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REVIEW ARTICLE

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Targeting autophagy in cardiac ischemia/reperfusion injury: A novel therapeutic strategy

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Abstract

Acute myocardial infarction (AMI) is one of the leading causes of morbidity worldwide. Myocardial reperfusion is known as an effective therapeutic choice against AMI. However, reperfusion of blood flow induces ischemia/reperfusion (I/R) injury through different complex processes including ion accumulation, disruption of mitochondrial membrane potential, the formation of reactive oxygen species, and so forth. One of the processes that gets activated in response to I/R injury is autophagy. Indeed, autophagy acts as a "double-edged sword" in the pathology of myocardial I/R injury and there is a controversy about autophagy being beneficial or detrimental. On the basis of the autophagy effect and regulation on myocardial I/R injury, many studies targeted it as a therapeutic strategy. In this review, we discuss the role of autophagy in I/R injury and its targeting as a therapeutic strategy.

KEYWORDS

acute myocardial infarction, autophagy, ischemia/reperfusion injury

1 | INTRODUCTION

Acute myocardial infarction (AMI) is one of the main causes of morbidity and mortality worldwide (Mozaffarian et al., 2015). Minimizing blood flow resumption time is critical to curing AMI (Bangalore, Pursnani, Kumar, & Bagos, 2013). However, Jennings (1960) described ischemia/reperfusion (I/R) injury as a phenomenon in which the restoration of blood flow leads to heart tissue damages. Many complex processes are involved in I/R injury including ion accumulation, disruption of mitochondrial membrane potential, the formation of reactive oxygen species (ROS), dysregulation of nitric oxide metabolism, endothelial dysfunction, platelet aggregation, immune activation, apoptosis, and autophagy (Turer & Hill, 2010).

The heart needs a constant energy supply for both diastolic relaxation and contractility, which is often provided by mitochondria

as adenosine triphosphate (ATP; Halestrap & Richardson, 2015). In the myocardial ischemia, a state of energy shortage and ATP depletion in the heart, mitochondrial function is considered as an important factor (Xia, Li, & Irwin, 2016). Following ischemia and loss of oxygen, anaerobic glycolysis is the most dominant metabolism pathway, which leads to the accumulation of lactate and hydrogen ions and subsequently results in intracellular acidosis (Buja, 2005). In contrast, although inner mitochondrial membrane (IMM) usually is impermeable, under ischemia injury and because of nutrient and oxygen deprivation inner mitochondrial permeability transition pore (mPTP) acts nonselectively, which leads to uncoupling of oxidative stress, ATP hydrolysis, and increase in mitochondrial inorganic phosphate (Di Lisa et al., 2011; Heusch, Boengler, & Schulz, 2010). Owing to the pH drop and ATP depletion, activation of Na^+/H^+ ion exchanger and Na^+/HCO_3^- transporter (Tani & Neely, 1989) and inhibition of Na^+/K^+-ATPase (Ibáñez, Heusch, Ovize, Van, & de Werf, 2015) resulting in intracellular Na^+ accumulation (Xia et al., 2016) occur. Sodium accumulation and changes in the Na^+/Ca^{2+} transporter system in the sarcolemma causes an increase in intracellular Ca^{2+} and mitochondria swelling (Di Lisa & Bernardi, 2006). When the blood flow restores and respiratory chain re-exposed to oxygen, ROS production exacerbates, which finally leads to cell death (Xia et al., 2016). Under I/R injury and in response to oxidative stress and energy depletion, the autophagy process is also activated. It has been shown that autophagy acts as a "double-edged sword" in the pathology of I/R injury (Ma, Wang, Chen, & Cao, 2015). In this review, we discuss the role of autophagy in I/R injury and its targeting as a therapeutic strategy.

2 | AUTOPHAGY

More than 50 years ago, the term "autophagy" ("self-eating" in Greek) was described by Christian de Duve. He described autophagy as a phenomenon in which the cells digest cytoplasmic materials within the lysosomes (Jiang & Mizushima, 2014). After several years, identifying autophagy-related proteins (ATGs) by Tsukada and Ohsumi (1993) has attracted attention again toward autophagy. Both Christian de Duve and Yoshinori Ohsumi earned the Nobel Prize in Physiology and Medicine in 1974 and 2016, respectively (Dikic & Elazar, 2018). Autophagy is an adaptive response to stressful situations including starvation (Dikic & Elazar, 2018), hypoxia (Wu, Huang, & Zhang, 2015), and infection (Choi, Bowman, & Jung, 2018). Indeed, autophagy is a critical energy homeostasis process for the survival of vital cells during a stressful situation by providing nutrients. In contrast, autophagy is a cytoprotective system, which selectively eliminates harmful cytosolic materials and damaged organelles (Dikic & Elazar, 2018). There are different forms of autophagy including chaperon-mediated autophagy, macroautophagy, and microautophagy (Ma et al., 2015). In addition to the three types, two other forms of autophagy called "DNautophagy" and "RNautophagy" have been introduced for the degradation of DNA and RNA in lysosomes, respectively (Fujiwara, Furuta, et al., 2013; Fujiwara, Kikuchi, et al., 2013). The most well-known form of autophagy is macroautophagy in which both intracellular organelles and cytoplasmic proteins are degraded (Ma et al., 2015).

There are several important signaling mechanisms in regulating autophagy of cardiomyocytes and in response to stressful conditions. Here we focus on two of the most important pathways.

2.1 | Mechanistic target of rapamycin (mTOR) pathway

The most dominant pathway of autophagy is the mTOR pathway (Figure 1). mTOR, a serine/threonine kinase, acts through two distinct multiprotein complexes, in which, only mTORC1 is directly involved in autophagy (Bar-Peled & Sabatini, 2014). In the normal conditions, Unc-51-like kinase 1 (ULK1) is phosphorylated at serine 757 by mTORC1, leading to inhibition of autophagosome formation. In response to stressful situations, such as starvation, the activity of mTORC1 is reduced and autophagy gets activated (Kim, Kundu, Viollet, & Guan, 2011). First, autophagy factors comprising of Atg13, Atg101, ULK1, and RB1-inducible coiled-coil protein 1 (FIP200) translocate to autophagosome formation site and form the ULK1 complex (Parzych & Klionsky, 2014). Phosphorylation of the class III PI3K (PI3KC3) by ULK1 complex on the endoplasmic reticulum structure called the omegasome leads to the generation of phosphatidylinositol 3-phosphate (PI3P). PI(3)P recruits the PI(3)P-binding proteins DFCP1 (double FYVE-domain-containing protein 1) and WIPI-1/2 (WD repeat domain phosphoinositide-interacting proteins 1/2) to the omegasome, further leading to growth and expansion of the omegasome (Ravikumar et al., 2010). In the expansion step, Atg9-containing vesicles also may deliver other components, such as proteins and lipids to contribute to omegasome growth (Karanasios et al., 2013; Manifava et al., 2016; Nishimura et al., 2017). The most important step of omegasome expansion is mediated by the Atg conjugation system, which requires an ubiquitin-like system (Nakatogawa, 2013). In one of them, Atg12 conjugates to Atg5, which is mediated by Atg7 and Atg10. Another one relies on Atg12-Atg5 complex and Atg16L1, which conjugate microtubule-associated protein 1 light chain 3β (LC3-II) to phosphatidylethanolamine (Ravikumar et al., 2010). As a result of omegasome expansion, autophagic bodies and materials are surrounded and enveloped into the double-membrane vesicle known as the autophagosome (Farré & Subramani, 2016). In the autophagosome maturation step, Atgs that are attached to the outer membrane of autophagosome are detached and lysosomal delivery machine including syntaxin 17, synaptosomal-associated protein 29, and vesicle-associated membrane protein 8 are recruited, and finally the autophagosomes fuse with lysosomes (Diao et al., 2015; Itakura, Kishi-Itakura, & Mizushima, 2012).

2.2 | 5′-AMP-activated protein kinase (AMPK) and glycogen synthase kinase-3β (GSK-3β) pathway

The AMPK and GSK-3β pathway are involved in activating autophagy (Dikic & Elazar, 2018). When the cellular ATP level depletes and AMP/

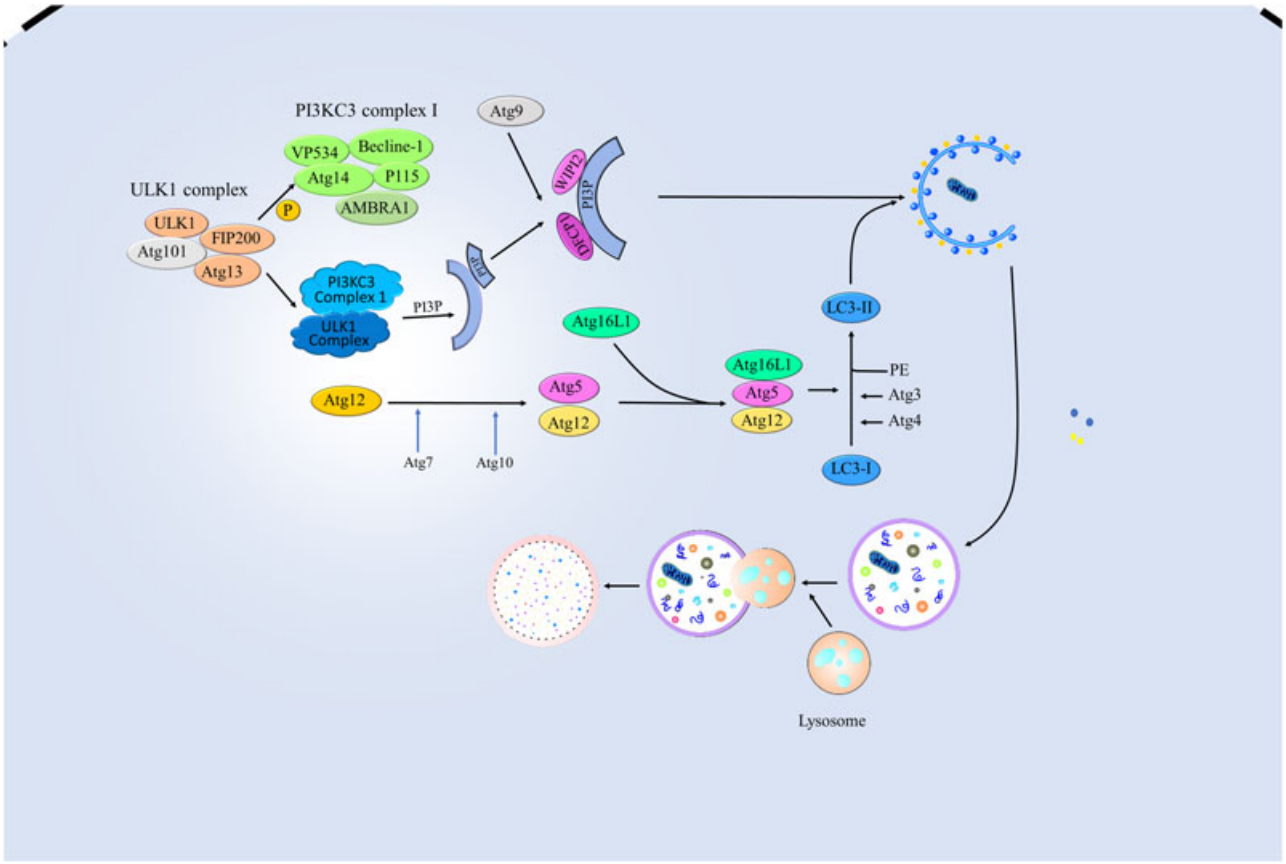

FIGURE 1 mTOR pathway and autophagy. LC3-II: microtubule-associated protein 1 light chain 3β; mTOR: mechanistic target of rapamycin; PI3KC3: phosphorylation of the class III PI3K; PI3K: phosphoinositide 3-kinase; ULK1: Unc-51-like kinase 1 [Color figure can be viewed at wileyonlinelibrary.com]

ATP ratio increases, liver kinase B1 promotes AMPK activation. The AMPK phosphorylates and activates tuberous sclerosis complex 1/2 (TSC1/2), which inhibits Ras homolog enriched in brain, an activator of mTORC1 (Esclatine, Chaumorcel, & Codogno, 2009). In a direct way, AMPK disassociates ULK1 from mTORC1 by phosphorylating ULK1 at serines 313 and 777 (Kim et al., 2011). During energy stress, very similar to AMPK, GSK-3β also phosphorylates TSC1/2, inhibits mTORC1, and finally stimulates autophagy (Inoki et al., 2006).

3 | MITOPHAGY

There is a subset of macroautophagy called "mitophagy" in which damaged mitochondria are cleared. Mitophagy is a protective form of autophagy in the cardiac I/R injury (Ma et al., 2015). Under normal conditions, where mitochondria are polarized, phosphatase and tensin homolog-induced putative kinase 1 (PINK1) is recruited to mitochondria and degraded by matrix processing peptidases (matrix metalloproteinase) and presenilin-associated rhomboid-like protein in the IMM, and then the cleaved PINK1 is released into the cytosol (Matsuda et al., 2010). In dysfunctional mitochondria that are depolarized, there is an insufficiency in the membrane potential of mitochondria in transferring PINK1 to IMM. In such cases, PINK1 degradation and cleavage are inactivated, thus PINK1 accumulates at the outer membrane of the mitochondria (OMM; Moreira, Estébanez, & Martínez-Florez, 2017). PINK1 recruits Parkin from the cytosol by phosphorylation of Parkin and ubiquitin. Then, Parkin ubiquitinates its substrates on the OMM, such as voltage-dependent anion channel-1 (VDAC1) and mitofusin 1 and 2. LC3 proteins on the omegasome recognize the polyubiquitinated proteins through adaptor proteins including p62, optineurin, and nuclear domain 10 protein 52 (Gkikas, Palikaras, & Tavernarakis, 2018). Furthermore, there is another pathway of mitophagy called receptor-mediated mitophagy in which OMM proteins including BCL2/adenovirus E1B 19 kDa interacting protein 3 (BNIP3), Nip3-like protein X, and FUN14 domain-containing protein 1 (FUNDC1) through their BH3 domain interact with LC3 (Yoo & Jung, 2018). Surprisingly, there are IMM proteins including prohibitin 2 and cardiolipin, which directly interact with LC3 (Chu et al., 2013; Shen, Li, Gasparski, Abeliovich, & Greenberg, 2017; Wei, Chiang, Sumpter, Mishra, & Levine, 2017). Under normal conditions, phosphorylation of FUNDC1 by Src kinase and casein kinase 2 inhibits its attachment to LC3 and its OMM localization (Hamacher-Brady, NRJC, & Sciences, 2016).

4 | AUTOPHAGY IN CARDIAC I/R INJURY

Owing to the critical role in controlling protein and organelle quality, autophagy is fundamental for cardiac development, structure, and function (Riquelme et al., 2016). It has been shown that knocking-down

of beclin-1, Atg5, or Atg7 during the development of cardiac embryo leads to structural aberrations of the heart (Lee et al., 2014). In the adult mice, inhibition of autophagy by knocking-out of Atg5 causes cardiomyopathy (Nakai et al., 2007). As autophagy is increased in some cardiovascular disease including heart failure, dilated cardiomyopathy, and anticancer drug-induced cardiomyopathy (Xie, Morales, Lavandero, & Hill, 2011) and diminished in some others, such as Danon disease (Endo, Furuta, & Nishino, 2015), an overall control of autophagy in the heart physiology is essential. About I/R injury, there is a controversy about autophagy being beneficial or detrimental. Basal levels of autophagy help the cell to maintain ATP levels by removing damaged mitochondria, organelles, and recycling of catabolites (Li & Lerman, 2012). It has been verified that preconditioning cardiomyocytes with autophagy inducers protect the cells after I/R injury (Khan et al., 2006). In contrast, excessive autophagy leads to the degradation of pivotal organelles and proteins, hence, result in organ dysfunction (Ma et anal., 2015). Thus, alleviation of excessive autophagy during I/R injury may prevent the death of cardiomyocytes, and thereby protect the cardiac function (Huang et al., 2015). It has been shown that the inhibition of BNIP3 and beclin-1 protects the cardiac function against I/R injury (Hamacher-Brady et al., 2007; Valentim et al., 2006). Thus, targeting autophagy to ameliorate I/R injury has attracted many attentions. Table 1 summarizes the previous studies on targeting autophagy as a therapeutic strategy in cardiac I/R injury.

4.1 | Targeting autophagy for its beneficial effects

Owing to antioxidant activity, pramipexole (PPX) inhibits the opening of mPTP, which occurs because of ATP and ADP depletion and Ca^{2+} overload (Cassarino, Fall, Smith, & Bennett, 1998; Sayeed et al., 2006). It has been shown that the number of autophagic bodies increases following I/R injury in the myocardium (Mo, Tang, Ma, & Wu, 2016) and autophagy is the main mechanism of cell death during I/R injury (Sadoshima, 2008). Mo et al. (2016) reported that pretreatment with PPX significantly reduced infarct size compared with the sham group by increasing autophagy in the mice model of I/R injury. In addition, PPX is able to reduce hypoxia/reoxygenation (H/R) injury and ROS generation in cultured H9c2 cells. Because activation of AMPK following PPX treatment was upregulated, its cardioprotective role could be related to the upregulation of autophagy through the AMPK-mediated pathway (Mo et al., 2016).

Coenzyme Q10 (CoQ10), which is structurally similar to vitamin E and vitamin K (Liang, Ping, & Ge, 2017), is a fat-soluble substance and a crucial component of mitochondria electron transport chain in the ATP production (Khan et al., 2017). CoQ10 plays a central role in mitochondria membrane integrity by preventing injuries due to oxidative stress (Farhangi, Alipour, Jafarvand, & Khoshbaten, 2014; Groneberg et al., 2005). It has been shown that CoQ10 has beneficial effects in the prevention and treatment of hypertension, chronic heart failure, arrhythmias, and ischemic heart disease (Kumar, Kaur, Devi, & Mohan, 2009; Littarru, Tiano, Belardinelli, & Watts, 2011). Liang et al. showed that intraperitoneal injection of CoQ10 in soybean oil solvent 3 days before ischemia and the following reperfusion until the end of the test led to improvements in cardiac function including cardiac systolic and diastolic functions, reduction of myocardial death and apoptosis, and reduction of antioxidant levels in the rat model of I/R injury. They also revealed that CoQ10 significantly enhanced autophagy proteins including beclin-1, Atg5, and LC-3II to LC-3I ratio (Liang et al., 2017). Although CoQ10 has protective effects against I/R injury, CoQ1, which is its derivative is more effective in restoring the cardiac contractile function after reperfusion but not the infarct size. This could be due to the high antioxidant potential of CoQ1 (Ashitey et al., 2016).

Visnagin, which is extracted from *Ammi visnaga*, has shown cardioprotective (Liu et al., 2014) and antihypertensive effects (Liu et al., 2014). Fu et al. encapsulated visnagin in NIPAAm-MMA nanoparticle (NP) to investigate its impact on myocardial I/R injury. Following intravenous injection of visnagin-loaded NP in a rat model of I/R, optical bioluminescence imaging data revealed that the nanoparticle targeted encapsulated visnagin to the ischemic area for protecting cardiomyocytes against I/R injury (Fu, Li, & Tan, 2018). It has been shown that the reoxygenation of heart after I/R could increase NP uptake via endocytosis (Cabigas et al., 2012). Visnagin-loaded NP was able to improve the systolic and diastolic function and pressure and inhibit fibrosis. Further analyses demonstrated that visnagin-loaded NP improved cardiac function and reduced I/R injury by enhancing autophagy and inhibiting apoptosis. The cardioprotective nature of visnagin could be related to the aryl hydrocarbon receptor (AHR). As AHR is upstream of the beclin-1:Bcl-2 autophagy regulatory complex, visnagin may inhibit the interaction between beclin-1 and Bcl-2 by promoting AHR signaling (Fu et al., 2018).

Cellular repressor of E1A-stimulated genes (CREG), which is a secreted glycoprotein, could improve cardiac functions by weakening myocardial fibrosis and inhibiting ventricular remodeling, suggesting protective property of CREG against cardiac injury (Journet, Chapel, Kieffer, Roux, & Garin, 2002; Xu, Liu, & Chen, 2004; Yan et al., 2015). It was reported that the expression of CREG markedly decreases in cardiac I/R mice model, whereas treatment with exogenous CREG protein protects the heart from I/R injury by reducing infarct size and apoptosis. Both in vivo and in vitro results demonstrated that the overexpression of CREG increases autophagy. CREG is able to induce autophagy by promoting early autophagosome formation (Song et al., 2017). Many studies revealed that CREG is a lysosome-regulated protein that proteolytically matured through lysosomal cysteine proteases (Qian, Sleat, Zheng, Moore, & Lobel, 2008; Schähs et al., 2008). CREG has a mannose 6-phosphate (M6P) recognition marker, which targets it toward lysosome and interaction with M6P/insulin-like growth factor 2 receptor for efficient delivery to the lysosome (Sacher et al., 2005). Also, CREG is able to enhance the acidic pH of lysosomes, which is essential for the enzymatic digestion (Song et al., 2017).

Histone deacetylases (HDACs) are involved in the transcription of DNA. Studies have reported their essential functions in the remodeling of cardiac pathology including contractility, ventricular hypertrophy, necrosis, fibrosis, and apoptosis (McKinsey, 2012). For example, valproic acid and tributyrin as HDAC inhibitors reduce collagen deposition and cardiomyocyte hypertrophy in a rat model of myocardial infarction (Lee,

TABLE 1 Autophagy-based therapeutic agents against cardiac I/R injury

Therapeutic agents	In vitro/ in vivo	Dose	Effect on heart/ cardiomyocytes	Effect on autophagy	Mechanism of action	References
PPX	In vivo In vitro	1 mg/kg 10 μM	↓ Infarct size ↓ CK and LDH ↑ Cell viability	↑	AMPK pathway	(Mo et al., 2016)
Berberine	In vivo In vitro	10 mg/kg 20 μM	↓ Infarct size ↑ Cardiac function ↑ Cell viability	↓	AMPK and mTORC2 pathway	(Huang et al., 2015)
TSA	In vivo	0.1 mg/kg	↓ Infarct size ↑ Cardiac function	↓	NM	(Zhang et al., 2018)
Crocin	In vivo In vitro	50 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ 10^{-5} mol/L	↑ Cardiac function ↓ Infarct size ↓ Apoptosis ↓ CK and LDH ↑ Cell viability	↑	Akt/mTOR pathway	(Zeng et al., 2016)
CoQ10	In vivo	6 $mg{\cdot}kg^{-1}{\cdot}ml^{-1}$	↑ Cardiac function ↓ Infarct size ↓ Antioxidant levels ↓ Apoptosis	↑	NM	(Liang et al., 2017)
Danshensu	In vitro	10 μM	↑ Cell viability ↓ CK and LDH ↓ Apoptosis	↓	mTOR pathway	(Fan et al., 2016)
Visnagin-loaded NP	In vivo	2 mg/kg	↑ Cardiac function ↓ Infarct size ↓ Apoptosis	↑	NM	(Fu et al., 2018)
miR-21	In vitro	50 nM	↓ Apoptosis	↓	Akt/mTOR pathway	(Huang et al., 2017)
Choline	In vivo	5 mg/kg	↓ Apoptosis ↓ Myocardial fibers	↓	Akt/mTOR pathway	(Hang et al., 2018)
CREG	In vivo	0.3 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$	↓ Infarct size ↓ Apoptosis	↑	NM	(Song et al., 2017)
BCF	In vivo	20 mg/kg	↓ CK and TNF-α ↓ mPTP ↑ NO content ↓ Apoptosis	↓	PI3K/Akt pathway	(Jian et al., 2015)
SAHA	In vivo	30–300 mg/kg	↓ Infarct size ↑ Cardiac function ↓ Apoptosis	↑	NM	(Xie et al., 2013)
bFGF	In vivo In vitro	2 μg 40 ng/ml	↑ Cardiac function ↓ Apoptosis ↓ Fibrosis	↓	PI3K/Akt/mTOR pathway	(Wang et al., 2015)
MHBFC	In vivo	10 mg/kg	↓ CK and TNF-α ↓ NO content ↓ Apoptosis	↓	PI3K/Akt pathway	(Xuan et al., 2017)
2% H_2	In vivo	above 10 ppm/g	↓ Infarct size ↓ Troponin I ↓ ROS	↓	NM	(Gao et al., 2017)
Vitexin	In vitro In vivo	50–100–200 μM 2–4–6 mg/kg	↓ Apoptosis ↓ CK and LDH ↓ MDA ↑ SOD	↓	PI3K/Akt/mTOR pathway	(Tang et al., 2017)
Spermine	In vitro	50 μM	↓ Apoptosis ↑ Cell viability ↓ CK	↑	mTOR pathway	(Duan et al., 2016)

Note. AMPK: 5′-AMP-activated protein kinase; BCF: Bauhinia championii flavone; bFGF: basic fibroblast growth factor; CK: creatine kinase; CoQ10: coenzyme Q10; CREG: cellular repressor of E1A-stimulated genes; H_2: hydrogen gas; LDH: lactate dehydrogenase; MDA: malondialdehyde; MHBFC: 17-methoxyl-7-hydroxy-benzene-furanchalcone; mPTP: mitochondrial permeability transition pore; mTORC: mammalian target of rapamycin complex; NM: not mentioned; NO: nitric oxide; NP: nanoparticle; PI3K: phosphoinositide 3-kinase; PPX: Pramipexole; ROS: reactive oxygen species; SAHA: suberoylanilide hydroxamic acid; SOD: superoxide dismutase. TNF-α: tumor necrosis factor α.

Lin, & Chang, 2007). It has been shown that the overexpression of cardiomyocyte-specific HDAC increases I/R injury (Zhang et al., 2018) and HDAC inhibitors reduce infarct size and increase cardiac function (Granger et al., 2008). Xie et al. have examined the efficacy of suberoylanilide hydroxamic acid (SAHA) or vorinostat, an HDAC inhibitor, in a mice and rabbit model of I/R. They revealed that SAHA is able to reduce infarct and preserve the heart function by promoting autophagy and inhibiting apoptosis in the infarct border zone (Xie et al., 2013). The beneficiary effect of SAHA may relate to the production of ROS in promoting autophagy (Zhang & Ren, 2014). SAHA is a Food and Drug Administration-approved agent for treatment of cutaneous T-cell lymphoma. In cancer cells, SAHA is able to induce autophagy through inhibition of mTOR (Bánréti, Sass, & Graba, 2013) and this mechanism also could be related to protection against cardiomyocytes (Xie et al., 2013). Thus, HDAC inhibitors could act as a therapeutic agent for myocardial I/R injury.

4.2 | Targeting autophagy for its detrimental effects

There is increasing evidence showing that ROS are important activators of autophagy (Morales, Pedrozo, Lavandero, & Hill, 2014). It has been shown that increased H_2O_2 generation during reperfusion is a major inducer of autophagy, which leads to overexpression of beclin-1. Treatment with *N*-2-mercaptopropionyl glycine (MPG), an antioxidant, vigorously downregulated beclin-1 expression, suggesting that oxidative stress may play a key role in mediating autophagy and upregulation of beclin-1 during I/R injury (Hariharan, Zhai, & Sadoshima, 2011). Zeng et al. reported that the transcription factor NF-κB mediated upregulation of beclin-1 during perfusion. They showed that ROS release following cardiac I/R injury activated NF-κB p65 and beclin-1 in the area at risk zone of the rabbit heart. Pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB, intervention significantly suppressed the p65 expression and inhibited the extension of the area at risk zone (Zeng et al., 2013). In another study, it has been found that the activity of toll-like receptor 4 (TLR4)/NF-κB p65 signaling pathway vigorously promoted autophagy in myocardial I/R injury, and the inhibition of TLR4 inhibited apoptosis and autophagy, therefore, attenuated I/R injury (Guo et al., 2016).

Protective effect of berberine, an isoquinoline alkaloid, against I/R injury has been shown in the animal models (Chang et al., 2012; Huang et al., 2015; Qin-Wei & Yong-Guang, 2016). Pretreatment of rats with berberine ($100\,mg{\cdot}kg{\cdot}^{-1}{\cdot}d^{-1}$ for 14 days) before induction of cardiac I/R led to alleviation of myocardial I/R injury through suppression of PI3K/AKT signaling and inflammatory responses (Qin-Wei & Yong-Guang, 2016). In addition to inhibiting inflammatory responses, berberine attenuated cardiac I/R injury by reducing oxidative stress (Yu et al., 2016). In another study, Chang et al. (2012) revealed that the treatment with berberine reduced infarct size and arrhythmias by decreasing AMPK concentration and ADP/ATP and AMP/ATP ratio in the risk area. Although AMPK acted as a chief regulator of energy metabolism of myocardial during I/R by promoting glycolysis and fatty acid oxidation (Chang et al., 2012), its persistent activation during perfusion led to high levels of fatty acid oxidation, accumulation of harmful by-products of glycolysis including lactate and protons, and finally further myocardial injury (Kudo, Barr, Barr, Desai, & Lopaschuk, 1995). However, Huang et al. showed that the cardioprotective property of berberine could be related to its inhibitory effects on autophagy. Berberine is able to reduce the expression of autophagy markers including beclin-1, BNIP3, and sirtuin 1. Furthermore, elevated levels of p-AMPK and p-mTORC2 during H/R in H9c2 cells are decreased by berberine (Huang et al., 2015). It has been shown that the activation of AMPK led to the inhibition of mTORC1 (Gwinn et al., 2008), which thereby induced autophagy (Rodríguez-Vargas et al., 2012). Unlike mTORC1, inhibition of mTORC2 alleviated autophagy (Gurusamy et al., 2009).

Thermal conditions also affect cardiac I/R injury by mediating autophagy. It has been shown that hypothermia is an effective intervention in limiting cardiac injury during organ transplantation and open heart surgery (Lampe & Becker, 2011). According to this fact, Cheng et al. tested the hypothermia effects on I/R cardiomyocytes injury and autophagy. They revealed that the ischemic and hypoxic cells under hypothermia culture condition (32°C) had longer viability when significantly compared with the cells under normoxic culture condition (37°C). Because the hypothermia condition significantly reduced autophagy in parallel with cell death, they claimed that the cardioprotective effect of hypothermia is due to the alleviation of autophagy (Cheng et al., 2013). Furthermore, hypothermia protects cardiomyocytes from I/R injury through reduction of cell metabolism, reduction of enzymatic reaction rates, maintenance of ATP, reduction of gene expression and protein synthesis, improved pH management, and enhanced ion management (Lampe & Becker, 2011). In contrast, Chien et al. (2014) reported that progressive thermal preconditioning reduced cardiac I/R injury by upregulating antiautophagic, antioxidant, and antiapoptotic mechanisms.

Danshensu (DSS) is a water-soluble constituent of the Chinese plant, *Salvia miltiorrhiza*, with antioxidant activities (Tang et al., 2011), which make it as a therapeutic option in several cardiovascular disorders in Asian countries (Wang et al., 2017). It has been shown that DSS has cardioprotective property against I/R injury, which is exhibited by inhibiting apoptosis and decreasing ROS generation (Zhao, Jiang, Zhao, Hou, & Xin, 1996; Zhou et al., 2012). Because the opening of mPTP is the fundamental culprit of I/R injury and c-subunit of ATP synthase is the main constituent of mPTP, Gao et al. (2017) reported that DSS protects against I/R injury by inhibiting the expression of c-subunit of ATP synthase. Other study demonstrated that preconditioning of isolated rat cardiomyocytes increases cell viability. Further analyses revealed that DSS attenuates dysfunction of cardiomyocytes and protects rat hearts during H/R injury by inhibiting excessive autophagy and apoptosis. Upregulation of Bcl-2, an antiapoptotic protein and downregulation of beclin-1, LC3, p62, caspase-3, and Bax following the DSS treatment have suggested a cross-talk between the autophagy and apoptosis during ischemic conditions. Therefore, upregulation of Bcl-2 may affect the cardioprotective effect of DSS against I/R injury by inhibiting apoptosis and preventing autophagy activation (Fan et al., 2016). It has been shown that the formation of beclin-1:Bcl-2 complex leads to negative regulation of autophagy (Pattingre et al., 2005).

Furthermore, pretreatment with DSS vigorously increase mTOR phosphorylation and its downstream targets, S6 and S6K, when compared with the I/R group (Fan et al., 2016).

Dual-specificity protein phosphatase (DUSP), which is also referred as mitogen-activated protein kinase (MAPK) phosphatase, can phosphorylate dephosphorylated MAPKs in a conserved Thr-Xaa-Tyr motif and, therefore, inactive them (Li, Yang, Guo, Wang, & Li, 2015). Jin et al. demonstrated that the expression of DUSP1 is downregulated after I/R injury. They also showed that the size of the infarcted area was significantly reduced in DUSP1 transgenic mice compared with the control group. DUSP1 deficiency led to an increase of JNK phosphorylation and activation. Activated JNK phosphorylates BNIP3 and promoted excessive mitophagy, leading to the destruction of mitochondrial energy production. However, the reintroduction of DUSP1 alleviated mitophagy and protected the hearts following I/R injury by inactivating the JNK signaling (Jin et al., 2018). Confirming these findings, Xu et al. (2015) stated that JNK activation aggravated heart injury following I/R injury through the activation of apoptosis and autophagy.

Choline, a precursor of acetylcholine (ACh), exhibited advantageous effects on several heart disorders including ischemic arrhythmias (Wang et al., 2012), myocardial infarction (Yang et al., 2005), and I/R injury (Zhao et al., 2010). Hang et al. examined the effects of choline on cardiac I/R injury and its underlying mechanisms. They reported that pretreatment with choline significantly attenuated myocardial I/R injury by inhibiting apoptosis and autophagy. Moreover, choline led to higher expression of Akt/mTOR compared with the control group, whereas rapamycin reversed the choline effects. The results suggested that choline alleviated myocardial I/R injury by activating Akt/mTOR-dependent autophagy (Hang et al., 2018). In addition to the choline, ACh also attenuated H/R injury by activating mitophagy through the PINK1/Parkin pathway. As methoctramine (METH), an antagonist of the M2 receptor, suppressed mitophagy activation by ACh, M2 receptor mediated the effect of ACh on mitophagy (Sun et al., 2016).

microRNAs (miRNAs) are endogenous, single-stranded, and non-coding RNAs with a length of 21–25 nucleotides, which are involved in regulating physiological and pathological processes (Goradel et al., 2018). Many studies have examined the effects of miRNAs on the cardiac I/R injury (Fan & Yang, 2015). For example, the cardioprotective property of miR-34a in I/R injury is related to the inhibition of autophagy (Shao et al., 2017). According to the significantly lower expression of miR-34a in I/R and H/R models, Shao et al. (2017) transfected hearts and isolated cardiomyocytes with miR-34a over-expressed adenovirus. Subsequent overexpression of miR-34a led to a decrease in apoptosis and autophagosome formation. Further analyses revealed that the tumor necrosis factor α (TNF-α) is the direct target of miR-34a in reducing autophagy during myocardial damage. These results suggested the interaction between autophagy and inflammation (Shao et al., 2017). To understand the association between autophagy and inflammation in myocardial I/R injury, Meng et al. (2017) reported that short-hairpin RNA interference of nod-like receptor protein 3 inflammasome reduced I/R injury by activating autophagy. However, the miR-34a role in cardiac I/R injury is contradictory. A study contrary to Shao et al. demonstrated that the suppression of miR-34a could rescue myocardial I/R injury (Fu et al., 2017). Also, Wang et al. (2019) found that the expression of miR-34a was markedly increased in cardiac I/R injury. Furthermore, it has been shown that I/R resulted in down-regulation and upregulation of miR-204 and autophagy, respectively. Transfection of miR-204 mimic into cardiomyocytes attenuated autophagy (Xiao et al., 2011). Studies reported that miR-21 expression was downregulated in the infarcted hearts and vigorously increased in the border area (Tu et al., 2013; Van Rooij et al., 2008). Huang et al. showed that the expression of endogenous miR-21 was decreased in H9c2 cells following H/R injury. They also showed that the transfection of cells under H/R injury with miR-21 precursor led to significant increase in miR-21 expression, which attenuated autophagy and apoptosis induced by H/R injury through PTEN/Akt/mTOR pathway (Huang et al., 2017). It has been shown that miR-21 induced Bcl-2 expression by targeting Bcl-2 mRNA (Dong, Zhao, Zhou, Zhang, & Chen, 2011), which could result in the inactivation of beclin-1. Furthermore, Seca et al. (2013) reported that treatment with an anti-miR-21 antibody increased expression of ATGs including beclin-1, LC3-II, and Vps34 through the reduction of Bcl-2 expression. Yang et al. demonstrated that miR-410 expression was meaningfully upregulated in a mice model of cardiac I/R injury. Overexpression of miR-410 during I/R injury was correlated with inhibition of mitophagy and induction of apoptosis. They identified that miR-410 suppressed high-mobility group box 1 protein (HMGB1) so that transfection of cardiomyocytes with pcDNA3.1-HMGB1 promoted autophagy, reduced apoptosis, and improved mitochondria function by modulating heat shock protein β1 (Yang, Li, Dong, & Mi, 2018). Moreover, HMGB1 in collaboration with the TNF promoted apoptosis of cardiomyocytes under I/R by JNK activation (Shvedova, Anfinogenova, Atochina-Vasserman, Schepetkin, & Atochin, 2018). miR-30e is another miRNA involved in I/R injury through autophagy, which was downregulated in patients with myocardial I/R injury. Suppression of miR-30e reduced apoptosis and increased autophagic proteins including beclin-1, LC3, and p62 in H9c2 cells. Thus, miR-30e exhibited a protective effect on cardiac I/R injury by modulating autophagy and apoptosis (Zheng, Li, Kou, Yi, & Shi, 2018).

5 | CONCLUSION

In patients with AMI, myocardial reperfusion is an effective and timely choice for ameliorating ischemia injury and reducing myocardial infarct size. However, myocardial reperfusion can lead to cardiomyocyte death through cardiac I/R injury. Unfortunately, there is no effective therapeutic choice to prevent I/R injury. Some studies have revealed autophagy as a main modulating process during myocardial I/R injury. Therefore, the relationship between autophagy and I/R injury is unclear and whether autophagy is beneficial or detrimental is controversial. Several factors are involved in this controversy including timely intervention, experimental models and their variability, and methods for assessing autophagy. For this reason, further studies or discussions are needed to reveal the underlying mechanisms of autophagy in

reperfusion injury and to introduce it as a novel therapeutic strategy to reduce myocardial I/R injury.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Conception and design: A. M. A., V. K., M. A., and S. G. Collection and assembly of data: M. A., M. M., A. J., A. E., M. R., M. P., and A. M. Manuscript writing: M. A., M. M., A. E., M. R., M. P., and A. M. Made critical revisions and approved final version: A. M. A., V. K., S. G., and A. J. All authors reviewed and approved of the final manuscript.

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