Autophagy, a term coined by Christian de Duve, describes an evolutionarily conserved process involved in the digestion of long-lived proteins and organelles. This process is characterized by the engulfment of cargo into double membrane autophagosomes, which then fuse with lysosomes for degradation. Autophagy is important for maintaining cardiac homeostasis and is a survival mechanism that is upregulated during stress or starvation. Accumulating evidence suggests that dysregulated or reduced autophagy is associated with heart failure and aging. Thus, modulating autophagy represents an attractive future therapeutic target for treating cardiovascular disease. Activation of autophagy is generally considered to be cardioprotective, whereas excessive autophagy can lead to cell death and cardiac atrophy. It is important to understand how autophagy is regulated to identify ideal therapeutic targets for treating disease. Here, we discuss the key proteins in the core autophagy machinery and describe upstream regulators that respond to extracellular and intracellular signals to tightly coordinate autophagic activity. We review various genetic and pharmacological studies that demonstrate the important role of autophagy in the heart and consider the advantages and limitations of approaches that modulate autophagy. (Circ Res. 2015;116:489-503. DOI: 10.1161/CIRCRESAHA.116.303791.)

Key Words: autophagy ■ heart failure ■ mitochondria

Autophagy is an evolutionarily conserved process by which long-lived proteins and organelles are sequestered by autophagosomes and subsequently degraded by lysosomes for recycling. Autophagy is important for maintaining cardiac homeostasis and is a survival mechanism that is upregulated during stress or starvation. Accumulating evidence suggests that dysregulated or reduced autophagy is associated with heart failure and aging. Thus, modulating autophagy represents an attractive future therapeutic target for treating cardiovascular disease. Activation of autophagy is generally considered to be cardioprotective, whereas excessive autophagy can lead to cell death and cardiac atrophy. It is important to understand how autophagy is regulated to identify ideal therapeutic targets for treating disease. Here, we discuss the key proteins in the core autophagy machinery and describe upstream regulators that respond to extracellular and intracellular signals to tightly coordinate autophagic activity. We review various genetic and pharmacological studies that demonstrate the important role of autophagy in the heart and consider the advantages and limitations of approaches that modulate autophagy. (Circ Res. 2015;116:489-503. DOI: 10.1161/CIRCRESAHA.116.303791.)

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Key Words: autophagy ■ heart failure ■ mitochondria
excessive activation of autophagy can also lead to development of cardiac atrophy.34,35

Because of its role in disease, there is great interest in understanding the fundamental mechanisms and function of autophagy. Substantial progress has been made in uncovering pathways that tightly coordinate autophagy. This has allowed the selective manipulation of autophagy using genetic and pharmacological approaches. Studies in the heart that have modulated the autophagy pathway have confirmed its importance in cardiovascular health. Thus, the autophagy pathway is emerging as a promising and appealing therapeutic target for diseases in humans. There are three types of autophagy in mammalian cells: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy (Figure 1). Although these are distinct processes, they all merge at the lysosome with the delivery of cargo for degradation and recycling.

**Mechanisms of Autophagy**

There are three types of autophagy in mammalian cells: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy (Figure 1). Although these are distinct processes, they all merge at the lysosome with the delivery of cargo for degradation and recycling.

**Chaperone-Mediated Autophagy**

CMA plays a role in the selective degradation of cytosolic proteins containing exposed KFERQ motifs. In this process, heat shock cognate 70 selectively binds these proteins and facilitates their transport inside lysosomes through the lysosome-associated membrane protein type 2A (LAMP-2A) receptor (Figure 1A). CMA is important for maintenance of cellular homeostasis and adaptation to stress.32 For instance, CMA is activated during prolonged starvation, which contributes to recycling of amino acids to maintain synthesis of critical proteins. Interestingly, CMA is activated after macroautophagy (discussed below) and activation persists for days.33 In contrast, macroautophagy is rapidly activated and peaks at around 4 to 6 hours in response to starvation. This suggests that CMA coordinates with macroautophagy to allow cells to adapt to extended periods of unfavorable conditions. CMA is increased during oxidative stress and contributes to protein quality control by degrading oxidized proteins.32 Abrogating CMA results in increased oxidative damage and cell death.34 Few studies have addressed the functional role of CMA in vivo. However, a recent study reported that many metabolic enzymes are degraded via CMA, and conditional disruption of LAMP-2A in the liver leads to hepatosteatosis and altered glucose metabolism.35 Similar to macroautophagy, CMA is reduced with age,36 and it is possible that accumulation of oxidized proteins observed in aged tissues and cells may be as a result of reduced activity by both macroautophagy and CMA. The role of CMA in cardiovascular disease has not been explored, and future studies need to address whether the regulation of cell metabolism by CMA is extended to the heart. However, it is possible that CMA activity is altered in the heart and that this contributes to development of cardiovascular disease and aging.

**Microautophagy**

In microautophagy, cytosolic cargo is directly internalized into lysosomes through invaginations of the lysosomal membrane (Figure 1B). These invaginations are also known as autophagic tubes and their formation is dependent on ATG7-dependent ubiquitin-like conjugation.37 Vesicles pinch off at the top of the tube into the lumen of the lysosome where the vesicle and its cargo are subsequently degraded by lysosomal hydrolases. Although this process has been mainly studied in yeast, there is evidence that microautophagy also occurs in mammalian cells.38 Microautophagy can be nonselective or selective. Nonselective microautophagy involves the degradation of randomly sequestered cargo and is present in both yeast and mammalian cells.37 Selective microautophagy involves degradation of specific organelles, such as nucleus, mitochondria, and peroxisomes, but has been mainly described in yeast.39 Unfortunately, the molecular mechanisms that mediate microautophagy and its functional role in mammalian cells are poorly understood, partly because of the limited number of techniques to monitor microautophagy. Hence, it is unknown whether alterations in microautophagy contribute to various diseases in humans.

**Macroautophagy**

Macroautophagy (herein referred to as autophagy), the most prevalent form of autophagy, is the process by which cytosolic materials and organelles are engulfed by autophagosomes and then subsequently delivered to the lysosomes (Figure 1C). Although the origin of the autophagosome membrane is controversial, the endoplasmic reticulum (ER)–mitochondria contact site seems to be the primary site for initial autophagosome formation.40 During the nucleation step, BECLIN1 forms a complex with vacuolar protein sorting 34 (VPS34) and VPS15 to initiate formation of the phagophore (Figure 1C).41 Once the phagophore is formed, other autophagy proteins are recruited to facilitate elongation of the membrane. Many
### TABLE 1. Cardiac Phenotype Associated With Modulation of Autophagy Genes

<table>
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AMPK indicates AMP-activated protein kinase; ATG, autophagy; BNIP3, BCL2/adenovirus E1B nineteen kDa protein-interacting protein 3; FOXO, forkhead box O; LV, left ventricular; mTOR, mammalian target of rapamycin; PINK1, PTEN-induced putative kinase 1; PRAS40, Proline-rich Akt substrate of 40 kDa; RAGA/B, Ras-related GTP-binding protein A/B; RAPTOR, Regulatory-Associated Protein of mTOR; RHEB, Ras homolog enriched in brain; SIRT1, sirtuin1; TAC, transverse aortic constriction; and VPS, vacuolar protein sorting.
Autophagy proteins are conserved between yeast and mammals and are involved in ubiquitin-like conjugation systems to form autophagosomes. As the membrane elongates, proteins on the autophagosome bind to receptors or adaptor proteins on the cargo to facilitate the engulfment of materials into the autophagosome. These mechanisms are discussed in further detail in the following section.

The mature autophagosome fuses with a lysosome which degrades its contents. However, the molecular mechanism of the fusion event is still not fully understood. In general, fusion of membranes is regulated by soluble NSF attachment protein receptor (SNARE) proteins. Several of these proteins have been identified on lysosomes where they facilitate fusion with the autophagosomes. Syntaxin 17 (STX17) was identified as the SNARE protein involved in the fusion with lysosomes in mammals. Syntaxin 17 is never present on phagophore membranes, which might explain why lysosomes only fuse with the autophagosome after sequestration of the cargo is complete. Once syntaxin 17 is localized to the autophagosome, it interacts with other SNARE receptors, such as lysosomal vesicle-associated membrane protein 8 (VAMP8) and lysosomal vesicle-associated membrane protein 8 (VAMP8) to mediate autophagosome–lysosome fusion.

The critical role of autophagy in mammals has been demonstrated by multiple groups using different mouse models. For instance, global deletion of BECLIN1 or VPS34 is embryonic lethal in mice. Mice with cardiac-specific VPS34 ablation are viable but have impaired autophagic flux, reduced contractility, and early mortality. There are no published studies using a cardiac-specific BECLIN1 knockout mouse line to specifically investigate the role of BECLIN1 in cardiac myocytes. Unfortunately, this represents a major limitation in the field, and it will be critical to fully elucidate the function of BECLIN1 in the heart before it can be targeted therapeutically.

Figure 1. Mechanisms of autophagy. A, Chaperone-mediated autophagy; import of proteins into lysosomes. B, Microautophagy; direct internalization of cargo into lysosomes. C, Macroautophagy; autophagosome membrane as it engulfs and sequesters cargo. Syntaxin 17 (STX17) is recruited to mature autophagosome and interacts with cytosolic synaptosomal-associated protein 29 kDa (SNAP29) and lysosomal vesicle-associated membrane protein 8 (VAMP8) to mediate autophagosome–lysosome fusion.
This is particularly important because studies have found that elevated levels of BECLIN1 contribute to maladaptive autophagy in the heart. In contrast, specific disruption of the autophagy pathway by deletion of autophagy proteins is not embryonic lethal, indicating that both BECLIN1 and VPS34 have additional roles besides autophagy. Instead, autophagy-deficient mice die within one day of birth as a result of an inability to adapt to starvation by inducing autophagy. For instance, ATG5, ATG7, and ATG16L-deficient neonates all die within 1 day of birth.

ATG5 has been selectively deleted in the heart and these mice have an intriguing phenotype. Deletion of ATG5 in the adult heart results in rapid accumulation of dysfunctional mitochondria, ventricular dilatation, and impaired contractility. In contrast, mice with deletion of ATG5 during development have no cardiac phenotype under baseline conditions, but are more sensitive to pressure overload. This suggests that cardiomyocytes can compensate for defects in the autophagy pathway when the loss occurs early, but this compensation is not activated when autophagy is abrogated in the adult heart. It will be interesting to determine whether this phenotype is limited to ATG5 or whether it occurs in other cardiac-specific autophagy knockout mice.

The autophagosomal contents are degraded inside the lysosomes and the catabolites are subsequently exported to the cytosol for recycling. It is not surprising that lysosomes are critical for cellular homeostasis and survival. Defects in lysosomal function have severe consequences for the cell as revealed in patients with Danon disease. This disease is caused by a LAMP-2 defect because of mutations in the gene encoding LAMP-2. These patients develop a lethal hypertrophic cardiomyopathy, demonstrating the importance of a functional autophagy-lysosomal degradation pathway. Studies in LAMP-2-deficient mice reveal that there is an accumulation of autophagic vacuoles in many tissues, including the heart because of a defect in the fusion between autophagosomes and lysosomes. The accumulation of autophagosomes becomes cytotoxic, which leads to cardiac dysfunction.

### Recognition of Cargo by Autophagosomes

Proteins and organelles that need to be removed by autophagosomes are marked for degradation by ubiquitination mediated by ubiquitin ligases. Most studies have focused on how mitochondria are cleared by autophagosomes in cells. The PTEN induced putative kinase 1 (PINK1)/Parkin pathway is important in clearing dysfunctional mitochondria in the heart via autophagy. PINK1 is a serine/threonine kinase responsible for recruiting the E3 ubiquitin ligase Parkin to damaged mitochondria. Parkin-mediated ubiquitination of substrates promotes their degradation by autophagy (Figure 2A). PINK1 levels are reduced in end-stage heart failure in humans, and PINK1-deficient mice develop left ventricular dysfunction and cardiac hypertrophy at 2 months of age. Parkin-null mice accumulate dysfunctional mitochondria in the heart after myocardial infarction, which leads to reduced survival. Parkin-deficient mice also accumulate abnormal mitochondria in the heart with age, which correlates with a decline in cardiac function. Thus, this pathway clearly plays a role in mitochondrial quality control both under baseline conditions and in response to stress. It is unknown whether there are other E3 ubiquitin ligases that can serve the same function as Parkin.

To recruit the autophagosome, adaptor proteins bind to ubiquitinated proteins on the cargo and to microtubule-associated protein 1 light chain 3 (LC3) on the autophagosome. The most studied adaptor protein is p62/Sequestosome 1, which contains a ubiquitin-associated domain and an LC3-interacting region that binds to LC3 and gamma-aminobutyric acid A receptor-associated protein. The p62 protein is important for cardiac homeostasis, and p62-deficient mice have enlarged tissues, including a 2.5-fold increase in heart weight. Interestingly, p62 is not required for Parkin-mediated mitochondrial clearance, suggesting that other adaptor proteins can compensate for lack of p62. Neighbor of BRCA1 gene 1 (NBR1) is another autophagy adaptor protein that has been identified in cells. Similar to p62, neighbor of BRCA1 gene 1 contains a ubiquitin-associated domain and an LC3-interacting region. Neighbor of BRCA1 gene 1 forms a complex with LC3 to sequester misfolded and ubiquitinated proteins. However, whether neighbor of BRCA1 gene 1 plays a role in Parkin-mediated clearance of mitochondria is unknown.

There are autophagy receptors present on organelles that are independent of ubiquitin and adaptor proteins. These receptors contain an LC3-interacting region and can directly tether the organelle to the autophagosome by binding to LC3. The most well-known autophagy receptors are BCL2/adenovirus E1B nineteen kDa protein-interacting protein 3 (BNIP3) and Nip3-like protein X (NIX)/BNIP3-like (BNIP3L), which function to mediate selective removal of mitochondria in cells, including cardiomyocytes (Figure 2B). BNIP3 can also function as an autophagy receptor on the ER. NIX is present on the endoplasmic/sarcoplasmic reticulum where it can promote activation of necrosis by perturbing calcium release. However, it has not been investigated whether NIX can also function as an autophagy receptor on the endoplasmic/sarcoplasmic reticulum.

The roles of BNIP3 and NIX in mediating cell death in the myocardium have been extensively studied. However, their important role in regulating cardiac mitochondrial turnover was revealed in mice deficient in these proteins. NIX-deficient mice accumulate dysfunctional mitochondria with age and develop cardiac enlargement and left ventricular dysfunction at 60 weeks of age. Interestingly, mice deficient in both NIX and BNIP3 develop the same cardiac phenotype as the NIX-null mice but at 30 weeks of age, indicating that loss of both proteins leads to accelerated progression of cardiac dysfunction. These data suggest that NIX and BNIP3 are involved in mitochondrial clearance under normal conditions and that they have redundant functions. Although BNIP3 and NIX serve dual functions as regulators of mitophagy and cell death, it is currently unknown how and when these proteins switch between these 2 seemingly opposing functions. It is also important to note that as BH3-only proteins, they can also directly activate autophagy by disrupting the interaction between BECLIN1 and BCL-2 or BCL-XL (as discussed later).
Additional studies are needed to understand the complex functions of these atypical BH3-only proteins in the heart.

FUN14 domain-containing protein 1 is another autophagy receptor that has been identified in the outer mitochondrial membrane (Figure 2B). This autophagy receptor is negatively regulated by Src kinase phosphorylation of a residue in its LC3-interacting region. During hypoxia, Src is inhibited, leading to FUN14 domain-containing protein 1 dephosphorylation, activation, and induction of mitophagy. Cardiolipin can also function as an autophagy receptor on mitochondria (Figure 2B). Cardiolipin is a phospholipid localized to the inner membrane of mitochondria and translocates to the outer mitochondrial membrane in response to mitophagy stimuli. Cardiolipin directly binds to LC3 to mediate mitophagy in neurons. There are likely additional autophagy receptors on organelles that have not yet been identified. Another unanswered question is whether autophagy receptors on other organelles are different and whether they have different E3 ubiquitin ligases that mark them for degradation.

**Autophagy Signaling Pathways**

**mTOR**
Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that integrates growth factor and nutrient signals from various pathways to promote cell growth and inhibit autophagy (Figure 3). The mTOR kinase is part of the mTORC1 signaling complex, which inhibits autophagy. This complex includes mammalian lethal with SEC13 protein 8, DEP domain-containing mTOR-interacting protein, regulatory-associated protein of mTOR, and proline-rich Akt substrate of 40 kDa. Studies using genetic mouse models have established the importance of the mTORC1 complex in cardiac homeostasis and disease (Table 1). Cardiac-specific deletion of mTOR is embryonic lethal, whereas deletion of mTOR in the adult heart leads to increased apoptosis and autophagy and reduced survival. Regulatory-associated protein of mTOR is a scaffold protein that regulates assembly of the mTORC1 complex, and cardiac-specific ablation of regulatory-associated protein of mTOR in adult mice disrupts mTORC1 activity and leads to development of dilated cardiomyopathy and heart failure within 6 weeks after deletion of the gene. Proline-rich Akt substrate of 40 kDa is a negative regulator of mTORC1 activity, and overexpression in myocytes inhibits pathological remodeling and protects cardiac function after pressure overload. These studies highlight the importance of autophagy and mTORC1 in cardiac development and growth, as well as their role in maintaining normal cardiac function in the adult heart. However, mTORC1 regulates many different processes in cells, and the cardiac phenotypes in these mice are most likely caused by the effect on several pathways, including autophagy.

The presence of growth factors inhibits autophagy via activation of the phosphoinositide 3-kinase (PI3K)/AKT pathway. Activated AKT directly phosphorylates and inactivates the tuberous sclerosis protein 1/2 (TSC1/2) complex, resulting in Ras homolog enriched in brain (RHEB) activation and promotion of mTORC1 signaling. The TSC1/2 complex acts as a GTPase activator and converts active RHEB-GTP to inactive RHEB-GDP. GTP bound RHEB associates with and facilitates activation of mTORC1. RHEB is an important regulator of mTORC1 in the heart, and cardiac ablation of RHEB results in abrogated mTORC1 signaling, development of heart failure, and premature death. AKT can also regulate mTORC1 independently of TSC1/2 by phosphorylating proline-rich Akt substrate of 40 kDa, leading to proline-rich Akt substrate of 40 kDa dissociation from the mTORC1 complex. This relieves mTOR inhibition, allowing mTOR to phosphorylate its downstream targets. Although growth factors activate mTORC1 through the PI3K/AKT/TSC1/2 pathway, amino acid–mediated activation of mTORC1 is mediated via Ras-related GTP-binding protein GTPases located on the lysosomal surface. They suppress autophagy via recruitment and activation of mTORC1 in response to abundant amino acids.
Regulation of Autophagy and Mitophagy by ULK1/2
The mTORC1 complex relays nutrient signals to the autophagy machinery via the UNC-51-like kinase (ULK1/2), ATG13, and focal adhesion kinase family–interacting Protein of 200 kDa complex. In the presence of abundant amino acids, mTOR phosphorylates ULK1 and ATG13, which leads to inhibition of the complex (Figure 4).63 As discussed earlier, ULK1 is activated by AMP-activated protein kinase (AMPK), and the activated ULK1 complex activates the VPS34–BECLIN1–VPS15 complex to initiate autophagy.62 ULK1 can also directly activate mitophagy by phosphorylating and activating the autophagy receptor FUN14 domain-containing protein 1 on the mitochondria.63 ULK1 and ULK2 share 78% homology in their kinase domains and have redundant functions in vivo.64 ULK1 is also involved in regulating mitophagy by clearing mitochondria during erythroid maturation.65 The mitophagy protein NIX also plays an important role in clearing mitochondria during erythrocyte maturation,66 and it will be interesting to investigate whether ULK1 is an upstream regulator of NIX.

AMPK
AMPK is an intracellular energy sensor that is activated in response to a low ATP/AMP ratio or increased energy demand. AMPK activates autophagy by at least 3 different mechanisms. First, AMPK inhibits the mTORC1 complex by phosphorylation of TSC2 and direct phosphorylation of regulatory-associated protein of mTOR,67 resulting in activation of TSC1/2 complex and inactivation of mTORC1 (Figure 3). Second, activated AMPK phosphorylates ULK1 on several sites, leading to ULK1 activation68 (Figure 4). Finally, AMPK activates the BECLIN1–VPS34–VPS15 complex (Figure 4). When Atg14L is present in the initiation complex, AMPK phosphorylates BECLIN1 and activates autophagy.69

FOXO Proteins
Autophagy can be regulated at the transcriptional level by the forkhead box O (FOXO) family of transcription factors. FOXO proteins are positive regulators of autophagy,70 and they activate transcription of several autophagy genes, such as Gabarapl1, Maplc3, Atg5, Atg12, Vps34, and Becn1.71 The FOXOs also regulate transcription of mitophagy proteins BNIP3 and PINK1.72,73 FOXO proteins are activated by AMPK when energy levels are low and inhibited by nuclear AKT when growth factors are present.74 PI3K phosphorylation of AKT leads to AKT translocation to the nucleus where it phosphorylates the FOXO proteins.75 Phosphorylation of FOXO by AKT at specific sites promotes FOXO translocation from the nucleus to the cytosol, where 14-3-3 proteins sequester FOXO proteins to inhibit their nuclear transcription activity.76 (Figure 5).

The FOXO proteins counteract cardiac hypertrophy by activating autophagy. FOXO1/3-mediated autophagy reduces myocyte size.77 In addition, FOXO3-deficient mice develop cardiac hypertrophy,78 whereas cardiac-specific transgenic mice develop atrophy.15 Similarly, cardiac-specific overexpression of FOXO1 results in increased autophagy and smaller

Figure 3. Regulation of mammalian target of rapamycin (mTOR). Hormones (insulin or IGF-1) and growth factors (IGF-1 signal through the PI3K/AKT pathway. AKT phosphorylates tuberous sclerosis protein (TSC2) and inhibits the TSC1/2 complex, leading to conversion of inactive Ras homolog enriched in brain (RHEB)-GDP to active RHEB-GTP and consequent activation of mTORC1. Abundance of amino acids activates mTORC1 through Ras-related GTP-binding protein (RAG) GTPases. AKT also activates mTORC1 by phosphorylating proline-rich Akt substrate of 40 kDa (PRAS40), which is a negative regulator of mTORC1. AMP-activated protein kinase (AMPK) is activated in response to low ATP levels and inactivates mTORC1 by phosphorylating TSC2 and RAPTOR. Resveratrol, metformin, and statins activate AMPK. Rapamycin inhibits mTOR activity.

Figure 4. Regulation of autophagy. Active mammalian target of rapamycin complex 1 (mTORC1) phosphorylates ATG13, UNC-51-like Kinase (ULK1), and ATG14 to inhibit autophagy. AMP-activated protein kinase (AMPK) activates autophagy by directly phosphorylating ULK1/2 and BECLIN1. BCL-2 and BCL-X, bind to BECLIN1 to inhibit autophagy. JNK phosphorylates BCL-2 to prevent its interaction with BECLIN 1, whereas MST1 phosphorylates BECLIN1 to increase its interaction with BCL-2 and BCL-X, inhibiting autophagy. 3-MA inhibits vacuolar protein sorting 34 (VPS34) and suppresses autophagy.
hearts. The mitophagy receptor BNIP3 has been implicated as a downstream effector of FOXO3-mediated cardiac atrophy because FOXO3-mediated cardiac atrophy is ameliorated in BNIP3−/− mice.

Although the heart undergoes hypertrophy in response to pressure overload, the hypertrophy can regress on unloading. Reversal of the cardiac hypertrophy involves FOXO-mediated activation of autophagy. FOXO1 and autophagy are rapidly increased in the heart on removal of transaortic constriction (DeTAC). However, heterozygous Beclin1 disruption in mice leads to reduced autophagy and attenuated regression of cardiac hypertrophy after DeTAC, indicating that autophagy plays a key role in regression of cardiac hypertrophy. Thus, activation of FOXO-mediated autophagy increases removal of cytotoxic protein aggregates and damaged organelles, as well as prevents or reverses hypertrophy. However, unregulated activation of FOXO proteins in the myocardium can lead to dramatic and progressive loss of cardiac mass, excessive autophagy, and early mortality. In addition, the FOXOs contribute to the pathogenesis of cardiomyopathy in a high fat diet. It has also been demonstrated that alterations of FOXOs and autophagy can contribute to myofiber degeneration and weakness in muscle disorders. Although FOXO proteins demonstrate good therapeutic potential, it is clear that off-target effects or excessive activation can have severe consequences for tissues.

**Sirtuins**
The sirtuin family of protein deacetylases is classified as class III histone deacetylases that deacetylate histones and many other nonhistone targets. Sirtuin1 is the most well studied isoform. Sirtuin1 protects against the pathogenesis of cardiomyopathy in a high fat diet. It has also been demonstrated that alterations of FOXOs and autophagy can contribute to myofiber degeneration and weakness in muscle disorders. Although FOXO proteins demonstrate good therapeutic potential, it is clear that off-target effects or excessive activation can have severe consequences for tissues.

Overexpression of sirtuin1 also leads to reduced expression of autophagy-related genes, mitochondrial dysfunction, and impaired cardiac energy metabolism. This suggests that chronic elevated levels of sirtuin1 are detrimental to myocytes. In contrast, transgenic mice with low overexpression of sirtuin1 have normal cardiac function under baseline conditions and have reduced age-mediated cardiac damage. However, these mice are much more sensitive to pressure overload even at a young age, suggesting that chronic overexpression of low levels of sirtuin1 leads to hidden cellular alterations that only manifest during stress. Clearly, there is strong evidence that sirtuin1 is cardioprotective and is a potential therapeutic target in the heart that can be targeted to enhance autophagy and many other beneficial cellular processes. However, additional studies must be performed to fully understand the function of sirtuin1 and when and at what levels it switches from a protective to a detrimental protein.

**BCL-2 Family Proteins**
The BCL-2 proteins are well known for their roles in regulating the mitochondrial or intrinsic pathway of cell death in
cells. Recent studies have demonstrated that the BCL-2 proteins are also important regulators of autophagy (Figure 4). Anti-apoptotic BCL-2"sx" and BCL-Xs interact with BECLIN1 to inhibit autophagy. The stability of the BCL-2/BECLIN1 complex is regulated by post-translational modifications of both BCL-2 and BECLIN1. JNK1-mediated phosphorylation of BCL-2 leads to release of BECLIN1 and activation of autophagy.87 MIST1 is a proapoptotic kinase that inhibits autophagy and impairs protein quality control. In myocytes, MIST1 phosphorylates BECLIN1, which increases the interaction between BECLIN1 and BCL-2 or BCL-Xs.88 The interaction between the antiapoptotic proteins and BECLIN1 can be disrupted by the proapoptotic BH3-only proteins. For instance, BAD and BIK can disrupt BCL-Xs-BECLIN1 and BCL-2-BECLIN1 interaction, respectively, to induce autophagy.

Alternative Autophagy

Recent studies have identified that an alternative autophagy pathway exists in cells that is independent of the autophagy ubiquitin-like protein conjugation systems.89 Cells lacking ATG5 or ATG7 can still form autophagic vesicles and accomplish autophagy-mediated protein degradation in response to stress.89 The alternative autophagy pathway is also involved in clearing mitochondria in maturing erythrocytes.90 The source for the autophagosome membrane in this pathway is derived from the trans-Golgi,90 whereas the membrane in traditional autophagy seems to be derived primarily from the ER90 (Figure 6). Similar to autophagy, initiation of the alternative autophagy pathway requires ULK1 and BECLIN1. The existence of this ATG5-independent autophagy pathway clarifies why BECLIN1−/− mice are embryonic lethal,11 whereas ATG5−/− mice are healthy until the perinatal period.8 It is likely that the alternative autophagy pathway compensates for the lack of ATG5/ATG7-dependent autophagy during embryonic development. Although alternative autophagy has not specifically been studied in the heart, activation of this pathway might explain why cardiac-specific ATG5-deficient mice are born normally and show normal cardiac function and structure for up to 10 weeks.2 The fact that cardiac function declines in aging cardiac-specific ATG5-deficient mice indicates that both pathways are needed in the heart to maintain homeostasis.91 Currently, our knowledge of the molecular mechanisms and in vivo function of alternative autophagy is limited. Additional studies are needed to further elucidate the role of this pathway in the heart and whether it contributes to pathology.

Modulation of Autophagy in Cardiovascular Disease

Ischemia/Reperfusion

Numerous studies have focused on understanding the role of autophagy in I/R injury where autophagy plays a paradoxical role. During ischemia, deprivation of nutrients leads to activation of autophagy via AMPK.4 The increase in autophagy during acute ischemia is primarily responsible for maintaining energy production by generating free fatty acids and amino acids.92 Inhibition of AMPK abrogates autophagy and increases cell death, demonstrating that AMPK activation promotes survival during ischemia.5 Similarly, caloric restriction is a potent inducer of autophagy and increases ATP content as well as reduces ventricular dilatation after myocardial infarction in mice. The beneficial effects of food restriction are abrogated in mice treated with chloroquine, an inhibitor of autophagic flux.93 Unfortunately, prolonged ischemia leads to inhibition of autophagic flux, likely because of an energy crisis in the cell.94 Many studies have reported that autophagic flux is restored or increased on reperfusion, and the primary role of autophagy during acute reperfusion is in cellular quality control. The restoration of oxygen leads to a significant increase in ROS production, which can damage proteins and organelles.95 Increased ROS also stimulates autophagic flux in myocytes,96 and the enhanced flux at reperfusion reduces loss of myocytes and acute I/R injury.97 However, increased ROS during reperfusion also induces expression of BECLIN1, and chronic activation of BECLIN1-mediated autophagic flux is detrimental and results in increased I/R injury.5 In contrast to these studies, Ma et al recently reported that autophagic flux is impaired during reperfusion, which contributes to loss of myocytes.98 Thus, additional studies are needed to determine exactly how and when autophagic flux is altered during ischemia and subsequent reperfusion. It is possible that the effect on flux will vary with the extent and severity of ischemia. Also, another major challenge will be to determine when autophagy switches from a beneficial to a detrimental role in I/R.

Pressure Overload

Autophagy is also increased in cardiac pressure overload but reports regarding its functional role are conflicting. For instance, autophagy is rapidly increased in the mouse heart after TAC where it remains elevated for weeks.12 This study also found that autophagy is a maladaptive response during pressure overload. Similarly, TAC-mediated cardiac fibrosis is reduced in mice treated with the autophagy inhibitor 3-MA,99 and downregulation of microRNA-30a activates autophagy and exacerbates cardiac hypertrophy in response to TAC.100 These studies suggest that enhanced autophagy is a detrimental response during pressure overload. In contrast, other reports suggest that autophagy is an adaptive response in this setting. Cardiac-specific abrogation of autophagy leads to increased susceptibility to TAC.2 Also, enhancing autophagy via treatment with AMPK activators AICAR or metformin reduces pressure overload-mediated cardiac hypertrophy.101 Overall, these studies indicate that a low or moderate level of autophagy is required to adapt to pressure overload, but that excessive autophagic flux can lead to unnecessary protein degradation of critical cellular components.

A recent study has provided novel insights into the mechanism underlying maladaptive autophagy in cardiac pressure overload. This study found that cytosolic acetyl-coenzyme A (AcCoA) is a negative regulator of autophagy by inhibiting deacetylation of autophagy proteins. They also found that maintaining high levels of AcCoA levels inhibits maladaptive autophagy after TAC and abrogates development of pathological hypertrophy.102 The importance of protein acetylation in mediating maladaptive autophagy has also been demonstrated by other studies where inhibition of histone deacetylases prevents cardiac remodeling in pressure overload by suppressing autophagy.103
Targeting Autophagy

Autophagy is upregulated in many cardiovascular diseases, where it can exert both protective and detrimental effects depending on the context and the disease. Thus, pharmacological modulation of autophagy represents a novel therapeutic approach to prevent or limit damage to myocardium during various stress or diseases (Table 2). As discussed in previous sections, activation of autophagy increases clearance of harmful protein aggregates and damaged organelles. It also provides cells with recycled catabolites that can be used to maintain energy production during periods of limited nutrients. As an mTOR inhibitor, rapamycin is a potent activator of autophagy (Figure 3) and has been reported to protect against pressure overload–induced hypertrophy, myocardial I/R injury, and improve cardiac function in diabetic mice.

Resveratrol is an antioxidant that enhances autophagy by activating both sirtuin1 and AMPK (Figure 3). Resveratrol administration reduces apoptosis and improves cardiac function in mice with diabetic cardiomyopathy. A combination of caloric restriction and resveratrol treatment in aging rats enhances autophagy and protects against doxorubicin-mediated cardiotoxicity. Metformin is a glucose-lowering drug that reduces the risk of heart attack in diabetic patients. Metformin activates autophagy via AMPK (Figure 3) and is a promising treatment for diabetic cardiomyopathy. In addition, metformin-mediated activation of AMPK inhibits pressure overload–induced cardiac hypertrophy and ameliorates cardiac dysfunction by promoting autophagy.

Many other well-known drugs have recently been identified to mediate some of their therapeutic effect via activation of autophagy. For instance, the antimicrobial compounds chloramphenicol and sulfaphenazole activate autophagy, which correlates with protection against I/R injury. In addition, statins are cholesterol-lowering drugs that target the HMG-CoA reductase. Recent studies have reported that these drugs can activate autophagy in macrophages and prostate cancer cells. Statins can also reduce infarct size by activating Parkin-mediated mitophagy.

Although there is abundant evidence that enhancing autophagy confers beneficial effects in heart, some studies have found that maladaptive autophagy is activated under certain conditions and that inhibiting autophagy is cardioprotective. Lu et al found that treatment with the autophagy inhibitor 3-methyladenine (Figure 4) reduces myocyte death and improves cardiac function in a rat model of doxorubicin–induced heart failure. Interestingly, these results contradict findings from Dutta et al who found that activating autophagy protects against doxorubicin-induced cardiotoxicity. This may be as a result of differences in the animal models and drug administration used in the 2 studies.

The histone deacetylase inhibitor Trichostatin A can prevent or reverse pathological cardiac remodeling elicited by pressure-overload stress via inhibition of autophagy. In contrast, suberoylanilide hydroxamic acid (SAHA), a different HDAC inhibitor, activates autophagy and reduces cardiomyocyte death and infarct size after I/R. Similarly, granulocyte colony-stimulating factor (G-CSF) treatment in hamsters with cardiomyopathy reverses excessive activation of autophagy, which correlates with increased survival, improved cardiac function, and reduced fibrosis. The antioxidant propofol reduces infarct size and cell death in rats subjected to myocardial I/R injury. In addition, although studies have found that rapamycin treatment can be cardioprotective, administration of rapamycin immediately before acute myocardial I/R is associated with reduced cardiac function and increased necrosis in pigs.

Unfortunately, all of the drugs discussed here do not specifically modulate the autophagy pathway. Instead, most affect multiple cellular processes, so it is difficult to discern exactly what pathways are contributing to the desired protective effect. Thus, development of pharmacological agents that directly target specific proteins in the autophagy pathway is desirable. Importantly, there is evidence that increasing expression of individual autophagy proteins is sufficient to increase autophagic activity in the heart and does not cause any apparent pathology or compromise in cardiac function. For instance, transgenic mice with a moderate overexpression of ATG5 have increased autophagy in most tissues, including the heart. These mice have extended life span compared with wild type mice, and aged ATG5-transgenic hearts have reduced fibrosis. Enhancing autophagic activity specifically in the heart is also beneficial. Cardiac-specific ATG7 transgenic mice have a milder desmin-related cardiomyopathy and prolonged survival because of increased clearance of protein aggregates.

Moreover, mitochondrial quality control plays an important role in preventing age-related cardiac pathologies. Parkin-deficient mice accumulate dysfunctional cardiac mitochondria and oxidative damage in hearts with age. Interestingly, transgenic mice overexpressing Parkin in cardiomyocytes are more resistant to age-dependent mitochondrial alterations compared with age-matched WT mice, suggesting that increasing mitophagy can delay aging. Thus, simply increasing baseline autophagy or mitophagy can ameliorate cardiac aging. It will be interesting to investigate whether these mouse lines are also protected against development of heart failure in response to pressure overload or myocardial infarction. In fact, it will be more informative to generate a conditional transgenic mouse line where the gene could be turned on after the injury to determine whether selectively activating autophagy can limit cardiac damage.

Autophagy in Vascular Cells and Fibroblasts

Although most studies on autophagy in the context of cardiovascular disease have centered on cardiac myocytes, recent studies are attempting to understand its role in vascular cells and cardiac fibroblasts. There is evidence that impaired autophagy contributes to endothelial dysfunction. Autophagy regulates secretion of von Willebrand factor, a glycoprotein important in hemostasis, and nitric oxide bioavailability in endothelial cells. Reduced autophagy also increases ROS and inflammatory cytokine production in response to shear stress. Conversely, activation of AMPK-mediated autophagy protects endothelial cells from oxidant-induced cell death. Accumulating evidence suggests that activation of autophagy in vascular smooth muscle cells (VSMCs) plays a role in the progression of vascular diseases. Under
normal conditions, VSMCs maintain vascular tone and distribute oxygen and nutrients to tissues. However, in vascular diseases, the VSMCs lose their contractile phenotype to a synthetic phenotype with increased proliferation and migration. Autophagy regulates the VSMC phenotype and that increased autophagy promotes loss of the contractile phenotype and increased VSMC proliferation. Overall, these findings indicate that alterations in autophagy affect vascular homeostasis. Additional studies are necessary to determine exactly how autophagy contributes to vascular dysfunction. Moreover, cardiac fibroblasts are responsible for synthesizing extracellular matrix, reduced autophagy in fibroblasts might contribute to increased fibrosis. It is not yet known how autophagy contributes to vascular dysfunction. However, transmission electron microscopy should always be done in conjunction to confirm the findings at the ultrastructural level.

**Table 2. Effect of Various Autophagy Modulators on Cardiovascular Diseases**

<table>
<thead>
<tr>
<th>Drug/Agent</th>
<th>Effect on Autophagy</th>
<th>Effect on Cardiovascular Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamycin</td>
<td>Activate</td>
<td>Reduces pressure overload-mediated cardiac hypertrophy</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protects against myocardioc I/R injury</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Improves cardiac function in diabetic mice</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduces cardiac function and increases necrosis after acute myocardial I/R</td>
<td>106</td>
</tr>
<tr>
<td>Sulfaphenazole</td>
<td>Activate</td>
<td>Protects against myocardioc I/R and reduces infarct size</td>
<td>107</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Activate</td>
<td>Attenuates myocardioc I/R injury and reduces infarct size</td>
<td>108</td>
</tr>
<tr>
<td>Statins</td>
<td>Activate</td>
<td>Reduces infarct size</td>
<td>109</td>
</tr>
<tr>
<td>Metformin</td>
<td>Activate</td>
<td>Improves cardiac function in diabetic mice</td>
<td>110</td>
</tr>
<tr>
<td>SAHA</td>
<td>Activate</td>
<td>Reduces infarct size after I/R</td>
<td>97</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Activate</td>
<td>Inhibits cardiac hypertrophy</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Protects against doxorubicin-induced cardiotoxicity</td>
<td>112</td>
</tr>
<tr>
<td>3-Methyladenine</td>
<td>Inhibit</td>
<td>Inhibits doxorubicin-mediated autophagy and cell death in myocytes, improves cardiac function</td>
<td>113</td>
</tr>
<tr>
<td>Trichostatin</td>
<td>Inhibit</td>
<td>Reverses pressure-overload-induced pathological cardiac remodeling</td>
<td>44</td>
</tr>
<tr>
<td>Propofol</td>
<td>Inhibit</td>
<td>Reduces infarct size</td>
<td>114</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Inhibit</td>
<td>Reduces autophagy and cardiomyocyte death</td>
<td>115</td>
</tr>
</tbody>
</table>

CSF indicates colony-stimulating factor; and SAHA, suberoylanilide hydroxamic acid.

but recent evidence indicate that it is involved in extracellular matrix turnover through intracellular degradation of Collagen-I. Another study found that increased autophagy correlates with reduced fibrosis after TAC. These data suggest that autophagy in cardiac fibroblasts regulates cardiac remodeling. A balance between synthesis and degradation of extracellular matrix proteins is crucial for maintaining tissue homeostasis, and if autophagy is important in degrading extracellular matrix, reduced autophagy in fibroblasts might contribute to increased fibrosis.

**Monitoring of Autophagy In Vivo by Imaging**

Monitoring the level of autophagy in vivo is critical in understanding its functional role in various cardiovascular diseases. Several mouse models have been developed to quantify autophagy in vivo, and these are useful in studying changes in this process in cardiovascular disease. Transgenic mice over-expressing green fluorescent protein (GFP)-LC3 are useful in assessing the number of autophagosomes in the heart. However, an increased number of autophagosomes does not always reflect an increase in autophagic activity but could also indicate reduced turnover. To confirm that autophagic flux is increased, the number of GFP-LC3-positive autophagosomes need to be evaluated in the presence or absence of an inhibitor of autophagosome–lysosome fusion, such as chloroquine or Bafilomycin A1. Recently, a tandem fluorescent-tagged LC3 transgenic mouse has been developed that can be used to assess autophagic flux without treating mice with inhibitors. GFP, but not red fluorescent protein (RFP), is quenched by the low pH in lysosomes. Thus, autophagosomes are positive for both GFP and RFP, whereas autophagosomes that have fused with lysosomes (autophagolysosomes) emit only RFP. These mouse models will be useful in assessing autophagic flux and how various pathological stimuli alter the process. However, transmission electron microscopy should always be done in conjunction to confirm the findings at the ultrastructural level.

**Conclusions**

It is clear that modulating autophagy provides a new avenue to treat or prevent cardiovascular diseases. However, there are still many questions that need to be resolved before modulation of autophagy can become clinically feasible. First, the precise role of autophagy in various cardiovascular diseases is still not completely understood, and inhibiting or activating autophagy at the incorrect time point might result in enhanced injury. In addition, it is often difficult to compare contradictory results because of experimental differences. This includes variations in species, mice with different genetic backgrounds, as well as differences in surgical techniques. Moreover, studies often use different doses of drugs and times of administration in their experiments. These variables likely contribute to differences in experimental outcomes. Second, current drugs that modulate autophagy do not directly or specifically modulate autophagy and instead act on upstream pathways that are involved in activating or inhibiting autophagy. Therefore, they also affect many other pathways in addition to autophagy, which can contribute to unwanted side effects.
In addition, modulating autophagy will also affect other organ systems. For instance, constitutive autophagy is critical for the maintenance of skeletal muscle mass, and excessive autophagy promotes muscle atrophy. Thus, enhancing autophagy to protect the heart might lead to reduced skeletal muscle mass as a side effect. This concern is also relevant to cancer where recent studies have reported that autophagy can act as a cellular adaptation pathway in cancer cells. This suggests that enhancing autophagy to protect the heart could also potentially promote tumorogenesis. Conversely, current therapeutic strategies to target certain cancers include inhibition of autophagy in combination with chemotherapeutic drugs, which could potentially induce cardiotoxicity. Therefore, it will be important to develop selective inhibitors and activators of autophagy, along with tissue-specific targeting. These strategies will help maximize the therapeutic effects and minimize potential side effects.

Finally, a major challenge will be the monitoring of autophagic flux in patients with cardiovascular disease. Because autophagy cannot be monitored directly in hearts, potential side effects.

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None

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