The role of autophagy in angiotensin II-induced pathological cardiac hypertrophy

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Abstract

Pathological cardiac hypertrophy is associated with nearly all forms of heart failure. It develops in response to disorders such as coronary artery disease, hypertension and myocardial infarction. Angiotensin II (Ang II) has direct effects on the myocardium and promotes hypertension. Chronic elevation of Ang II can lead to pathological cardiac hypertrophy and cardiac failure. Autophagy is an important process in the pathogenesis of cardiovascular diseases. Under physiological conditions, autophagy is an essential homeostatic mechanism to maintain the global cardiac structure function by ridding damaged cells or unwanted macromolecules and organelles. Dysregulation of autophagy may play an important role in Ang II-induced cardiac hypertrophy although conflicting reports on the effects of Ang II on autophagy and cardiac hypertrophy exist. Some studies showed that autophagy activation attenuated Ang II-induced cardiac dysfunction. Others suggested that inhibition of the Ang II induced autophagy should be protective. The discrepancies may be due to different model systems and different signaling pathway involved. Ang II-induced cardiac hypertrophy may be alleviated through regulation of autophagy. This review focuses on Ang II to highlight the molecular targets and pathways identified in the prevention and treatment of Ang II-induced pathological cardiac hypertrophy by regulating autophagy.

Key Words
- angiotensin II
- autophagy
- cardiac hypertrophy
- oxidative stress
- mTOR

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Introduction

The simple definition of cardiac hypertrophy is the enlargement of the heart (Hou & Kang 2012). The enlargement of the heart is closely matched to its functional load and, under normal conditions, is primarily constitutive in nature. The heart triggers a hypertrophic response, which is an adaptation to changes in wall stress so as to maintain cardiac output. Cardiac hypertrophy can be classified as either physiological or pathological hypertrophy (Bernardo *et al*. 2010, Abel & Doenst 2011). Pathological cardiac hypertrophy develops in response to disorders such as hypertension, coronary artery disease, myocardial infarction, metabolic and diabetic cardiomyopathy, and valvular heart disease, which eventually give rise to ventricular remodeling and cardiac dysfunction (Maillet *et al*. 2013). Pathological hypertrophy is a key risk factor for heart failure and commonly associated with the upregulation of fetal genes, fibrosis, cardiac dysfunction and increased mortality (Bernardo *et al*. 2010). Although there are many factors and regulators that affect myocardial

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hypertrophy, the renin-angiotensin system (RAS) and its primary effector peptide, angiotensin II (Ang II), are involved in the pathophysiology of cardiac hypertrophy and failure (Demos-Davies *et al*. 2014). Ang II has a role in stimulating total RNA, mRNA and protein synthesis levels in cardiomyocytes (Rohini *et al*. 2010). The classical effects of Ang II are mainly mediated via two G-protein-coupled receptors, type 1 receptors (AT_1R) and type 2 receptors (AT_2R) (Dasgupta & Zhang 2011, Araujo *et al*. 2011). The AT_1R mediates the major physiological actions of Ang II, such as stimulation of aldosterone secretion from the adrenal gland, vasoconstriction, retention of salt and water, cardiac contractility and growth stimulation, whereas the AT_2R is believed to induce opposing effects, namely hypotension, vasodilatation, anti-hypertrophic effects, antigrowth by apoptosis and the possible inhibition of AT_1R (Dasgupta & Zhang 2011). Ang II contributes to the pathogenesis of cardiac hypertrophy in four ways: (a) through Gq/11-1,2-diacylglycerol (DAG)-protein kinase C (PKC)-dependent activation of mitogen-activated protein kinases (MAPKs), c-Jun N-terminal kinases (JNKs) and Janus kinases (JAK)-signal transducers and activators of transcription (STAT) cascades; (b) Gq/11-inositol 1,4,5-triphophate (IP3)–calcium–calcineurin-dependent activation of MAPK; (c) activation of Rho A-Rho kinase (ROCK) via G12/13 protein and (d) activation of matrix metalloproteinases (MMPs) via the JAK/STAT pathway (Balakumar & Jagadeesh 2010).

Autophagy is characterized by an evolutionarily conserved process for the lysosome-dependent degradation of cytoplasm components and damaged organelles such as endoplasmic reticulum, peroxisomes and mitochondria, as well as eliminating intracellular pathogens (Wang *et al*. 2010, Jia & Sowers 2015, Mei *et al*. 2015). It was reported that constitutive autophagy in the heart is a homeostatic mechanism for maintaining cardiomyocyte size and global cardiac structure and function. For example, temporally controlled cardiac-specific deficiency of autophagy-related 5 (*Atg5*), led to cardiac hypertrophy, left ventricular dilatation and contractile dysfunction in the adult mice (Li *et al*. 2015*b*). In cardiomyocytes, autophagy is necessary for the continual process of removing, repairing and replacing damaged cellular materials. The association between autophagic activity and heart disease has been noted for almost 40 years. Until now, a recurring paradox in the study of autophagy is its dual nature (Schiattarella & Hill 2016). In the heart, autophagy functions mainly as a pro-survival pathway during cellular stress by removing protein aggregates and damaged organelles, protecting the heart against famine, ischemia and excessive β-adrenergic stimulation. However, when severely triggered, the autophagic machinery may lead to cell death (Gatica *et al*. 2015, Schiattarella & Hill 2016).

Accumulating evidence has revealed a tight link between cardiomyocyte autophagy and cardiac hypertrophy (Li *et al*. 2015*b*, Li *et al*. 2016). The role of autophagy in Ang II-induced cardiac hypertrophy has recently been explored; however, conflicting reports on the effects of Ang II on autophagy and its role in cardiac hypertrophy exist (Oyabu *et al*. 2013). This review is to describe the main mechanisms of autophagy and elucidate the role of autophagy in Ang II-induced cardiac hypertrophy. In addition, the possibility of autophagy as a therapeutic target for cardiac hypertrophy will provide novel therapeutic strategies for the treatment of heart failure.

Overview of autophagy

To form a mature autophagosome, autophagy proceeds in successive stages, which include induction, nucleation, expansion and maturation (Wang *et al*. 2010, Hale *et al*. 2013). Autophagy process is regulated by a system of autophagy-related gene (ATG) products (Fig. 1).

Induction of autophagy

Many stimuli and numerous upstream signaling pathways modulate autophagy. Phosphatidylinositol 3-kinase (PI3K)/Akt, growth factor signaling, AMP-dependent protein kinase (AMPK), MAPK, trimeric G proteins, small GTPases, IP3, calcium signaling and others regulate the process. Many of these pathways work through the mammalian target of rapamycin (mTOR) (Dunlop & Tee 2014). mTOR is one of the most important upstream regulators for the induction of autophagy. In response to ample nutrients and intact insulin signaling, class I PI3K is activated to phosphorylate its downstream target Akt. Akt then activates mTOR. Active mTOR phosphorylates Atg13 and inhibits its interaction with unc-51-like kinase 1 (ULK1), a critical step during autophagy induction. In response to starvation, the affinity of ULK1 for both ATG13 and FIP200 increases and promotes the formation of a trimeric ULK1/ATG13/FIP200 complex, which facilitates the induction of autophagy (Wang *et al*. 2010, Nemchenko *et al*. 2011, Maejima *et al*. 2016).

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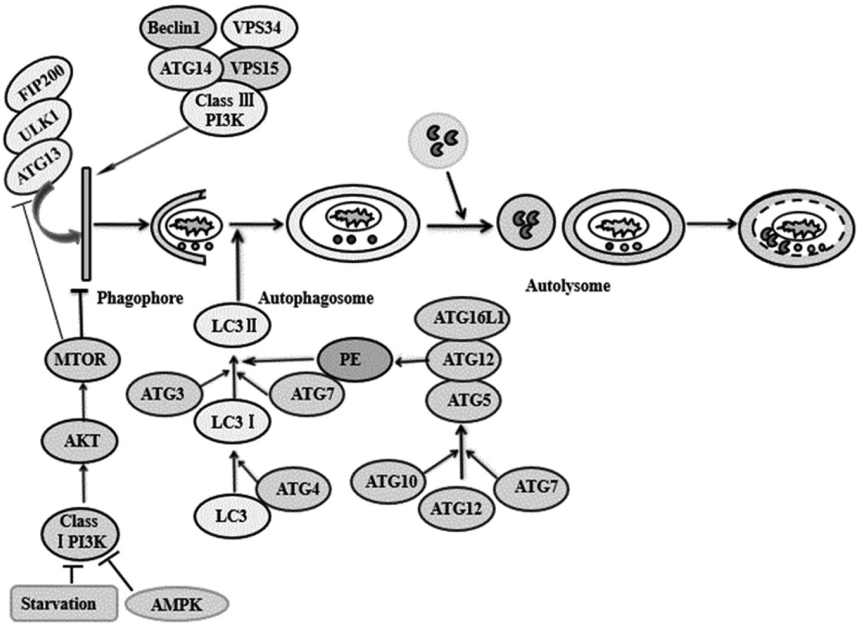

Figure 1
The core machinery of autophagy. Starvation and AMPK activation inhibit class I PI3K to phosphorylate its downstream target Akt, and then, inhibit mTOR. Inhibition of mTOR reduces the phosphorylation of Atg13. Atg13 combines with ULK1 and FIP 200 forming a ULK1 complex. The nucleation of phagophore is regulated by the Ulk1 complex and the PI3K complex (Beclin1-ATG14-VPS34-VPS15-Class IIIPI3K). The expansion of the phagophore and the formation of autophagosome require two complexes: the ATG16L1 complex (ATG12-ATG5-ATG16L1) and LC3-phosphatidyl ethanolamine (PE). LC3-II remains on mature autophagosomes until after fusion with lysosomes to generate autolysosomes, and the contents are then degraded by proteases, lipases, nucleases and glycosidase.

Nucleation of autophagy

Autophagy induction promotes ULK1 complex activation, which phosphorylates Beclin1-regulated autophagy 1 (Ambra1), thereby enhancing the activity of Beclin1-ATG14-VPS34-VPS15 class III PI3K core complexes to promote autophagosome nucleation (Mei *et al*. 2015). Additionally, in response to ample nutrient, the Bcl-2 family antiapoptotic proteins interact with Beclin1 and exert inhibitory effects on autophagy (Cheng *et al*. 2013).

Expansion of autophagy

The phagophore elongation and formation of complete autophagosomes require two ubiquitin-like protein conjugation systems: ATG12-ATG5-ATG16L1 and microtubule-associated light chain-3 (LC3)-phosphatidylethanolamine (PE) (Jia & Sowers 2015, Li *et al*. 2015*b*). ATG12 is first activated by the E1-like enzyme, ATG7, and it is then conjugated to ATG5 by ATG10, an E2-like enzyme. The ATG12–ATG5 conjugate further interacts with ATG16L1 to form a large multimeric complex. The ATG16L1 complex forms pre-autophagosomal structures and acts as an E3 ligase, allowing the second conjugation reaction to be completed (Mei *et al*. 2015). The second ubiquitin-like conjugation pathway involves LC3 lipidation, the mammalian homolog of yeast protein ATG8. ATG4B cleaves the C-terminal 22 residues of precursor LC3 (proLC3) to produce LC3-I. Following the combination of ATG3 (E2-like enzyme), the ATG16L1 complex (E3-ligase), ATG7, and LC3 are then conjugated to PE to produce LC3-PE (also called LC3-II). LC3-II specifically localizes to the autophagosomal membranes and so it is suited to serving as an autophagy-specific marker (Dirks-Naylor 2013, Hale *et al*. 2013, Jia & Sowers 2015).

Maturation of autophagy

A mature autophagosome can fuse directly to a lysosome or first fuse with an endosome before trafficking to the lysosome, forming an autolysosome (Rotter & Rothermel 2012). There, the inner membrane of the former autophagosome and the engulfed cargo are degraded by acid hydrolases. The resulting small molecules, including amino acids, sugars and lipids, are released into the cytosol through permeases (Wang *et al*. 2010).

Ang II-induced autophagy

Recently, a number of studies have shown that autophagy plays important roles in Ang-II induced cardiac hypertrophy. Some researchers have shown that Ang II treatment induces autophagy and others have shown that Ang II treatment inhibits autophagy. As autophagy has dual functions in cardiomyocytes, many investigators have used chemical means of manipulating autophagy to elucidate its role in Ang II-induced cardiac hypertrophy. However, it is significant to notice that a lot of the currently used chemical modulators of autophagy have off-target effects to be considered when interpreting results (Dirks-Naylor 2013). As discussed in the following sections, majority of studies have shown that Ang II

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Table 1 Roles of autophagy in Ang II-induced cardiac injury.

Ang II-induced cardiac injury	Autophagy changes	Models	Ang II administration	Targets and effects	References
Cardiac hypertrophy	HcRed-LC3 and autophagosomes increased	Neonatal cardiomyocytes	0.1 μmol/L Ang II (48 h)	AT_2R constitutively antagonizes AT_1R-mediated autophagy	Porrello and Delbridge (2009) Porrello *et al.* (2009)
Cardiac hypertrophy	LC-3 II/I ratio, numbers of autophagosomes and autolysosomes increased	Mice	1.1 mg/kg/day Ang II (4 weeks)	Mitochondrial oxidative stress increase, overexpression of mCAT, but not pCAT could block hypertrophy	Dai *et al.* (2011), Dai and Rabinovitch (2011)
Cardiac hypertrophy	LC-3 II/I ratio and mitophagy decreased	Macrophages from wild-type C57BL/6J or *Atg5*$^{+/-}$ mice	1 μmol/L Ang II (48 h)	*Atg5* deficiency-mediated mitophagy aggravates cardiac inflammation and injury	Zhao *et al.* (2014)
Cardiac hypertrophy	Autophagosomes and acidic vesicular organelles reduced	H9C2 cells	20 μmol/L Ang II (48 h)	Oxidative stress reduces autophagy, increases apoptosis, decreases connexin 43	Chen *et al.* (2014)
Cardiac hypertrophy	ATG5 and LC3 elevated	Human adult cardiac myocytes	200 nmol/L Ang II (4 days)	Regulator of calcineurin 1-1L rescued cell viability and growth	Duan *et al.* (2015)
Cardiac hypertrophy	LC3b-II and p62 increased; induction of autophagosome formation; not affect autophagic flux	Neonatal rat cardiomyocytes and C57BL/6 male mice	10^{-6} mol/L Ang II (48 h) 1000 ng/kg/min Ang II (4 weeks)	Ang-(1–7) decrease gross heart size and HW/BW ratio; downregulate ANP, Saa and sectional area of cardiomyocytes by Mas receptor	Lin *et al.* (2016)
Cardiomyocyte injury	Increased LC3-II/I ratio and LC3 mRNA	Male adiponectin knockout mice and wild-type C57BL/6 mice	3.2 mg/kg/day Ang II (14 days)	Adiponectin inhibits H_2O_2-induced autophagy by AMPK/mTOR/ERK pathway	Essick *et al.* (2013)
Cardiomyocyte hypertrophy	Autophagic vacuoles increased; Atg9A expression induced	Neonatal rat ventricular cardiomyocytes	1 μmol/L Ang II	miR-34a decreased the expression of ATG9A and cardiomyocyte surface area, also decreased ANP and β-MHC expression	Huang *et al.* (2014)
Cardiomyocyte hypertrophy	Autophagic vacuoles increased, Beclin-1, LC3-II/I increased	Neonatal rat ventricular myocytes	1 μmol/L Ang II	miR-30a decreased cardiomyocyte surface area, downregulated ANP and β-MHC expression	Pan *et al.* (2013)
Myocardial injury	miR-30a decreased, Beclin-1 increased	Rats	NP	miR-30a regulate Beclin-1 protein	Huang *et al.* (2015*a*)
Cardiomyocyte hypertrophy	LC3-II/I ratio increased	Neonatal rat cardiomyocytes	100 nM Ang II (48 h)	Calhex231 inhibits the Ca^{2+}/CaMKKβ-AMPK-mTOR pathway and increases cardiomyocyte survival	Liu *et al.* (2015)
Cardiac hypertrophy	Autophagic vacuoles, LC3B expression increased	Neonatal rat ventricular cardiomyocytes	1 μmol/L Ang II	Inhibit LC3B expression, rescued cell area, ANP and β-MHC level	Huang *et al.* (2015*b*)

Cardiac remodeling	Reduced LC3-II/I ratio	Male WT and HDAC6 null mice	1.0 μg/kg/min Ang II (8 weeks)	HDAC6 developed cardiac hypertrophy and fibrosis, HDAC6 deletion improves cardiac function	Demos-Davies *et al.* (2014)
Myocardial fibrosis	Increased LC3II, Beclin-1, and p62 autophagosome formation increased and autophagic flux decreased	Male *Creg1*$^{+/-}$ and *Creg1*$^{+/+}$ mice	1.5 mg/kg/day Ang II (14 days)	CREG1 protected the heart tissue against Ang II-induced fibrosis by activating autophagy	Yan *et al.* (2015*a*)
Cardiac hypertrophy	LC3 II decreased, ROS generation increased	Male Sprague-Dawley rats	200 ng/kg/min Ang II (4 weeks)	Decrease in HW/BW, cardiac myocyte cross sectional area, reversed heart contractility by chronic inhibition of class I PI3K pathway	Yan *et al.* (2015*b*)

AMPK, AMP-dependent protein kinase; ANP, atrial natriuretic peptide; CaMKK, Ca^{2+}/calmodulin-dependent protein kinase kinase; CREG1, cellular repressor of E1A genes; ERK, extracellular regulated protein kinases; HDAC6, histone deacetylases 6; HW/BW, heart weight to body weight ratio; mCAT, catalase targeted to mitochondria; mTOR, mammalian target of rapamycin; NP, not published; pCAT, peroxisomal-targeted catalase; PI3K, phosphatidylinositol 3-kinase; Saa, skeletal a-actin; WT, wild type; β-MHC, β-myosin heavy chain.

increases autophagy and, thereby, contributes to cardiac hypertrophy by enhancing cellular dysfunction and cell death (Table 1).

AT_1R and AT_2R in Ang II-induced autophagy

To date, majority of the physiological actions of Ang II are regulated by AT_1R, and the function of AT_2R is less well delineated. Porrello and coworkers provided the first link between Ang II and autophagy regulation in the heart (Porrello & Delbridge 2009). They found that Ang II increased autophagosome formation via the AT_1R in neonatal cardiomyocytes; however, co-expression of the AT_2R abrogated this response (Porrello & Delbridge 2009). That is, AT_2R antagonizes AT_1R-mediated cardiomyocyte autophagy (Porrello *et al.* 2009). This study proposed that the anti-autophagic functions of AT_2R are dependent on a PI3K signaling mechanism in cardiomyocytes (Porrello & Delbridge 2009, Porrello *et al.* 2009). In addition, hypertrophic heart rat neonatal cardiomyocytes display an elevated susceptibility to AT_1R-mediated autophagic vacuolization relative to normal heart rat neonatal cardiomyocytes *in vitro* (Porrello *et al.* 2009).

Oxidative stress and Ang II-induced autophagy

Studies have shown that Ang II-induced cardiac hypertrophy is associated with increased cardiac mitochondrial reactive oxygen species (ROS) generation and oxidative stress (Dai *et al.* 2011, Zablocki & Sadoshima 2013). Ang II can increase cardiac mitochondrial DNA deletions and protein oxidative damage (Dai *et al.* 2011). Oxidative stress is closely related to mitochondrial dysfunction; in addition, the turnover of mitochondria is dependent on autophagy (Lee *et al.* 2012). ROS-induced mitochondrial damage seemed to be connected with increased number of autophagosomes and autolysosomes. Mitochondrial autophagy was upregulated in Ang II-treated mice heart, accompanied with increased mitochondrial protein carbonyls and mitochondrial DNA deletions (Dai *et al.* 2011). Meanwhile, mice overexpressing catalase targeted to mitochondria (mCAT) challenged by Ang II infusion are resistant to mitochondrial damage, biogenesis, cardiac hypertrophy and fibrosis induced by Ang II (Dai & Rabinovitch 2011, Dai *et al.* 2011, Dikalov & Nazarewicz 2013). Moreover, Chen and coworkers also suggested that the reduced autophagic response to the oxidative stress which was induced by hydrogen peroxide in Ang II-induced hypertrophic H9C2 cells causes increased apoptotic cell death (Chen *et al.* 2014).

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Regulation of Ang II-induced autophagy

Regulation of various key molecules

As an important adaptive stress response, mitochondrial autophagy can be modulated by various key molecules. Regulator of calcineurin 1-1L (Rcan1-1L) promoted cell viability and mitochondrial autophagy in Ang II-treated human adult cardiac myocytes, with elevated ATG5 and LC3 expression and downregulation of phosphorylated p70S6K (Duan *et al*. 2015). Additionally, Rcan1-1L significantly inhibited calcineurin/nuclear factor of activated T cells (NFAT) signaling, which may protect human cardiomyocytes from Ang II-activated oxidative stress through the induction of mitochondrial autophagy and may be a method of cardiac protection (Duan *et al*. 2015).

Angiotensin1–7 (Ang-(1–7)) is formed from Ang II by angiotensin-converting enzyme 2 (ACE2) and has been shown to oppose the Ang II-AT_1R-axis-exerted deleterious effects. Ang-(1–7) retarded hypertrophy, oxidative stress and autophagy induced by Ang II treatment in the heart. Moreover, a Mas receptor antagonist A779 was used to assess the role of Mas receptor in Ang-(1–7)-mediated action, and the results indicated that Mas receptor offers protective effects of Ang (1–7) against Ang II-induced excessive cardiac remodeling and cardiac autophagy (Lin *et al*. 2016).

Adiponectin and Ang II-induced autophagy

Studies show that adiponectin (APN) ameliorates Ang II-induced oxidative stress (Essick *et al*. 2011, Nour-Eldine *et al*. 2016). Chronic Ang II infusion significantly increased the level of ROS in male APN knockout (APN-KO) mice vs wild-type (WT) mice; furthermore, excessive ROS caused cardiomyocyte autophagy (Essick *et al*. 2013, Qi *et al*. 2014). In APN-KO mice, Ang II infusion significantly increased LC3II/I gene and protein expression ratio. However, neither Ang II nor APN affected Beclin-1 expression between Ang II-infused APN-KO and WT mice. These findings suggest that APN protects against excessive ROS-mediated cardiomyocyte autophagy by suppressing the autophagic machinery via an AMPK/mTOR/extracellular-regulated protein kinases (ERK)-dependent mechanism (Essick *et al*. 2013). However, APN activates macrophage autophagy through the activation of AMPK in the heart, thereby reducing the extent of cardiac fibrosis and inflammation (Qi *et al*. 2014). These studies demonstrate that APN has cardioprotective actions by modulating oxidative stress-induced autophagy in cardiomyocytes (Essick *et al*. 2013).

Effects of microRNA in Ang II-induced autophagy

Recent studies have found that expression signatures of miRNAs are associated with pathological cardiac hypertrophy and heart failure (Wang & Yang 2012). Several miRNAs that induce pathological hypertrophy and heart failure have been identified, such as miR-195, miR-21, miR-23a and miR-208a, whereas miR-133 and miR-1 negatively regulate cardiac hypertrophy (Yang *et al*. 2011). Many studies have shown that miRNAs regulate both cardiac hypertrophy and cardiomyocyte autophagy, for example, miR-212/132 and miR-199a; induce cardiac hypertrophy; and attenuate the autophagic response in cardiomyocytes (Ucar *et al*. 2012, Li *et al*. 2015*a*). Ang II-induced cardiomyocyte hypertrophy in terms of cell area of cardiomyocytes and expression of the cardiomyocyte hypertrophy markers atrial natriuretic peptide (*ANP*) and β-myosin heavy chain (*β-MHC*) mRNA; moreover, the level of ATG9A protein and mRNA expression was upregulated. Ang II treatment increased the autophagic vacuoles and ratio of LC3II/I in cardiomyocytes. Overexpression of miR-34a suppressed the number of autophagic vacuoles and myocardial hypertrophy induced by Ang II. The study indicates that miR-34a alleviates Ang II-induced myocardial hypertrophy by the inhibition of autophagic activity and ATG9A expression (Huang *et al*. 2014). Pan and coworkers provide strong evidence that downregulation of miR-30 induced by Ang II leads to excessive autophagy in cardiomyocytes through the activation of beclin-1, thereby promoting myocardial hypertrophy. Treatment of cardiomyocytes with miR-30a mimics decrease in the cardiomyocyte surface area and attenuates the Ang II-induced upregulation of hypertrophy-related genes *ANP* and β-*MHC* *in vitro* (Pan *et al*. 2013). Consistent with the above study, Ang II-induced cardiomyocyte autophagy may correlate with the downregulation of miR-30a through upregulation of the Beclin-1 protein *in vivo* (Huang *et al*. 2015*a*). Taken together, several miRNAs are associated with Ang II-induced pathological cardiac hypertrophy and autophagy, and this would provide a novel strategy for the management of cardiac hypertrophy.

Autophagy-related protein

ATG5 is an E3 ubiquitin ligase that is necessary for the autophagy. *Atg5*-deficient mice develop left ventricular

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dilatation and cardiac dysfunction and show disorganized sarcomere structure after pressure overload. Compared with the saline controls, Ang II infusion significantly increased the *Atg5* mRNA expression level in the macrophages, but not the cardiomyocytes and cardiac fibroblasts. Ang II treatment significantly stimulated LC3 protein in cultured WT macrophages, which was further reduced in *Atg5*$^{+/-}$ macrophages. *Atg5* haplodeficiency markedly decreases cytosolic autophagic vacuole formation induced by Ang II, increases nuclear factor-κB (NF-κB) activity and mitochondrial ROS production in macrophages, which contribute to subsequent cardiac inflammation and cardiac injury (Zhao *et al*. 2014). These findings indicated that Ang II elevated ROS production and oxidative stress and induced autophagy, concomitant with cardiac injury, which also suggested that upregulation of autophagy may be a method of cardiac protection (Zhao *et al*. 2014, Duan *et al*. 2015).

As a marker of autophagic activity, growing evidence highlighted the importance of LC3B in monitoring autophagy in myocardial hypertrophy. Ang II (1 μmol/L for 48 h) induced neonatal rat cardiomyocytes hypertrophy, accompanied by a marked increase in cell area and ANP and β-MHC expression. In addition, LC3B-II/I protein and mRNA expressions were also upregulated. Moreover, LC3B overexpression resulted in upregulated levels of autophagic activity and increased *ANP* and β-*MHC* mRNA expression as well as increased cardiomyocyte area in Ang II-treated cardiomyocytes. LC3B-mediated autophagy plays a role in the regulation of myocardial hypertrophy induced by Ang II which may provide a therapeutic target to reverse myocardial hypertrophy induced by Ang II (Huang *et al*. 2015*b*).

Other signaling pathways in Ang II-induced autophagy

Many signaling pathways have also been found to be involved in Ang II-induced autophagy, for example, Calhex231 suppressed calcium-sensing receptor (CaSR) expression and downregulated autophagy by inhibiting the Ca^{2+}/calmodulin-dependent protein kinase-kinase-β (CaMKKβ)-AMPK-mTOR pathway to ameliorate cardiomyocyte hypertrophy induced by Ang II in neonatal rat cardiomyocytes (Liu *et al*. 2015). Ang II exposure induced significant increase in the expression of endogenous intermedin in H9c2 cell cultures and mouse hearts, and the sizes of cardiomyocyte, interstitial collagen, ANP and BNP expression were also increased. Intermedin supplementation could protect cardiomyocytes against hypertrophy induced by pressure overload or hypertrophic stimuli *in vivo* and *in vitro*. Intermedin protected hypertrophy-induced cardiomyocyte from apoptosis through the activation of autophagy in hypertrophic cultured H9c2 cells,

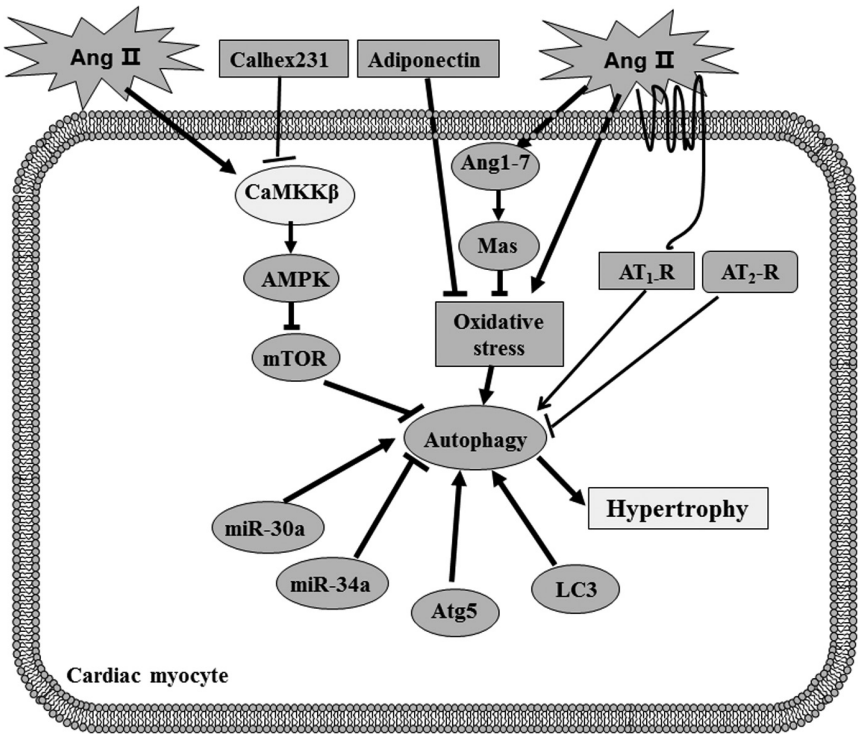

Figure 2
Mechanism of autophagy in Ang II-induced pathological cardiac hypertrophy.

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which was almost abrogated by 3-methyladenine (3-MA). Furthermore, intermedin supplementation remarkably augmented the cAMP contents in H9c2 cells stimulated by Ang II. Co-incubation of intermedin with Ang II only increased the ERK1/2 phosphorylation, whereas showed no obvious effect on Akt activation. PI3K inhibition by wortmannin, PKA inhibition by H89 or MAPK/ERK1/2 inhibition by PD98059 effectively reduced the intermedin-augmented autophagy level in Ang II-exposed H9c2 cells, but only H89 and PD98059 pre-incubation abolished the antiapoptotic action of intermedin. All these results indicate that the endogenous intermedin induced by Ang II may play an important role in cardiac hypertrophy, and the augmented autophagy level induced by intermedin supplementation is involved in its protection against cardiomyocyte hypertrophy through the activation of both MAPK/ERK1/2 and cAMP/PKA signaling pathways (Chen *et al*. 2013). Other signaling pathways are also found in the regulation of autophagy in the Ang II-induced cardiac hypertrophy; for example, class I PI3K, via the activation of the Akt/mTOR pathway, is involved in Ang II-induced impairment of autophagy, elevation of ROS, cardiac hypertrophy and fibrosis (Yan *et al*. 2015*b*). Histone deacetylases 6 (HDAC6) is also involved in Ang II-induced cardiac hypertrophy and autophagy, but the mechanisms are unknown (Demos-Davies *et al*. 2014). In addition, mouse with aortic arch constriction (TAC) induced marked heart hypertrophy and fibrosis, accompanied by high levels of Ang II in plasma and heart. Aliskiren ameliorates heart hypertrophy in the model by suppressing Ang II-PKCβI-ERK1/2-regulated autophagy (Weng *et al*. 2014, Zhang *et al*. 2014). Moreover, cellular repressor of E1A genes (CREG1) protected the heart tissue against Ang II-induced fibrosis by activating autophagy (Yan *et al*. 2015*a*). Induction of autophagy may serve as a cytoprotective mechanism to inhibit cardiac fibrosis induced by Ang II (Liu *et al*. 2016).

Conclusion

As mentioned previously, a large body of studies both *in vitro* and *in vivo* clearly reveals that Ang II increases autophagy. Upregulation or downregulation autophagy may play a protective role to antagonize Ang II-induced cardiac hypertrophy. Whether activation or inhibition of autophagy plays a protective role would depend on the model and the signaling pathway involved. As most of the studies used the rat neonatal cardiomyocytes, others used human adult cardiac myocytes, H9C2, or macrophage and so on. What is more, study found that the autophagy and apoptosis in HL-1 cardiomyocytes after exposure to Ang II were dependent on the concentration and duration of exposure of cells to Ang II (Wang *et al*. 2013). The mechanisms of autophagy in Ang II-induced pathological cardiac hypertrophy mainly included mediating AT_1R and AT_2R, affecting oxidative stress, microRNAs and others (Fig. 2). Whether autophagy functions as a pro-survival or pro-death program during the Ang II-induced cardiac hypertrophy is still not totally understood, it may finally be determined by the identity of the cellular components being degraded as well as the timing and magnitude of autophagic activity relative to other essential cellular processes (Rotter & Rothermel 2012, Li *et al*. 2015*b*). Future investigations will be given to elucidate the importance of the autophagic pathways in the cardiac hypertrophy, which will provide new therapeutic approaches to the cardiac failure.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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