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Autophagy as a Therapeutic Target in Cardiovascular Disease

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Abstract

The epidemic of heart failure continues apace, and development of novel therapies with clinical efficacy has lagged. Now, important insights into the molecular circuitry of cardiovascular autophagy have raised the prospect that this cellular pathway of protein quality control may be a target of clinical relevance. Whereas basal levels of autophagy are required for cell survival, excessive levels – or perhaps distinct forms of autophagic flux – contribute to disease pathogenesis. Our challenge will be to distinguish mechanisms that drive adaptive versus maladaptive autophagy and to manipulate those pathways for therapeutic gain. Recent evidence suggests this may be possible. Here, we review the fundamental biology of autophagy and its role in a variety of forms of cardiovascular disease. We discuss ways in which this evolutionarily conserved catabolic mechanism can be manipulated, discuss studies presently underway in heart disease, and provide our perspective on where this exciting field may lead in the future.

Keywords

heart failure; cardiac hypertrophy; remodeling

Introduction

According to World Health Organization estimates, cardiovascular diseases are the number one cause of death globally [1], a sad ranking which is expected to persist into the future. Costs deriving from cardiovascular disease morbidity and mortality are staggering, estimated to exceed $500 billion in the US alone [1]. In light of these sobering facts, there is urgent need to identify novel mechanisms of disease pathogenesis and therapeutic targets capable of stemming inexorable progression of disease.

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For three decades, it has been recognized that lysosomal pathways of protein degradation are prevalent in most forms of heart disease [2]. Until recently, however, it has been difficult to discern the role(s) of these catabolic pathways: whether they promote or antagonize disease pathogenesis. Now, based on molecular discoveries in yeast, a model has emerged of an intricate cascade of events leading to cargo sequestration and delivery to lysosomes. This process, termed autophagy, is an evolutionarily conserved mechanism of protein and organelle catabolism present within all eukaryotic cells [3, 4]. To date, 32 autophagy-related (*ATG*) genes have been identified which regulate autophagosome processing, including a subset termed the "core autophagy machinery" essential for autophagosome formation [5]. Now, armed with specifics regarding the molecular anatomy of the autophagic machinery, it is becoming possible to determine the role(s) of autophagy in numerous pathological processes [6], including cardiovascular disease [7, 8].

Autophagy, cellular cannibalization

Autophagy is a catabolic process whereby cells respond to energy stress by recycling intracellular components: proteins, ribosomes, lipids, and even entire organelles. [9]. In the presence of ample nutrient supply, anabolic reactions predominate within the cell, and autophagy is maintained at low levels critical for normal cellular homeostasis and survival [10, 11]. For example, basal levels of autophagic flux are required to degrade long-lived proteins, lipid droplets, and dysfunctional organelles, particularly in post-mitotic cells (e.g. cardiomyocytes, neurons), where the capacity for regeneration is limited. In response to starvation, induced either by inadequate nutrient supply or by defects in growth factor signaling pathways, autophagy is rapidly activated. The result is engulfment and degradation of portions of the cytoplasm.

Products derived from autophagic breakdown play a dual role: provision of biosynthetic substrates and replenishment of intracellular energy. With respect to the latter, the major contribution of autophagy to ATP pools derives from degradation of amino acids and fatty acids [12, 13]. This ATP is then used to build new macromolecules and to support cellular processes, including autophagy itself [14]. In addition, autophagic breakdown of RNA yields nucleosides, which are then degraded to ribose-phosphate. Six ribose-phosphate molecules are energetically equivalent to five glucose-phosphates and can yield ATP either aerobically or anaerobically [12, 13].

Apart from conditions of nutrient scarcity, enhanced levels of autophagy are observed in other clinically important circumstances, including neurodegenerative disorders, cancer, misfolded protein accumulation, microbial invasion, and cardiovascular diseases [7–9]. When these cells are exposed to stress, such as starvation and hypoxia, autophagic mechanisms are triggered to liberate energy substrates and eliminate defective organelles. However, taken too far, excessive and uncontrolled autophagic activation leads to depletion of essential molecules and organelles, provoking autophagic cell death [7, 8, 15, 16].

Three distinct types of autophagy have been described: microautophagy, chaperone-mediated autophagy, and macroautophagy [10]. Microautophagy refers to a process of engulfment of cytosolic materials directly into lysosomes. In chaperone-mediated autophagy, misfolded proteins are translocated by heat shock protein 70 (Hsp 70) to the lysosome for clearance. Macroautophagy (hereafter termed autophagy) is the major pathway to degrade and recycle long-lived proteins and the exclusive means of clearing dysfunctional organelles.

Whereas we have known for years that lysosomal pathways participate in the pathogenesis of virtually all forms of heart disease, it is only recently that we are equipped with the knowledge and tools to manipulate and dissect the autophagic apparatus. With this

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capability in hand, it has become apparent that autophagy in the heart can confer both adaptive and maladaptive actions depending on the context. Now, much work is underway to tease apart "good" autophagy from "bad" autophagy and to define underlying mechanisms. Together, these studies raise the exciting prospect of targeting and titrating the autophagic response in cardiac myocytes to effect therapeutic gain.

Molecular anatomy of autophagy

In overview, autophagy is a mechanism whereby cytoplasmic components are sequestered in a double-membrane vesicle (autophagosome) for delivery to the lysosome for breakdown [17]. However, the details of the process itself are quite intricate, involving membrane dynamics, vesicle trafficking, and cargo degradation. The autophagic cascade has been divided into distinct stages, *viz.* induction, cargo recognition and selection, vesicle formation, autophagosome-vacuole fusion, cargo breakdown and release of degradation products, and termination (Figure 1) [18–20]. The molecular architecture of the autophagic pathway comprises four subgroups:

Atg1 kinase complex

This signaling complex governs early steps in autophagosome formation and is regulated by nutrient availability via mTOR (TORC) [21]. In yeast, Tor integrates information from multiple upstream signaling pathways to negatively regulate Atg1 [22, 23]. In the setting of Tor suppression, either pharmacological (e.g. rapamycin) or as a result of nutrient scarcity, Atg1 kinase activity is triggered. As a consequence, the affinity of Atg1 for both Atg13 and Atg17 increases, promoting the formation of a trimeric, Atg1/Atg13/Atg17 complex. This, in turn, leads to recruitment of other Atg proteins to initiate autophagosome formation. (ULK1/2 is the mammalian orthologue of yeast Atg1 [24, 25].)

In mammalian cells exposed to nutrient-rich conditions, mTOR phosphorylates and inactivates ULKs [26, 27]. Upon starvation or rapamycin treatment, ULK1 and ULK2 are activated and phosphorylate Atg13 and FIP200, which are essential for the induction of autophagic flux [27].

mAtg9 signaling pathway

Double-membrane autophagosomes are assembled at the preautophagosomal structure (PAS) by addition of new membrane material. Then, the phagophore enlarges by Atg9-dependent delivery of new membrane [22]. Atg9 shuttles between the PAS and multiple sites within the cell, acting as a carrier to transfer membrane from the donor sites to the expanding phagophore [22, 28]. Anterograde movement of Atg9 to the PAS is governed by Atg11, Atg23, and Atg27; conversely, Atg1-Atg13, Atg2-Atg18, and Atg14 are involved in retrograde movement of Atg9 back to peripheral sites. Atg9 co-localizes with the Atg2-Atg18 complex at the PAS to promote retrieval of Atg9 from that site [9, 22, 23].

Class III phosphatidylinositol-3-kinase (PI3K)/Vps34 complex

Multiple Atg proteins are recruited to the phagophore to participate in autophagosome formation. Among them, Atg18, Atg20, Atg21, and Atg24 are recruited to the PAS via binding to phosphatidylinositol-3-phosphate (PIP) generated by Vps34 (PI3K in mammalian cells) [9, 22, 23]. Vps34 forms two distinct PI3K complexes: complex I (Vps34, Vps15/p150, Atg6/beclin 1, mAtg14) and complex II (Vps34, Vps15, Atg6, and Vps38). PI3K complex I activity (PI3P generation) is required for the targeting of several Atg proteins, such as Atg18, to the PAS. The autophagy-promoting activity of Beclin 1 is inhibited by Bcl-2 under nutrient-rich conditions; dissociation of Beclin 1 from Bcl-2 is required for the induction of autophagy [9].

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Two ubiquitin-like protein conjugation systems

Two cascades with features similar to the ubiquitin-conjugation cascade contribute to phagophore expansion and formation of the autophagosome. Atg12 is activated by a ubiquitin E1-like enzyme, Atg7, and subsequently transferred to a ubiquitin E2-like enzyme, Atg10. Atg12 is next covalently conjugated to Atg5, and the resulting Atg5-Atg12 complex interacts with Atg16. In the other ubiquitin-like cascade, LC3 (mammalian homolog of Atg8) is cleaved by Atg4 to expose a carboxyl terminal glycine. This processed LC3-I is then activated by Atg7, an E1-like enzyme. After being transferred by the E2-like enzyme Atg3, LC3-I is cleaved, covalently linked to a phosphatidylethanolamine molecule, and localized to the phagophore membrane. This cleaved/lipidated isoform, termed LC3-II, migrates faster than LC3-I on SDS-PAGE, and its levels correlate with autophagosome abundance. Thus, the autophagic flux pathway is governed by interlacing circuitry comprising two kinase systems (Atg1-Atg13, class III PI3K), two ubiquitin-like systems (Atg5-Atg12, LC3-II-PE), and a retrieval/maturation system.

Lysosomes

Expansion of the phagophore culminates in self-sealing to complete autophagosome formation; this structure, in turn, fuses with a lysosome to form an autolysosome. This process is mediated by the same machinery that is involved in homotypic vacuole membrane fusion. In mammalian cells, autophagosome-lysosome fusion requires the lysosomal membrane protein LAMP-2 and the small GTPase Rab7 [22]. After fusion, degradation of the inner vesicle, along with its contents, is dependent on a series of lysosomal/vacuolar acid hydrolases, including cathepsin B, D, and L [29]. The small molecule end-products of degradation, including amino acids, sugars, and nucleotides, are released to the cytosol through permeases. However prior to this step, fusion of the autophagosome with early and late endosomes lowers intra-vesicular pH.

Inactivation of the LAMP-2 gene is the causative lesion associated with Danon disease in humans [30]. In the absence of functional LAMP-2, fusion of autophagosomes with lysosomes is blocked, leading to accumulation of long-lived proteins, accretion of unprocessed autophagosomes, and consequent myopathy.

In addition to the aforementioned core Atg proteins, secretory and endocytic pathways, along with the cytoskeleton, are also required during autophagy, providing membrane substrate, facilitating autophagosome transport, and enabling clearance of degraded autophagic cargo. In light of this, both the molecular circuitry of the autophagic pathway itself and downstream lysosomes are points of potential therapeutic manipulation (Figure 1).

Signaling pathways governing autophagy

mTORC1

A central nexus of autophagy control is mTOR, a protein kinase that regulates cell growth and metabolism in response to nutrients, growth factors, ATP, and stress [31] (Figure 2). mTOR exists as two distinct multiprotein complexes, TORC1 and TORC2. TORC1 (comprising mTOR, Raptor, and mLST8) is rapamycin sensitive and mediates the temporal control of cell growth by transcription, translation, and autophagy. TORC2 (formed by mTOR, Rictor, mLST8, and Protor) is rapamycin insensitive and governs spatial control of cell growth by regulating the actin cytoskeleton. Abundance of ATP and growth factors activates TORC1 to maintain macroautophagy at low basal levels [31]. In contrast, TORC1 inhibition by nutrient starvation or rapamycin (a macrolide molecule that blocks mTOR through its interaction with FKBP12) triggers robust macroautophagy [31]. Upstream of TORC1, a regulated balance between protein phosphatases and kinases controls its

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activation. The pro-growth insulin/IGF-1 (insulin-like growth factor-1) pathway inhibits autophagy through activation of class I PI3Ks. This group of enzymes produces phosphatidylinositol-3,4,5-trisphosphate [PIP3], a molecular signal that activates Akt, and ultimately mTOR, thereby inhibiting autophagy [32]. Conversely, the tumor suppressor PTEN (phosphatase and tensin homologue) antagonizes the insulin/IGF-1 pathway via its PIP3 phosphatase activity and stimulates autophagy [33]. TORC1 regulatory factors are Rheb (Ras homolog enriched in brain, a protein which directly associates with TOR to promote its signaling activity) and the tuberous sclerosis complex 1 (TSC1) and TSC2 proteins (which together inhibit Rheb through the GAP activity of TSC2) [31]. As a result, the TSC-Rheb-TOR complex is a point of convergence of multiple signals that ultimately regulate autophagic activity [31].

IP3 receptors

Inositol-1,4,5-trisphosphate (IP3) and its receptor (IP3R) have emerged as endogenous negative regulators of autophagy [34, 35]. IP3R plays a major role by fostering microdomains of intracellular Ca^{2+} accumulation to transmit specialized signals from intracellular Ca^{2+} stores to specific sites within the cell, such as mitochondria [36, 37]. Inhibition of the IP3R with the specific antagonist xestospongin B, or knockdown of different IP3R isoforms with small interfering RNAs, triggers robust autophagy [35, 38]. For example, xestospongin B and nutrient starvation induce autophagy by disrupting a molecular complex formed by the IP3R and Beclin 1. Recent work has shown that the IP3R at the endoplasmic reticulum provides Ca^{2+} to mitochondria constitutively, which in turn promotes conversion of pyruvate into acetyl-coA, tricarboxylic acid cycle activity, and production of ATP by the electron transport chain [37]. When the IP3R is not activated, energy levels drop, AMPK is activated, and autophagy is engaged to preserve energy homeostasis and cell survival [37].

AMPK

This protein is a heterotrimeric kinase comprising αβγ subunits which is a critical integrator of multiple signals in the control of energy balance [39]. In some contexts, AMPK serves as a positive regulator of autophagy mainly via inhibition of the mTOR complex [40]. AMPK is activated in response to low ATP levels through the upstream LKB1 kinase. Activated AMPK, in turn, promotes the inhibition of Rheb by the TSC1/TSC2 complex and consequent inhibition of mTORC1 activity. TSC1/TSC2 phosphorylation events mediated by AMPK and AKT have opposite effects on mTORC1 and connect mTORC1 with energy and growth factor signaling, respectively. However, AMPK can also regulate mTORC1 by an alternative mechanism, as this kinase directly phosphorylates Raptor, leading to inhibition of mTORC1 [40]. The upstream kinase CaMKKβ also phosphorylates AMPK in an AMP-independent and Ca^{2+}-dependent manner [39]. Cytokines and increases in intracellular Ca^{2+} each activate AMPK and autophagy via this mechanism [40].

We have found that AMPK can also serve as an inhibitor of cardiomyocyte autophagy under conditions of ample energy supply (A.N., S.L., J.A.H. unpublished observations). Manipulations of AMPK activity in cultured neonatal rat cardiomyocytes strongly supported a link between AMPK and suppression of autophagy. For example, strong suppression of autophagy was observed when AMPK was activated by the AMPK activator, AICAR. Conversely, compound C, an inhibitor of AMPK, activated autophagy. Further, knockdown of AMPK using two sequence-independent siRNA constructs triggered robust activation of autophagy (A.N., S.L., J.A.H. unpublished observations). Clearly, the role(s) of AMPK in the governance of cardiomyocyte autophagy are complex and warrant careful scrutiny.

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PKA

Cyclic adenosine 3′,5′-monophosphate (cAMP) is a universal second messenger that directs numerous physiological events following activation of G protein-coupled receptors [41]. Its primary effector is cAMP-dependent protein kinase A (PKA), a heterotetramer consisting of two regulatory and two catalytic subunits [41]. Following dissociation, the catalytic subunits phosphorylate multiple substrates to regulate a wide range of cellular processes [41]. For example, PKA governs cell growth in response to extracellular nutrients and cellular stress. In the setting of high concentrations of glucose, Ras assumes a GTP-bound active form that up-regulates cAMP concentrations with consequent PKA activation. PKA is a negative regulator of autophagy, acting primarily on Atg1, Atg8 and Atg13 [42] (Figure 2). cAMP also inhibits autophagy in a PKA-independent manner via Epac/Rap2B/phospholipase C-ε [43].

p53

Accumulating evidence indicates that the human tumor suppressor protein p53 modulates autophagy in a dual fashion, depending on its subcellular localization [44]. p53 functions as a nuclear transcription factor and can induce autophagy through transcriptional effects, including transactivation of the human Damage-Regulated Autophagy Modulator (DRAM) family of genes [44, 45]. Indeed, p53-dependent induction of autophagy occurs in response to DNA damage, Arf activation, or re-expression of p53 in p53-negative tumor cells [44]. On the other hand, cytoplasmic p53 can act as a repressor of autophagy via poorly characterized mechanisms [44]. Inhibition of p53 triggers autophagy in enucleated cells, indicating that the cytoplasmic, non-nuclear pool of p53 is capable of governing autophagy [44].

Chromatin remodeling enzymes

During pathological cardiac remodeling, both anabolic and catabolic pathways are activated, and complex cascades of protein modification and protein degradation are triggered. Among the major post-translational modifications that take place is protein acetylation, a powerful regulator of function that may rival protein phosphorylation in terms of ubiquity and importance [46]. In the case of histone proteins, acetylation of ε-lysine groups leads to relaxation of chromatin structure, enhanced accessibility to DNA-binding proteins, and consequent activation of transcription. This epigenetic mechanism is a powerful regulator of tumor responses to chemotherapy and adaptation to environmental triggers (e.g. hypoxia).

Acetylation and deacetylation reactions are controlled by the antagonistic actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs are divided into five classes based on phylogenetic and functional criteria [47]: class I (HDACs 1, 2, 3, 8), class IIA (HDACs 4, 5, 7, 9), class IIB (HDACs 6, 10), class III (sirtuins), and class IV (HDAC 11). Class I, II, and IV HDACs are termed "classical" HDACs and are targeted by small molecule inhibitors currently in clinical development for cancer [48–50] and which have been shown to have efficacy in animal models of heart disease [51]. Sirtuin-1 is required for the autophagic response to nutrient deprivation but not for autophagy triggered by downstream signals such as the inhibition of mTOR or p53 [52]. HDAC6 interacts with ubiquitin to modulate aggresome function and autophagy [53].

Recently, HDAC biology has been shown to be critically important in governing myocardial development, metabolism, and responses to stress [46]. Furthermore, a series of animal models, including those from our laboratory, have attributed potent cardioprotective benefits to HDAC inhibitors (HDACi) in the setting of myocardial ischemia. Other investigations by our group and others, using murine models, have demonstrated HDACs to be important mediators of the myocardial hypertrophic response to hemodynamic stress (e.g. from

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thoracic aortic constriction, TAC) [54]. Furthermore, we found that this pathologic left ventricular remodeling could be blunted by administration of the HDAC inhibitor Trichostatin A (TSA). The downstream effect of TSA in heart involves activation of pro-survival kinase pathways [55, 56]. At the same time, HDACi can provoke both mitochondria-mediated apoptosis and caspase-independent autophagic cell death in tumors [50, 57]. Collectively, this suggests a novel window of benefit with this class of therapies not seen with other drugs.

We have demonstrated previously that cardiomyocyte autophagy elicited by pressure-overload stress is maladaptive and hence is a potential target for therapeutic intervention [58]. Given this, combined with strong evidence of cardioprotective actions of HDACi [51, 54], we hypothesized that HDAC-dependent pathological autophagy may contribute to the disease process. We further posited that suppression of pathological autophagy by HDACi may contribute to their beneficial effects. To test this, we recently employed small molecule HDAC inhibitors in a TAC model of afterload stress. Consistent with prior observations [54], HDACi was, in fact, capable of profoundly suppressing load-induced cardiomyocyte autophagy [59]; further, we found that this autophagic response is required for much of the pathological hypertrophic growth response [59]. Our studies went on to demonstrate that blunting of autophagy with HDACi is capable of reversing pre-existing systolic dysfunction and myocyte hypertrophy, a scenario with potential clinical implications. (McKinsey et al in this issue sheds additional light on HDACi as a cardioprotective therapeutic [60].)

Based on these and other data, we suggest that HDACi has potential in the therapeutic targeting of cardiac autophagy. Further studies, however, are needed to define the actions of HDACs and HDACi in the heart. Historically, HDACs were thought to act via histone acetylation-dependent control of gene expression. Accumulating evidence, however, demonstrates that HDAC-mediated deacetylation targets exist well beyond histone proteins [60, 61]. For example, the report from the Gupta lab in this issue [62] reviews NAD-dependent class III HDACs, collectively called sirtuins, which are suggested to manifest activity at cytoplasmic protein targets. Indeed, the genomic and cytoplasmic actions of HDACs and HDACi are areas of ongoing investigation.

Autophagy in cardiovascular biology

Activation of autophagic flux pathways occurs across a spectrum. At one end, low-level constitutive autophagic flux is fundamental to cell survival. At the other end of the spectrum, over-active autophagy can deplete a cell of elements required for life, thereby triggering cell death. In between these two extremes, the actions of autophagy are complex and potentially pro- or anti-survival (Figure 3).

Basal autophagy

Cardiomyocyte function and survival rely critically on the presence of basal levels of cardiomyocyte autophagy. Indeed, autophagic recycling of damaged cellular components in nutrient-rich conditions constitutes a major means of protein and organelle quality control, ridding the cell of defective (e.g. misfolded or oxidized) proteins and dysfunctional organelles. This fact is highlighted by the observation that abrogation of autophagic pathways in adult heart by conditional inactivation of either the *Atg5* or *Atg7* genes triggers rapid-onset cardiac hypertrophy, left ventricular dilation, and diminished cardiac output [63, 64]. Danon disease, a condition marked by severe and progressive myopathy, stems from defective fusion of autophagosomes with lysosomes [65, 66]. In early cardiac development, *Atg5* disruption provokes *in utero* defects and embryonic lethality [67]. At the other end of the age spectrum, age-related declines in the efficiency of autophagic clearance likely contribute to progressive accumulation of defective proteins and organelles which ultimately

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lead to functional deterioration over time [64, 68]. Normal aging is associated with loss of cardiac function mainly due to impaired relaxation during diastole [69]. Varying formulations of caloric restriction (CR) can prolong life span and improve LV diastolic function; underlying mechanisms may involve induction of autophagy [70–72], possibly by reduced insulin/PI3K signaling [71, 72]. Together, these facts highlight the vital housekeeping role for cardiomyocyte autophagy as a mechanism of protein and organelle surveillance and quality control.

Afterload-induced autophagy

While the critical necessity of basal levels of autophagy is well established, the role(s) of stress-activated autophagy in cardiac disorders is more complex. As noted earlier, conditions of clear-cut pathological stress, such as nutritional paucity or hypoxia, elicit rapid increases in autophagic flux which serve to clear defective organelles and misfolded proteins and replenish scarce nutrients [9, 21, 73]. Consistent with this, suppression of autophagy during fasting reduces intracellular ATP levels and diminishes cardiac performance [67]. Beyond this, cardiac pathology elicited by multiple stressors, including elevated afterload, chronic ischemia, and ischemia/reperfusion (I/R), are associated with robust inductions of autophagy [8]. At present, a consensus is emerging in the field that induction of autophagy can either antagonize disease pathogenesis or contribute to the progression of disease depending on the context and amplitude of induction [7, 8]. For example, activation of autophagy is protective during ischemia, when the cell is “starved” of energy [74]. By contrast, activation of autophagy is maladaptive in the load-stressed heart [58] and during post-ischemic reperfusion [74] (Figure 3).

In a surgical model of pressure overload *in vivo*, the amplitude of autophagic flux correlates with the degree of pressure stress [58, 59]. When load stress-induced autophagy is augmented by cardiomyocyte-specific over-expression of Beclin 1, a molecular element critical to autophagic flux [75], a rapid transition to cardiac failure was observed. Conversely, diminishing the autophagic response by 50% in *Beclin 1* haploinsufficient mice attenuated pathological remodeling induced by afterload stress [58]. Such dualism of autophagy can be explained in part by evidence that excessive autophagy can lead to depletion of key molecules and organelles, triggering autophagic cell death [7, 8, 16].

By contrast, cardiomyocyte autophagy is adaptive in a model of proteotoxic cardiomyopathy, where a mutation in the gene coding for the protein chaperone αB-crystallin confers a dominant-negative effect to inhibit protein folding [76]. In this context, autophagic flux is activated to rid the cell of toxic, misfolded, oxidized proteins. Importantly, these studies of proteotoxicity deriving from afterload stress or chaperone protein dysfunction were performed by manipulating the same gene, *BECN1*, lending additional credence to the notion that the functional differences in the actions of autophagy derive from differences in the underlying cardiomyopathic stimuli.

Autophagy in myocardial ischemia and ischemia/reperfusion

Induction of autophagic flux in response to ischemic insult has been reported in multiple systems [56, 74, 77]. Despite this, there is disagreement regarding whether the effects of autophagy in ischemia/reperfusion (I/R) injury are protective or maladaptive. For example, during mild ischemic stress, activation of cardioprotective autophagy depends on AMP-activated protein kinase (AMPK)-mediated inhibition of mammalian target of rapamycin (mTOR) [74, 78]. Pharmacological inhibition of autophagy in ischemia-mimicking conditions (e.g. glucose and oxygen withdrawal) enhances cardiomyocyte death, suggesting pro-survival effects [79]. However, when oxygen and nutrients are restored, myocyte autophagy is up-regulated dramatically *in vivo* (rat [80], rabbit [81], swine [82]), in cultured

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cell lines (H9c2 [83], HL-1[84]), and in primary cultured neonatal cardiomyocytes [74, 79]. Ischemia, where nutrient and oxygen supply to the myocardium are limited, is a state reminiscent of starvation, a context where autophagy is adaptive. Reperfusion, by contrast, is a very different situation and the associated activation of autophagic flux can be adaptive or detrimental (depending on the model system) and involves Beclin 1 independent of the AMPK/mTOR pathway [74]. Short, repetitive ischemic episodes – which elicit beneficial ischemic preconditioning effects [82, 85] – induce autophagy, as well, and when this autophagic response is suppressed, the protective effects of preconditioning are lost [82, 85].

Cardiac autophagy as a therapeutic target

Despite significant advances in cardiovascular therapeutics, both pharmacologic and device-based, the incidence of heart failure remains distressingly high [1]. A significant reason for this apparent failure is the fact that our understanding of cardiac plasticity and pathological remodeling is incomplete [86]. Recent scientific advances, however, have raised the tantalizing prospect of targeting the myocyte autophagic reaction as a novel means of achieving therapeutic gain.

In the setting of cell growth, both anabolic and catabolic processes are activated. During the initial phase, the former predominates and cell growth ensues. Ultimately, however, a new steady state emerges where levels of autophagic flux are increased. And depending on the strength of the growth stimulus – and the genetic context where autophagy is either suppressed completely, suppressed partially, or amplified – the resulting autophagic activity is either adaptive or maladaptive. Indeed, consensus is coalescing around the notion that cardiomyocyte autophagy triggered by elevations in afterload has both adaptive and maladaptive features. Complete abrogation of autophagic flux is incompatible with cell survival. Activation of autophagy in the setting of pressure stress may be beneficial up to a point, but over-activation of autophagic flux is maladaptive. At one level, this is not surprising, as the dual nature of autophagy is a recurring theme in other organ systems and disease states [87]. Indeed, we have postulated that the (patho)physiological impact of autophagy exists as a continuum, and a window of optimal autophagic activation (“adaptive” zone of autophagy) is critical to the maintenance of cellular homeostasis and function (Figure 3). Another (not mutually exclusive) model holds that different types of autophagic flux exist within the cardiomyocyte – e.g. selective versus nonspecific, mitochondria-targeting, etc. – which contribute to the differential actions of autophagy.

In any event, the prevalence of autophagic activation in the vast majority of cardiac disorders suggests the existence of a common cellular pathway which can be targeted for therapeutic gain. Pause is warranted, however, as the challenge we face will be to remain cognizant of the widespread actions of autophagy in numerous cell types. Further, we must envision tuning the autophagic response within a physiological range without abolishing it altogether. Happily, some evidence suggests this may be possible [59]. Indeed, a growing number of drugs in clinical use already or in development hold promise in this regard (Figure 1, Tables 1, 2).

Future of cardiovascular autophagy research

As noted, recent findings by our group demonstrate that, at least in the context of cardiac hypertrophy, HDACi blocks pathological cardiomyocyte autophagy and blunts hypertrophic growth [59]. Given the ever-expanding burden of cardiac pathology worldwide, these findings merit further study in patients with heart disease.

Two HDAC inhibitors, Zolinza® (vorinostat) (a hydroxamic acid derivative structurally related to TSA) and romidepsin (Istoda®) have been granted FDA approval for the

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treatment of cutaneous T-cell lymphoma. Meanwhile, clinical trials are underway evaluating a number of other small molecules with inhibitory actions on HDACs. Based on these facts, we are moving swiftly to a first-in-man study of HDACi in ischemic heart disease. Large animal studies of surgical I/R are presently underway, as is a safety trial in patients with stable coronary disease presenting for coronary angiography. With these data in hand, we anticipate moving quickly toward a proof-of-concept trial in patients presenting with ST-segment elevation myocardial infarction (STEMI).

Meanwhile, HDACi is being evaluated actively in the context of a number of cancers. As noted earlier, HDACi promotes tumor cell death in some instances and antagonizes it in others. Thus, in addition to HDACi trials aimed at promoting cell death, two anti-autophagic drugs, chloroquine and hydroxychloroquine, are being tested in combination with various other therapeutic agents (Figure 1). The rationale here is that tumor cell autophagy promotes chemotherapy resistance in some instances, and blocking this response may re-sensitize cancer cells to toxic effects of concomitant therapy.

Only 11 patents were found relating to autophagy in the cardiovascular field (Table 2). Gottlieb et al described the use of fluorescent cadaverine to label autophagic vesicles in cardiac myocytes [88]. Tanida et al pioneered the use of GFP-GABARAP and GFP-GATE16 transgenes as markers of autophagosomes in the heart [89]. The remaining 9 patents disclosed the use of autophagy activators or inhibitors for the treatment of several cardiovascular diseases, including myocardial ischemia, myocardial infarction, vascular hyperplasia, cardiac hypertrophy, congestive heart failure, cardiomegaly, restenosis, atherosclerosis, hypertension and angina pectoris [90–98].

Concluding remarks

Autophagic “self-eating” is a critical pro-survival response in cardiomyocytes exposed to diverse forms of pathological insult. In this light, the growing number of cardioprotective therapies affecting autophagic activity is encouraging. For example, a recent report showed that chloramphenicol succinate activates autophagy in I/R-stressed swine heart, and this therapy was associated with benefit [99]. Looking to the future, it is possible to envision harnessing therapeutic agents already in clinical use to modulate cardiac autophagy for therapeutic benefit. This modulation of the autophagic processes can be achieved by either direct manipulation of the autophagic “core” machinery or through the exploitation of signaling pathways linked to autophagy (Figure 4). As the benefits afforded by autophagic activation depend on pathological context, vigilance for extra-cardiac effects will be critical. Further, it will be critical to titrate the stress-triggered autophagic response within an “optimal” zone, where proteostasis is promoted and yet the fundamentally important role of basal autophagy-dependent protein quality control is maintained. A comprehensive view of myocardial autophagy will be obligatory, as strategies for suppressing excessive activation of pathological pathways must always be precisely regulated to avoid disrupting homeostatic mechanisms. Major challenges remain, but patients with heart disease are likely to benefit from these efforts.

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Autophagic Stage	Molecular Component	Therapeutics	Reference
Isolation Membrane	Atg1/ULK1	Rapamycin	[100, 101]
		RAD001	[100, 101]
		CCI-779	[100, 101]
		AP23573	[101, 101]
		Torin1	[102]
	mAtg9		
Elongation Step	PI3K/Vsp3	3-methyladenine	[103]
		Wortmannin	[103]
		LY294002	[103]
	Beclin1	Urocortin	[79]
		Tamoxifen	[104]
		TSA, SAHA	[105,106]
		As_2O_3	[107]
		Xestospongin B	[14, 62]
		BH3-peptides (ABT737)	[108, 109]
		2-methoxyestradiol (2-ME)	[110]
		FoxO1, FoxO3	[111, 112]
Autophagosome	Atg5-Atg12	Fluspiriline	[113, 114]
	Atg4	ROS/antioxidants	[72]
	Atg7	Epothilone	[115]
	Atg8	FoxO1, FoxO3	[111, 112]
	Fusion with Lysosomes	Bafilomycin A	[103, 116]
		Vinblastin	[116]
		Nocodazole	[116]
Autolysosome	Lysosomal Enzymes	Chloroquine	[103]
		Hydroxy chloroquine	[103]
		E64d	[103, 116]
		Pepstatin A	[103, 116]
		Nigericin	[113]
		Wiskostatin	[113]
		Monensin	[103]
		ROS/antioxydants	[72]

Figure 1. Therapeutic manipulation of core autophagic machinery

Therapeutic agents that target specific molecular components of the core autophagic machinery. Abbreviations: TSA – Trichostatin A; SAHA – Suberoylanilide hydroxamic acid; ROS – reactive oxygen species

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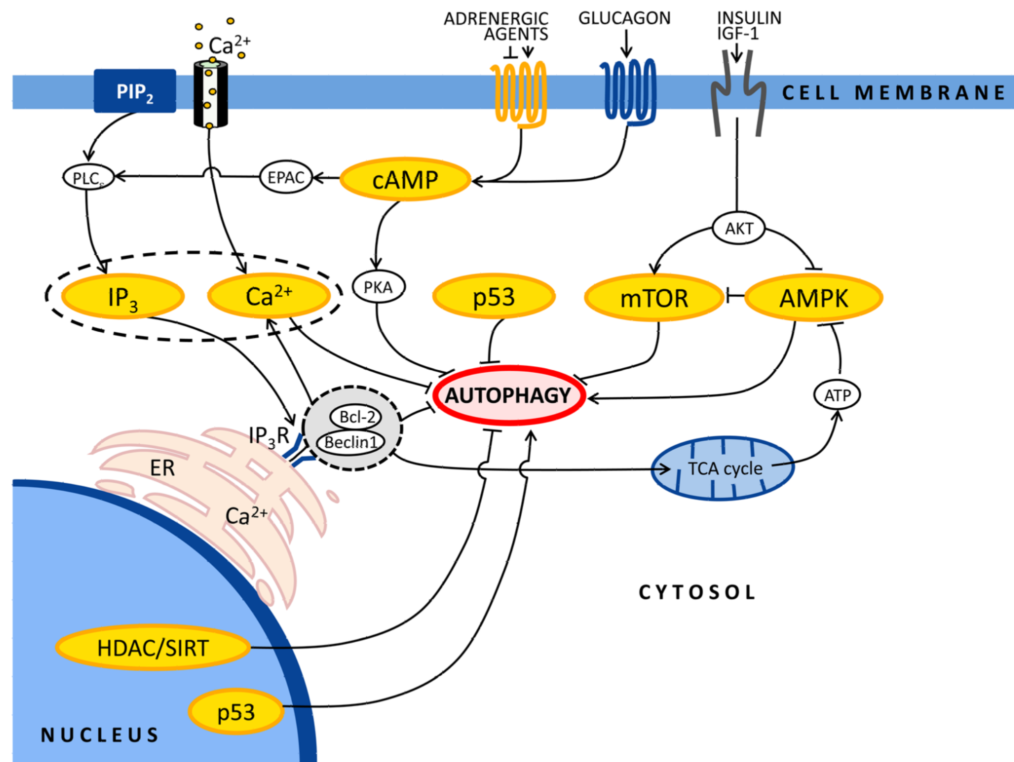

Figure 2. Main regulatory pathways governing autophagy

Simplified scheme of the major cellular pathways governing autophagic responses in ventricular cardiomyocytes. See text for details. Arrow denotes stimulation, and T-shaped indicators denote inhibition.

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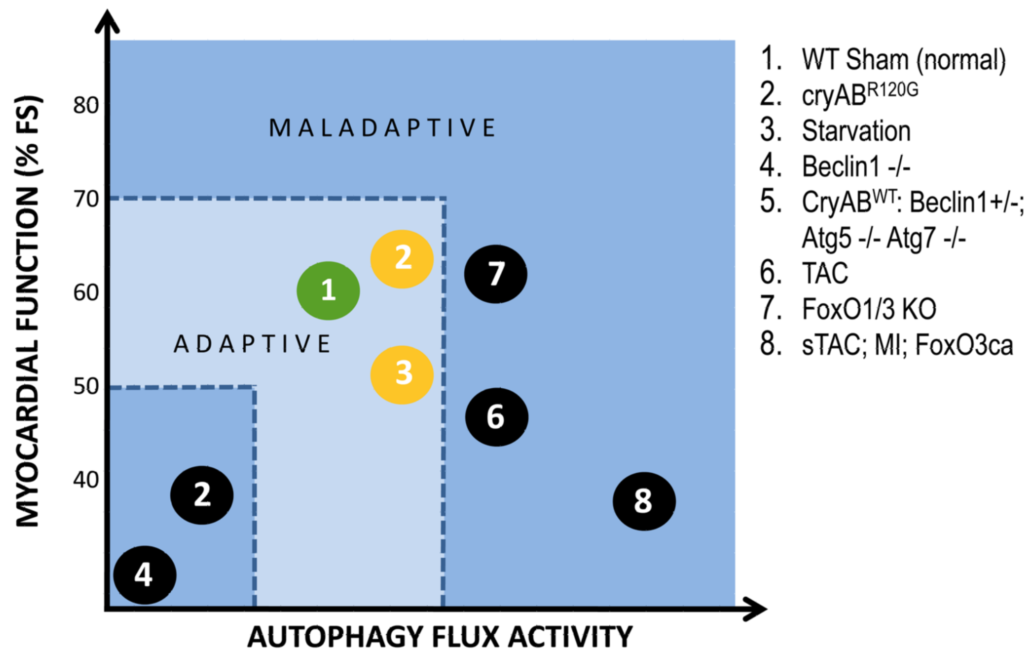

Figure 3. Relationship between autophagic flux activity and myocardial function

Working model of instances where changes in cardiac function (expressed as % fractional shortening, FS) were associated with alterations in autophagic flux. 1) WT – wild type; 2) cryAB^{R120G} – αB-crystallin mutant; 3) starvation – 48 h food deprivation; 4) *Beclin 1 −/−* knockout; 5) CryAB^{WT} – over-expresser of WT αB-crystallin, Autophagy-related gene (*Atg*) 5 and 7 knockout; 6) TAC – afterload stress induced in WT mice by thoracic aortic constriction; 7) forkhead box transcription factors, O *Fox*O1 knockout, *FoxO3* knockout; 8) sTAC – severe TAC, MI – myocardial infarction induced in WT mice by ligation of left anterior descending artery. FoxO3 – constitutive over-expression of FoxO3;

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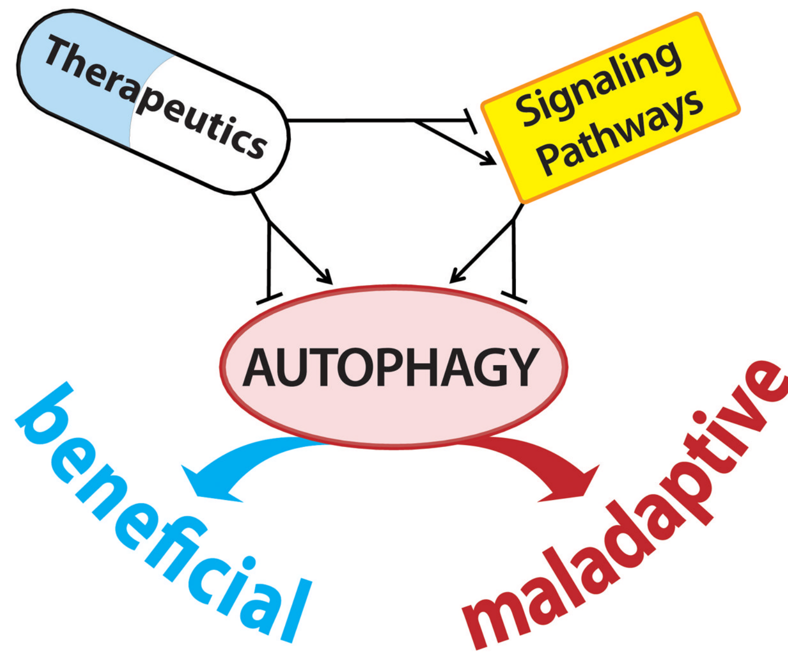

Figure 4. Therapeutic manipulation of cardiac autophagy

Targeting cardiac autophagy using FDA-approved therapeutics could be accomplished by direct regulation of the core autophagic machinery (Figure 1) or by manipulation of key upstream regulatory pathways (Figure 2 and Table 1) (see text for details).

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Table 1

Therapeutic manipulation of autophagy through regulatory signaling pathways.

Signaling pathway	Therapeutics	CVS effect	Autophagy
$IP3/Ca^{2+}$	• IP3 receptor antagonist: xestospongins[38] • Decrease IP_3 levels: carbamazepine, lithium, IMPase inhibitors[117, 118] • L-type Ca^{2+} channel antagonists (verapamil, nitrendipine (*in vitro*)[43]	ND	Activation
	• Myoinositol[117, 118]	ND	Inhibition
cAMP	• Glucagon (*in vivo*)[119] • AR agonists & antagonists: adrenaline (*in vivo*)[119], isoproterenol & salbutamol (*in vitro*)[120], propranolol & carvedilol (*in vivo*)[121, 122] • Minoxidil & clonidine (*in vitro*)[43]	Yes[119] Yes[119–122] ND	Activation
	• β-AR antagonists (*in vitro*)[120]	Yes[120]	Inhibition
p53	• Etoposide induces nuclear p53[123]	ND	Activation
	• Nutilin-3, HDM2, RITA[124]	ND	Inhibition
AMPK	• AMPK activator: Metformin (*in vitro e in vivo*)[125, 126]	Yes[126]	Activation
	• AMPK inhibitors: Ara A (*in vitro*)[74], compound C (*in vitro*)[90], IGF-1 (*in vitro & in vivo*)[78] • AMPK activators: AICAR[127, 128]	Yes[74, 78] ND	Inhibition
mTOR	mTOR inhibitors: • Rapamycin (in vivo)[129], torin 1[130], • Perhexiline, amiodarone, niclosamide (*in vitro*)[131]	Yes[129]	Activation
	Akt activators • IGF-1 (in vitro)[132]	ND	Inhibition
Chromatin remodeling	• HDAC inhibitors: Trichostatin A (TSA)[59], suberoylanilide hydroxamic acid (SAHA)[133], valproic acid[134], OSU-HDAC42[135], Curcumin[136], ATRA[137], spermidine[138] • Sirtuin activator: Resveratrol[138, 139]	ND	Activation
	• HDAC inhibitors: TSA[59]	Yes[59]	Inhibition

AR = adrenergic receptor; HAT = histone acetyltransferases; HDAC = histone deacetylase; ND = not determined

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Table 2

Autophagy-related patents and putative applications in cardiovascular disease.

Patent category	Therapeutics/Target	Remarks	Patent Number	Ref
Autophagy induction	8-methylchroman-7-ol derivatives	Atherosclerosis, myocardial ischemia.	US2010173983	[98]
Proteinopathy treatment	Farnesyl transferase inhibitor	Myocardial ischemia, MI, vascular hyperplasia, cardiac hypertrophy, CHF, restenosis, atherosclerosis, hypertension, angina pectoris,	US2010160372	[97]
Autophagy regulation	Compounds that regulates ATG14L and Rubicon which binds Class III PI3K/Vps34-Beclin 1 complex,	Inflammatory cardiac diseases	WO2010030936	[96]
Autophagy induction	Glycosylated anti-tumor ether lipids (GAEL) are small molecules that induce and/or enhance autophagy in cells	Ischemic/reperfusion injury	WO2009092170	[95]
Autophagy regulation	Autophagy modulators identified by a high- throughput phenotypic screen	Reperfusion injury, ischemic cardiac disease	WO2008122038	[94]
Cardiac autophagy death regulation	siRNA against ANT isoforms which selectively regulates autophagic cell death	Treating cardiac ischemia	US20060210535	[93]
Autophagy regulation	A phosphorus-rapamycin analog, AP23573	Hyperproliferative vascular diseases (restenosis; graft vascular atherosclerosis; and cardiovascular disease, cerebral vascular disease, and peripheral vascular disease,	US20040073024	[90]
Anti-cardiac autophagic degeneration/death	Long-term administration of a colony-stimulating factor (G-CSF)	Ischemic cardiac failure, myocardial fibrosis, left ventricular remodeling	US2006051318	[92]
Cardiac atrophy	Agent that increases the expression of the runx1 gene to prevent or decrease cardiac autophagy	Treatment of a heart condition whereby heart muscle is destroyed	US2006003959	[91]