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FoxO, Autophagy, and Cardiac Remodeling

Anwarul Ferdous,

Department of Internal Medicine (Division of Cardiology), University of Texas Southwestern Medical Center, NB11.200, 6000 Harry Hines Boulevard, Dallas, TX 75390-8573, USA

Pavan K. Battiprolu,

Department of Internal Medicine (Division of Cardiology), University of Texas Southwestern Medical Center, NB11.200, 6000 Harry Hines Boulevard, Dallas, TX 75390-8573, USA

Yan G. Ni,

Department of Internal Medicine (Division of Cardiology), University of Texas Southwestern Medical Center, NB11.200, 6000 Harry Hines Boulevard, Dallas, TX 75390-8573, USA

Beverly A. Rothermel, and

Department of Internal Medicine (Division of Cardiology), University of Texas Southwestern Medical Center, NB11.200, 6000 Harry Hines Boulevard, Dallas, TX 75390-8573, USA

Joseph A. Hill

Department of Internal Medicine (Division of Cardiology) and Department of Molecular Biology, University of Texas Southwestern Medical Center, NB11.200, 6000 Harry Hines Boulevard, Dallas, TX 75390-8573, USA

Joseph A. Hill: joseph.hill@utsouthwestern.edu

Abstract

In response to changes in workload, the heart grows or shrinks. Indeed, the myocardium is capable of robust and rapid structural remodeling. In the setting of normal, physiological demand, the heart responds with hypertrophic growth of individual cardiac myocytes, a process that serves to maintain cardiac output and minimize wall stress. However, disease-related stresses, such as hypertension or myocardial infarction, provoke a series of changes that culminate in heart failure and/or sudden death. At the other end of the spectrum, cardiac unloading, such as occurs with prolonged bed rest or weightlessness, causes the heart to shrink. In recent years, considerable strides have been made in deciphering the molecular and cellular events governing pro- and anti-growth events in the heart. Prominent among these mechanisms are those mediated by FoxO (Forkhead box-containing protein, O subfamily) transcription factors. In many cell types, these proteins are critical regulators of cell size, viability, and metabolism, and their importance in the heart is just emerging. Also in recent years, evidence has emerged for a pivotal role for autophagy, an evolutionarily conserved pathway of lysosomal degradation of damaged proteins and organelles, in cardiac growth and remodeling. Indeed, evidence for activated autophagy has been detected in virtually every form of myocardial disease. Now, it is clear that FoxO is an upstream regulator of both autophagy and the ubiquitin-proteasome system. Here, we discuss recent advances in our understanding of cardiomyocyte autophagy, its governance by FoxO, and the roles each of these plays in cardiac remodeling.

Correspondence to: Joseph A. Hill, joseph.hill@utsouthwestern.edu.

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Keywords

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Introduction

Heart disease is unsurpassed as the greatest noninfectious health hazard ever to confront the human race. It is estimated that five million Americans have heart failure, a syndrome with mortality of approximately 50% at 5 years [1]. For some years, heart failure has remained the leading cause of death in industrialized nations, and the epidemic is rapidly expanding to include the developing world. Accordingly, heart failure is responsible for a huge societal burden of morbidity, mortality, and cost.

In many instances, heart failure is the end result of longstanding cardiac hypertrophy. This latter phenomenon is one of several manifestations of the heart's substantial ability to alter its structure in the face of changes in workload. Indeed, a hallmark feature of the heart is its capacity of robust remodeling in response to changes in environmental demand. A variety of stimuli can induce the heart to grow or shrink by eliciting an array of molecular and cellular responses, some adaptive and others maladaptive [2]. For example, cardiac growth triggered by exercise, postnatal development, or pregnancy is adaptive, facilitating the heart's ability to meet these physiological burdens. In other instances, such as persistent hypertension, myocardial infarction, and neurohumoral activation, cardiac remodeling is maladaptive, predisposing to contractile dysfunction and ultimately heart failure [2]. In the setting of prolonged bed rest, cancer, and weightlessness, the heart actually decreases in size.

There is great interest in deciphering mechanisms that control and regulate cardiac remodeling, and a number of major insights have been discovered in recent years. Here, we discuss the role of cardiomyocyte autophagy and one of its major, upstream triggers, FoxO transcription factors. These mechanisms have emerged as key elements in cardiomyocyte homeostasis under both basal and stressed conditions, ageing, and ultimately heart disease.

FoxO Family Transcription Factors

FoxO (*Forkhead box*-containing protein, *O* subfamily) transcription factors are a subfamily of the large Forkhead family of transcriptional regulators. They are characterized by a conserved 110-amino-acid DNA-binding motif called the "forkhead box" or "winged helix" domain [3–5]. Based on homologies within the forkhead box domain, the 39 distinct members of the human Forkhead family are divided into 19 subclasses (FoxA–FoxS). The FoxO subgroup contains four members (FoxO1, FoxO3, FoxO4, and FoxO6) [5,6].

FoxO proteins were initially identified at chromosomal breakpoints in human tumors (rhabdomyosarcomas for FoxO1, and acute myeloid leukemias for FoxO3 and FoxO4), a fact that highlights their important roles in cancer development [7,8]. Subsequently, important roles for FoxO proteins have been uncovered in a wide array of diverse cellular functions, including cell cycle regulation, differentiation, metabolism, proliferation, and survival (Fig. 1) [9,10]. A great deal of research has been dedicated to understanding the cellular mechanisms underlying FoxO transcriptional activity and downstream target effects in a variety of pathophysiological settings.

FoxO factors regulate diverse cellular functions by modulating gene expression in specific cell types (Fig. 1). The forkhead domain of FoxO recognizes a consensus DNA-binding motif (5'-TTGTTTAC-3') in numerous genes, and the fact that this motif is recognized by all FoxO factors suggests that aspects of their functions overlap. However, targeted disruption

of *FOXO1*, but not *FOXO3* or *FOXO4*, causes embryonic lethality due to abnormal angiogenesis [11,12]. Conversely, disruption of *FOXO3* leads to age-dependent female infertility [12,13]. These observations point to specific roles for FoxO1 in vascular development and for FoxO3 in ovarian follicle maturation, which cannot be compensated by other FoxO family members [11–13]. In contrast, expression of constitutively active mutants of either FoxO1 or FoxO3 in fully differentiated skeletal and cardiac muscle cells is sufficient to trigger muscle atrophy without activating apoptosis. This reduction of cell size is seemingly due to increased protein degradation through FoxO-mediated activation of two muscle-specific ligases, atrogin-1 (MAFbx) and MuRF-1 [14–16]. FoxOs have been shown to either promote or inhibit myoblast differentiation [17,18]. Although the specific mechanisms underlying these opposing roles for FoxOs in muscle cell differentiation are not fully understood, it was hypothesized that isoform-specific temporal changes in FoxO, occurring in the context of different cell types, positively or negatively regulates cell differentiation. Consistent with this hypothesis, FoxO1 induces p21^{CIP1} expression to prevent differentiation, while FoxO3 induces B-cell translocation gene-1 which promotes erythroid differentiation [19,20]. Cardiomyocyte-specific overexpression of FoxO1 is embryonic lethal, and skeletal myocyte-specific overexpression of FoxO1 and FoxO3 triggers severe muscle atrophy, both of which are due to aberrant cellular proliferation [15,16,21].

FoxO factors promote or inhibit differentiation of non-muscle cell types, including adipocytes and beta cells [19]. Interestingly, the inhibitory role of FoxO1 in adipocyte differentiation is tightly regulated by the insulin-Akt signaling pathway [19]. FoxO transcriptional activity is also crucial in liver. Under fasting conditions, a primary function of the liver is to provide energy by releasing glucose into the circulation. Indeed, under conditions of prolonged starvation, gluconeogenesis is the major source of circulating glucose, and FoxO factors up-regulate this process by activating the expression of genes encoding key enzymes for gluconeogenesis [22]. In some cell types, such as cardiac, hepatic, and beta cells, FoxO gain-of-function impairs insulin signaling, decreases cell mass, and alters cellular energy homeostasis and glucose metabolism [23]. FoxO-dependent transcriptional control of gluconeogenic gene expression is potentiated by Sirt1, an NAD-dependent histone deacetylase [22]. Regulation of FoxO activity by Akt and Sirt1 suggests that posttranslational modifications are critical mechanisms controlling FoxO transcriptional activity.

Regulation of FoxO by Posttranslational Modifications

The expression of a gene and the activity of its encoded protein (e.g. enzyme, receptor, transcription factor) are governed by a host of interlacing regulatory cascades, including transcriptional, translational, and posttranslational mechanisms. With respect to transcription factors, posttranslational regulation by covalent modification of the protein itself (e.g. phosphorylation, acetylation, ubiquitylation) encompasses the predominant and best characterized mechanisms governing their actions. Posttranslational modifications regulate transcriptional activity by modulating protein stability, DNA-binding activity, protein–protein interactions, and subcellular localization. In the case of FoxO factors, posttranslational modifications are the predominant mechanism regulating their transcriptional activity (Fig. 1) [24].

The most prevalent and well-characterized posttranslational modification for FoxO is protein phosphorylation. Stimulation of insulin or insulin-like growth factor (IGF) receptors by their corresponding ligands triggers the recruitment and activation of phosphoinositide-3-kinase (PI3K) and phosphoinositide-dependent kinases (PDKs). These molecules, in turn, activate several other serine/threonine kinases, including Akt (also known as protein kinase

B, PKB) and serum and glucocorticoid-regulated kinase (SGK), to phosphorylate distinct serine and threonine residues on FoxOs [25–31]. This growth factor-mediated, phosphorylation-dependent regulation of FoxO activity is highly conserved through evolution [32]. In addition to Akt and SGK, casein kinase 1 [33], dual tyrosine phosphorylated regulated kinase 1 [34], extracellular signal-regulated kinases 1 and 2 (ERK1/2) [35], and I κ B kinase β (IKK β) [36] are also capable of phosphorylating FoxO factors.

Phosphorylation of FoxO proteins is also stimulated by other growth factors, such as epidermal growth factor and nerve growth factor [37]. The general consequence of FoxO factor phosphorylation by Akt and SGK is to promote FoxO translocation to the cytoplasm with consequent quenching of transcriptional activity [25,26,38]. Phosphorylated FoxOs bind specifically with 14-3-3 proteins, which act as a chaperone to escort FoxO proteins out of the nucleus, either by inducing a conformational change in FoxO that promotes interaction with exportin/Crm1 [26,33,39] or by impeding nuclear translocation by masking a nuclear localization signal [40,41].

In contrast to the FoxO-inhibitory actions of Akt and SGK kinases, several other kinases, such as the stress-activated c-jun-NH₂-kinase (JNK) [42,43], the mammalian orthologue of the Ste20-like protein kinase (MST1) [44], and AMP-activated protein kinase [45], are known to activate FoxO. Although the precise mechanism of FoxO activation by these kinases is unknown, phosphorylation of 14-3-3 by JNK has been shown to reduce its interaction with FoxO protein [26,46]. Thus, phosphorylation of FoxO proteins represents a unique regulatory mechanism governing their transcriptional activity by modulating their subcellular localization. In many cases, specific residues on FoxO have been mapped, which are phosphorylated by specific kinases [37,47]. That being said, mechanisms regulating the activity of the most recently identified FoxO family member, FoxO6, are not well understood. FoxO6 lacks the C-terminal Akt phosphorylation site and primarily resides in the nucleus. Nevertheless, phosphorylation by Akt has been shown to be necessary to modulate FoxO6 activity [48,49].

The transcriptional activity of FoxO factors is also regulated by protein acetylation and deacetylation. For example, mammalian FoxOs are acetylated by several histone acetyltransferases, including p300, CREB-binding protein (CBP), and p300/CBP-associated factor (P/CAF) [43,50–52], while the NAD-dependent histone deacetylase (HDAC) Sirt1, the mammalian homologue of silent information regulator-2.1, removes acetyl groups from FoxO and p300/CBP [43,51–54]. FoxOs may also be regulated by other HDACs, as treatment with trichostatin A, an inhibitor of HDACs, induces FoxO acetylation and subcellular translocation [55,56]. Despite the fact that the acetylated residues within FoxO factors have been identified using deletion mapping and mass-spectroscopy techniques [37,57], the precise physiological roles of acetylation and deacetylation of FoxOs remain obscure. This complexity is due, in part, to the observations that both acetylation and deacetylation can either inhibit or activate FoxO activity depending on context [43,57]. The apparent discrepancies could also be due to the complex nature of gene regulation by acetylation, either by affecting the phosphorylation status of FoxO factors or by modulating chromatin structure. At this time, the general consensus is that acetylation inhibits and deacetylation activates FoxO factors, most likely by modulating their DNA-binding activity [37,56,57].

FoxO factors are also regulated by ubiquitylation, adding yet another layer of complexity to their control. Several studies reveal that phosphorylation of FoxOs by Akt and IKK β not only induces their cytoplasmic localization but also triggers polyubiquitylation and subsequent proteasome-mediated degradation [58–60]. For example, FoxO1 is

polyubiquitylated by Skp2, an E3 ligase, in an Akt phosphorylation-dependent process that leads to FoxO1 degradation by the proteasome [60]. In the case of FoxO3, Akt, ERK1/2, and IKK β -dependent phosphorylation occurs, but the specific E3 ligase that mediates FoxO3 ubiquitylation has yet to be identified. The mechanism of degradation of other FoxO members (FoxO4, FoxO6) is not clearly understood. In contrast to FoxO degradation by polyubiquitylation, oxidative stress-induced monoubiquitylation of FoxO factors potentiates their transcriptional activity [61]. Taken together, it is apparent that unique combinations of phosphorylation, acetylation, and ubiquitylation control FoxO transcriptional activity by modulating subcellular localization and proteasomal degradation. Thus, three types of posttranslational FoxO modifications afford a cascade of interlacing layers of control and fine tuning of FoxO's ability to orchestrate transcriptional programs in response to environmental cues.

Autophagy and Its Control by FoxO

Autophagy is an evolutionarily ancient lysosome-dependent mechanism of degradation of damaged proteins and organelles. It was first described by Christian de Duve as a process of self-(auto) eating (phagy) [62]. Unlike the well-known ubiquitin-proteasome pathway that targets polyubiquitylated proteins for degradation, autophagy eliminates misfolded proteins and intracellular organelles, such as mitochondria, which may or may not be tagged by ubiquitin. Three major types of autophagy have been described: macroautophagy, microautophagy, and chaperone-mediated autophagy [63–66]. Among them, macroautophagy (hereafter referred to as autophagy) has received the greatest attention and is best understood. Autophagy is activated by diverse stimuli, including nutrient deprivation and growth factor withdrawal, hyperthermia, and hypoxia (Fig. 2) [63–66].

Greater than 30 autophagy-related gene (ATG) products orchestrate the autophagic cascade. The pathway is subdivided into distinct steps: initiation, elongation, vesicle formation, autophagosome–vacuole fusion, and degradation of the cellular components followed by release of the degradation products (Fig. 2). Upon stimulation, the process of autophagy initiates with formation of the phagophore by activation of a class III phosphatidylinositol-3-kinase (PI3K III) complex containing several Vps (vacuolar protein sorting) proteins, Atg14, and Atg6/Beclin 1. Activation of this pathway, also called nucleation, mediates autophagosome formation by recruiting to the phagophore two unrelated ubiquitylation-like conjugation systems: Atg5-Atg12-Atg16 and Atg8/MAP-LC3. These processes play essential roles in regulating membrane elongation, expansion of the nascent autophagosome, and substrate recognition. A recent study has identified a new protein phosphatase, Jumpy, which negatively regulates the early initiation process of autophagy [67]. When formation of the resulting double-membrane structure (autophagosome) is complete, Atg8 at the outer membrane is cleaved off by Atg4, and the autophagosome fuses with a lysosome to form an autolysosome. This is followed by degradation of the sequestered materials and their subsequent release in the form of essential building blocks (amino acids, fatty acids) to maintain cellular homeostasis under conditions of stress.

Recent work has uncovered an important role for FoxO factors in the control of autophagy. Forced expression of FoxOs up-regulates autophagic and atrophic responses in muscle cells [14–16]. Gene expression analyses revealed that FoxOs promote expression of several autophagy-related genes, suggesting that FoxO and autophagic activities are intimately related; yet, the precise molecular mechanisms underlying these events remain obscure. Regulation of autophagy by FoxO factors and the established, essential roles of Atg proteins in autophagy suggested that *ATG* genes might be regulated at the transcriptional level. Consistent with this, several studies have shown that FoxO factors induce expression of

multiple *ATG* genes, including *ATG8*, *Gabarapl1*, *ATG12*, *ATG4B*, *VSP34*, and *BECLIN1* during muscle atrophy [16,68,69]. FoxO factors bind to a consensus DNA-binding motif in the promoter sequences of *BECLIN1* and *ATG8* to activate their expression. Expression of these and several other *ATG* genes, such as *ATG16* and *ATG5*, is essential for the processes of initiation and autophagosome formation (Fig. 2). These findings highlight FoxO factors and *ATGs* as potential therapeutic targets in the governance of cellular homeostasis and disease pathophysiology.

FoxOs and Autophagy in Physiological Cardiac Plasticity

The four-chamber adult heart comprises three major cellular compartments: epicardium, myocardium, and endocardium. Only the myocardium is populated with long-lived and terminally differentiated myocytes. Therefore, despite the ongoing controversy regarding the regenerative capacity of adult heart, cellular mechanisms underlying myocyte function, viability, and cellular homeostasis in adult heart remain poorly understood. Several lines of evidence suggest critical regulatory functions of autophagy and FoxO factors in myocyte function and ageing [70]. For example, basal levels of autophagy are essential to efficient removal and recycling of damaged cytoplasmic contents, processes critical to cellular homeostasis (Fig. 3). FoxO factors play essential roles in regulating expression of genes, which are essential to cellular homeostasis and survival (Figs. 1 and 3). Importantly, dysregulation of the autophagy-lysosome degradation pathway in myocytes, due either to overexpression or to inactivation of specific *ATG* and *FOXO* genes, is capable of eliciting either beneficial or detrimental effects depending on the pathophysiological context. These findings highlight the fact that dysregulation of *ATG* and *FOXO* gene expressions and activities evokes critical actions on myocyte function, homeostasis, and survival.

Ageing is an inevitable process. Incomplete elimination, and consequent progressive accumulation, of obsolete cellular components is considered a critical factor in the biology of ageing. Over a decade ago, a role of FoxO factors in regulating longevity was described in *Caenorhabditis elegans* [71,72]. Loss-of-function of DAF-16 (worm FoxO orthologue) reverts a longevity phenotype in nematodes mutant in DAF-2, the insulin/IGF-1 receptor. Subsequently, this antiageing role of FoxOs was found to be conserved in flies and mammals [37,73,74]. Thus, insulin/IGF-1 signaling governs life span by regulating the activities of FoxO proteins. A working hypothesis is that life span and augmented stress resistance are linked. Consistent with this notion, generation of reactive oxygen species (ROS) from mitochondria increases with age and in the setting of prolonged cardiomyocyte starvation. Excessive production of ROS in aged and stressed myocytes leads to oxidative stress and mitochondrial damage. Indeed, accumulating evidence directly links ROS and autophagy to ageing. For example, ROS inactivates Atg4 to cleave Atg8/LC3 from the autophagosome and promotes Atg8 lipidation as an essential step in the autophagic cascade. Consistent with this, overexpression of Atg8 in the *Drosophila* nervous system extends life span by 50% [75]. Although the underlying mechanisms whereby FoxO factors prolong life span are not known, it is plausible that oxidative stress activates either JNK/MST1 and Sirt1, or both, to potentiate FoxO transcriptional activity and induce expression of genes required in the stress response. Consistent with this model, a role for Sirt1 in regulating longevity further links FoxO factors and autophagy, most likely through Sirt1-dependent regulation of FoxO transcriptional activity [43,76]. As FoxOs and autophagy are coupled in several age-related diseases, such as diabetes, FoxO factors and autophagy may contribute importantly to the complicated interplay between life span and disease.

FoxOs and Autophagy in Pathological Cardiac Plasticity

Current thinking holds that cardiac atrophy is a catabolic response, where the size of cardiac myocytes decreases due to increases in autophagy- and proteasome-mediated proteolysis. Thus, it is not surprising that increases in lysosomal, as well as proteasomal, degradative activities are seen in atrophied cardiac and skeletal muscle cells [16]. Of interest is that these increases in proteolytic activity, both proteasomal and autophagy-dependent, are directly regulated by FoxO factors [16] through induction of muscle-specific ubiquitin ligases, atrogin-1 and MuRF1.

Whereas these studies have established a clear contribution of FoxO factors and autophagy in the setting of muscle atrophy, the precise role of FoxOs and autophagy in the setting of more common pathological cardiac remodeling events (e.g. hypertrophy, ischemia) is incompletely understood. As mentioned above, increased FoxO activity and autophagy in the setting of mild stress, such as ischemia and starvation, affords protective effects by inducing specific gene expressions, by recycling obsolete cellular constituents to maintain energy production, and by removing and preventing accumulation of toxic protein aggregates. In contrast, autophagic activity induced during chronic ischemia, ischemia-reperfusion injury, and pressure overload in most cases is detrimental to cardiac homeostasis [77–79].

Growth and remodeling of the adult heart in response to pressure overload (e.g. persistent hypertension), following myocardial infarction, chronic ischemia, or ischemia-reperfusion injury is pathological in that it is associated with structural and functional deterioration. Prolonged stress on the heart culminates in ventricular dilation, contractile dysfunction, and ultimately clinical heart failure. Interestingly, increased lysosomal activity is readily detected in tissue samples from diseased and failing hearts [80,81], implicating autophagy in the pathophysiology of heart failure. Additional evidence in support of this notion has come from patients with cardiomyopathy who manifest hallmark features of marked accumulation of autophagosomes in cardiac myocytes and increased cell death [82]. However, the role of autophagy in these conditions is under intense investigation to determine whether it serves a protective role or contributes to disease pathogenesis. Our current understanding is that autophagy plays dual, beneficial and detrimental, roles in the myocardium depending on the degree of activation and the context of its induction [79]. Consistent with this notion, we have reported robust and prolonged activation of autophagic activity in cardiomyocytes from hearts subjected to pressure overload by thoracic aortic constriction [77]. Subsequently, we reported that pressure overload-induced autophagy is blunted in mice haploinsufficient for Beclin 1 (*Beclin 1*^{+/-}), a rate-limiting protein in the molecular cascade of autophagy [77]. Conversely, pressure overload provoked severe cardiac hypertrophy and enhanced autophagic activity in cardiomyocytes overexpressing Beclin 1 [77]. Thus, these data suggest that the level of autophagic activity is an essential parameter to dictate the beneficial-to-detrimental switch on cardiac pathology. In other words, too little or too much autophagy will each be detrimental to the heart (Fig. 3). Indeed, one group has reported significant cardiac hypertrophy and profound heart failure after cardiac-specific ablation of *ATG5* in adult heart, and pressure overload further amplified these changes [83]. Taken together, we conclude that basal autophagy is fundamental to the regulation of cardiac myocyte function and homeostasis, whereas either the complete absence of autophagic activity or its activation to excessive levels is each capable of eliciting maladaptive and untoward effects [82].

As *BECLIN1* and many other autophagy-related genes are downstream targets of FoxO [84], it is conceivable that FoxO factors play a role in modulating myocyte autophagic activity and cardiac plasticity. Whereas overexpression of FoxO triggers muscle atrophy primarily

by promoting proteolysis by proteasomal and autophagic mechanisms [16], the actions of FoxO in adult cardiomyocytes in a variety of contexts are poorly characterized. We and others have shown that overexpression of FoxO in isolated neonatal rat ventricular myocytes blunts agonist-induced myocyte hypertrophy and induces autophagy [69,85]. Constitutively active mutants of FoxO trigger apoptotic cell death by inducing FasL, Bim-1, and Bnip3 expression [37], whereas fewer apoptotic cells were detected in areas of the heart with increased autophagic activity [83]. These data raise the intriguing possibility that FoxO-dependent and FoxO-independent autophagic activities exist in cells to modulate cardiac function independently or cooperatively in a context-dependent manner. Consistent with this notion, we have demonstrated that FoxO factors are inactivated in load-stressed heart [85] and yet autophagy is up-regulated robustly [77]. Although these findings seem difficult to reconcile at first, we suggest that early pressure overload mimics conditions of relatively mild stress, where myocyte hypertrophy is adaptive and compensated by increased FoxO and autophagic activities. However, prolonged stress and super-activated autophagy might trigger other signaling events that not only inactivate FoxOs but also regulate FoxO-independent autophagic activity, ultimately leading to maladaptive consequences. In other words, during the course of cardiac remodeling, multiple events converge to regulate autophagic and FoxO activities in a differential, only partially overlapping, manner. In addition to load-stressed heart, increased autophagic activity has also been reported in the settings of ischemia and ischemia/reperfusion in humans, and in swine and rodent models. However, it is presently unknown whether this autophagic activity is cardioprotective or modulated by FoxO factors.

A recent study reported that the transcriptional regulator p8 interacts with FoxO3 and represses its activity. Moreover, suppression of p8 impaired cardiac function due to increased cardiomyocyte autophagy and apoptosis [86]. Moving forward, it will be interesting to determine whether p8 expression is regulated in pathophysiologically relevant animal models and whether it modulates the activity of other FoxO isoforms.

Conclusion

The molecular and cellular functions of the FoxO family of transcription factors and autophagy overlap in diverse pathophysiological conditions within the heart. Both activities are evolutionarily ancient and tightly regulated in the settings of basal cellular homeostasis, ageing, and in virtually all forms of heart disease. Both activities can confer beneficial or deleterious actions, dependent on the context. As too little or too much autophagy are each detrimental, major goals for the future will be to differentiate “good autophagy” from “bad autophagy” and to tease out the role of FoxO in each. As FoxO factors are involved in a variety of cellular functions, it is yet to be uncovered whether FoxO1, FoxO3, FoxO4, and FoxO6 target different subsets of genes or share similar target genes in different cell types. It is our expectation that identification of novel FoxO target genes that may be specific to certain tissues and cell types, as well as detailed understanding of the signaling pathways that govern both the FoxO and autophagy axes, will enable us to titrate myocyte autophagy and FoxO-dependent events in a disease-specific manner. Clearly, much work remains to explore the continuum over which FoxO factors and autophagy function in cardiac health and disease. It is also important to understand whether the multiple members of the FoxO family function similarly in these events. Careful dissection of this fascinating and intricate biology will be required to accomplish a vision of meaningful therapeutic manipulation of autophagy in patients with heart disease. Indeed, deciphering molecular mechanisms underlying FoxO factor-dependent and FoxO factor-independent autophagic activities is likely to lead to significant insights into a mechanism that is at once prevalent, poorly understood, and clinically important.

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Fig. 1.

FoxO activity is regulated by multiple posttranslational modification events. Insulin/IGF-1 activates the insulin receptor to recruit the PI3K/PDK complex which, in turn, phosphorylates Akt/SGK leading to FoxO phosphorylation and nuclear exclusion via 14-3-3- and Crm-1-dependent mechanisms. Cytoplasmic FoxO can be phosphorylated by IKK β and ERK1/2, leading to FoxO polyubiquitylation by Skp2 and an unknown E3 ligase, and subsequent proteasomal degradation. On exposure to oxidative stress, phosphorylation of FoxO by JNK/MST1 and monoubiquitylation by an unknown E3 ligase promote FoxO nuclear translocation to potentiate its transcriptional activity. Stress-induced deacetylation of FoxO by Sirt1 counteracts p300/CBP-dependent FoxO inactivation and results in prolonged FoxO promoter occupancy. Concomitantly, deubiquitylases (e.g. USP7) and phosphatases (PPase) antagonize these pathways

Fig. 2.

FoxO governs the autophagic pathway at multiple steps. Numerous stimuli, such as starvation and oxidative stress, activate the class III PI3 kinase/Beclin 1/Atg14 complex to trigger the autophagic process. First, an isolated, double-membrane structure (phagophore) is formed (nucleation) followed by membrane elongation and substrate recognition. This is followed by Atg8 lipidation, cargo engulfment, and membrane fusion to form the distinctive double-membrane autophagosome. The autophagosome then fuses with a lysosome to form an autolysosome where the engulfed substrate is degraded. The resulting small molecule by-products, including amino acids, energy mediators, and lipids, are released, providing fuel to preserve cellular function and viability. The transcriptional activity of FoxO regulates expression of several autophagy-related genes (*ATGs*) that participate in autophagy at multiple points

Fig. 3.

Graded levels of autophagic activity, each regulated by FoxO, contribute importantly to cardiac homeostasis and disease. Under physiological conditions, basal autophagic activity participates in protein quality control, efficiently removing damaged proteins and organelles, and recycling each element. Under conditions of mild stress and during ageing, FoxO factors induce the expression of diverse genes to detoxify ROS and eliminate damaged proteins and organelles, thereby promoting stress responsiveness. In the setting of severe or chronic stress, such as biomechanical load (e.g. hypertension), autophagy is activated yet further, which can lead to cellular demise and consequent heart failure. In other instances, such as ventricular unloading (e.g. prolonged bed rest), increased catabolic activity through both autophagy- and proteasome-mediated protein degradation reduces myocyte size, leading to cardiac atrophy. Complete abrogation of autophagic activity in the myocyte through inactivation of critical autophagy-related genes is also maladaptive, triggering rapid cardiac dysfunction