Chapter

The Importance of Autophagy and Proteostasis in Metabolic Cardiomyopathy

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Abstract

Metabolic cardiomyopathy and other heart disorders are associated with proteostasis derailment and subsequent autophagy. Proteostasis is a process of protein homeostasis, and autophagy is a mechanism of self-degradation for surviving cells facing stressful conditions. Metabolic challenges have been linked to excess reactive oxygen species. Cardiomyocyte proteotoxicity, an important underlying pathologic mechanism in cardiac disease, is characterized by chronic accumulation of misfolded or unfolded proteins that can lead to proteotoxic formation or aggregation of soluble peptides. Autophagic processes are mediated by the ubiquitin-proteasome and autophagy-lysosome systems, fundamental for cardiac adaptation to physiological and pathological stress. Cellular proteostasis alterations in cardiomyopathy are represented by myocardial remodeling and interstitial fibrosis with reduced diastolic function and arrhythmias. Autophagy regulation may be a potential therapeutic strategy for metabolic cardiomyopathy necessary for the treatment of fibrosis and cardiac tissue remodeling alterations. Furthermore, autophagy has been shown to be active in the perimeter of cardiovascular fibrotic tissue as mechanism of fibrosis recovery and scarring secondary to cell apoptosis. In the present work, we review the current knowledge on the role of autophagy and proteostasis in the pathogenesis of heart failure to resolve the ever-expanding epidemic of metabolic cardiomyopathy and heart failure associated with substantial morbidity and mortality.

Keywords: autophagy, proteostasis, cardiac hypertrophy, metabolic cardiomyopathy, myocardial interstitial fibrosis, heart failure

1. Introduction

Metabolic cardiomyopathies can be caused by disturbances in metabolism and may develop in the context of a broad spectrum of pathological conditions. These disorders include a number of inherited metabolic diseases in early childhood affecting the heart and other organs. Cardiomyopathies are associated with systemic

metabolic diseases acquired during adulthood, such as metabolic syndrome, dyslipidemia, obesity, hypertension, diabetes mellitus and cardiomyopathy by alcoholism [1], which are also considered important causes of cardiovascular diseases. Furthermore, abnormal mitochondrial function related to mitochondrial ATP-producing capacity and high cardiac energy demand is linked to several of these cardiovascular diseases. The heart is a high-energy-demanding organ and mitochondria are important organelles that provide its source of cellular energy by oxidative phosphorylation; however, enzyme deficiency related to mitochondrial beta-oxidation leads to cardiac disorders. Another key point is that autophagic activity has been found to decrease with age resulting in intracellular protein aggregate accumulation, unfolded protein response activation and subsequent cardiomyocyte apoptosis, likely contributing to the accumulation of damaged macromolecules and organelles during aging. Equally important, several forms of heart failure are progressive disorders associated with substantial morbidity and mortality, and of these, cardiovascular pathologies are the leading cause of death in the elderly. Autophagy, a lysosome-mediated degradation pathway, plays a critical role in proteostasis by removing potentially toxic cytosolic protein aggregates and damaged organelles within cells [2]. Cardiomyocyte proteostasis is the gradual derailment of cellular protein homeostasis important to protein quality control [3]. The dysfunction in proteostasis leading to the accumulation of protein aggregates is the hallmark of cardiovascular disease and many chronic and age-related diseases [4].

The metabolic syndrome has become one of the most important topics in recent decades because of the marked increase in cardiovascular risk associated with the clustering of risk factors [5]. Obesity is a major independent risk factor for cardiovascular disease, including cardiac hypertrophy and heart failure. Leptin, an adipocyte-derived hormone, acts through its receptors (LepRs) on hypothalamic neurons that regulate body weight and energy homeostasis. LepRs are also expressed on cardiovascular cells, and leptin has also been shown to promote cardiomyocyte hypertrophy, endothelial proliferation, migration and angiogenesis, and fibrosis.

The effects of the mechanistic target of rapamycin (mTOR) are mediated through its activity as a central inhibitor of autophagy, a highly conserved cell survival mechanism. Cardiac hypertrophy is associated with increased energy demands, and cellular stressors like ischemia or nutrient deprivation, which result in the rapid regulation of myocardial autophagy. In this context, endothelial cells are particularly sensitive to metabolic stress, and defective or maladaptive endothelial autophagy may contribute to the rarefaction of the cardiac microvasculature during hypertrophy, a critical event in the transition toward heart failure [6]. In the present work, we review the current understanding of the role of autophagy and proteostasis in the pathogenesis of heart disease, considering the essential involvement of both degradation processes to find a novel therapeutic target to resolve the ever-expanding epidemic of metabolic cardiomyopathy and heart failure associated with significant morbidity and mortality.

2. Proteostasis

A complex proteostasis network functions to ensure the maintenance of proteostasis, consisting of molecular chaperones and proteolytic machineries and their regulators in healthy cells. Each type of these molecules with a precise amino acid sequence has important physical properties to determine specific protein structure and a three-dimensional conformation to proteins, which is important in order to regulate cellular performance and balance. Protein structures are made by

the formation of peptide bonds that build the polypeptide long chains of alpha-amino acids, a common property of all proteins. Disturbed proteostasis in postmitotic cell types, such as cardiomyocytes and neurons, produces an accumulation of misfolded and aggregated proteins resulting in disease. These factors coordinate protein synthesis with polypeptide folding for the conservation of protein conformation and protein degradation. In particular, maintaining proteome balance is a challenging task against external and endogenous stresses that accumulate during chronic cardiovascular disease and aging, which lead to the decline of the proteostasis network capacity and proteome integrity [4].

2.1 Balance and integrity of the proteome

The protein flux of the cell must remain in balance to ensure proper cell and tissue function. The protein homeostasis, also known as proteostasis, leads to the accumulation of protein aggregates and it is the cause of several diseases. In view of this, protein aggregation is a common characteristic of many chronic diseases. Proteome balance is a task in defiance of external and endogenous stresses that accumulate in a lifetime, such as chronic cardiovascular diseases and aging. Moreover, regulated proteolysis mediated by proteases of damaged proteins is fundamental for protein quality control of eukaryotic cells that require the ubiquitin-proteasome system (UPS). The UPS activity can be executed by ubiquitin-protein ligases or chaperones and the first crucial step is recognition of a specific degradation signal (degron). Degrons are portions of a protein that when exposed create a signal that is recognized by target proteins to the UPS pathway [7].

From this perspective, after several steps of substrate polyubiquitylation followed by substrate unfolding and degradation, proteins with specific degrons are recognized by the proteasome and targeted for degradation.

The cellular proteome is exceedingly complex and large-scale proteomic studies have identified thousands of modification sites (common modifications include phosphorylation, ubiquitylation, methylation and acetylation) in roughly 50% of proteins in humans, the combinatorial nature of which is mostly unknown [8]. Individual proteins often exist in several modified forms and they also engage in numerous dynamically regulated protein complexes during their life cycle. It is estimated that about 100,000 distinct protein isoforms can be generated through alternative splicing from all the pool of protein-coding genes. Nonetheless, the mechanisms that underlie the dynamics, interactions, stoichiometry and turnover of most individual protein species are poorly understood at the global level [8].

2.2 Proteostasis and its network

In the cell, the proteome is a wide surveillance and regulatory network of the biogenesis process and protein degradation, which intervenes when these processes develop in a suboptimal way [8]. Proteome imbalance often results in complex and chronic diseases; therefore, it is a continuous process in order to meet the dynamic of proteomic needs of the cell [8]. In healthy cells, a complex proteostasis network (PN), comprised of molecular chaperones and proteolytic machineries and their regulators, operates to ensure the maintenance of proteostasis. These factors coordinate protein synthesis with polypeptide folding, the conservation of protein conformation and protein degradation [4]. The PN is performed by mechanisms controlling protein biosynthesis, cotranslational folding process, trafficking, neofunctionalization and degradation of proteins in vivo, among others, to maintain proteome balance and conform to the PN [9].

The proteome must have the ability to generate adequate synthesis, folding and protein expression and at the same time to detect abnormalities during this process by identifying the characteristics that force protective degradation when a component lacks quality. Human cells have more than 10^3 proteins per cell, and 5% of these are involved only in protein synthesis and turnover, and 60–80% of the etiologies of some diseases are associated with misfolding proteins. Therefore, it is clear that the constantly dynamic and complex eukaryote proteome requires a tightly regulated process [10].

The description of cellular proteomes requires an understanding not only of how proteins and their multimeric assemblies are built and their mechanisms established but also of the rules that determine how proteins are selected for degradation when they are unable to assemble properly with components of cognate networks. The network is constantly regulating the proteome, but it responds to conditions of proteotoxic stress by addressing the triage decision of fold, hold or degrade [11]. Consequently, the PN is constantly regulating the proteome and influences several cellular functions by affecting their physiology and readapting through transcriptional and translational changes within the biology of the cell [10, 11].

Numerous biological pathways affecting protein synthesis, folding, misfolding, trafficking, disaggregation and degradation may adapt the PN by using proteostasis regulators that can partially correct protein impairment, resulting in human diseases by cell stress and aging. The main PN components include several modules like protein synthesis machinery and the major mammalian protein degradation: UPS that is central to the unfolding protein response (UPR), which is activated when unfolded or misassembled proteins accumulate in the endoplasmic reticulum (ER), and the armada of intra- and extracellular chaperones including proteases, which detoxify cells from nonrepairable proteins [10, 11].

The structure of a determined protein is crucial for its function; hence, molecular chaperones are important components of the PN. Chaperones and other proteins like oxidoreductases and glycosylating enzymes bind nascent proteins and assist in proper folding into the correct structure and cellular location throughout their life cycle [12].

Diverse agents modify the structure of proteins like aging, oxidative, and thermal stress or misfolding-prone mutations. In this context, misfolded, damaged, unnecessary or aggregated proteins should be degraded, or their interactions could cause cell instability. There are two major intracellular proteolysis pathways: the autophagy-lysosomal pathway and UPS [13]. The difference between these two processes is the nature of the targeted protein degradation: in the case of autophagy, it mainly acts in the cytoplasm, and for UPS, considered the main route of protein degradation in mammalian cells, it acts on both cytoplasm and nucleus [14].

A deficient PN allows the disruption of cellular membranes by damaged proteins or toxic aggregates, which interfere with cell function, and as a result, many metabolic, oncological, cardiovascular and neurodegenerative disorders could appear in the individual [15].

The UPS is a complex machine formed by numerous subunits that degrade ubiquitin-attached proteins. This proteolysis pathway is critical for the quality control of proteins by eliminating damaged proteins and also maintaining the concentration of many regulatory proteins of apoptosis, inflammation, signal transduction and cell cycle [12]. The other proteolysis pathway, autophagy that is in charge of degraded proteins, is not detected by the UPS, and it has an important role in the immune response and starvation stage [16]. Autophagy eliminates several dysfunctional cell components or catabolizes them when the cell is under starvation and stress to maintain optimal levels of energy and nutrients [12]. ROS, DNA damage or starvation activates this autoproteolysis pathway engulfing organelles in

the autophagosome that are later fused with lysosomes, and by doing so, the amino acids and fatty acids produced by the catabolism of the organelles are recycled in the cytoplasm. However, three ways of delivering target proteins to the lysosome have been identified, and based on this, autophagy is classified into three distinct types: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy [16].

In microautophagy, the cellular contents are invaginated directly by the lysosome. The major cytosolic chaperone systems are HSP70 and HSP90, which are connected to the UPS pathway. The proteasome complex contains the proteolytic active sites in the core particle (20S) and the regulatory activity of the holo-complex in the regulatory particle (19S). The UPS pathway only recognizes polyubiquitination proteins, a process that requires three enzymes: E1 ubiquitin activator, E2 conjugase and E3 ligase, which act sequentially. The polyubiquitylated proteins are recognized by the core particle for their degradation by the regulatory particle (19S) [17]. Meanwhile, CMA uses the molecular chaperone, known as heat shock cognate 71 kDa protein (Hsc70), for recognition of the KFERQ sequence motif in cytosolic proteins that must be degraded, and drives them to the lysosome membrane [18]. The transmembrane receptor or docking protein is a lysosomal-associated membrane protein-2A (LAMP-2A) that transports the unfolded cytosolic proteins into the lysosome [18].

Macroautophagy involves the formation of the autophagosomes, defined as special structures that invaginate cellular contents or target proteins and then transport them to the lysosome. Besides eliminating pathogens, autophagy is also required for antigen presentation by the major histocompatibility complex (MHC) class II. The major autophagy pathway used by cells is the MHC class II [16].

2.3 Cardiomyopathy, cellular proteostasis alterations and myocardial remodeling with interstitial fibrosis

Diabetic cardiomyopathy (DC) is a specific heart muscle disease that increases the risk of heart failure and mortality in diabetic patients independent of vascular pathology. Basal level autophagy plays a housekeeping role to maintain cellular homeostasis. However, autophagy mechanisms are impaired in diabetic hearts. In this sense, diminished autophagy limits cardiac injury in type 1 diabetes and inhibited autophagy contributes to cardiac injury in type 2 diabetes. In this context, protein homeostasis is a necessity for the correct function of the cell, in other words, an interaction between protein synthesis, transport, post-translational modification and degradation [17]. However, an accumulation of defective proteins results in proteotoxicity or disturbed proteostasis. Progression of cardiovascular diseases due to proteostasis alterations has been related with interstitial fibrosis and altered myocardial remodeling. Recent evidence indicates that the progression of ventricular dysfunction may be associated with changes in the process of autophagy and impaired proteostasis.

Autophagy in the mitochondria is a necessary process for maintaining a healthy mitochondrial network, also known as mitophagy. Under pathological conditions, mitochondrial dysfunction and enhanced ROS generation associated to cardiac hypertrophy and impaired left ventricular function with increased aggregation of abnormal proteins and enlarged or collapsed mitochondria can be found, such as structural and functional remodeling with changes in composition of the extracellular matrix, which are characterized by fibrotic tissue, impaired vascular and coronary microvascular function or effects on subcellular cardiomyocyte composition (**Figure 1**). Thus, mitophagy has been shown to be essential for myocardial protection [19]. In addition, calorie restriction is sufficient to accelerate cardiac

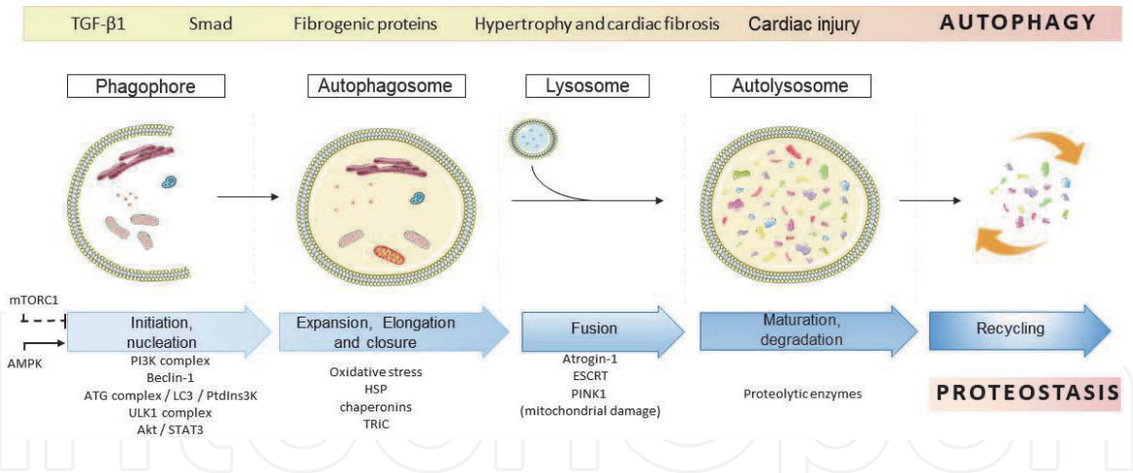

Figure 1.
Factors involved in autophagy and proteostasis in metabolic cardiomyopathy. Many proteins participate in the activation and formation of the autophagosome. TGF-β1 induces the activation of signaling pathways such as Smad, which in turn activates the formation of fibrogenic proteins such as type 1 collagen and fibronectin, and these induce hypertrophy and cardiac fibrosis generating cardiac damage and activating autophagy in cardiomyocytes. Modified of Kobayashi et al. [19].

autophagic flux and reduce mitochondrial oxidative damage in the heart, results that suggest the important role of autophagy for maintaining optimal mitochondrial structure and function [20].

Proteostasis and autophagy are related to various heart diseases; however, both mechanisms can be beneficial or harmful depending on age and pathology. From this standpoint, heart diseases linked to autophagy due to degradation of contractile heart proteins are associated with cardiac aging, inherited cardiomyopathy, diabetic cardiomyopathy (DC), atherosclerosis, heart failure (HF) and atrial fibrillation (AF) [20]. The quality of cardiomyocytes depends on the efficient elimination of damaged proteins by autophagy. The mechanism performed by chaperone proteins, particularly heat shock proteins (HSP70/HSP40/HSP110) and chaperonins like the T-complex protein 1 ring complex (TRiC), takes place to a greater extent in the heart in response to oxidative stress [21]. HSPs are found in specific protein regions to prevent aggregation; these HSPs regulate oxidative stress (OS) and metabolism and maintain proper cell proliferation. The imbalance in the degradation of damaged intracellular proteins induces aging of the heart muscle fibers as a result of OS, the deterioration of the Ca^{+2} transits and the excessive generation of ROS. This process affects remodeling, favoring hypertrophy and cardiac fibrosis [22].

In cardiomyopathies, the accumulation of incorrectly folded proteins or acquired dysfunction of protein quality control has been implicated in impaired proteostasis. The cellular function in the myocardium follows the regulation of proteostasis and autophagy in order to control the quality of new synthesized proteins and removal of unfolded/misfolded proteins. When UPS targets are too large to be degraded by the proteasome, the autophagy system must control degradation through the selection between UPS and autophagy. Among autophagy regulators, the endosomal sorting complex required for transport protein complexes (ESCRT) affects the lysosome-autophagosome fusion. Part of ESCRT is the charged multivesicular body protein 2B (CHMP2B), which is required for autophagy. The work of Zaglia et al., in 2014, identified a novel link between UPS and autophagy and showed that the muscle-specific ubiquitin ligase atrogin-1 controls turnover of the ESCRT-III family protein CHMP2B, which controls the autophagy signaling pathways [23].

Transforming growth factor β1 (TGF-β1) is an important regulator of fibrogenesis. Its expression is regulated by biochemical stimuli, as a humoral response to infections, glucose and pH [24]. Binding of TGF-β1 to specific cellular

receptors, such as TGF-β type II and RII, activates phosphorylation for intracellular signaling pathways, such as Smad2 and Smad3 [25], which induce the expression of fibrogenic proteins like type I collagen and fibronectin [26]. These pathways trigger an inappropriate deposition of collagen in cardiac fibers, causing impaired heart function [27]. However, in other cell lineages, TGF-β1 is also capable of inducing autophagy, so the regulatory mechanisms between the two events are unknown [26].

Many target molecules are involved in fibrosis including the multiprotein complex formed by phosphoinositide 3-kinase class III (PI3K) dependent on Beclin 1, which regulates vesicular autophagy by activation of signaling pathways, such as Akt, and, in turn, increases the expression of TGF-β for the development of fibrosis [28]. HSP25 and alpha B-crystallin are expressed to a lesser extent in the heart; however, they fulfill the function of chaperone proteins that favor stability between actin and desmin, thus avoiding cardiotoxicity [29]. PINK1 (PTEN-induced putative kinase 1) is another protein involved in autophagy. It is located in the outer membrane of defective mitochondria, and it favors autophagy through the recruitment of the Parkin protein to depolarized mitochondria of cardiomyocytes [30].

These mechanisms can induce deregulation of autophagy by apoptosis in type II cells in cardiac tissue, which leads to the development of myocardial infarction (MI) [31]. Moreover, autophagy has been shown to be active in the perimeter of cardiovascular fibrotic tissue as a mechanism for fibrosis recovery and scarring secondary to cell apoptosis [32]. Many molecules protect against type 1 diabetes–induced cardiac dysfunction by activating autophagy. Lastly, the inhibition of autophagy has a beneficial effect on type 2 diabetes–induced cardiomyopathy [33].

2.4 Mitophagy and redox dysregulation

Cardiomyopathies as a result of excessive ROS production and protein modifications in the mitochondria involve abnormal mitochondrial function resulting in cardiac disorders due to the high energy demand of the heart through this organelle, considered as a source of cellular energy production and mitochondrial ATP production achieved by oxidative phosphorylation and beta-oxidation. As a result of mitochondrial damage, the process of autophagy, known as mitophagy, is essential for myocardial function and protection [19].

The physiological performance of endothelial nitric oxide synthase enzyme (eNOS) is important for NO production, which is dependent on L-arginine through its reaction with O_2 and the constitutive eNOS dependent on Ca^{2+}/calmodulin, as well as the cofactors (6R-)5,6,7,8-tetrahydrobiopterin (BH_4), nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). Nitric oxide (NO) produced by the endothelium from eNOS, which is oxidized to L-citrulline and NO, works through the transference of electrons from NADPH via FAD and FMN. Both eNOS constitutive activation events are dependent, and in caveolae, they are Ca^{2+}/calmodulin concentration dependent [34].

Under pathological situations and in the presence of uncoupled eNOS, increased OS is produced, instead of producing NO after eNOS activation due to the reaction with reduced BH_4 levels and upregulated NADPH. As a result of these cardiovascular (CV) risk factors, NO is not produced, but there is ROS production. These abnormal reactions due to CV risk factors reduce bioactive NO [34].

The biological abnormalities produced by excessive ROS production such as superoxide anion (—O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (—OH) species [35], including the rapid interaction of O_2^- with NO, result in the loss of NO bioavailability and increased production of peroxynitrite ($ONOO^-$) [34].

The harmful overproduction of these ROS and protein mitochondrial modifications as a result of impaired redox and pathological signaling in the CV system mediate regulation of the most important ion channels, transporters and kinases related to heart diseases.

These mechanisms lead to selective cardiac dysfunction and decreased energy production due to reductions in mitochondrial respiration, increased OS and defective contractile Ca^{2+} regulatory proteins. These types of changes and alterations in mitochondrial biogenesis, content and function related to an heterogeneous group of cardiovascular disease risk factors like metabolic syndrome, have been documented. Damaged mitochondria are degraded through mitophagy, the main protective function of autophagy that is for myocardial protection and the target of successful drug development emerging in the cardiovascular space. These strategies may be applied upon several redox targets, such as the membrane caveolae region where key cardiovascular redox proteins, such as eNOS, calmodulin and NADPH oxidase, among other important cardiovascular-related receptors, are located. Thus, calorie restriction is sufficient to accelerate cardiac autophagic flux to help improve mitochondrial oxidative damage and to maintain a healthy mitochondrial network [11, 18, 19].

3. Autophagy

Dr. Christian de Duve was the first to use the term "autophagy," meaning "self-eating" in Greek, at the Ciba Foundation Symposium on Lysosomes, which took place in London on February 12–14, 1963 [36]. When there is a functional decline in the cardiovascular system and aging, cardiomyocytes need a cellular control mechanism to minimize damage and prevent cardiac malfunction. In this context, autophagy may degrade and recycle long-lived proteins, cytoplasmic components and organelles [37]. The notion of autophagy as cell death is a phenomenon that has been controversial and remains mechanistically undefined. It should be noted that when autophagy promotes cell death, there is an association of autophagy with the different cell death pathways [38].

The biogenesis of an autophagosome is orchestrated by the so-called autophagy-related (ATG) proteins, which act in a hierarchical order to first generate the phagophore and then expand it into an autophagosome. The mammalian homologs of ATG1 are the uncoordinated-51-like kinases 1 and 2 (ULKA1 and ULK2) ULK complex, the ATG9A cycling system and the autophagy-specific class III phosphatidylinositol 3-kinase (PtdIns3K) complex, which are key in generating the phagophore upon induction of autophagy [39]. Besides, knockdown of EP300 and the inhibition of histone acetylases potentially induce autophagy indicating that protein deacetylation may play a role in the autophagic cascade. EP300 acetylates several autophagy-related proteins, including autophagy-related 5 (ATG5), ATG7, ATG12 and microtubule-associated protein 1 light chain 3 β (LC3). Lastly, protein deacetylation influenced by several proteins controls autophagy at diverse levels from the modification of autophagy core proteins to transcriptional factors controlling autophagic genes [39].

3.1 Autophagy basics

Autophagy is also considered an evolutionarily conserved process critical for cellular homeostasis [3]. Implication of either the pathogenesis or the response to a wide variety of diseases by autophagy has been related to the pathogenesis of various disease states and to the basic molecular pathways that regulate autophagy [40].

Basal levels of autophagy maintain cellular homeostasis, and under stress conditions, high levels of autophagy are induced. However, the pro-death role of autophagy is complicated due to the extensive cross-talk between different signaling pathways [38]. Autophagy is a process by which cytoplasmic components are sequestered in double membrane vesicles and degraded upon fusion with lysosomal compartments. Depending on the stimulus, autophagy can degrade cytoplasmic contents nonspecifically or it can target the degradation of specific cellular components. Higher eukaryotes have adopted both of these mechanisms and account for the expanding role of autophagy in various cellular processes, as well as they contribute to the variation in cellular outcomes after induction of autophagy. As the basic molecular pathways that regulate autophagy are elucidated, the relationship of autophagy to the pathogenesis of various disease states becomes apparent [40].

Autophagy is a highly conserved eukaryotic pathway responsible for the lysosomal degradation (and subsequent recycling) that is rapidly growing and elucidating an intriguing mechanistic complexity as well as a tremendous range of cargo substrates. Imbalances in proteostasis are connected to aging and multiple (age-associated) disorders [41]. Several pathologies including cardiovascular disease and stress-related disorders are associated with autophagy dysregulation. Moreover, excessive or insufficient levels of autophagic flux have been characterized in cardiomyocytes, cardiac fibroblasts, endothelial cells and vascular smooth muscle cells within the cardiovascular system [42]. Damaged and potentially cytotoxic mitochondria elicit an autophagic response termed mitophagy. Depending on the initiating stimulus, the substrate selection could differ. Thus, mitophagy takes part in physiological processes like the removal of paternal mitochondria during egg fertilization, and it is also a key process for the removal of damaged mitochondria in toxic conditions [43]. Furthermore, autophagy stimulation may result in reduced accumulation of misfolded and aggregated proteins; however, the overactivation of autophagy can trigger autophagy-mediated apoptosis.

3.2 Autophagy and heart diseases

The cardiovascular system has the ability to adapt to a wide range of environmental stresses. The myocardium itself manifests robust plasticity for both physiological and pathological stimuli. From this perspective, autophagy is an intracellular process required to maintain cardiovascular homeostasis, and it is also an evolutionarily ancient process of intracellular catabolism in response to a wide variety of stresses. In the case of postmitotic cells, where cell replacement is not an option, finely tuned quality control of cytoplasmic constituents and organelles is especially critical [41]. Mitochondrial DNA has an important role at inducing and maintaining inflammation in the heart that escapes from autophagy. These autophagic mechanisms degrade damaged mitochondria through fusion of autophagosomes and lysosomes. Lastly, the impairment of mitochondrial cristae affecting cardiac morphology and function is induced by pressure overload [44].

3.3 Regulation of autophagy in the heart

Excessive caloric intake results in obesity, a major independent risk factor for cardiovascular disease, including cardiac hypertrophy and heart failure. From this standpoint, cardiac remodeling is modulated by overnutrition or starvation. The adipokine leptin mediates energy balance between adipose tissue and the brain. Leptin and its receptors (LepRs) are expressed in the heart. LepRs belong to the class I cytokine receptor family signaling via JAK (Janus kinase)-2 and signal transducer and activator of transcription (STAT)-3. In addition, nutrient signaling

mediators, such as mTOR (mammalian target of rapamycin), induce LepR-mediated activation of Akt. Cellular hypertrophy, proliferation and survival play an important role in cardiovascular function and pathology mediated by the Akt/mTOR pathway [45]. To examine the importance of endothelial leptin signaling in cardiac hypertrophy, transverse aortic constriction was used in mice with inducible endothelium-specific deletion of leptin receptors (End.LepR-KO) or littermate controls (End.LepR-WT). Histology and quantitative polymerase chain reaction analysis confirmed reduced cardiomyocyte hypertrophy. STAT3 activation was reduced, and Akt (protein kinase B) and mTOR phosphorylation after transverse aortic constriction were blunted in End.LepR-KO mice hearts [46].

For normal cardiac physiology in response to pressure overload (PO), mTORC2 is also required to ensure cardiomyocyte survival. It has been observed that dysregulation of autophagy in cardiomyocytes is implicated in various heart disease conditions. In these cases, vigorous protein quality control (PQC) systems are essential for maintaining the long-term well-being of nonproliferating mammalian cells, such as neurons and cardiomyocytes (CMs) [47]. Similarly, PO activates autophagy in at least an acute phase and the suppression of PO-induced autophagy that alleviates pathological cardiac remodeling. Recent investigations revealed that enhancing autophagy ameliorates desmin-related cardiomyopathies, which are inherited cardiomyopathies that result in severe heart failure due to protein aggregation and myofibrillar disarray in CMs [47].

3.4 New therapeutic objective of metabolic cardiomyopathy in autophagy

Perturbations in autophagy are involved in virtually all stages of cardiovascular disease. Research in the last decade has revealed that autophagy in cardiomyocytes plays a protective role, but not only during hemodynamic stress, but also in homeostasis during aging, resulting in mitochondrial damage. These damaged mitochondria are degraded through mitophagy and this process could be the main protective function of autophagy in the heart. From this standpoint, the mTORC1 complex regulates numerous biological processes, including proliferation, protein synthesis and autophagy inhibition. In addition, the mTORC1 pathway inhibits phosphorylation of the ULK1 protein (Ser 757) [48] considered an important element of autophagy activation.

The effects of mTOR are mediated through its activity as a central inhibitor of autophagy, a highly conserved cellular survival mechanism by which nutrient-deprived cells refresh the bioavailability of metabolic precursors [6]. In the cardiovascular system, the mTOR pathway regulates the physiological and pathological processes in the heart. In this regard, mTORC2 is necessary to maintain normal cardiac physiology and it ensures the survival of cardiomyocytes that have been subjected to PO. However, partial genetic or pharmacological inhibition of mTORC1 has been shown to reduce cardiac remodeling and heart failure in response to PO and chronic myocardial infarction. Therefore, mTOR may be a therapeutic strategy to confer cardioprotection [45].

Nonetheless, depending on the context, autophagic flux may be biased up or down. A large body of preclinical evidence suggests that autophagy is a double-edged sword in cardiovascular disease, acting in either beneficial or maladaptive ways, depending on the context. Modulation of Beclin 1 significantly influences both autophagy and apoptosis, thereby deeply affecting the survival and death of cardiomyocytes in the heart. This is the reason why it is important to discuss the signaling mechanism of autophagy modulation through Beclin 1, including the therapeutic potential of Beclin 1 in heart diseases [49]. In light of this, the autophagic machinery in cardiomyocytes and other cardiovascular cell types has

been proposed as potential therapeutic targets. Autophagy mediators hold promise as targets for cardiovascular disease therapy; however, recent evidence suggesting that titration of autophagic flux holds potential as a new therapeutic goal for cardiovascular diseases, and heart failure, needs to be analyzed further [40].

3.5 Treatment for autophagy

The use of pharmacological modulators can be beneficial for the treatment and prevention of autophagy. It is known that many agents or procedures induce or reduce autophagy activity; among these are spermidine, carvedilol, trehalose, resveratrol, metformin, caloric restriction, exercise training, intermittent fasting and ischemia/reperfusion.

Fasting and calorie restriction are the most potent nongenetic autophagy stimulators related to autophagy promotion. Regarding the upregulation of autophagy, the evidence overwhelmingly suggests that autophagy has to be induced in a wide variety of tissues and organs in response to food deprivation. From a mechanistic point of view, age-related vascular remodeling is driven by a greater accumulation of ROS. Thus, the induction of autophagy per se is sufficient to extend the shelf life in various species ranging from yeast to mammals [50]. Therefore, in addition to preserving the homeostasis of organisms in baseline physiological conditions, autophagy also contributes to metabolic fitness and the adaptation to stressful conditions, such as nutrient deprivation, hypoxia, OS or physical exercise.

Autophagy is a critical process for cell homeostasis and survival, and it is also implicated in the reduction of OS and inflammation. Furthermore, autophagic processes have been associated with a greater expression of eNOS and bioavailability of the protein. Long-lived, damaged, dysfunctional and potentially harmful cellular components break down for detoxification, energy production and cell renewal, providing building components and stimulating anabolic processes for effective cell recycling.

Vascular induction of NO production as a response to shear stress during exercise with augmented blood flow and increased flux sanguin over endothelial cells (EC) result in eNOS activation and NO production. Autophagic process has been related to greater expression of eNOS and bioavailability of the protein. ATG3 is an important autophagy pathway mediator; in contrast with a reduction of 85% by knockdown of ATG3 protein expression using control siRNA upon exposure to shear stress showed impairment of eNOS activation and as a result were incapable of produce NO as a response to shear stress. Autophagy is a critical process for cell homeostasis and survival is also implicated. Long-lived, damaged, dysfunctional and potentially harmful cellular components break down for detoxification, energy production and cell renewal, providing building components and stimulating anabolic processes for effective cell recycling as a result of autophagy [51].

3.5.1 Exercise-mediated regulation of autophagy

Substantial evidence indicates that exercise training plays a beneficial role in the prevention and treatment of CV diseases. The regulation of autophagy during exercise is a bidirectional process. Autophagy is a physiologic process that is a defense mechanism for cells in adverse environments and it is also involved in several pathological processes [52]. Autophagy normal levels confer cell protection versus environmental stimuli to balance and protect organisms [53]. In this context, various diseases are the response to excessive or insufficient autophagy. Exercise training, referring generally to the cardiac adaptation to exercise, which has to be in an appropriate intensity as a chronic stimulation process, can reduce the risk of CV

diseases and improve the prognosis of patients after CV events. This type of training can also reduce the production of ROS, reduce the inflammatory response, regulate collagen metabolism, moderate the imbalance of extracellular matrix synthesis and degradation, and alleviate cardiac fibrosis [54].

3.5.2 Intermittent fasting

Calorie restriction and stimulation of autophagy have healthy effects on the lifespan and cardioprotection in humans. Intermittent fasting induces adverse ventricular remodeling and cardiomyocyte death in null mice with LAMP2 (lysosome-associated membrane protein 2) associated with an impaired autophagic flow. The study of Godar et al. [54] highlights that intermittent fasting conferred cardioprotection in wild-type female mice, with an ∼50% reduction in infarct size compared to controls matched without fasting, and this cardioprotection was lost in heterozygous null mice for LAMP2. One of the characteristics of these heterozygous null mice is the accumulation of damaged mitochondria with a deteriorated basal autophagic flux even on a fed day fed after 6 weeks, which probably results in the loss of cardioprotection observed with this regimen in wild-type mice. Intermittent fasting modulates OS from the myocardium through the effects on the mitochondria, where it is lost in the context of LAMP2 ablation due to the deterioration of mitochondrial autophagy [55]. Recent studies have discovered a potential mechanism for transcriptional replacement of autophagy-lysosome machinery with starvation. In addition, a central role was attributed to dephosphorylation and the cytoplasm induced by rapid hunger to nuclear translocation of TFEB (EB transcription factor) [55]. The endogenous TFEB-mediated stimulation of the autophagic flow is essential for the cytoprotective effects of repetitive hunger in hypoxia-reoxygenation injury. The research group suggests the hypothesis that the transcriptional replenishment of the autophagy-lysosome machinery by fasting (and hunger as described earlier) may be a critical determinant of beneficial autophagy, which allows living organisms to survive in what has probably been one of the first evolutionary stresses that accompanied the origin of life [56].

Therefore, starvation (total caloric restriction) is a potent stimulus for the induction of myocardial macroautophagy (called “autophagy”) [57–59]. It is already known that autophagy is essential for cardiac homeostasis in the period of perinatal hunger at birth; this effect is observed before the establishment of breast milk supply [60]. In experiments using mice with genetic ablation of autophagy proteins ATG5 and ATG7, autophagosomes could not be formed and fatal myocardial ischemia developed [60, 61]. In this respect, autophagy is also essential for the maintenance of cardiac structure and function during prolonged starvation in mice, since the concomitant deterioration of autophagy with FOXo1 genetic ablation, Becn1 haplo-insufficiency [57] or pharmacological inhibition with bafilomycin A1 [62], an inhibitor of acidification and lysosome function, produces a rapid development of cardiomyopathy with starvation.

3.5.3 Ischemia/reperfusion

The different roles of autophagy in cardiomyocytes exposed to varying degrees of ischemia/reperfusion injury (I/R) or severe anoxia (S/A) were explored, and it was observed that the autophagic activity of cardiomyocytes increased with an increment in ischemia that was dependent on the duration of anoxia, undergoing ischemia, or severe ischemia [63].

During the process of cardiac ischemia, the restriction in the blood supply and the reduction of ATP leads to an imbalance in the amount of blood and energy,

causing cell heart dysfunction and myocardial damage, inflammation and excess of ROS production leading to cardiomyocyte death. It should be noted that ATP levels can be monitored by adenosine monophosphate-activated protein kinase (AMPK), which functions as a nutrient deprivation sensor in response to a decreased ATP level during cardiac ischemia [64].

In the initial phase of ischemia, a low level of ATP activates AMPK in cardiomyocytes. Once activated, AMPK directly phosphorylates and activates ULK1 resulting in the induction of autophagy by modifying ULK1 directly or indirectly [48]. The pathway by which AMPK activates autophagy is through AMPK/mTORC1 signaling. AMPK inhibits mTORC1 through phosphorylation of TSC2 and the raptor site, followed by indirect activation of ULK1 [48]. Recent studies revealed new pathways through which AMPK activated autophagy. Also, AMPK directly phosphorylates and activates activated ULK1, allowing the onset of autophagy [65–67]. Also, in the early I/R process, ROS modify the function of Ca^{2+} channels and exchangers, which triggers a decrease in available ATP, and thus, directly affect the autophagy process [68].

Beclin 1 is an important autophagic protein that has been shown to regulate both the formation and processing of autophagosomes, especially in the reperfusion phase. An in vitro study revealed that autophagic response to nutrient deprivation mediated by Beclin 1 is modulated by the Bcl-2 protein in cardiac cells [69]. Moreover, it has been observed that ROS can also be strong inducers of Beclin 1 in mediating autophagy during the reperfusion phase [70]. In addition to regulating Beclin 1 expression, ROS could also oxidize and decrease ATG4 activity, contributing to LC3 lipidation at the start of autophagy [71].

Cellular stress by ischemia, hypoxia, depletion of intracellular Ca^{2+} stores, induced OS and ROS, and the accumulation of unfolded/misfolded proteins induce ER dysfunction known as ER stress, and then the unfolded protein response (UPR) is generated to deal and play a critical role in cell death after myocardial I/R injury. Several transcription factors are induced by ER stress and the UPR whose branch includes ATF6, inositol-requiring enzyme 1 (IRE1) and PKR-like ER kinase (PERK) activated by I/R injury, which is the mediated signal pathway of UPR. The activating transcription factor 6 alpha (ATF6) is an ER transmembrane protein and most ATF6-induced proteins localize to the ER [72].

Catalase is an enzyme that has been shown to decrease damaging ROS in the heart. ATF6 induces catalase known to decrease ROS and reduce I/R damage in the heart. Catalase is a component of peroxisomes that has also been found in the cytosol and cardiac mitochondria, and it neutralizes H_2O_2 and also serves to oxidize ONOO—, NO and organic peroxides; however, it has not be found in the ER. In the study by Jin et al. [72], they examined the effects of blocking ATF6-induced proteins in the ER stress response on I/R injury in cardiac myocytes and mouse hearts. The role of ATF6 as a link between ER stress and OS and its effect on I/R myocardial injury show an important function for ATF6, which binds to specific elements in the regulatory elements of the catalase gene inducing its transcription [72].

Myocardial I/R injury negatively regulates protein synthesis, leading to the activation of signaling pathways from the ER to the cytosol and nucleus, representing UPR and ER-associated protein degradation (ERAD). Most of I/R damage is caused by ROS generated outside the ER. The study by Zhang et al. revealed that all the three branches of UPR pathway are involved. Moreover, they demonstrated reduced myocardium damage in I/R surgery, while the activation of UPR had opposite effects. The results of this study were shown after the inhibition using a standardized animal model with Sprague-Dawley rats that were pretreated with UPR stimulator dithiothreitol (DTT) and UPR inhibitor 4-phenylbutyrate (4PBA) and then subjected to myocardial I/R surgery [73].

Under the cardiac I/R condition, increased autophagic activity compensated for impaired UPS function, thereby maintaining proteolysis at an appropriate level. However, cooperation between UPS (short-lived proteins) and autophagy (long-lived proteins) is considered a housekeeping mechanism for protein quality control in I/R injury. Thus, this increased autophagic response helps to maintain an adequate proteolysis level and proteostasis in order to compensate impaired UPS function under cardiac I/R condition, which ultimately results in degradation by the proteasome as well as autophagy.

3.5.4 Spermidine

Spermidine (SPD) is a type of polyamine that has been shown to enhance heart function to delay cellular and organismal aging and provide cardiovascular protection in humans. Initially, the cardioprotective effects of SPD were explored in rodent models of physiological cardiac aging (mice) and congestive heart failure induced by high salt concentration (rats) [74]. SPD in the diet of mice delays cardiac aging by improving diastolic function.

Furthermore, the evidence demonstrated that a high intake of SPD in the diet was correlated with a reduction in the incidence of cardiovascular diseases. In humans, high levels of SPD (natural polyamine) in the diet, as assessed by food questionnaires, correlated with reduced blood pressure and a lower incidence of cardiovascular disease. Subsequently, SPD was identified as a potent inducer of autophagy [74]. SPD by increasing autophagic and mitophageal activity improves mitochondrial respiratory function. SPD also inhibited kidney damage and fibrosis. It is suggested that the effect of SPD on the improvement of cardiac function is mediated by the promotion of autophagy and mitophagy in the heart and by the reduction of the systemic chronic inflammatory response. This natural polyamine is importantly involved in maintaining cellular homeostasis, and it affects several processes including cell growth, proliferation and tissue regeneration; it also stimulates the antineoplastic immune response and anti-aging properties, including transcriptional and transductional modulation through several enzymes and nucleic acid enzymes. Moreover, SPD promotes chaperone activity and ensures proteostasis through anti-inflammatory and antioxidant properties, and it also enhances mitochondrial function and cellular respiration [75].

Therefore, the effect of exogenous SDP administration was examined in aged rat hearts [76]. SPD was shown to improve mitochondrial biogenesis by increasing nuclear expression of PGC-1α (peroxisome proliferator-activated receptor gamma coactivator alpha), which is mediated by enhanced NAD^+-dependent deacetylase activity of SIRT1 (sirtuin-1). These results suggest that SIRT1 is an essential intermediary in the mechanism by which SPD stimulates mitochondrial biogenesis and function in cardiac cells. In addition, findings showed that the administration of SPD in vivo increased the activity of the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and improved mitochondrial respiratory activity in the myocardium [76]. To date, there are not enough clinical trials to evaluate the effects of SPD in reducing cardiovascular diseases. These findings could guide new therapeutic strategies to counteract cardiac aging and prevent age-related cardiovascular disease and, as a result, lay the foundation for better heart disease treatments related to mitochondrial dysfunction [76].

3.5.5 Carvedilol

Carvedilol (CVL) belongs to the so-called α, β blockers, used to treat high blood pressure and congestive heart failure, which are generally used for the treatment of

cardiovascular disorders. CVL blocks sympathetic neural activation through antagonism of the β1, β2 and α1 adrenoceptors and it has demonstrated greater cardiovascular benefits than traditional β blockers in both humans and animals. However, some benefits beyond decreased blood pressure were observed clinically, suggesting the potential anti-inflammatory activity of CVL [77]. In addition, CVL is a known membrane "fluidizer" that alters membrane structure and protein-lipid interactions [78]. The most widely characterized inflammasome sensor in the heart is activated in response to noninfectious stimuli, such as cell debris during acute myocardial infarction. The NOD-like receptor (NLR) family, pyrin domain–containing protein 3 (NLRP3) inflammasome is a component of the inflammatory process. Activation of the NLRP3 inflammasome triggers further myocardial damage indirectly through the release of IL-1β and directly through the promotion of inflammatory cell death via pyroptosis [79]. Pyroptosis is a type of caspase-1–dependent cell death, which is often associated with inflammasome activation and IL-1β production characterized by a loss of cell membrane integrity that leads to fluid influx and cell swelling [77]. Experimental studies have shown that strategies inhibiting the activation of the NLRP3 inflammasome in the early reperfusion period after acute myocardial infarction reduce the overall size of the infarct and preserve normal cardiac function [79]. There is also evidence supporting the therapeutic value of NLRP3 inflammasome-targeted strategies in experimental models and data supporting the role of the NLRP3 inflammasome in AMI and its consequences on adverse cardiac remodeling, cytokine-mediated systolic dysfunction and heart failure [79]. Mechanistic analysis revealed that CVL prevented lysosomal and mitochondrial damage and reduced apoptosis-associated speck-like protein containing a CARD (ASC) oligomerization. Additionally, CVL caused autophagic induction through a SIRT1-dependent pathway, which inhibited the NLRP3 inflammasome [77].

CVL activates survival signaling of p-AKT and pluripotential markers in cardiomyocytes (CM) after I/R. Cardioprotective actions of CVL are associated with higher levels of the miR-199a-3p and miR-214 cardioprotective miRNAs [79]. CVL stimulates the processing of microRNA (MIR)-199a-3p and miR-214 in the heart through β-arrestin-1–biased β-1 adrenergic receptor (β1 AR) for cardioprotective signaling. Studies show that using cultured cardiomyocyte and primary cardiomyocyte cell lines, carvedilol is regulated by an increase in miR-199a-3p and miR-214 in ventricular and atrial cardiomyocytes undergoing reperfusion ischemia (I/R) injury.

3.5.6 Trehalose

It is known that trehalose, a natural disaccharide, protects cells against various stresses. Trehalose is a natural disaccharide formed from two glucose molecules with an α-type glycosidic junction. It is widely distributed in nonmammalian species, such as fungi, yeasts, bacteria, invertebrates, insects and plants. Trehalose acts to provide energy sources and protect the integrity of cells exposed to various environmental stresses. Furthermore, it has also been shown that trehalose protects against apoptosis in an autophagy-dependent manner. This natural disaccharide improves cardiac remodeling, fibrosis and apoptosis after myocardial infarction and attenuated heart dysfunction [80]. The cardioprotective effect of trehalose was not observed in the heterozygous elimination of Beclin 1 in mice, indicating that these protective effects are mediated by autophagy [81]. In this connection, trehalose induces autophagy by facilitating the recruitment of LC3B to the autophagosomal membranes in an mTOR-independent manner. The basal level of autophagy plays a unique housekeeping role in the regulation of cardiac geometry and impaired autophagy function and may contribute to various end-organ complications in

insulin resistance and diabetes, including cardiomyopathy and nephropathy [82]. Autophagy is usually regulated by both mTOR-dependent and -independent mechanisms. The mTOR pathway is considered the classic autophagy regulation route, which negatively regulates autophagy involving two functional complexes: mTORC1 and mTORC2, with a much more predominant role for mTORC1. Research findings suggest that trehalose may rescue the contractile myocardial defect induced by insulin resistance and apoptosis, through autophagy associated with the dephosphorylation of p38 MAPK and FOXo1 without affecting the phosphorylation of Akt [82]. Moreover, it was observed that trehalose not only activated autophagy but also increased the expression of p62. In addition, the expression of antioxidant genes regulated by trehalose through enhanced nuclear translocation of Nrf2 in a p62-dependent manner leads to the suppression of OS. Therefore, a new antioxidant action target for trehalose was proposed [83].

3.5.7 Lysosomal inhibitors blocking autophagy

Several lysosomal inhibitors such as bafilomycin A1 (BafA1), protease inhibitors and chloroquine (CQ) have been used interchangeably to block autophagy in vitro for lysosomal degradation. Only CQ and its derivate hydroxychloroquine (HCQ) are FDA-approved drugs currently considered the principal compounds used in clinical trials aimed for treating tumors through autophagy inhibition by impairing autophagosome fusion [84]. They focus on how CQ inhibits autophagy and directly compare its effects to those of BafA1. CQ mainly inhibits autophagy by impairing autophagosome fusion with lysosomes rather than by affecting the acidity and/or degradative activity of this organelle. Furthermore, CQ induces an autophagy-independent severe disorganization of the Golgi and endolysosomal systems, which impair autophagosome fusion. These results of Mauthe et al. suggest not using these compounds (CQ and HCQ) for in vivo experiments because of multiple cellular alterations caused by these drugs [84].

3.5.8 Resveratrol

Human clinical studies differ markedly in terms of the administered doses of resveratrol, as well as in the duration of treatment. Overall, the most pronounced effects of resveratrol include reduced body weight in obese patients and a partial decrease in systolic blood pressure, as well as fasting blood glucose levels and HbA1c in patients with diabetes mellitus in some clinical trials. Studies show that resveratrol attenuates high glucose-induced cardiomyocyte apoptosis through AMPK, a serine/threonine kinase that detects the state of cellular energy and regulates energy homeostasis [85]. Activation of AMPK is involved in the determination of multiple cellular processes including cell growth, apoptosis [86] and autophagy [87]. It is known that AMPK activation could inhibit mTOR, the best characterized protein kinase that negatively regulates autophagy [88]. Diabetic cardiomyopathy has shown inhibition of autophagy and increased apoptosis in cardiac cells. The study of Xu et al. demonstrated that using resveratrol in H9c2 cardiac myoblast cells exposed to high glucose combined with palmitate suppressed autophagic activity and increased apoptotic cell death. The H9c2 cells showed restored autophagy and attenuated apoptosis in cells with diabetic stimuli when treated with resveratrol [89, 90].

3.5.9 Metformin

Metformin is a first-line antidiabetic drug that also activates autophagy and it has cardiovascular protective effects [91], although a recent study reported

otherwise, since metformin did not achieve the cardioprotective effect in an I/R model in nonaged pigs [92]. This was proven because the protective effect of metformin was abolished by treatment with chloroquine. This treatment inhibits the fusion of lysosomes with autophagosomes and a high lysosomal pH, avoids the final digestion stage and inhibits lysosomal activity [93].

However, a recent study by Chen Li et al. showed protection with metformin on both cellular and animal models of aging and I/R injury. During aging, failure of organelles results in the accumulation of macromolecules and impaired proteostasis that result in the death of cardiac tissue. Necroptosis is a programmed cell death involving receptor-interacting protein kinases 1 and 3 (RIP1, RIP3) that form the necrosome and mixed lineage kinase domain-like protein (MLKL), which are subsequently phosphorylated [94]. Besides, metformin treatment was able to restore autophagy and reduce the accumulation of p62 in the aged myocardium, as well as decrease the cardiac junction of p62-RIP1-RIP3 complexes and the RIP3 and MLKL-induced phosphorylation. Therefore, metformin can break the unfavorable chain mechanism of aging-related autophagy decrease that induces necroptosis [94].

3.6 Development of new autophagy modulators

Diabetes is a metabolic disorder that contributes to the development of cardiac fibrosis and cardiomyopathy. Aminoguanidine (AG) inhibits advanced glycation end products (AGEs) and advanced oxidation protein products (AOPP) accumulated as a result of excessive oxidative stress in diabetes. In a recent work, we investigated whether AG supplementation mitigates oxidative-associated cardiac fibrosis in rats with type 2 diabetes mellitus (T2DM). In vivo experiments were performed in a model of T2DM, and in vitro we used primary rat myofibroblasts to confirm the antioxidant and antifibrotic effects of AG to determine if blocking the receptor for AGEs (RAGE) prevents the fibrogenic response in myofibroblasts. Diabetic rats exhibited an increase in cardiac fibrosis resulting from a high-fat, high-carbohydrate diet (HFCD) and streptozotocin (STZ) injections. In contrast, AG treatment significantly reduced cardiac fibrosis, alfa-smooth muscle actin (αSMA) and oxidative-associated NOX4 and NOS2 mRNA expression [95]. In vitro challenge of myofibroblasts with AG under T2DM conditions reduced intra- and extracellular collagen type I expression and platelet-derived growth factor (PDGF), transforming growth factor beta (TGFβ1) and collagen type 1 a 1 (COL1A1) mRNAs, albeit with a similar expression of tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) mRNAs. This was accompanied by reduced phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and SMAD2/3 but not of AKT1/2/3 and signal transducer and activator of transcription (STAT) pathways. RAGE blockade further attenuated collagen type I expression in AG-treated myofibroblasts. Thus, AG reduces oxidative stress-associated cardiac fibrosis by reducing pERK1/2, pSMAD2/3 and collagen type I expression via AGE/RAGE signaling in T2DM [95]. However, clinical studies need to be performed in order to evaluate if AG treatment is useful and well-tolerated in human cardiac disease and leads to a significant reduction in cardiac fibrosis as well as it modulates the expression of oxidative and fibrogenic response in myofibroblasts like in this disease model.

Although the autophagy modulators described above have great potential, there are currently no interventions aimed at modulating autophagy for human use. Despite this, there are already licensed medicines for use in humans, which activate or inhibit autophagy, such as rapamycin, chloroquine and HCQ, among others, that were not developed for this purpose [96]. The main clinical obstacle is that they have low pharmacological specificity for their objective, which is the autophagic

Increase AMPK		Decrease mTORC1
Caloric restriction Physical exercise H_2S Metformin Simvastatin A-769662	Initiation	Caloric restriction Physical exercise Everolimus Rapamycin Temsirolimus Torins
Increase ROS		Increase nuclear RF-1 Activation of MAPK
Antimycobacterial antibiotics Carbon monoxide Melatonin	Pre-phagophore induction	IFNγ
Increase MAPK		Inositol 1,4,5-triphosphate receptor
Carbamazepine Lithium	Nucleation	BECN1 activating peptide
Chromosome maintenance region-1 (CRM-1)		
Hydroxycitrate Resveratrol Spermidine	Elongation	
		Unknown
	Fusion	Chloramphenicol Retinoic acid
Unknown		
Trehalose Trichostatin A	Degradation	

Examples of autophagy activators. A-769662, a new activator of AMP-activated protein kinase (AMPK); BECN1, Beclin 1; H_2S, hydrogen sulfide; mTORC1, target of rapamycin complex 1.

Table 1.
Autophageal processes susceptible to therapeutic modulation.

process [84]. However, they have allowed us to know the main pathways by which the autophagy process is activated or inactivated. Several pharmacological and nutritional interventions are available to inhibit autophagy in the initiation, nucleation, elongation, fusion or degradation phase [97]. In addition, several agents modulate autophagy through multiple molecular mechanisms that are not yet characterized (**Table 1**).

4. Conclusions

Alteration of proteostasis in heart tissue leads to diabetic cardiomyopathy characterized by myocardial remodeling and interstitial fibrosis. Cardiomyocyte proteotoxicity frequently faces the chronic accumulation of misfolded or unfolded proteins that can lead to proteotoxic formation or aggregation of soluble peptides with reduced cardiac function and arrhythmias. However, under pathological conditions, autophagic flux may be an important strategy to prevent the progression of various cardiovascular diseases due to risk of dysfunctional endothelial cells. Autophagy is insufficient in endothelial cells isolated from individuals with diabetes

mellitus. Moreover, it has been demonstrated that intact autophagy is essential for eNOS signaling in endothelial cells. Nitric oxide-mediated vasodilation was promoted by the induction of autophagy.

Autophagy has been shown to be a mechanism of fibrosis recovery and scarring secondary to cell apoptosis and active in the perimeter of cardiovascular fibrotic tissue. Autophagy inhibition has a beneficial effect on type 2 diabetes–induced cardiomyopathy. These findings suggest that autophagy is diversely altered in different types of diabetes-induced cardiac pathologies. Therefore, targeting autophagy regulation may be a potential therapeutic strategy for diabetic cardiomyopathy.

Moreover, the animal model of T2DM induced by STZ plus HFCD whose diabetic response evokes pro-oxidative and profibrotic attenuated reactions in the presence of AG suggests that this molecule may be part of autophagy therapy for diabetic cardiomyopathy. Thus, AG reduces oxidative stress-associated cardiac fibrosis by decreasing pERK1/2, pSMAD2/3 and collagen type I expression via AGE/RAGE signaling in T2DM.

The knowledge of the molecules involved in mechanisms of proteostasis and autophagy in cardiac cells and the role they play in various signaling pathways will serve as an opportunity for the future design of therapeutic targets for the treatment of fibrosis, alterations of cardiac tissue remodeling and cardiomyopathy.

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Conflict of interest

The authors declare no conflict of interest.

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