

Cardiovascular autophagy

Concepts, controversies, and perspectives

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Abbreviations: AMPK, AMP-activated protein kinase; ATG, autophagy-related; ER, endoplasmic reticulum; GSK3B/GSK-3 β , glycogen synthase kinase 3 beta; HDAC, histone deacetylase; HDACi, HDAC inhibitor; IP₃, inositol 1,4,5-trisphosphate; I/R, ischemia/reperfusion; ITPR, inositol 1,4,5-trisphosphate receptor; LC3, microtubule-associated protein 1 light chain 3 (homolog of yeast Atg8); MTOR, mechanistic target of rapamycin; NAD⁺, nicotinamide adenine dinucleotide; PIK3C3/VSP34, phosphatidylinositol 3-kinase, catalytic subunit type 3; PKA, cAMP-dependent protein kinase; PtdIns3K, phosphatidylinositol 3-kinase; ULK, unc-51 like autophagy activating kinase; VSMC, vascular smooth muscle cells; WDFY3/ALFY, WD repeat and FYVE domain containing 3

Despite recent scientific and technological advances, cardiovascular disease remains the leading cause of morbidity and mortality worldwide. Autophagy, an evolutionarily ancient response to cellular stress, has been implicated in the pathogenesis of a wide range of heart pathologies. However, the precise role of autophagy in these contexts remains obscure owing to its multifarious actions. Here, we review recently derived insights regarding the role of autophagy in multiple manifestations of cardiac plasticity and disease.

Introduction

Heart disease, the greatest noninfectious health hazard ever to confront the human race, is rampant around the globe. Its prevalence in the developed world is longstanding, and it is expanding rapidly and unchecked in the developing world. The end result of many forms of cardiovascular disease is heart failure, a syndrome where the heart is unable to meet the metabolic demands of the body. It is estimated that five million Americans have heart failure marked by a five-year mortality rate of ~50%;¹ more than 64 million Americans have some type of cardiovascular disease. According to the World Health Organization, cardiovascular diseases are the leading cause of death in the world.² Although cardiovascular disease deaths have declined in the past 50 years, the effect of the leading killer and disabler varies greatly based on gender, age, race, location, and type.³ Indeed, heart failure has remained the leading cause of hospitalization in industrialized nations for several years. Accordingly, cardiovascular disease,

including heart failure, is responsible for a huge societal burden of morbidity, mortality, and cost.

The cardiovascular system comprises the heart and blood vessels, including arteries, veins, and capillaries, both systemic and pulmonary. The cardiovascular system is a closed tubular system in which the blood is propelled by the heart. The heart is a mechanical pump whose main function is to pump blood throughout the tissues of the body, carrying nutrients and oxygen and removing carbon dioxide and other metabolic waste, and through the lungs, delivering carbon dioxide and accepting oxygen. The heart is an incredibly resilient organ, marked by approximately 2.5 billion contractions over a 70-year lifetime. As a consequence, the heart is a robust consumer of energy, requiring a constant supply of oxygen and metabolic fuels in order to sustain contractile function. Energy reserves in the heart are limited, sufficient only to support contraction for a very few seconds; as a result, energy must be produced continually by catabolism of a variety of energy substrates.⁴ The heart is a “metabolic omnivore,” capable of metabolizing free fatty acids, glucose, lactate, pyruvate, ketone bodies, and amino acids. Under normal resting conditions, metabolism is mainly oxidative, with free fatty acids and glucose being the major sources of energy. The preferred substrate depends on arterial substrate concentrations (dietary conditions), hormonal factors (mainly insulin), and workload. However, glycolytic ATP production through conversion of glucose to lactate is independent of oxygen; thus, glucose is the preferred substrate under hypoxic conditions such as ischemia and increased workload.⁴

Much of cardiovascular disease centers on blood vessels, which can be afflicted by atherosclerotic change, calcification, inflammation, vasomotor dysfunction, hypertrophic thickening of the vessel walls, and more. Arteries, which carry blood from the left heart to the body and from the right heart to the lungs, are thick-walled, with a muscular contraction that helps propel blood downstream.

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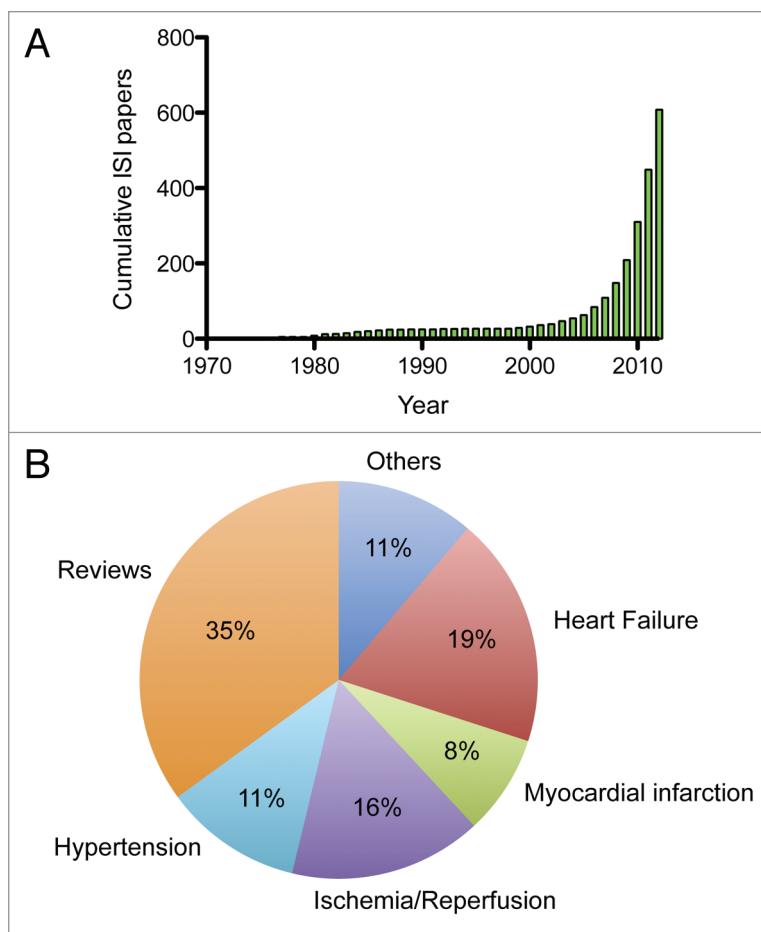


Figure 1. Autophagy in cardiovascular disease. **(A)** Number of published papers per year related to autophagy and cardiovascular disease listed in PUBMED. **(B)** Pie chart shows the percent of papers focusing on the major topics of cardiovascular research.

Smaller caliber arteries are termed arterioles, which feed ultimately into capillaries where gas and small molecule exchange takes place. Conversely, veins carry blood back to the heart.

Cardiovascular Plasticity

Virtually every element of the cardiovascular system is capable of robust plasticity in response to exogenous exposures. This plasticity can be either physiological, occurring in the context of normal changes in workload demand, or pathological, occurring in the context of disease. Exercise, pregnancy, and other physiological conditions promote physiological cardiovascular remodeling, whereas neurohumoral activation, hypertension, hypercholesterolemia, etc. cause pathological cardiovascular remodeling.⁵ These alterations in the size, morphology, and function of cardiovascular tissues have important functional consequences.⁵⁻⁷ Indeed, the concept of cardiovascular remodeling can be extended to denote molecular, structural, and compositional changes in subcellular organelles, such as sarcoplasmic reticulum, sarcolemma, myofibrils, mitochondria, the nucleus, and extracellular matrix.⁸ The term “metabolic cardiovascular remodeling” has been coined to

describe the response to chronically altered workload and substrate availability seen in several forms of heart disease, including heart failure.⁹

Vascular remodeling entails restructuring of the wall of arteries, arterioles, and coronary resistance vessels.¹⁰ This process is characterized by changes in lumen diameter, wall thickness and stiffness, and cellular and extracellular matrix components. For example, thickening and fibrotic change of the medial wall occur commonly in hypertension and atherosclerotic vascular disease.¹⁰ Medial thickening is caused by hypertrophy and/or hyperplasia of vascular smooth muscle cells.

The heart itself is also a highly plastic organ capable of robust growth or shrinkage in response to changes in physiological or pathological demand. When workload increases, the heart compensates through hypertrophic growth of individual cardiomyocytes to normalize ventricular wall stress and increase cardiac output.^{11,12} This cellular process is characterized by enhanced protein synthesis and an increase in the size and organization of cardiomyocyte sarcomeres.^{11,12} In the setting of chronic stress, the heart manifests apparently irreversible decompensation, culminating in dilation, and contractile dysfunction.¹³ This is accompanied by thinning of the ventricular walls through a combination of proteolysis and/or death of cardiomyocytes. Recent evidence has implicated autophagy, a process of protein and organelle recycling, in the cardiomyocyte response to stress and in the transition to cardiac failure.¹⁴⁻²⁰

Cardiovascular Autophagy

Recent years have witnessed rapid growth in studies seeking to define the role of autophagy in cardiovascular homeostasis and disease, focusing on virtually every cell type within the cardiovascular system. It was in the 1970s when the first reports of cardiomyocyte autophagy emerged.^{21,22} Then, we had to wait until 2000 to witness robust growth in the investigation of cardiovascular autophagy.²³⁻²⁵ These studies have been designed to dissect the regulatory circuitry governing autophagic activation, flux, and lysosomal processing, and to determine the functional implications of changes in autophagic pathways.

We searched MEDLINE (1976 to May 2013) for animal and human studies of autophagy and cardiovascular diseases, including early work on lysosomal degradation events. We found 616 publications related to autophagy and cardiovascular diseases (5.5% of the papers on cardiovascular disease) (Fig. 1A). Nearly 85% of these papers (525) were published during the past 5 years. Heart failure, ischemia/reperfusion, and cardiac hypertrophy have been the main topics investigated (Fig. 1B). Critical reviews represent 36% of the total published papers (219). A number of pivotal observations have emerged as a result of these efforts (Fig. 2). Here, we summarize current concepts regarding the regulation and consequences of cardiovascular autophagy, point to remaining knowledge gaps, and discuss possible directions for future research.

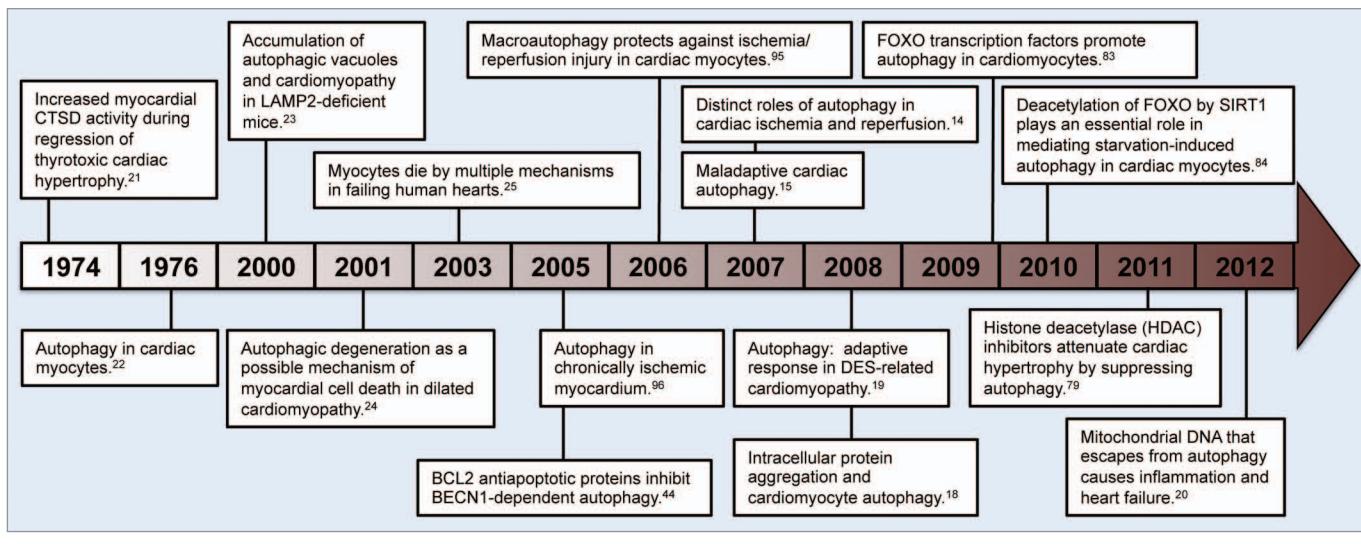


Figure 2. Timeline depicting principal findings in cardiac autophagy.

Autophagy

The term “autophagy” was coined by Christian de Duve, who discovered the lysosome.²⁶ de Duve chose this term to distinguish lysosomal degradation of the cell’s own components from lysosomal degradation of extracellular material (heterophagy).²⁷ Etymologically, autophagy derives from the Greek words auto (self) and phagy (eating). This term originally reflected observations made by electron microscopy of novel single- and double-membrane vesicles harboring organelles and components of the cytoplasm at different stages of degradation.²⁸

Autophagy is an evolutionarily conserved catabolic mechanism that is critically required for cellular homeostasis.²⁹ Under basal conditions, autophagy is required to degrade long-lived proteins and dysfunctional organelles. Under conditions of stress (e.g., starvation or hypoxia), autophagy is activated, promoting cell survival by releasing energy substrates via degradation of cellular constituents and by eliminating defective or damaged organelles.³⁰ However, taken too far, excessive and uncontrolled autophagic activation can lead to depletion of essential molecules and organelles, triggering autophagic cell death.^{31,32} Importantly, autophagy also appears to operate in an organelle-selective manner, e.g., selectively targeting mitochondria (mitophagy), endoplasmic reticulum (reticulophagy), portions of the nucleus (nucleophagy), peroxisomes (pexophagy), microorganisms (xenophagy), ribosomes (ribophagy), lipid droplets (lipophagy), or protein aggregates (aggrephagy).³³

At least three different types of autophagy have been described, which differ in the mode of cargo delivery to lysosomes.^{33,34} The most extensively investigated is macroautophagy. A second type of self-eating is microautophagy, in which cargo engulfment takes place directly by the lysosomal membrane. A third type of self-eating is chaperone-mediated autophagy. This pathway exhibits no bulk engulfment by membranes and a selective recognition of substrates. At this point in time, the roles of these alternative forms of autophagy in heart have not been elucidated.

Interestingly we recently discovered that RYR2 (ryanodine receptor 2 [cardiac]) sequence harbors six KFERQ motifs, and is degraded via chaperone-mediated autophagy.³⁵ As RYR2 plays a key role in cardiomyocyte excitation-contraction and its dysfunction can contribute to cardiac failure, this observation may have pathophysiological relevance.

Macroautophagy is divided into specific steps, including induction, cargo recognition and selection, vesicle formation, autophagosome-vacuole fusion, breakdown of the cargo, and release of the degradation products into the cytosol (Fig. 3). The core autophagic machinery consists of evolutionarily conserved signaling modules encoded by autophagy-related (*ATG*) genes that govern these steps:^{30,36,37}

- The Atg1 kinase complex (composed of at least Atg1-Atg13-Atg17-Atg31-Atg29) and the mammalian ULK1/2 (unc-51 like autophagy activating kinase) complex (including ULK1/2-ATG13-RB1CC1-C12orf44/ATG101).
- The phosphatidylinositol 3-kinase (PtdIns3K) complex composed of Vps34-Vps15-Vps30/Atg6-Atg14 and the mammalian counterpart (including PIK3C3/VPS34-PIK3R4/VPS15-BECN1-ATG14).
- The ubiquitin-like protein conjugation cascades involving Atg3, Atg4, Atg5, Atg7, Atg8 (and the mammalian LC3 and GABARAP subfamilies), Atg10, Atg12, and Atg16 (mammalian ATG16L1) and the corresponding mammalian homologs, which are required for phagophore expansion, autophagosome maturation and cargo recruitment.
- A recycling protein complex consisting of Atg2, Atg9, Atg18, and Atg21 (and the mammalian homologs including WIPI1/2), which participates in the transfer and recycling of components between the phagophore and/or the phagophore assembly site and membrane donor sites.

The molecular elements of the autophagic machinery are more complex in mammalian cells, with several congeners of yeast Atg proteins having multiple mammalian family members. Systematic proteomic analysis of the human autophagy system

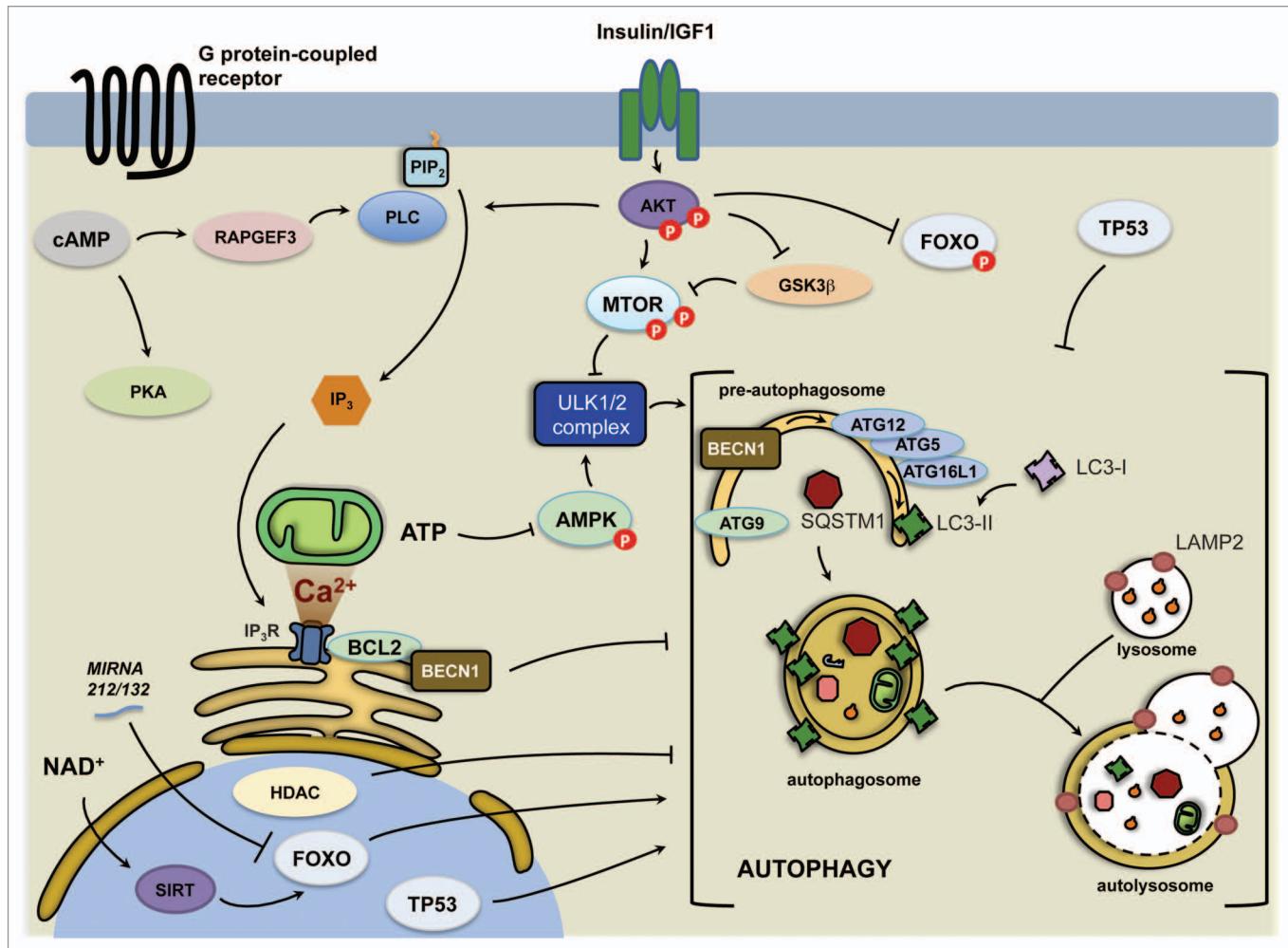


Figure 3. Main pathways regulating the autophagic machinery. Simplified scheme of the major cellular pathways governing autophagic responses in cardiomyocytes. See text for details. Arrows denote stimulation, and T-shaped indicators denote inhibition.

coupled with a functional analysis of a subset of genes in the pathway has revealed for the first time the global architecture of the autophagy interaction network, its complexity and the unappreciated level of interconnectivity between the different modular components of the autophagy system.²⁹

The ULK1/2 (and Atg1) kinase complex controls early steps in autophagosome formation. This complex is regulated by nutrient availability via the mechanistic target of rapamycin (MTOR) kinase.³⁸ Basal-level autophagic activation is low under nutrient-rich conditions. MTOR integrates input information from different upstream signaling pathways and negatively regulates ULK1/2.³⁹ AMP-activated protein kinase (AMPK) acts as a cellular energy sensor during starvation, mediating phosphorylation of ULK1 to promote its release of MTOR and subsequent localization in the autophagosome formation region.^{40,41} Presently, unresolved questions include how the ULK1/2 complex is activated and whether changes in ULK1/2 phosphorylation are essential for activation of the protein complex and consequent autophagy induction. Also, the physiological mechanism for downregulating autophagy remains unclear. DAP (death-associated protein) participates in this process;⁴² however, it remains to

be determined whether this protein targets the ULK1/2 complex, or other components of the cascade.

In yeast, the double-membrane autophagosome is assembled at the phagophore assembly site by addition of new membranes.²⁹ Similarly, in mammalian cells several ATG proteins are recruited to the phagophore to participate in autophagosome formation. Next, nucleation and assembly of the initial phagophore membrane requires the class III PtdIns3K complex formed by PIK3C3 the catalytic subunit of the lipid kinase, PIK3R4/VPS15 a myristoylated serine/threonine kinase that is a presumed regulatory subunit, ATG14 and BECN1. The autophagy-promoting activity of BECN1 is regulated by BCL2 and BCL2L1.^{43,44} The PtdIns3K complex recruits two interrelated ubiquitin-like conjugation systems, which generate ATG12–ATG5–ATG16L1 and LC3/GABARAP–phosphatidylethanolamine, and together these play essential roles in regulating membrane elongation and expansion of the nascent phagophore.²⁹

The autophagic pathway has a key role in the clearance of cytosolic ubiquitinated substrates or protein aggregates. This degradative process is also selective and mediated through SQSTM1/p62.⁴⁵ SQSTM1 directly binds both poly- or mono-ubiquitin,

via its ubiquitin-associated domain, and LC3, thereby linking ubiquitinated cargos to the autophagy machinery for autophagic degradation.⁴⁶ A new partner has been described in selective autophagy, the large scaffolding protein WDFY3/ALFY (WD repeat and FYVE domain containing 3). WDFY3 interacts with SQSTM1 and ATG5, as well as PtdIns3P found in autophagic membranes, reminiscent of the function of a scaffolding protein. WDFY3 in normal conditions is localized in the nucleus and translocates to the cytoplasm during conditions favoring formation of protein aggregates.⁴⁷ WDFY3 is required for aggrephagy, but not for cytoplasmic cargo degradation in response to starvation.⁴⁸

The last event in the autophagy cascade occurs when the autophagosome fuses with a lysosome to form an autolysosome. The same machinery that is involved in homotypic vacuole membrane fusion mediates this process.²⁹ Autophagosome-lysosome fusion requires the lysosomal membrane protein LAMP2 and the small GTPase RAB7.⁴⁹ After fusion, degradation of the inner vesicle, along with its contents, is dependent on a series of lysosomal/vacuolar acid hydrolases. Amino acids, sugars, and nucleotides are released to the cytosol through permeases.⁴⁹ Prior to this step, fusion of the autophagosome with early and late endosomes lowers intra-vesicular pH. The 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.

Autophagy Signaling Pathways in the Cardiovascular System

MTOR

A central node of autophagy regulation is the serine/threonine protein kinase MTOR, a highly conserved protein that regulates cell growth, protein synthesis, and metabolism (Fig. 3).⁵⁰ Two different multiprotein MTOR complexes exist, MTORC1 and MTORC2. The MTORC1 complex includes RPTOR, AKT1S1/PRAS40, and MLST8.⁵¹ The MTORC2 complex which comprises RICTOR, MAPKAP1/SIN1, and MLST8,⁵² is relatively rapamycin insensitive, and governs spatial control of cell growth by regulating the actin cytoskeleton. By contrast, MTORC1 is highly rapamycin sensitive and mediates temporal control of cell growth by transcription, translation, and autophagy. On the one hand, MTORC1 maintains autophagy at basal levels by binding and phosphorylating the autophagy kinase complex ULK1/2 to inhibit phagophore formation.⁴¹ On the other hand, MTORC1 is dissociated from the ULK1/2 complex when inactivated, inducing robust autophagy. Upstream of MTORC1, a finely tuned circuitry regulates MTOR activity. Insulin and IGF1 (insulin-like growth factor 1) control autophagy through activation of the class I PI3K and production of PtdIns(3,4,5)P₃, a molecular signal that activates the serine/threonine kinase AKT, which governs the function of MTOR through phosphorylation, thereby inhibiting autophagy.⁵³ Interestingly, cardiomyocyte-specific deletion of MTORC1 leads to development of a lethal dilated cardiomyopathy.⁵⁴

AMPK

Depletion of cellular energy (e.g., low ATP levels) activates AMPK and stimulates autophagy.⁵⁵ This protein is a heterotrimeric kinase comprising $\alpha\beta\gamma$ subunits, serving as a critical integrator of multiple signals in the control of energy balance. A variety of cellular stressors decrease intracellular ATP/AMP ratios, activating STK11/LKB1 kinase, which in turn activates AMPK to phosphorylate the TSC1/2 complex leading to MTOR inhibition through RHEB.⁵⁶ However, AMPK can also regulate MTORC1 by an alternative mechanism, as this kinase directly phosphorylates RPTOR, leading to MTORC1 inhibition.⁵⁷ The upstream kinase CAMKK2 also phosphorylates AMPK in an AMP-independent and Ca²⁺-dependent manner.⁵⁸ Cytokines and increases in intracellular Ca²⁺ each activate AMPK and autophagy via this mechanism.⁵⁷ Finally, AMPK can directly phosphorylate ULK1 on serine 317 and 777 leading to its dissociation from the MTORC1 complex, enhancing autophagic turnover in response to glucose deprivation. Under basal conditions, MTOR phosphorylates ULK1 on serine 757 preventing its activation and its interaction with AMPK.⁵⁹ AMPK is an essential regulator of glucose deprivation-induced protective autophagy in neonatal cardiomyocytes.¹⁴ Also, transgenic mice overexpressing a dominant negative AMPK exposed to ischemia manifest reduced autophagy in vivo.¹⁴ Moreover, IGF1 inhibits autophagy by increasing ATP levels, resulting in AMPK activation in glucose-deprived cultured cardiomyocytes.⁶⁰

Inositol 1,4,5-trisphosphate (IP₃) and ITPR (inositol 1,4,5-trisphosphate receptor)

IP₃ and the ITPR each play a key role in the control of autophagy.⁶¹ Inhibition of IP₃ with the antagonist xestospongin B or knockdown of different ITPR isoforms each trigger robust autophagy.⁶² Xestospongin B and nutrient starvation disrupt a molecular complex formed by the ITPR, BECN1, and BCL2.⁶³ Recently, Cárdenas et al. showed that constitutive ITPR-dependent Ca²⁺ transfer to mitochondria is an essential cellular process required for efficient mitochondrial respiration.⁶⁴ When the ITPR is not activated, oxygen consumption and ATP levels drop, activating AMPK and promoting autophagy to maintain cell homeostasis.⁶⁴ In cardiomyocytes, depletion of IP₃ by over-expressing a selective phosphatase or treatment with an ITPR antagonist (e.g., 2-APB) promotes autophagy.⁶⁵

Transcription factor TP53

The protein TP53 (tumor promoter p53) has a double function in autophagy regulation depending on its subcellular location.⁶⁶ In the nucleus, TP53 functions as a pro-autophagic factor in transcription-dependent or -independent manners.⁶⁷ TP53 regulates various targeted genes to activate AMPK or inhibit MTOR, each promoting autophagy.⁶⁸ In addition, TP53 functions as a nuclear transcription factor and can transactivate the DRAM1 (DNA-damage regulated autophagy modulator 1) family of genes, stimulating accumulation of autophagosomes by regulating autophagosome-lysosome fusion.⁶⁹ However, in the cytoplasm, TP53 suppresses the induction of autophagy.⁶⁷ Cytoplasmic TP53 controls autophagy through inhibiting AMPK and activating MTOR.⁷⁰ Also, TP53 modulates C12orf5/TIGAR, which reduces glycolysis and intracellular levels of reactive oxygen

species leading to autophagy inhibition.⁷¹ Recent evidence indicates that the TP53-C12orf5 axis attenuates mitophagy to exacerbate cardiac damage after ischemia.⁷²

Cyclic AMP-dependent protein kinase A (PKA)

PKA is able to sense nutrient availability and control cell growth⁷³ and exists as a heterotetramer of two regulatory and two catalytic subunits. Following dissociation, the catalytic subunits phosphorylate multiple substrates to regulate a wide range of cellular processes.⁷³ PKA is a negative regulator of autophagy, which in yeast acts primarily on Atg1, Atg8, and Atg13.⁷⁴ cAMP also inhibits autophagy in a PKA-independent manner via RAPGEF3/EPAC-RAP2B-PLCE1/phospholipase C, epsilon 1.⁷⁵

Histone acetyltransferases and histone deacetylases (HDACs)

Reversible acetylation and deacetylation of proteins is controlled by the antagonistic actions of histone acetyltransferases and HDACs. HDACs are targeted by small molecular inhibitors, and these molecules manifest efficacy in the suppression of pathological cardiac remodeling.⁷⁶ class III HDACs (sirtuins) are required for the autophagic response to nutrient deprivation but not for autophagy triggered by downstream signals such as TP53 activation or MTOR inhibition.⁷⁷ class IIB (HDAC6) deficiency leads to failure of autophagosomal maturation in the context of basal autophagy, thus disrupting mitophagy and protein aggregate removal.⁷⁸ On the other hand, HDAC inhibition is capable of profoundly suppressing load-induced cardiomyocyte autophagy, and this autophagic response is required for much of the pathological growth response.⁷⁹⁻⁸¹

GSK3B (glycogen synthase kinase 3 β)

The serine/threonine kinase GSK3B is involved in regulating gene transcription, protein translation, apoptosis, and hexose metabolism.⁸² In the heart, inhibition of GSK3B is cardioprotective. Recent evidence demonstrates that GSK3B inhibits MTOR and activates autophagy in prolonged ischemia.⁸³ However, GSK3B inhibition protects against ischemia/reperfusion (I/R) injury through modulation of mitochondrial permeability transition pore opening.⁸³

Nicotinamide adenine dinucleotide (NAD⁺)

NAMPT (nicotinamide phosphoribosyltransferase) is a rate-limiting enzyme in the mammalian NAD⁺ salvage pathway.⁸⁴ Preventing downregulation of NAMPT protects from myocardial injury through increasing NAD⁺ and ATP levels, inhibits apoptosis and stimulates autophagic flux.⁸⁵ Moreover, increased NAD⁺ levels activate SIRT1, which induces autophagy through nuclear localization of FOXO1.⁸⁶ Also, exogenous NAD⁺ blocks phenylephrine and angiotensin II-induced cardiac hypertrophy via the SIRT3-STK11-AMPK pathway.⁸⁷ Thus, cellular control of NAD⁺ levels is an efficient means of regulating cellular homeostasis.

MicroRNAs

These molecules are small, endogenous RNA molecules that regulate their target gene expression post-transcriptionally.⁸⁸ microRNAs govern a wide array of cellular processes, including cell size, survival, electrophysiology, mitochondrial function, and energetics.⁸⁹ Recent work has demonstrated that the *MIRNA212/132* family regulates both cardiac hypertrophy and

autophagy through directly targeting of FOXO3. Ablation of *MIR212/132*, or injection of an antagomir, protects from pressure overload-induced heart failure.⁹⁰

Autophagy in Cardiovascular Medicine

The myocardium comprises long-lived, largely post-mitotic cardiomyocytes. Therefore, despite ongoing controversy regarding the regenerative capacity of adult heart, elucidation of cellular mechanisms underlying cardiomyocyte function, viability, and cellular homeostasis has a pivotal role in the design of new therapeutics in cardiovascular medicine. Autophagy is fundamental to maintain cardiomyocyte function and viability. Also, autophagy provides a critical means for intracellular self-renewal, energy repletion, and substrate recycling through degradation of dysfunctional or misfolded proteins and aged and/or damaged organelles.

Cardiac basal autophagy

Cardiomyocyte function and survival rely critically on the presence of basal levels of autophagy. On the one hand, in a model of controlled cardiomyocyte-specific ATG5 deficiency, abrogation of basal autophagy provokes precipitous declines in cardiac structure and performance.¹⁶ In this context where autophagic flux is silenced, pressure overload triggers rapid-onset cardiac hypertrophy, left ventricular dilation, and diminished cardiac output.¹⁶ Thus, constitutive autophagy controls cardiomyocyte size and function and is a protective mechanism in hemodynamic stress.¹⁶ Further, mutation of the LAMP2 protein, characteristic of Danon disease, triggers a severe and progressive cardiomyopathy, stemming from defective fusion of autophagosomes with lysosomes.⁹¹ On the other hand, the long-term consequences of ATG5-deficiency in the heart include cardiac hypertrophy and diminished cardiac output with age, resulting from accumulation of defective proteins and organelles.⁹² Together, these facts highlight the vital housekeeping role for cardiomyocyte autophagy as a mechanism of protein and organelle surveillance and quality control.

Autophagy in the stressed heart

The role of stress-activated autophagy in cardiac disorders is more complex than basal autophagy. Stress-activated autophagy is induced during fasting with effects on heart weight, consistent with activation of catabolic pathways. Moreover, 72 h of starvation diminishes myocardial ATP content without altering cardiac function; however, suppression of autophagy with baflomycin A₁ during starvation results in pronounced ATP reduction and impaired heart performance.⁹³ Similar results have been reported in glucose-deprived cultured cardiomyocytes.^{14,60} In contrast, afterload stress, a common clinical scenario leading to heart failure, induces autophagy, which is required for hypertrophic growth of the myocardium.^{16,79} Thus, these data reinforce the emerging idea of a new paradigm of mechanisms governing cell growth and protein degradation, each of which is required for cardiac plasticity.

Robust autophagy has been seen in the context of multiple stressors that induce cardiac pathology, including elevated afterload, chronic ischemia, and ischemia/reperfusion injury.⁹⁴

Autophagy functions as a double-edged sword; it can either antagonize disease pathogenesis or contribute to the progression of disease, depending on the context and amplitude of induction.¹⁷ Autophagy induction is protective when the cells are starved during ischemia.¹⁴ On the other hand, autophagy can be maladaptive in load-stressed heart¹⁵ and during reperfusion.¹⁴

Cardiomyocyte-restricted overexpression of BECN1 in an in vivo model of surgical pressure overload promotes rapid transition to cardiac failure.¹⁵ Conversely, diminishing the autophagic response by 50% in BECN1 haploinsufficient mice attenuates pathological remodeling induced by afterload stress.¹⁵ By contrast, in a study of hypertrophy regression by release of aortic constriction, FOXO1-mediated autophagy plays an important role in unloading-induced regression of cardiac hypertrophy.⁹⁵ Moreover, autophagy is adaptive in a model of proteotoxic cardiomyopathy where mutations in genes encoding DES/desmin or the CRYAB chaperone protein (crystallin, α B) lead to accumulation of protein aggregates and profound heart failure.¹⁸ Autophagic activity is enhanced and confers an adaptive function to facilitate clearance of aggregates.¹⁸

Mechanical support has emerged as an important and rapidly expanding therapy in advanced heart failure, eliciting in some patients a beneficial reverse remodeling response. As part of this, myocyte atrophy and autophagy are prominent features. Mechanistic analyses have uncovered FOXO3, a transcription factor activated in samples from preclinical models and in diseased human tissues, as a master regulator that governs both the autophagy-lysosomal and ubiquitin-proteasomal pathways to orchestrate cardiac muscle atrophy.⁹⁶

The functional effects of autophagy in I/R injury remain unclear, with some evidence suggesting protective effects and some pointing to maladaptive effects. During mild ischemic stress, activation of cardioprotective autophagy depends on AMPK-mediated inhibition of MTOR.^{14,60} Pharmacological inhibition of autophagy in ischemia-mimicking conditions (e.g., glucose and oxygen withdrawal) enhances cardiomyocyte death, suggesting prosurvival effects.⁹⁷ Ischemia, where nutrient and oxygen supply to the myocardium are limited, is a state reminiscent of starvation, a context in which autophagy is adaptive. Reperfusion, by contrast, is a very different situation, and the associated autophagic response can be adaptive or detrimental and involves BECN1, independent of the AMPK-MTOR pathway.¹⁴ In HL-1 cells, I/R impairs autophagic flux at the level of both induction and degradation, and enhancing autophagy constitutes a powerful protective mechanism against I/R injury in that cell line.⁹⁸ On the one hand, short, repetitive ischemic episodes, which elicit beneficial ischemic preconditioning effects, depend on autophagy induction, and when this response is suppressed, the protective effects of preconditioning are lost.^{99,100} On the other hand, chronic ischemia provokes autophagic activation, which inhibits apoptosis and mitigates deleterious effects of ischemia.⁹⁹ Moreover, autophagosomes have been detected in surviving cardiomyocytes in chronic stages of myocardial infarction. Exposure to baflomycin A₁ exacerbates cardiac dysfunction and remodeling.¹⁰¹

Recently, a novel role for BECN1 and LAMP2 in IR injury was reported.¹⁰² Cardiac autophagy is upregulated in response

to I/R injury, but autophagosome clearance is impaired, which contributes to cell death. Reperfusion increases BECN1 levels, which impair autophagosome processing and reduce LAMP2 levels, which is critical for autophagosome-lysosome fusion. Partial BECN1 knockdown restores autophagy processing and protects from I/R injury-induced cell death, whereas a more complete BECN1 knockdown impairs autophagosome formation and increases cell death.¹⁰² Thus, BECN1 abundance can determine the final fate of autophagy, ensuring cellular homeostasis and survival or triggering cell death, which is consistent with its role as a tumor suppressor.¹⁰³ Whereas, it is clear that partial reduction of BECN1 levels protects the heart in TAC (thoracic aortic constriction)-induced hypertrophy¹⁵ or I/R injury,^{14,102} but the actions of BECN1 at both early and late points of the autophagic cascade complicate the interpretation of these studies.

Chemotherapeutic drugs, such as doxorubicin, can induce cardiomyopathy.¹⁰⁴ However, the precise mechanism involved is unknown. Cardiomyocyte death by apoptosis and necrosis may participate in doxorubicin-induced cardiomyopathy. An early study showed that pharmacological suppression of autophagy by 3-methyladenine is associated with a significant rescue of myocardial function.¹⁰⁵ Moreover, overexpression of GATA4 inhibits doxorubicin-induced cardiotoxicity through the inhibition of autophagy by modulating the expression of BCL2 and autophagy-related genes.¹⁰⁶ In addition, conflicting reports have emerged on the role of autophagy in the cardiotoxicity of doxorubicin. Caloric restriction and resveratrol, both known to induce autophagy, prevent doxorubicin-induced cardiomyocyte death in cultured neonatal cardiomyocytes.^{107,108} By contrast, starvation mitigates acute doxorubicin cardiotoxicity in mice through an increase in myocardial autophagy via an AMPK-dependent mechanism.¹⁰⁹ These conflicting results could be related to differences in study design or the stimuli used, and more research is required to assess the role of autophagy in doxorubicin-induced cardiomyopathy.

Autophagy in atherosclerosis

Several studies have reported potential triggers of autophagy present in atherosclerotic plaques, including reactive oxygen species production,¹¹⁰ oxidized lipoproteins,¹¹¹⁻¹¹⁴ ER stress,¹¹⁵ and hypoxia.¹¹⁶ Despite the protective actions of autophagy in several human pathologies, the role of autophagy in atherosclerosis remains poorly understood. Under basal conditions, autophagy protects plaques against oxidative stress through degradation of intracellular components, principally depolarized mitochondria.¹¹⁷ In addition, the autophagy inducer 7-ketosterol attenuates cell death induced by statins in vascular smooth muscle cells (VSMCs).¹¹² Moreover, autophagy activation in VSMCs by free cholesterol or 4-hydroxycholesterol plays a protective role.^{111,118} Comparable to VSMCs, autophagy induction by oxidized lipoproteins or advanced glycation end products is protective against endothelial cell injury.^{119,120} Recently, it was shown that macrophages harboring a specific deletion of *Atg5* in a mouse model of low density lipoprotein receptor knockout promote plaque necrosis, with increased oxidative stress.¹²¹ In concordance, basal autophagy becomes dysfunctional in advanced stages of atherosclerosis, which stimulates hyperactivation of

inflammasomes to promote atherogenesis.¹²² Altogether, these reports support the beneficial role of basal autophagy in atherosclerosis and the potential merit of a drug promoting autophagy for plaque stabilization.¹²³

Basal autophagy can be stimulated by several drugs.¹²⁴ Rapamycin is the most widely employed inducer of autophagy, but it has poor solubility in aqueous solution. In response, analogs have been developed, including everolimus, temserolimus, deforolimus, and zotarolimus.¹²⁴ Everolimus has pleiotropic anti-atherosclerotic properties, including inhibition of VSMC migration and proliferation,¹²⁵ suppression of monocyte chemotaxis,¹²⁶ and diminution of lipid accumulation in both macrophages and VSMCs.^{114,127,128} Moreover, local stent-based delivery of everolimus leads to reduction in macrophage abundance within atherosclerotic plaques from cholesterol-fed rabbits.^{129,130} Depletion of macrophages by everolimus has been related to autophagic cell death, in part through the inhibition of protein synthesis and cell proliferation.¹²⁴

Thus, everolimus inhibits several basic mechanisms that control plaque growth. Indeed, everolimus induces several indicators of autophagy, including degradation of long-lived proteins, LC3 processing, and cytoplasmic vacuolization.^{129,130} By contrast, ligands of TLR7, such as imiquimod, induce autophagy through MYD88, which acts as an adaptor protein that targets BECN1.^{131,132} The presence of TLR7 has been reported in macrophages but not in VSMC, which likely explains why imiquimod induces autophagy only in macrophages.¹³³ However, local delivery of imiquimod in atherosclerotic plaques of cholesterol-fed rabbits does not deplete macrophages.¹³³ In addition, imiquimod induces VCAM1 in endothelial cells and promotes infiltration of T-lymphocytes, contributing to increases in plaque area.¹³³ The effects of imiquimod on inflammation and plaque progression are dependent on NFkB activation and cytokine production, but are not related to autophagy.¹³³ The contradictory effects of everolimus and imiquimod highlight our incomplete understanding of how different compounds promote induction of MTOR-dependent or -independent autophagy. Stimulation of MTOR-dependent autophagy in macrophages (via everolimus or analogs) leads to inhibition of protein synthesis and triggers autophagy-mediated cell death, macrophage depletion, and formation of a stable plaque phenotype. On the other hand, induction of the MTOR-independent type of autophagy in macrophages via imiquimod seems detrimental, because it is associated with inflammation and plaque progression.¹²⁴

Cardiac and Vascular Autophagy as a Therapeutic Target

Presently, 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, uncontrolled levels can contribute to pathogenesis. A critical focus will be to distinguish mechanisms of adaptive and maladaptive autophagy to manipulate those pathways for

therapeutic gain. In addition, Sciarretta et al.¹³⁴ have proposed several criteria that should be considered:

- Activators or inhibitors of autophagy should be chosen based on the pathological context.
- The extent of autophagy modulation should be taken into account.
- Autophagy is activated through different signaling mechanisms in different cardiac diseases.
- Pharmacological modulators of autophagy may exert autophagy-independent functions, which should be considered.

Besides these factors, the role of autophagy in cardiac stress *in vivo* remains incompletely characterized. Particularly, the contribution of autophagy in afterload stress, a setting where both anabolic and catabolic processes are activated, is complex. 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 overactivation 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.¹³⁵

As autophagy is critical to protein and organelle quality control, beneficial effects from its inhibition are at first glance counterintuitive. However, as discussed above, emerging evidence points to a requirement that autophagic activation remain fixed within a zone where its adaptive effects can take place; when autophagic flux is activated to excessive levels, or when it drops below a certain threshold, pathological effects emerge. Also, one might speculate that suppressing overactive autophagy in the setting of cardiomyocyte remodeling would lead to accumulation of cellular debris (e.g., protein aggregates, dysfunctional organelles) that escape the now-suppressed autophagic response.

New Perspectives in Cardiovascular Autophagy Research

Autophagic “self-eating” is a critical prosurvival response in cardiomyocytes exposed to diverse forms of environmental insult; in the setting of heart disease, involvement of autophagosomes/lysosomes mechanisms is long-established. A great deal of data from preclinical models demonstrates that excessive autophagy elicited by pathological stimuli, such as pressure overload or I/R, is maladaptive and promotes cell death. Conversely, basal levels of constitutive autophagy are essential to maintain proteostasis, and elimination of this means of protein quality control triggers rapid cell death. In other words, understanding of the context-dependent role of autophagic flux in disease promotion

and disease antagonism is emerging. These insights follow precedents in oncology, where a similar requirement of finely tuned autophagic activation exists.

Our vision for the future includes elucidation of the autophagic circuitry in the heart such that precise tuning of its actions can be accomplished for therapeutic gain. 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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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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