Published in final edited form as:

Cardiovasc Toxicol. 2013 September ; 13(3): 220–229. doi:10.1007/s12012-013-9200-8.

Cardiovascular Autophagy: Crossroads of Pathology, Pharmacology and Toxicology

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Abstract

Cardiovascular disease (CVD) remains the leading cause of death worldwide, despite significant advances in medicine. Autophagy, a process of self-cannibalization employed by mammalian cells for the recycling of cellular contents, is altered not only in a number of CVDs, but in other diseases, as well. Many FDA-approved drugs are known to induce autophagy-mediated side effects in the cardiovascular system. In some cases, such drug-induced autophagy could be harnessed and used for treating CVD, greatly reducing the duration and cost of CVD treatments. However, because induction of autophagy in cardiovascular targets can be both adaptive and maladaptive under specific settings, the challenge is to determine whether the changes stimulated by drug-induced autophagy are, in fact, beneficial. In this review, we surveyed a number of CVD in which autophagy is known to occur, and we also address the role of FDA-approved drugs for which autophagy-mediated side effects occur within the cardiovascular system. The therapeutic potential of using small molecule modulators of autophagy in the management of CVD progression is discussed.

Keywords

autophagy; cardiovascular disease; drug-eluting stents; LC3; rapamycin; verapamil

II. Introduction

Despite advances in modern medicine, cardiovascular disease (CVD) remains the leading cause of mortality in both the United States and worldwide. According to the CDC, the total CVD-related healthcare costs exceeded \$450 billion in 2008 and are expected to increase in the future [1]. Thus, there is an urgent need to identify disease mechanisms and to develop novel therapies that could be used to treat and curb the burden of CVD. Of course, along with new therapeutic modalities comes the potential risk of drug-induced cardiovascular toxicity, which must also be identified, quantified and mitigated at the clinical level.

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Autophagy is a process of self-cannibalization employed by mammalian cells for the recycling of cellular contents. Systematic studies in yeast have identified a number of autophagy-related (ATG) genes (and their proteins) that are evolutionarily conserved and have distinct roles in the autophagic program. Autophagy is generally activated under stress conditions, such as during starvation (i.e., increased AMP/ATP ratio) or during oxidative stress (Figure 1). Under such stress conditions, phosphorylation of ULK1 (ATG1), the first ATG protein in the autophagic pathway, results in ATG1 activation and dissociation from the mammalian target of rapamycin complex (i.e., mTOR complex). ATG1 subsequently triggers downstream events, ultimately leading to the assembly of a semi-circular double membrane vesicle called the phagophore. The precise link between ATG1 and phagophore formation, however, has yet to be defined. Phagophore formation and maturation into the autophagosome (i.e., the characteristic double membrane vesicle) depends on the interaction of the beclin 1/PI3KIII complex and other ATG proteins. During the first step of this maturation, ATG7 and ATG10 catalyze the conjugation of ATG12 to ATG5 [2, 3]. ATG16L stabilizes the ATG12–ATG5 complex, forming ATG12–ATG5–ATG16L. The stabilized ATG12–ATG5 complex then stimulates a second conjugation, which involves two steps: 1) ATG4 first primes LC3 (ATG8) by exposing a glycine residue at the -COOH terminal [4]; and, 2) this primed LC3 or LC3-I is then modified by phosphatidylethanolamine (PE) to form LC3-PE or LC3-II. The reaction is catalyzed by ATG7 and ATG3 [3, 5]. LC3-II is subsequently recruited to both outer and inner faces of the growing autophagosome and stabilizes it. This autophagosome then fuses with the lysosome leading to the lytic degradation of autophagosomal contents. Protein degradation yields amino acids and other building blocks that can be re-utilized by the cell for the biosynthesis of essential macromolecules or energy production.

Autophagy has also been shown to play an important role in the pathogenesis of several diseases such as cancer, Alzheimer's, and ischemic heart disease [6]. It is also known to have a positive effect on longevity in animals [7–9]. The importance of autophagy and the signaling cascades that modulate it have been eloquently reviewed elsewhere [7–15]. However, the role of autophagy in specific cardiac diseases and the potential to harness drug-induced autophagy side effects in the cardiovascular system have only been modestly addressed. As such, this review is focused on autophagy in the cardiovascular system, and its role not only in CVD, but in drug-induced cardiovascular toxicity, as well. For example, even non-CVD drug treatments are known to induce autophagy (and have autophagy-mediated side effects) in the cardiovascular system. Finally, this review examines some small molecule modulators of autophagy as these could be potential drugs for treating cardiovascular diseases.

III. Autophagy in the Cardiovascular System

III.A. The heart

Basal cardiomyocyte autophagy is a recurring process required for proper functioning of the heart. Inhibition or over-activation of autophagy, however, is also associated with various cardiac diseases. A survey of specific cardiac pathologies thus reveals the importance of autophagy in various cardiomyopathies.

III.A.1. Cardiomyocyte hypertrophy and hypertensive heart disease—

Hypertrophy is an increase in cardiomyocyte size that is chronically accompanied by extensive cardiac remodeling and increased protein expression. Several studies show evidence of autophagy in hypertrophy. For example a study by Nakai *et al*, shows that induction of hypertrophy by trans-aortic constriction (TAC) in mice led to suppression of the autophagic response [16]. In this same study, the team also suppressed the expression of key autophagic genes, including *Atg5* and *Atg7*, and found that the loss of functional autophagic

targets induced cardiomyocyte hypertrophy. Other recent studies in both experimental animals and humans have also reached similar conclusions to those of Nakai *et al* [17–19]. McMullen *et al* [20], for example, experimentally-induced autophagy using the mTOR inhibitor and found that rapamycin promotes regression of cardiac hypertrophy. In their study, 12-week-old male FVB/N mice were subjected to pressure overload (ascending aortic constriction; AAC) for 1 week, and then treated with rapamycin ($2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for 1 week. Rapamycin decreased the heart weight/body weight (HW/BW) ratio in AAC mice with either compensated or decompensated hypertrophy by 68% and 41%, respectively [20]. In mice with decompensated cardiac hypertrophy, improved cardiac function – as measured by fractional shortening and ejection fraction – was observed. In 2012, Garcia *et al*, induced chronic hypertrophy (5 weeks of TAC) using the same FVB/N mice strain used by McMullen *et al*, and found that rapamycin had similar beneficial outcomes. Thus, supposing that animals are pre-treated before pressure overload, these studies suggest that the beneficial effects of rapamycin-induced autophagy could prevent and combat cardiac hypertrophy [21].

III.A.2. Cardiac arrhythmias—Cardiac arrhythmias, in general, occur when the normal pattern of depolarization, which begins at the SA node and ends in the ventricular myocardium, is disrupted. This disruption could lead to a slow, fast or irregular depolarization, and can be due to direct effects on nodal (pacemaker) and/or indirectly via damaged myocardial tissue. Cardiac arrhythmias can indicate not only potential alterations in cardiac remodeling, but the presence of both ischemia and heart block on an ECG. While there is limited literature to support the involvement of autophagy in arrhythmias, a recent study by Garcia *et al.*, [22] did find defective autophagy in patients with post-operative atrial fibrillation (POAF). Samples of the right atrial appendages in patients with POAF showed accumulation of autophagic vacuoles and decreased expression levels of the autophagy marker, microtubule-associated protein light chain 3 (LC3), suggesting impaired cardiac autophagy in the development of POAF. In a separate study, Chen *et al.* [23] showed that induction of autophagy occurs in atrial cardiomyocytes of most cases with severe mitral and tricuspid regurgitation. In their study of 20 patients, LC3 positive myocytes were detected in approximately 90% of patients with fibrillation in the right atria. Interestingly, the majority of LC3 positive myocytes had a moderate-to-severe myolysis (cardiomyocyte degeneration), which suggests that, in patients with mitral regurgitation, autophagy could play a significant role in the cardiomyocyte myolysis. In the context of cardiac arrhythmias, however, whether or not autophagy is protective has yet to be elucidated.

III.A.3. Ischemia reperfusion and heart failure—Evidence for the involvement of autophagy in ischemia-reperfusion (I-R) injury and heart failure abound. In the mid-1970s, Sybers *et al*, reported the up-regulation of autophagy in fetal mouse hearts when exposed to glucose deprivation and hypoxia [24]. Since then, controversial findings have evolved regarding the importance of autophagy in I-R injury, as well as in heart failure. For example, Matsui *et al*, [25] as well as Valentim *et al*, [26] showed that ablation of the proautophagic gene, beclin 1, or the inhibition of PI3KIII using 3-methyl adenine (3-MA) treatment, respectively, prevented cardiomyocyte death induced by I/R injury in mice. These studies suggest that autophagy activated during I-R may be maladaptive. On the contrary, Hamacher-Brady *et al.*, [27] demonstrated that enhancing autophagy through overexpression of beclin 1 actually protects cardiomyocytes against I-R injury. They also showed that the overexpression of dominant-negative ATG5 led to increased cellular injury, and thus, demonstrated that autophagic flux constitutes a powerful mechanism against I-R injury. Similarly, activation of autophagy with rapamycin [28] or chloramphenicol succinate [29] treatment leads to a cardioprotective phenotype in mice and swine, respectively. Although these results oppose those of Matsui *et al.*, [25] and Valentim *et al.*, [26] the discrepancy is

likely due to the use of different treatment protocols and models of I-R injury. Nevertheless, these findings not only highlight the intrinsic importance of autophagy during ischemic heart disease, but also suggest that whether the activation or inhibition of autophagy will be adaptive or maladaptive ultimately depends on the conditions.

Likewise, the role of autophagy in heart failure can be observed to be adaptive or maladaptive as well. A study by Shimomura *et al.*, supports the potential for dual autophagic outcomes during heart failure. This team found that cardiac autophagy in patients with end-stage heart failure is not only responsible for the removal of damaged organelles but is also involved in the progressive destruction of cardiomyocytes [30]. In a follow-up study of patients suffering from dilated cardiomyopathy, Kostin *et al.*, confirmed a similarly maladaptive role of autophagy. They found that while cardiomyocytes in the failing heart die by multiple mechanisms, autophagy was a major instigator [31]. Interestingly, Kostin and colleagues identified autophagic myocytes that stained positive for monodansylcadaverine, which is a specific marker of autophagic vacuoles [32]. Yet, other studies have suggested a more adaptive role of autophagy during I-R injury and progressive heart failure. In a study of 9 patients with idiopathic dilated cardiomyopathy, Kassiotis *et al.*, showed that the implantation of a left ventricular assist device (LVAD) led to a decreased expression of most autophagic markers [33]. They reasoned that, because this decrease occurs upon LVAD implantation, the heightened autophagy observed beforehand is likely an adaptive mechanism used to generate more energy for the failing heart – a main function of autophagy. It is not clear whether this difference in findings is due to the small sample size or perhaps the peculiar form of heart failure. Thus, additional human studies, using appropriate case controls, are needed to truly understand the contribution of autophagy during heart failure. If autophagy could in fact be adaptive within the context of heart failure, then the pharmacological regulation or modulation of autophagy may be a clinically relevant target.

III.B. Arterial Media and Smooth Muscle Cells

Arteries are efficient and flexible blood delivery tubes, and in the adult human body, these span >60,000 miles. Blood vessels can be both conduits and resistors because of their highly specialized, but relatively simple, 3 layer structure. The single cell inner lining, known as the endothelium, allows for gas and nutrients exchange between blood and the underlying cells. Around the endothelium is a medial layer composed of vascular smooth muscle cells (VSMCs) that are interspersed with layers or lamellae made of extracellular matrix (ECM), including collagen and elastin. Its principal function is the regulation of blood vessel tone (contraction and relaxation), which determines vascular resistance, the primary determinant of blood flow. Surrounding the media is the adventitial layer composed mainly of fibroblasts and ECM, and, in aorta elements, of the *vasa vasorum* and perivascular nerves. The adventitia functions on a structural and secretory level. In the diseased artery, one or more of these layers is affected, and autophagy can play a role in vascular remodeling during physiological adaptation or pathogenesis.

III.B.1. Atherosclerosis and restenosis—While atherosclerosis is an inflammatory disease of the aorta and great vessels, it can also affect the peripheral microvasculature. Atherosclerosis is typically characterized by a buildup of cholesterol in the sub-intimal space, wherein engulfment of cholesterol by monocytes/macrophages and vascular smooth muscle cells (VSMC) eventually leads to the production of foam cells, oxidative stress, and inflammation, as well as VSMC proliferation and neointima formation. Decades of such buildup can lead to plaque formation, cellular necrosis, necrotic core and plaque rupture [34]. Interestingly, both VSMCs and macrophage autophagy are known to be critical during the process of atherogenesis, and current research has focused on the modulation of

autophagy in one or both of these cell types [35–37]. However, because the role of autophagy during atherosclerosis could be, in general, either protective or detrimental, there is a need for caution when exploring potential therapeutic application.

The beneficial effects of autophagy have been well-documented. It has recently been reported, for example, that inhibiting autophagy in macrophages by silencing ATG 5 or other autophagy mediators led to both increased apoptosis and plaque instability in advanced lesions [38], which increases the risk of both MI and stroke following plaque rupture. It is possible that ablation of autophagy in these macrophages led to the accumulation of damaged materials (e.g., misfolded proteins, damaged mitochondria) that triggered apoptosis. Such a finding indicates a beneficial role of autophagy in atherosclerosis. However, despite the potential benefit autophagy offers during atherosclerosis, excessive induction of autophagy might eventually trigger autophagy-induced cell death or type 2 cell death, i.e., resulting from prolonged activation of autophagy, and lead to plaque destabilization [39].

In the event of plaque rupture and artery occlusion, angioplasty is commonly used to restore blood flow. Previously opened (restored) arteries, however, often re-occlude (termed restenosis) with the excessive proliferation of underlying VSMCs that migrate lumenally and form the neointima [40]. For example, in a study of 1,012 patients randomly assigned to treatment with either Sirolimus- (n = 503) or Paclitaxel-eluting (n = 509) stents, approximately 28% of subjects needed revascularization at a later date [41]. Although the outcomes of clinical studies such as this one ultimately depend on demographics (e.g., diabetic patients), it does highlight that restenosis remains a significant, ongoing health concern. The majority of drug-eluting stents (DES) designed to prevent restenosis are rapamycin-based (e.g., Sirolimus, Everolimus) and these drugs are known not only as inhibitors of the mTOR pathway but as inducers of autophagy as well [42]. It is not clear what role autophagy plays in endogenous restenosis, but clearly DES induces autophagy to both benefit and detriment as discussed below.

III.B.2. Aortic Aneurysm—The involvement of autophagy in aneurysm formation has also been documented. Zheng *et al.*, have shown that the protein levels of key autophagic genes, such as LC3, Atg4b, Beclin1/Atg6, Bnip3 and Vps34, are markedly upregulated in abdominal aortic aneurysm (AAA) tissues [43]. mRNA microarray analysis revealed that osteopontin, a proinflammatory cytokine, was found to be the gene most often induced in these tissues. Thus, to determine the mechanism of induced autophagy in AAA tissues, the team treated cultured smooth muscle cells with recombinant osteopontin and found a p38-MAPK-dependent induction of autophagy – a finding that implicates autophagy as a mechanism of vessel weakening in AAA. A summary of autophagy and its CVD-related roles can be found in Table 1.

IV. Targeting Autophagy in CVD

IV.A. Harnessing autophagy-mediated (side) effects of cardiovascular drugs

Since it was first identified in the 1960s, the therapeutic manipulation of autophagy has been utilized for the treatment of CVD and other diseases, such as cancer. Although many clinically-approved drugs used for the treatment of CVD were not previously thought to stimulate autophagy, some drugs have recently been shown to induce autophagy independent of their conventional mechanism of action. These drugs could thus be carefully regimented for the treatment of different cardiovascular complications. Verapamil, for example, is a L-type calcium channel blocker (CCB) that has been used for over 30 years in the treatment of hypertension, angina pectoris and cardiac arrhythmia [44–47]. However, we recently showed that either individual pure Verapamil stereoisomer (as well as racemic

Verapamil) alone induces autophagy and is antiproliferative in cultured VSMCs, indicating a pro-autophagic mechanism entirely independent of calcium channel blocker action [48]. Such a finding is potentially significant because it reveals a heretofore undefined mechanism of action – one that, when better understood, could be targeted for more efficacious anti-atherosclerotic and/or anti-restenosis therapy. And because the effects of Verapamil on VSMC proliferation are stereo-independent, utilization of higher dosages without calcium channel blocking activity could eliminate the risk of hypotensive crises. Moreover, study of this antiproliferative and autophagic mechanism of action could reveal a novel therapeutic target in proliferating smooth muscle cells, much like mTOR inhibitors that promote the stabilization of atherosclerotic plaque. Interestingly, Verapamil and the beta-blocker propranolol were each shown to separately induce autophagy in the rat heart *in vivo*, and yet, at that time, the mechanism of action was attributed to a cardiodepressive or a hypotensive effect [49].

The introduction of drug eluting stents (DES) with rapamycin also greatly reduced the frequency of restenosis after angioplasty [42, 50]. Yet the efficacy of DES therapy does suffer one major setback – DES that induce autophagy also lead to the inhibition of endothelial cell repair and re-endothelialization in the stented blood vessel lumen [51]. Thus, when weighing the beneficial effects of inhibiting VSMC proliferation vs preventing re-endothelialization, the more adverse effects must also be carefully considered, and newer generation of stents (absorbable) is being developed. Nonetheless, because the principal benefit of rapamycin is its autophagy-inducing effect, it could also potentially be used in the treatment of other CVDs. For example, studies using experimental animals have shown that rapamycin reduces infarct size after MI [52], indicating a potential for cardioprotective effects in humans.

It is known that some widely used cardiovascular drugs, such as α -receptor agonists (isoproterenol, norepinephrine) and β -receptor blockers (propranolol, salbutamol), have also been shown to alter autophagy in the cardiovascular system, and perhaps autophagy induction is a part of their beneficial effects [53–55] (see Table 2). If so, a better understanding of their autophagic inducing mechanism of action could yield new cellular targets for “old drugs.”

IV.B. Harnessing autophagy of non-CVD targeted drugs

Although known principally for its use in type II diabetes, metformin, a glucose-lowering drug that suppresses hepatic gluconeogenesis, has beneficial effects in the cardiovascular system. Metformin is a 5' adenosine monophosphate-activated protein kinase (AMPK) activator that induces autophagy. Experimental studies in both animals [56–58] and humans [59–61] have shown that metformin treatment plays a protective role not only in heart failure, but also in diabetic cardiomyopathy. A study of Xie *et al.*, [56] shows that following metformin treatment, diabetic OVE26 mice had improved cardiac function as a result of enhanced cardiac autophagy. Additional beneficial cardiovascular effects of metformin could also be attributed to its eNOS-activating capabilities [57]. Despite these well-documented and beneficial effects, however, metformin is only prescribed for use in the treatment of type II diabetes and Polycystic Ovary Syndrome (PCOS). What proportion of beneficial effects in these conditions is autophagy-derived is unclear.

Some drugs have no autophagic effect on their target tissues whatsoever, but have been shown to alter autophagy in the cardiovascular system – a side effect that when better understood could be manipulated to a therapeutic advantage in the right setting. A number of such drugs are listed in Table 2. For example, the proteasome inhibitor drug, bortezomib (Velcade), and the tyrosine kinase inhibitor (TKI), imatinib (Gleevec), are used in the treatment of multiple myeloma [62, 63]. Unfortunately, cardiotoxicity is reported with both

bortezomib and imatinib and, interestingly enough, autophagy is also activated during cardiotoxicity [63, 64]. Whether bortezomib- or imatinib-induced autophagy and cardiotoxicity are causally linked is not entirely clear, but a better understanding of the specific mechanisms of autophagy induction by either bortezomib or TKI is needed. This information could facilitate developing new targeted use of these drugs (or structurally-related compounds) to effectively modulate autophagy in the setting of cardiac disease, for example, and thus, potentially turn “drug toxicity into therapy.”

V. Modulation of Autophagy as an Emerging Strategy -- Targets and Toxicology

At present, there are many small molecules currently used in laboratories for the inhibition or activation of autophagy. Such molecules have great potential to be therapeutically utilized in the treatment of CVD (see Table 2). Like many of the therapies discussed in this review, however, their use could also alter autophagy in untargeted organs or tissues. Because basal physiological autophagy is necessary for normal growth and development, alteration of autophagy can also be detrimental to overall health. Moreover, some autophagy inhibitors, such as wortmannin and 3-methyl adenine (3-MA), inhibit certain upstream signaling molecules (i.e., the PI3K/Akt pathway) that are critical in the signaling of many other non-autophagy targets (e.g., eNOS). However, this limitation has not prevented the discovery of novel downstream inhibitors of autophagy. Spautin-1, for example, is an inhibitor of the autophagic pathway that could be rendered tissue-specific. Indeed, spautin-1 has recently been shown to promote beclin-1 degradation [65]. It also has one major advantage over other autophagy inhibitors; because of its specificity, spautin-1 is far less likely to inhibit other signaling processes, e.g., PI3K/Akt pathway. In recent work from our laboratory, spautin-1 was used to effectively modulate phenotype conversion in VSMCs, and thus, it could potentially be used to combat vasculoproliferative disorders such as restenosis *in vivo* (Salabei, unpublished data). Chloroquine and Bafilomycin A1 have also been shown to modulate autophagy [66, 67], but the effects of these compounds on cardiovascular-related complications have yet to be tested. A summary of these and other potentially useful autophagy-related compounds is in Table 2.

VI. Concluding Remarks

It is clear that autophagy is a significant physiological process, one that is altered in a number of cardiovascular diseases and drugs specifically designed to modulate autophagy under these disease conditions prove to be very promising.. Both the cost and time spent developing these new autophagy-directed therapies, however, could be greatly reduced by reutilizing existing FDA-approved drugs. Existing drugs that, as a side effect, induce autophagy in the cardiovascular system could be harnessed for additional therapeutic use. Small molecule inhibitors/activators of autophagy, such as spautin-1, show similar promise and should be further investigated. In any case, whether exploring the side effects of approved drugs or developing new chemical modulators for the treatment of CVD, great care must be taken when modulating autophagy. Perhaps a fine-tuning of autophagy could be most beneficial, such that its essential homeostatic roles are preserved, while modulating its over- or under-activation. And although such an approach involves substantial challenges, the potential therapeutic benefits of controlled autophagy should not be discounted.

Acknowledgments

This work was supported in part by NIH funds: NIGMS RR024489 and HL89380. We thank Ms. Samantha R. Clausi, M.A., for excellent editorial assistance.

Abbreviations and Acronyms

VSMC	vascular smooth muscle cells
Spautin 1	specific and potent inhibitor of autophagy 1
3-MA	3-methyl adenine
CVD	cardiovascular disease
mTOR	mammalian target of rapamycin
ATG	autophagy-related gene
TAC	trans-aortic constriction
ECG	electrocardiogram
POAF	post-operative atrial fibrillation
AAA	abdominal aortic aneurysm
DES	drug-eluting stent
MI	myocardial infarction
FDA	Food and Drug Administration
LVAD	left ventricular assist device
eNOS	endothelial nitric oxide synthase
ULK1	UNC-like kinase 1
ECM	extracellular matrix
AMPK	AMP-activated protein kinase
LC3	microtubule-associated protein light chain 3
TKI	tyrosine kinase inhibitor

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Figure 1. Autophagy in mammalian cells

In the presence of amino acids, growth factors and ample energy, phosphorylated Ser-2448 of the mammalian target of rapamycin (mTOR) by PKB (Akt) [68, 69] inhibits autophagy via Ser-757 phosphorylation in ULK1 [70]. The phosphorylation and activation of mTOR simultaneously induce growth and proliferative responses. In contrast, in the absence of amino acids and growth factors or during starvation (i.e., increase in the AMP-to-ATP ratio) or during oxidative stress, phosphorylation of ULK1 at Ser-317, -467, -555, -637, -777 or Thr-574 is associated with ULK1 activation and dissociation of the raptor complex from the ULK1 complex leading to autophagy activation [70, 71]. In addition to phosphorylating ULK1, AMPK directly phosphorylates the mTOR binding partner raptor on two well-conserved serine residues, Ser-722/Ser-792, and this phosphorylation induces 14-3-3 binding with raptor. The phosphorylation of raptor by AMPK is required for the inhibition of mTORC1 and cell-cycle arrest induced by energy deprivation [72]. The phosphorylation of ULK1 and raptor on multiple residues ensures tight control of autophagy activation, and thus, prevents inappropriate activation of autophagy. It is suggested that ULK1 acts as a focal point for multiple signals that control autophagy [73], and it can bind to other autophagy-related (Atg) proteins. However, the mechanism that links the ULK1 complex to downstream complexes such as the PI3KIII complex during autophagy induction is not well known. Beclin 1 in the PI3KIII complex functions as a scaffold molecule that recruits other molecules, such as PIST, UVRAG and Bif-1, important for the nucleation or maturation of

the phagophore into the autophagosome. The formation of the autophagosome relies on 2 ubiquitin-like conjugation systems. In the first, ATG7 and ATG10 catalyze the conjugation of ATG12 to ATG5 [2, 3]. ATG16L stabilizes the ATG12-ATG5 complex, which is required for the stimulation of the second conjugation reaction. At this time, cellular components primed for degradation are starting to be enclosed. The second conjugation involves 2 steps: first, ATG4 primes LC3 (ATG8) by exposing a glycine residue at the COOH-terminus [4], and, second, primed LC3 (or LC3-I) is then conjugated with the polar head of phosphatidylethanolamine (PE) to form LC3-PE (or LC3-II). This reaction is catalyzed by ATG7 and ATG3, and LC3-II is recruited to both the outer and inner faces of the growing autophagosome. Formation of LC3-II is used as a measure of autophagy induction because LC3-I is converted to LC3-II only during autophagy [74, 75]. The mature autophagosome then fuses with the lysosome leading to degradation of the contents of the autophagosome by lysosomal enzymes.

Table 1

Role of autophagy in cardiovascular complications.

CVD Model	Autophagy in the CVS	Consequence(s)
Cardiac hypertrophy	Inhibited	<ul style="list-style-type: none"> Cardiomyocyte growth [16–18, 76–80]
Ischemia/reperfusion	Inhibited Activated	<ul style="list-style-type: none"> Cardiomyocyte survival [81, 82] Cardiomyocyte survival [83]
Heart failure	Activated	<ul style="list-style-type: none"> Removal of damaged organelles [30] Progressive destruction of cardiomyocytes [30, 31] Adaptive mechanism to the failing heart [33]
Arrhythmias	Inhibited	<ul style="list-style-type: none"> Atrial fibrillation [22] Mitral and tricuspid regurgitation [23] Myolysis of cardiomyocytes [23]
Atherosclerosis	Activated	<ul style="list-style-type: none"> Inhibition of autophagy leads to apoptosis of macrophages and plaque instability Activation leads to VSMC death [84] Over activation may lead to cell death in macrophages and VSMCs [39]
Restenosis	Not determined	<ul style="list-style-type: none"> Activation of autophagy leads to inhibition of VSMC proliferation [42] Activation with rapamycin prevents endothelial healing [51, 85]
Aortic aneurysm	Activated	<ul style="list-style-type: none"> Activation of autophagy leads to VSMC death and rupture of vessels [43]

Table 2
Potential applications of drug-mediated modulation of autophagy in the cardiovascular system.

Drug	Current Use(s)	Autophagy	Potential Future Use(s)
Verapamil	Hypertension, angina, arrhythmia	Activates	Atherosclerosis and restenosis [48]
Rapamycin	Restenosis	Inhibits	Myocardial infarction [52] preconditioning
Metformin	Diabetes	Activates	Heart failure and diabetic cardiomyopathy [56-58, 60, 61, 86]
Isoproterenol, norepinephrine, salbutamol (salbutamol)	Brachycardia and asthma (isoproterenol) and asthma (salbutamol)	Activates	Cardiac fibrosis [53]
Bortezomib	Myeloma and mantle cell lymphoma	Activates	Hypertrophy?
Doxorubicin	Cancer chemotherapy	Activates	Hypertrophy?
Paclitaxel	Restenosis, ovarian, lung and breast cancers and Kaposi's sarcomas	Inhibits	Myocardial infarction?
Granulocyte Colony stimulating factor	Stimulation of WBCs	Inhibits	Heart failure [87, 88]
Urocortin	None	Inhibits	Ischemic heart disease and chronic heart failure [26]
Sulfaphenazole	None	Activates	Ischemic heart disease [89]
3-Methyl adenine *	None	Inhibits	Inhibition of autophagy in conditions where autophagy is hyperactivated *
Wortmannin *	None	Inhibits	Inhibition of autophagy in conditions where autophagy is hyperactivated *
Spautin-1 #	None	Inhibits	Inhibition of autophagy in conditions where autophagy is hyperactivated
Bafilomycin A1	None	Inhibits	Inhibition of autophagy in conditions where autophagy is hyperactivated
Chloroquine	Anti-malarial	Inhibits	Heart failure? [90] cardioprotection
Tamoxifen	Breast cancer	Activates	Useful in conditions with insufficient autophagy
Lithium, carbamazepine, sodium valproate	Abnormal mood changes and seizures	Activates	Useful in conditions with insufficient autophagy

* Side effects are more likely to occur with 3-MA and Wortmannin because these are inhibitors of PI3K activity. Both inhibitors inhibit VSMC proliferation after platelet-derived growth factor (PDGF) stimulation, and thus, these drugs could have anti-restenotic properties.

Spautin-1 is a recently discovered autophagy inhibitor that targets degradation of both PI3KIII and beclin 1.