contains at least one Bcl-2 homology (BH) region (54). Bcl-2 binding molecules have been recently shown to regulate autophagy activation (55). Transgenic mice with a cardiac-specific overexpression of Bcl-2 are protected from I/R injury (56,57). Autophagy is disrupted when Bcl-2 binds to Beclin-1 (58). In addition, when a mutant of Beclin-1 that lacks the Bcl-2 binding domain is overexpressed in cells, excessive autophagy and cell death are induced (47). Bcl-2 can also inhibit Beclin-1/Vsp34 PI-3K complex formation and the activity of Beclin-1-associated class III PI-3K (53). Furthermore, the class III PI-3K autophagic pathway is inhibited by combining the BH3 hydrophobic groove in Bcl-2 and the BH3-like amphipathic α-helix in Beclin-1 (59). However, the interaction with Bcl-2 (and Bcl-xL) in the ER, rather than in the mitochondria, inhibits the Beclin-1 activity in autophagy (60). The interaction between Bcl-2 and Beclin-1 maintains

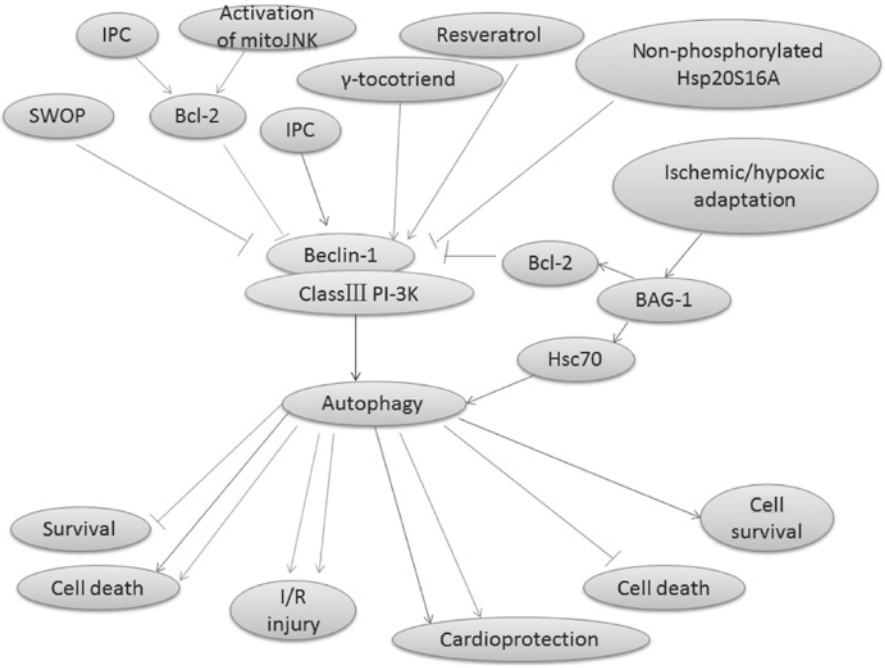

Figure 1. Beclin-1/class III PI-3K pathway-regulated autophagy and autophagy-mediated function in I/R injury. Under I/R condition, autophagy is activated by the class III PI-3K complex, which contains class III PI-3K Vps34 and Beclin-1. SWOP induces cardioprotection by reducing Beclin-1 expression, increasing the survival rate of cells, and reducing their apoptosis percentage. IPC protects rat heart against myocardial I/R injury by inhibiting Bcl-2 dissociation from Beclin-1. MitoJNK activation, instead of JNK mitochondrial localization, induces triggering of Bcl2-regulated autophagy, which further causes cell death and aggravates myocardial I/R injury. However, IPC can exert cardioprotection via autophagy through the activation of PI-3K. Resveratrol and γ-tocotrienol can enhance autophagy through the induction survival pathway, which depends on class III PI-3K, thereby synergistically providing an increased degree of cardioprotection. Non-phosphorylated Hsp20S16A increases cell death by suppressing autophagy, and Beclin-1 is a potential target of phosphorylated Hsp20 in regulating autophagy. Ischemic/hypoxic adaptation induces improvement in cardiac cell survival mediated by BAG-1. BAG-1 can bind to both Bcl-2 and Hsc70 molecules, and may activate autophagy via Hsc70. PI-3K, phosphatidylinositol-3 kinase; I/R, ischemia/reperfusion; JNK, C-Jun N-terminal kinase; IPC, ischemic preconditioning; Hsp20, heat shock protein.

autophagy at levels (47). Blocking the interaction between the BH3 domains of Beclin-1 and Bcl-2 increases autophagic activity (53). Recent studies indicate that the increase in the interaction between Beclin-1 and Bcl-2 is caused by IPC (53). C-Jun N-terminal kinase (JNK), which is a member of an evolutionarily conserved subfamily of mitogen-activated protein kinases, is critical for the cellular responses of multiple environmental and cellular stimuli (61,62). I/R can trigger Bcl2-regulated autophagy by inducing a dominant increase in mitoJNK activation, which causes cell death (63). Xu *et al* reported that mitoJNK activation, and not JNK mitochondrial localization, induced autophagy, which further aggravates I/R injury (63). In addition, the mitoJNK phosphorylate Bcl2, which antagonizes Bcl2 anti-apoptotic and anti-autophagic activities, may contribute to the deleterious role of mitoJNK in I/R injury (64,65).

Heat shock protein (Hsp20) is the only member of the sHsps family that contains the consensus peptide motif RRAS for protein kinase A-/protein kinase G-dependent phosphorylation at Ser16 (66). Qian *et al* demonstrated that non-phosphorylated Hsp20S16A is detrimental in I/R injury because it suppresses autophagy and further increases cell death (36). Ischemic/hypoxic adaptation improves cardiac cell survival by suppressing the BAG-1 protein expression (67). BAG-1 can bind with both Bcl-2 and Hsc70 molecules (67). Autophagosomal membrane contains a significantly higher amount of Hsc70 proteins (68). BAG-1 has been shown exhibit numerous functions through its interaction with Hsc70 (69). The treatment of rats with wortmannin, an inhibitor of class III PI-3K, has been used to suppress autophagy in many studies (70,71), and attenuates both the LC3-II and BAG-1 protein expressions (67). Zheng *et al* (72) reported that the activated PI3K/Akt pathway contributes to the berbamine postconditioning-induced cardioprotection through modulating autophagy. The Beclin-1/class III PI-3K pathway-regulated autophagy and autophagy-mediated function in I/R injury are shown in Fig. 1.

AMPK/mTOR pathway. AMPK, which is activated in response to stress that exhaust cellular ATP supplies, such as ischemia and hypoxia, plays a crucial role as a master regulator of cellular energy homeostasis (73). AMPK is ubiquitously expressed in metabolically active tissues, such as cardiac muscles, and activated upon the depletion of energy stores by functioning as an intracellular fuel sensor (74). Ischemia has been proposed to stimulate autophagy via an AMPK-dependent mechanism (53), which is one of the most significant approaches in upregulating autophagy (75,76). During I/R injury, intracellular ATP stores are rapidly consumed and cannot be supplemented with decreasing glucose supply (77). AMPK signaling can positively regulate autophagy by activating Ulk1 via the phosphorylation of Ser 317 and Ser 777 or indirectly by inhibiting mTOR signaling (78,79). Moreover, AMPK functions as a master regulator of the autophagy pathway through inactivating mTOR (80).

High mTOR activity negatively regulates autophagy by inhibiting the activation of Ulk1, which is one of the

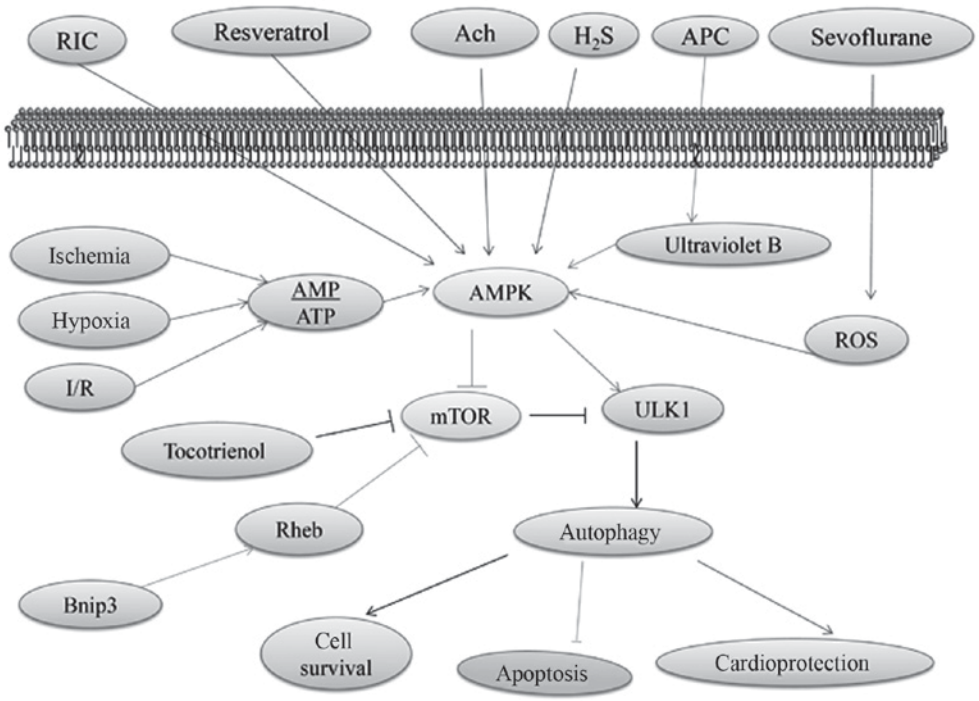

Figure 2. AMPK/mTOR pathway-regulated autophagy and autophagy-mediated function in I/R injury. Tocotrienol induces autophagy through the mTOR pathway, which subsequently leads to cell survival and cardioprotection. mTOR activity negatively regulates autophagy by inhibiting Ulk1 activation, which initiates the nucleation of the autophagic membrane. Bnip3 can inhibit the mTOR pathway and induce autophagy by directly binding to Rheb and protect cardiac myocytes against I/R injury-related apoptosis. Under the condition of ischemia, hypoxia, or I/R, AMPK is activated by the increased levels of AMP/ATP. Then, autophagy triggered by AMPK can resist cardiac injury, and AMPK signaling can positively regulate autophagy by activating Ulk1, or indirectly, by inhibiting mTOR signaling. RIC, resveratrol, Ach, and H_2S can induce the autophagic AMPK pathway, which is cardioprotective. Ultraviolet B is a critical mediator of cardioprotection via APC, and ultraviolet B-induced autophagy activates AMPK by inhibiting the phosphorylation of GSK3β. Sevoflurane provides cardioprotection against I/R injury via ROS-mediated upregulation of autophagy. mTOR, mammalian target of rapamycin; I/R, ischemia/reperfusion; Rheb, Ras homolog that is enriched in the brain.

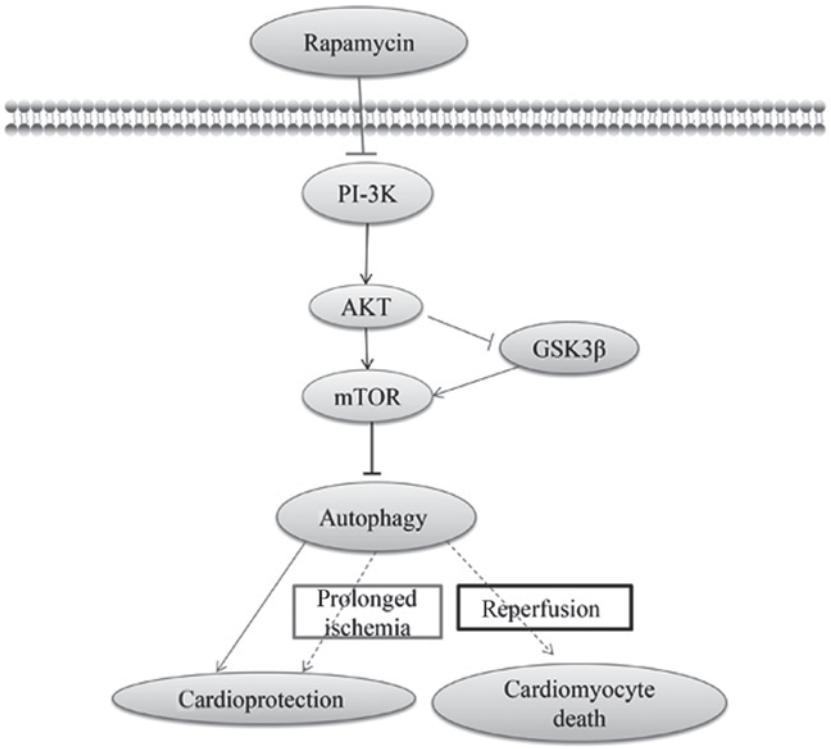

Figure 3. PI-3K/Akt/mTOR pathway-regulated autophagy and autophagy-mediated function in I/R injury. Class I PI-3K (PI-3K) will inhibit the induction of autophagy via the phosphorylation of Akt and mTOR, and achieve additional benefit against I/R injury. Rapamycin exerts beneficial effects against cardiomyocyte I/R injury through autophagy that depends on the PI-3K-Akt signaling pathway. GSK-3β is the downstream of PI-3K/Akt, and GSK-3β causes cardiomyocyte death during reperfusion but mediates the survival of cardiomyocytes during prolonged ischemia through mTOR-dependent autophagy attenuation. PI-3K, phosphatidylinositol-3 kinase; mTOR, mammalian target of rapamycin.

Table I. Summary of various autophagy modulators on cardiac ischemia/reperfusion injury.

Author, year	Drugs	Mechanism of action	Effect on cardiac ischemia/reperfusion injury	(Refs.)
Huang *et al*, 2010	Sulfaphenazole	Activate protein kinase C	Protects against myocardial I/R and reduces infarct size	(39)
Xie *et al*, 2015	Sevoflurane	Inhibition Beclin 1-mediated autophagic cell death	Delayed cardioprotection	(52)
Zheng *et al*, 2017	Berbamine	Activate PI3K/Akt pathway	Improved post-ischemic myocardial function and attenuated cell death	(72)
Lekli *et al*, 2010	Tocotrienol	Activate the mTOR pathway	Reduces cardiomyocyte apoptosis	(84)
Gurusamy *et al*, 2010	Resveratrol	Activate mTOR-Rictor survival pathway	Attenuates myocardial I/R injury and reduces infarct size	(91)
Zhao *et al*, 2013	Acetylcholine	Activate AMPK-mTOR pathway	Reduces cardiomyocyte death	(92)
Xie *et al*, 2015	H_2S	Activate AMP-activated protein kinase	Protects against myocardial I/R injury	(93)
Zhong *et al*, 2017	Trimetazidine	Activate AMPK-mTOR pathway	Reduces hypoxia/reoxygenation injury	(99)
Wang *et al*, 2015	Rapamycin	Activate PI3k/Akt pathway	Attenuates anoxia/reoxygenation injury	(103)

mTOR, mammalian target of rapamycin; I/R, ischemia/reperfusion.

mammalian autophagy-initiating kinases that is important for membrane nucleation via the phosphorylation of Ulk1 Ser 757 (1,81,82). The association between ATG1 and ATG13 is negatively regulated by mTOR, which inhibits autophagy (83). In addition, the activation of Ulk1 and its combination with other molecules, such as ATG13 and FIP200, initiate the nucleation of the autophagic membrane (1). Lekli *et al* proposed that tocotrienol could induce autophagy through the mTOR pathway, which would consequently lead to cell survival and cardioprotection (84). The overexpression of Bnip3, a hypoxia-inducible Bcl-2 homology 3 domain-containing protein (85) and the pro-apoptotic molecule present in the mitochondrial membrane; can upregulate autophagy and protect cardiac myocytes against I/R injury-related apoptosis (86). The high-mobility group box 1 protein (HMGB1)-mediated activation of mTOR inhibits hypoxia and reoxygenation injury in rat cardiomyocytes (87,88). Moreover, Bnip3 can inhibit the mTOR pathway and induce autophagy by directly binding to the Ras homolog that is enriched in the brain (Rheb), which is a Ras-related small guanosine triphosphatase (85,89).

The cardioprotection effect of resveratrol has been shown to induce autophagy by facilitating AMPK activation (90,91). In addition, AMPK expression is elevated with ACh during H/R (92). ACh activates cytoprotective autophagy through the AMPK-mTOR-dependent pathway that is activated by a muscarinic receptor (92). Xie *et al* found that the post-reperfusion AMPK activation induced by a slow-releasing organic H_2S donor that could restore I/R impaired autophagic flux; is critical to H_2S cardioprotection (93). Accordingly, studies have proven that autophagy activation through the AMPK/mTOR pathway plays a cardioprotection role (94). Recently, ultraviolet B-induced autophagy has been found to activate AMPK by inhibiting the phosphorylation of GSK3β (95), which is a critical mediator of cardioprotection via anesthetic preconditioning (96). Sevoflurane provides cardioprotection against I/R injury via the ROS-mediated upregulation of autophagy (97). Hariharan *et al* reported that oxidative stress triggers autophagic flux during MI/R injury (98). In addition, trimetazidine (99) and thioredoxin-2 (100) protect against hypoxia/reoxygenation injury by promoting the AMPK-dependent autophagic flux in H9c2 cardiomyocytes. The AMPK/mTOR pathway-regulated autophagy and autophagy-mediated function in I/R injury are illustrated in Fig. 2.

PI-3K/Akt/mTOR pathway. PI-3K/Akt/mTOR signaling may also provide an additional benefit against I/R injury (101). In addition, class III PI-3K focuses on the formation of autophagosomes, whereas class I PI-3K will inhibit the induction of autophagy through the phosphorylation of Akt and mTOR (70). Thus, the interaction of Akt with mTOR is multifaceted and bidirectional (101). Moreover, the self-regulation of autophagy has been postulated to be regulated by the autophagy-induced inhibition of mTOR (102). Furthermore, rapamycin provided a strong beneficial effect against cardiomyocyte anoxia/reoxygenation injury, which would mediate cardioprotection via autophagy that probably depended on the PI-3K/Akt signaling pathway (103). During prolonged ischemia and I/R, the differential effects of GSK-3β, which is the downstream of PI-3K/Akt, on myocardial injury has been suggested to be determined by changes in autophagy (104). GSK-3β inhibition modulates mTOR-dependent attenuation of autophagy, thereby causing the death of cardiomyocytes during prolonged ischemia while mediating their survival during reperfusion (104). In addition, mTOR activation via GSK-3β has been suggested to provide cardioprotection via autophagy (104). The PI-3K/Akt/mTOR pathway-regulated

autophagy and autophagy-mediated function in I/R injury are illustrated in Fig. 3.

Others. The p53 transcription factor is a major regulator of cellular response to acute stress (105). Knockdown of p53 can activate autophagy in cardiomyocytes, thereby protecting the myocardium against ischemic injury (106). Autophagy is inhibited with STAT1 to modulate stress response to I/R in STAT1$^{-/-}$ null hearts (32). In I/R, STAT1 can interact directly with p53 and regulate its functional activity (107), thereby suggesting that STAT1 can act with p53 to modulate autophagy (32). The mitochondrial permeability transition pore (MPTP) plays an important role in myocardial I/R injury, and the opening of MPTP has also been shown to trigger autophagy (108). Making the heart more tolerant to subsequent I/R injury is a crucial step in transient MPTP opening before prolonged ischemia (109,110). Moreover, PKC has been reported to trigger the phosphorylation of a regulatory sub-unit of VPATPase, which subsequently induces autophagy (111-113).

5. Autophagy as a therapeutic target for I/R injury

Microarray analysis showed that autophagy-associated genes and the unfolded protein response were upregulated under the condition of repetitive coronary occlusion achieved during chronic local ischemic conditioning in mice (114,115). In another study, inhibiting mTOR decreased infarct size in mice (116). Rapamycin (116), caloric restriction (117), exercise (118), nitric oxide (119), and lipopolysaccharide (120) has been identified as cardioprotective interventions for triggering autophagy. Gurusamy *et al* used isolated rat heart models and demonstrated that the induction of IPC via repeated I/R cycles immediately enhanced the expression of LC3-II and Beclin-1 (67). *In vivo* swine models, infarct size was limited after chloramphenicol succinate was used before ischemia (121). Han *et al* found that cardioprotection induced by remote limb ischemic postconditioning was associated with elevated autophagy 3 h post-reperfusion (2). A similar phenomenon was observed by Hamacher-Brady *et al* in HL-1 myocytes; they found that simulated I/R-mediated cell death was prevented by strengthening autophagy, whereas its inhibition caused cell death (40). Other researchers have observed that blocking autophagy via cell-permeable Tat-Atg5K130R concurrently increased infarct size in hearts when treated with SUL (39). Moreover, Tibetan patients with coronary heart disease resist I/R injury during cardiac surgery better than patients living at sea level, which is possibly correlated with the upregulation of basal autophagy resulting from chronic hypoxia (28).

Autophagy has been determined as a significant element of the endogenous defense mechanisms activated by various preconditioning types. Induction of autophagy may represent a novel therapeutic approach to myocardial protection in humans (39). The identification of agents that can rapidly induce autophagy can contribute to the discovery of new cardioprotective drugs (122). In addition, induction of autophagy can preserve heart function during I/R injury (91,121,123). Other studies have suggested that autophagy is detrimental because it contributes to cell death (15,124). The beneficial or detrimental role of autophagy may be a consequence of balance, depending on the extent of autophagy (7). Thus, for autophagy to be effective, searching for a candidate cardioprotective drug that can induce autophagy in a target population is important, together with the appropriate timing and response magnitude (16). Various autophagy modulators on cardiac ischemia/ injury are summarized in Table I.

6. Conclusions

Recent studies have shown that autophagy plays an important role in I/R injury. Moreover, evidence has emerged that autophagy plays various roles in I/R through multiple mechanisms. Autophagy can trigger a survival signal in the case of myocardial ischemia, whereas defective autophagy during reperfusion is detrimental. Although we have obtained substantial knowledge about the function of autophagy in I/R injury, the autophagy pathway is highly complex and remains far from being understood completely. Additional studies are necessary to identify the molecular components of the autophagy pathway, characterize the role of autophagy in I/R injury, elucidate the diverse processes that regulate autophagy expression and activity, and determine the contribution of autophagy to myocardial infarction protection in humans. Such studies will provide additional insights into the role of autophagy in I/R injury and potentially discover novel therapeutic strategies for treating the diseases.

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Authors' contributions

X-LL and M-HL conceived and designed this review. X-LL, L-LX and W-JX contributed the central idea, analyzed most of the data, and wrote the initial draft of the paper. L-LX and M-HL revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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