CHAPTER 6

FoxO Proteins and Cardiac Pathology

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Abstract

The FoxO family of transcription factors mediate a wide range of cellular responses from cell death to cell survival, growth inhibition and glucose utilization. This complex array of responses is regulated by an equally complex regulatory system, involving phosphorylation, ubiquitinization and acetylation, in addition to interactions with other transcription factors and transcriptional modifiers. In heart, FoxO proteins have been shown to be involved in development, in limiting hypertrophic growth responses and in cardioprotection provided by silent information regulator 1 (Sirt1). However, the range of responses mediated by FoxO proteins and the clear evidence for involvement of FoxO regulators in cardiac pathology, suggest that further pathological actions of FoxO family members remain to be elucidated.

The FoxO Family

FoxO proteins are members of the forkhead family of transcription factors characterized by the presence of a forkhead box or Fox, which binds DNA at GTAAACA consensus sequences.[1,2] Genes encoding the FoxO proteins were initially identified at chromosome break points in tumour cells and shown to be homologues of the *Caenohabditis elegans* DAF 16 protein that regulates longevity.[3] Thus, from their initial discovery, the FoxO proteins have been associated with cell survival and cell death responses. There are currently four FoxO proteins known to be expressed in mammalian tissues; FoxO1, FoxO3, FoxO4 and the more recently described FoxO6.[4,5] The first 3 FoxO proteins are expressed in heart,[6] show strong sequence similarity and are regulated similarly. FoxO6 is expressed only in the central nervous system and will not be discussed further here. While FoxOs 1, 3 and 4 are expressed in cardiomyocytes, there is relatively little information about their functional roles in the heart. However, a number of factors that regulate FoxO activity have been shown to have major roles in protecting the heart under pathological conditions or in some cases in causing cardiac damage. This chapter will examine the evidence for an involvement of FoxO proteins in cardiac pathology and will also examine the roles of known FoxO effectors and suggest ways in which their cardiac responses may be mediated by FoxO transcription factors.

The Spectrum of Transcriptional Responses Mediated by FoxO Family Members

FoxO proteins are transcription factors that mediate a bewildering range of cellular responses, which in some cases appear to be opposing. In worms and flies the FoxO homologues, DAF 16 and dFoxO respectively, extend longevity by promoting resistance to stressors, including infectious agents and oxidative stress.[7] The functions of the FoxOs are more complex in mammalian tissues. FoxO 1 and 3 are widely expressed in mammalian cell types and responses observed depend to an extent on the cell type studied. Expression of FoxO4 is more restricted, but it is expressed in the myocardium

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along with FoxOs 1 and 3. Even within the one cell type, responses generally differ between the FoxO subtypes.[8,9] Furthermore, there is evidence that FoxOs can have different functions depending on the nature or the magnitude of the stimulus, as well as on the presence other transcriptional effectors.[9-14] In various mammalian cell types, FoxOs can promote resistance to oxidative stress by transcriptional activation of catalase and MnSOD that serve to remove reactive oxygen species.[15,16] However, FoxO members are also able to initiate apoptosis via transcriptional increases in apoptotic effectors such as Fas-L and Bim.[14,17-19] FoxO proteins increase DNA repair via growth arrest and DNA damage-inducible 45 (GADD 45) and damaged DNA binding protein 1 (DDB1),[20] cause cell cycle arrest via p21, p27, p130 cyclin-dependent kinase inhibitors, as well as cyclin G2[21-23] and regulate glucose and energy homeostasis via glucose 6-phosphatase, phosphoenolpyruvate carboxy kinase, Agouty related peptide (AgRP) and neuropeptide Y (NPY).[24-27]

Regulation of FoxO Proteins

Phosphorylation

In keeping with the array of responses associated with FoxO transcription factors, their regulation, both positive and negative, involves multiple mechanisms. Initially FoxO proteins were shown to be phosphorylated by the protein kinase Akt (or protein kinase B, PKB).[28] Akt itself is activated by phosphorylation subsequent to phosphatidylinositol 3-kinase (PI 3-kinase) activation following stimulation with growth factors or G protein coupled receptor agonists.[29] Akt phosphorylates FoxOs 1, 3 and 4 at three specific sites, as outlined in Figure 1. This phosphorylation results in nuclear exclusion and association with 14-3-3 proteins[30] and thereby inhibits FoxO from functioning as a transcription factor. As shown in Figure 1, one of the Akt phosphorylation sites is in the DNA binding domain and the nuclear localization sequence. Phosphorylation of this site (S^{253} in mouse FoxO1) generally occurs prior to phosphorylation occurring at the other two sites.[31] It is now also clear that phosphorylation of S^{253} displaces DNA-bound FoxO and thereby directly inhibits transcriptional activity,[32] in addition to facilitating exclusion from the nucleus. Thus, there is a defined hierarchy between the Akt phosphorylation sites in FoxO. Removal of phosphorylated FoxO from the nucleus is a complex process involving both the nuclear exclusion sequence (NES) exposed following phosphorylation and the 14-3-3 association.[28,33] In heart,

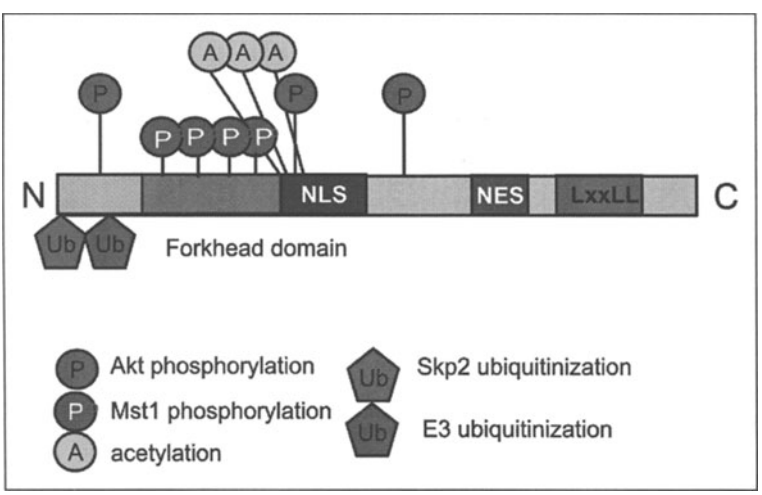

Figure 1. The structure of FoxO family members. FoxO1, FoxO3 and FoxO4 follow a similar pattern of phosphorylation, ubiquitinization and acetylation sites. NLS, nuclear localization sequence; NES, nuclear exclusion sequence; LxxLL is s sequence associated with binding of nuclear hormone receptors.

phosphorylation of FoxO by Akt has been demonstrated[6] and, under basal conditions, FoxO proteins are largely cytoplasmic.

In marked contrast, more recent studies have revealed that FoxO family members can also be activated by phosphorylation, although on different sites from those phosphorylated by Akt, as shown in Figure 1. Activating phosphorylation is mediated by mammalian sterile 20 like kinase 1 (Mst1) a homologue of the *Drosophila* sterile 20 kinase.[15] Mst1 is a Ser, Thr directed protein kinase activated by stressors, including oxidative stress.[15,34] The biology of Mst1 remains to be fully investigated. Mst1 is activated by phosphorylation downstream of K-Ras signalling[35] and K-Ras can be activated downstream of NADPH oxidase 1 (Nox1).[36] Mst1 can also be activated following cleavage by caspase 3.[37] Under this scenario, activation by caspase 3 cleavage could serve to perpetuate Mst1 activation and consequent cellular damage following initiation of apoptosis.[38] Mst1 phosphorylates FoxO family members on Ser/Thr residues within the DNA-binding forkhead domain to enhance DNA binding and therefore FoxO transcriptional activity.[39] Mst1 phosphorylation also disrupts association with 14-3-3 proteins and thus facilitates nuclear retention.

Ubiquitinization

FoxO proteins, phosphorylated by Akt and shunted into the cytoplasm, are subsequently polyubiquitinated and degraded via the proteosome system,[40] by a process that absolutely requires phosphorylation of Akt phosphorylation sites. The ligase most prominent in this response is Skp2 and overexpression of Skp2 can reverse FoxO mediated responses.[41] Thus, polyubiquitinization and proteosomal destruction is the end point of Akt initiated FoxO inhibition (Fig. 2).

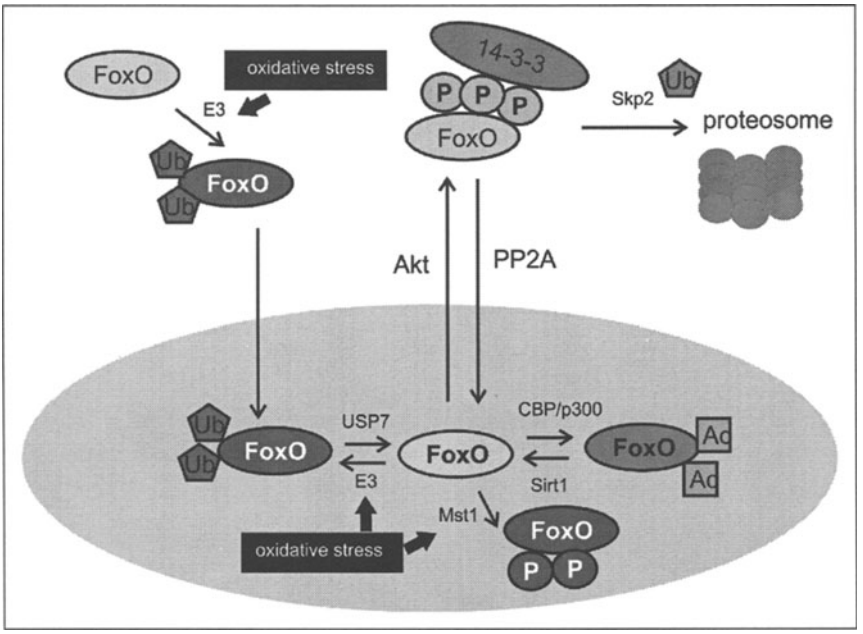

Figure 2. Regulation of FoxO family members under conditions of ischemia and reperfusion. FoxO proteins are phosphorylated by Akt and dephosphorylated by protein phosphatase 2A (PP2A). Skp2 ubiquitinates Akt-phosphorylated, 14-3-3-bound FoxO and targets it for degradation. E3 ubiquitin ligase activates FoxO and this is reversed by herpes virus-associated ubiquitin specific protease (HAUSP or USP7). Mst1 phosphorylation activates transcriptional activity of FoxO. Additionally, FoxO activity is regulated by acetylation by cAMP response element binding protein (CBP) and p300 histone acetyltransferase. Deacetylation is achieved by silent information regulator 1 (Sirt1).

In addition to this polyubiquitinization that is required for proteosomal degradation, FoxO proteins can also be selectively ligated with monomers of ubiquitin. This mono-ubiquitinization, (on K^{63} in mouse FoxO1) mediated by ligases such as E3-ubiquitin ligase, enhances nuclear localization and transcriptional activity.[42] This increases nuclear localization and enhances transcriptional activity of FoxO.[43] Removal of these ubiquitin residues is achieved by herpes virus-associated ubiquitin specific protease (HAUSP). The balance between mono-ubiquinylated and nonubiquinylated FoxO determines transcriptional activity and this balance is regulated by reactive oxygen species.

Acetylation

In addition to regulation by phosphorylation and ubiquitinization, FoxO proteins are regulated by acetylation. Acetylation is mediated by histone acetyl transferases including p300 and the cAMP response element binding protein (CBP) and involves critical lysine residues (K^{242}, K^{245}, K^{262}, in mouse FoxO1).[44] Such acetylation reduces the positive charge on the FoxO protein reducing DNA binding and thereby reducing transcriptional activity.[39] Acetylation also facilitates Akt phosphorylation of S^{253}, further limiting FoxO functioning, as described above.[44] Deacetylation is achieved primarily by class III histone deacetylases, particularly silent information regulator 1 (Sirt1), a homologue of Sir2 in *C. elegans*. Inhibition, rather than activation, of FoxO activity by deacetylation has also been reported.[45,46] The reason for the apparently opposing effects of FoxO acetylation is not known. Given that acetylation of positively charged lysine residues inhibits DNA binding, it is possible that increased transcriptional responses reflect FoxO acting as a transcriptional partner rather than a direct DNA binding transcription factor at those particular promoters.

Transcriptional Partners of FoxO

FoxO family members have direct transcriptional activity by binding forkhead consensus sequences, but in addition these proteins also interact with other transcription factors and transcription modifiers to regulate transcription. There are a number of different ways in which this can be accomplished. In some cases, FoxO and its transcriptional partner both bind their respective DNA sequences, as occurs for the interaction between FoxO and Smads.[47] In other cases FoxO-DNA interaction is not involved. This mechanism is exemplified by the inhibitory interaction with myocardin, where FoxO4 reduces the association between myocardin and serum response factor.[48] There are also examples of FoxO functioning by simply soaking up a transcriptional cofactor and reducing its availability. The interaction between FoxO and β-catenin removes β-catenin from another transcription factor (TCF) and reduces its activity[49] (Fig. 3).

FoxOs interact strongly with nuclear hormone receptors via its LxxLL domain (Fig. 1), resulting in altered activity of both proteins.[9] Among these, interactions with peroxisome proliferator-activated receptors (PPAR), PPARα and PPARγ are likely to be important in heart, where these transcription factors are protective.[50,51] FoxO proteins also interact with the PPARγ co-activator PGC-1α; in this case the interaction enhances FoxO activity.[52] PGC-1α is deacetylated by Sirt1. This suggests a complex relationship between PPAR and FoxO family members.

The transcriptional modifier, myocardin, is active only in smooth and cardiac muscle where it plays critical roles in development and in postnatal growth, via association with serum response factor.[53] Myocardin interacts with FoxO4 in a mutually inhibitory manner,[48] but this interaction has not been reported in heart, to date.

The Smad family of transcription factors is activated by phosphorylation downstream of transforming growth factor β (TGFβ) receptors. Phosphorylated Smad3 translocates to the nucleus where it associates with Smad4 and the Smad3/4 complex is transcriptionally active. The Smad3/4 complex can form a larger complex with FoxO family members and this heightens responses to both FoxO and Smad transcription factors.[9,54] As shown in Figure 3, both FoxO and Smad bind to their respective consensus sequences, but with heightened activity. Thus some of the responses initiated by FoxO family members may result from interaction with Smads.

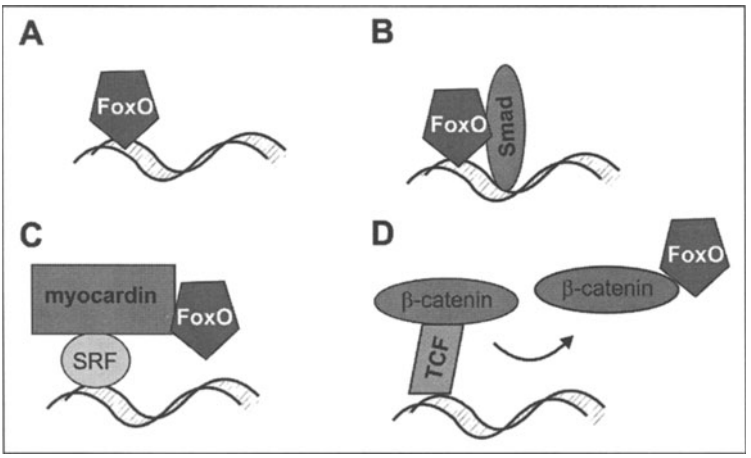

Figure 3. Transcriptional activity of FoxO transcription factors. A) FoxO family members can bind DNA consensus sequences to activate transcription. B) FoxO proteins can associate with other transcription factors, with both factors binding their respective consensus sequences, as shown for the Smad family of transcription factors. C) FoxO proteins can interact with transcriptional enhancers to reduce their activity, as shown for myocardin enhancement of serum response factor (SRF) activity. D) FoxO can bind other transcriptional activators removing them from other effectors, as shown for β-catenin activation of TCF responses.

β-catenin is another factor that associates with FoxO family members, in this case in a mutually inhibitory fashion.[49] Under conditions of cell stress, β-catenin translocates from the plasma membrane to the nucleus and initiates cell survival responses by binding its partner, the transcription factor TCF. FoxO inhibits these responses by sequestering β-catenin away from TCF[55] (Fig. 3).

FoxO Transcription Factors in Cardiac Pathology

Myocardial Ischemia and Post-Ischemic Reperfusion

By definition, myocardial ischemia involves a critical reduction in the blood supply to the myocardium. Clinically, this generally involves blockage of the coronary arteries supplying the ventricle. In the experimental situation, isolated hearts are subjected to reduced flow of perfusate delivering oxygen and nutrients,[56] or isolated cardiomyocytes are subjected to low oxygen together with changes in media composition.[57] Reperfusion is achieved by re-instating blood flow, or in the experimental situation, by re-introducing oxygen.[56,58] While reperfusion is essential to prevent irreversible tissue damage, it introduces further damage to the myocardium, mediated, in part, by the generation of reactive oxygen species (ROS). Ischemia and postischemic reperfusion are major initiators of cardiac pathology. Acutely, ischemic episodes cause arrhythmia often leading to sudden cardiac death.[59,60] Ischemic episodes that are not immediately fatal cause myocardial infarction that is followed by compensatory cardiomyocyte hypertrophy, leading eventually to heart failure.[61] Damage to cardiomyocytes during ischemia and subsequent reperfusion involves both necrotic and apoptotic cell death[62] and at least in animal models, reduction in cardiomyocyte apoptosis improves functional outcomes.[63]

In model systems, ischemic injury can be ameliorated by activation of PI 3-kinase and subsequent activation of the protein kinase Akt, a FoxO inhibitor described above.[64,65] Akt protection results in reduced infarct size, indicating improved cell survival, as well as improved functional recovery.[64,65] Akt phosphorylates a number of targets including pro-apoptotic effectors. Important among these are the pro-apoptotic proteins BAD, Mst1, caspase 9[66] and all of the FoxO proteins expressed in heart.[30] FoxO proteins promote apoptosis via transcriptional activation of apoptotic effectors such as tumor necrosis factor related apoptosis inducing ligand (TRAIL), Bim and Fas ligand.[67-69] Cardiomyocytes

are relatively resistant to apoptosis and, in particular, the extrinsic pathway of apoptosis is though to be only minimally involved in cardiomyocyte damage.[70,71] However, there have been reports of Fas activation and its possible involvement in ischemic injury.[72,73] Cardiomyocyte apoptosis most commonly involves the mitochondrial, intrinsic pathways.[74] Bim, a proapoptotic BcL-2 protein, is a transcriptional target of FoxO proteins and thus increased Bim expression, subsequent to FoxO activation, is a possible contributor to Akt-reversible cardiomyocyte cell death. This, of course, leaves open the question of how FoxOs would be activated by ischemia/reperfusion. This question is discussed further below.

One possible mechanism by which FoxO proteins might be activated under conditions of ischemia/reperfusion involves the activating protein kinase Mst1, alluded to earlier. Mst1 is activated by stressors, including oxidative stress,[15,34] as occurs under conditions of ischemia/reperfusion.[75] As noted above, Mst1 phosphorylates FoxO family members within the forkhead domain to enhance DNA binding and therefore FoxO transcriptional activity.[39] Thus, Mst1 phosphorylation of FoxO acts in opposition to the inhibitory phosphorylation mediated by Akt. Recently, Ste20/oxidant stress response kinase-1 (SOK1), a close relative of Mst1, has been shown to be directly activated by interaction with reactive oxygen species.[34] However, there are no similar reports of direct activation by ROS for Mst1 itself. Overexpression of Mst1 in heart in vivo (Tg-Mst1) causes severe dilated cardiomyopathy,[75] by inhibiting hypertrophy and autophagy while activating apoptosis. Importantly, inhibiting Mst1 activity by expressing a dominant negative Mst1 mutant in heart reduced apoptosis and dysfunction following myocardial infarction.[76] This suggests that Mst1 is an important contributor to heart failure following ischemic insult.

However, it is less clear that FoxO family members are the mediators of Mst1-induced cardiac pathology. Mst1 inhibits hypertrophy and autophagy, while increasing apoptosis. Inhibition of hypertrophy by FoxO3 has been demonstrated in heart[77] and initiation of apoptosis by FoxO family members has been described in other tissues[67,69,78] FoxO3, however, is associated with increased autophagy in the myocardium,[79,80] seemingly opposite to responses initiated by Mst1. However, it is clear that transcriptional responses mediated by the FoxOs vary depending on the cell type, the nature of the stimulus and the intensity of the stimulus. Therefore, it is possible that FoxO members mediate the apoptotic and antihypertrophic actions of Mst1, but not the inhibition of autophagy. The question of FoxO mediation of Mst1-induced cardiac pathology will only be answered satisfactorily, with FoxO knock-out animals, or by expressing dominant negative FoxO.

As discussed earlier, FoxO family members are subject to acetylation/deacetylation reactions mediated by histone acetyl transferases (HATs) and class III histone deacetylases (HDACs), respectively. In heart, FoxO3 is acetylated by cAMP response element binding protein (CBP)[81] and p300 acetylase[39] and deacetylated by silent information regulator-1 (Sirt1, a homologue of yeast Sir2).[82-84] In mammalian heart, Sirt1 is a cardioprotective factor activated following oxidative stress.[10,16] Moderate increases in Sirt1 expression in heart are protective under conditions of pathological growth or under ischemic challenge and this is related to increased expression of detoxifying enzymes such as catalase and manganese superoxide dismutase (MnSOD). This protective response was prevented by dominant negative FoxO, pointing to a role for FoxO in ischemic protection.[10] As dominant negative FoxO inhibits the activity of all members of the FoxO family, the FoxO subtype responsible for this response was not identified in this study. This apparently protective action of FoxO members is opposite to what would be expected based on effects of Akt and Mst1. However, in addition to its transcriptional activation of potentially apoptotic factors, FoxO proteins increase transcription of catalase and MnSOD,[10] factors that aid in removing ROS and would be expected to ameliorate ischemic damage. Higher levels of expression of Sirt1 in heart caused rapid development of hypertrophy, followed by heart failure.[10] It is not clear whether this deleterious response to Sirt1 was also mediated by FoxO family members.

From these data, it appears that FoxO proteins can have both advantageous and disadvantageous effects on the heart during ischemic episodes. It should be noted that there are also reports that deacetylation by Sirt1 can inhibit FoxO1 and specifically can reduce FoxO-mediated apoptosis, although these observations were not made in cardiomyocytes.[16,46,84,85]

FoxO proteins are regulated positively and negatively by ubiquitination. Ubiquitinization by Skp2 is essentially the end point of Akt mediated FoxO inhibition, by targeting FoxO for proteosomal degradation.[41] On the other hand, ubiquitinization is also a mechanism of FoxO activation and this process is enhanced under ischemic conditions.[86] By this E3 ligase mediated mechanism, FoxO proteins are ubiquitinated on K^{63} in mouse FoxO1,[42] enhancing nuclear localization and transcriptional activity.[43] Removal of these ubiquitin residues is achieved by herpes virus-associated ubiquitin specific protease (HAUSP). The balance between mono-ubiquinitated and nonubiquinitated FoxO determines transcriptional activity and this balance is regulated by reactive oxygen species generated under conditions of ischemia/reperfusion.[43]

FoxO proteins interact with a number of critical factors, often in a mutually inhibitory fashion. Important among these is β-catenin.[49] β-catenin translocates from the sarcolemma to the nucleus of cardiomyocytes under ischemic conditions and protects from cardiomyocyte apoptosis.[87] Expression of β-catenin reduces infarct size following myocardial infarction and furthermore, inhibition of β-catenin by cardiac-targeted knock-out or by expression of a dominant negative mutant leads to growth failure in response to challenge and thus precipitates heart failure.[87,88] The interaction between β-catenin and FoxO is heightened under ischemic conditions.[89] As this is a mutually inhibitory association, FoxO would be expected to reduce the beneficial effects of β-catenin. However, there are also reports that β-catenin is required for adaptive cardiac hypertrophy,[88] but it is not certain that this involves FoxO family members.

Hypertrophy

Cardiomyocytes are terminally differentiated and do not undergo cell division to any significant extent after birth. However, heart size can be induced to increase by a process of hypertrophy whereby the size of the individual cells increases without their undergoing mitosis. The heart undergoes hypertrophic growth in response to increased work demand on the cardiomyocytes. Essentially there are two apparently distinct types of hypertrophy; physiological hypertrophy that accompanies exercise and pathological hypertrophy. Physiological hypertrophy results in a larger more powerful heart that does not degenerate into heart failure.[90] Pathological hypertrophy, on the other hand, is initially a compensatory response to produce a larger more powerful heart, but in this scenario, increased growth is associated with arrhythmia and sudden death and in the longer term degenerates into heart failure.[91,92] Pathological hypertrophy follows loss of myocytes due to infarction, as mentioned above, or when there is pressure or volume overload exerted on the heart, e.g., by increased blood pressure or renal impairment, respectively. FoxO transcription factors are associated with inhibition of growth in many cell types. This involves transcriptional activation of the cell cycle regulators, p21 and p27, as well as other intermediates and this maintains cells in the G_0 state.[7] In terminally differentiated cardiomyocytes, FoxO3 has been shown to inhibit hypertrophic growth. FoxO3 induces transcription of atrogin-1,[77] a muscle F-box protein. Atrogin-1 associates with calcineurin promoting its degradation via the proteosome,[93] thereby inhibiting the calcineurin/nuclear factor of activated T-cells (NFAT) response pathway that is pivotal in pathological hypertrophy. In addition, atrogin-1 and E3 ubiquitin ligase cause ubiquitinization on K^{63} of FoxO1 and FoxO3, promoting nuclear localization and transcriptional activity. This ubiquitinization serves to oppose the actions of Akt and by this mechanism FoxO members can limit physiological hypertrophy,[86,93,94] that depends on PI 3-kinase and Akt activation.[95]

Other antihypertrophic mechanisms involving FoxO have been reported also. Statins, cholesterol-lowering drugs that inhibit HMG CoA reductase, have a direct action to limit cardiac hypertrophy, in addition to their lipid lowering activity. Studies by Hauck et al (2007)[96] show that statins facilitate the recruitment of FoxO3 to the p21 promoter and thereby initiate growth-suppression via p21 signaling pathways.

FoxO4 interacts in a mutually inhibitory manner with myocardin[48] and myocardin is a powerful activator of cardiac hypertrophy.[97] However, the interaction between FoxO4 and myocardin has not been demonstrated in heart as yet.

Development

Unlike post natal growth, the fetal development of the heart requires cell growth and division and FoxO family proteins are involved in this process. This was demonstrated in studies where FoxO1, FoxO3 and FoxO4 were expressed under a β-myosin heavy chain promoter to initiate expression during fetal development.[98] Overexpression of FoxO3 caused death at embryonic day 18 due to restricted mitosis, whereas embryonic overexpression of FoxO1 was lethal by 10.5. FoxO4 overexpression was not lethal during prenatal growth. Knockout of the FoxO1 gene is embryonic lethal at E 10.5 due to restricted vascular development. Deletion of either FoxO3 or FoxO4 was not lethal during development.[99]

As noted earlier, the transcriptional partner of serum response factor, myocardin, is a critical regulator of heart specification.[100] In addition to being negatively regulated by FoxO4, myocardin is a transcriptional target of FoxO, which, in this case, acts together with myocyte enhancer factor 2 (Mef2) to activate myocardin gene transcription.[101] This being the case, it is unclear why deletion of FoxO does not prevent early heart development. The answer may reside in functional redundancy between family members. This possibility will only be addressed by expressing a dominant negative mutant FoxO in early embryos to interfere with the transcriptional activity of all family members.

Conclusion

The FoxO family of transcription factors clearly mediate a wide range of cellular responses and this is achieved by an even more complex regulatory network responsible for FoxO activity. To date, the only cardiac effects definitively ascribed to FoxO are developmental regulation and growth inhibition. However, given the number of cardiac effectors that are FoxO regulators, it seems inevitable that further functions will be described for FoxO family members in the myocardium.

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References

1. Burgering B. A brief introduction to FOXOlogy. Oncogene 2008; 27:2258-2262.
2. Biggs WH 3rd, Cavenee WK, Arden KC. Identification and characterization of members of the FKHR (FOX O) subclass of winged-helix transcription factors in the mouse. Mamm Genome 2001; 12:416-25.
3. Guarente L, Kenyon C. Genetic pathways that regulate ageing in model organisms. Nature 2000; 408:255-62.
4. Richards JS, Sharma SC, Falender AE et al. Expression of FKHR, FKHRL1 and AFX genes in the rodent ovary: evidence for regulation by IGF-I, estrogen and the gonadotropins. Mol Endocrinol 2002; 16:580-99.
5. Van der Heide LP, Jacobs FMJ, Burbach JPH et al. FoxO6 transcriptional activity is regulated by Thr(26) and Ser(184), independent of nucleo-cytoplasmic shuttling. Biochem J 2005; 391:623-629.
6. Morris JB, Kenney B, Huynh H et al. Regulation of the proapoptotic factor FOXO1 (FKHR) in cardiomyocytes by growth factors and α_1-adrenergic agonists. Endocrinology 2005; 146:4370-6.
7. Burgering BM, Kops GJ. Cell cycle and death control: long live Forkheads. Trends Biochem Sci 2002; 27:352-60.
8. Moylan JS, Smith JD, Chambers MA et al. TNF induction of atrogin-1/MAFbx mRNA depends on Foxo4 expression but not AKT-Foxo1/3 signaling. Am J Physiol 2008; 295:C986-993.
9. van der Vos KE, Coffer PJ. FOXO-binding partners: it takes two to tango. Oncogene 2008; 27:2289-2299.
10. Alcendor RR, Gao SM, Zhai PY et al. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circ Res 2007; 100:1512-1521.
11. Accili D, Arden KC. FoxOs at the crossroads of cellular metabolism, differentiation and transformation. Cell 2004; 117:421-6.
12. Brunet A. The multiple roles of FOXO transcription factors. M S-Medicine Sciences 2004; 20:856-859.

13. Jonsson H, Peng SL. Forkhead transcription factors in immunology. Cell Mol Life Sci 2005; 62:397-409.
14. Lam EWF, Francis RE, Petkovic M. FOXO transcription factors: key regulators of cell fate. Biochem Soc Trans 2006; 34:722-726.
15. Lehtinen MK, Yuan ZQ, Boag PR et al. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. Cell 2006; 125:987-1001.
16. Kobayashi Y, Furukawa-Hibi Y, Chen C et al. SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress. Int J Mol Med 2005; 16:237-243.
17. Fu Z, Tindall DJ. FOXOs, cancer and regulation of apoptosis. Oncogene 2008; 27:2312-2319.
18. Yan L, Lavin VA, Moser LR et al. PP2A Regulates the Pro-apoptotic Activity of FOXO1. J Biol Chem 2008; 283:7411-20.
19. Urbich C, Knau A, Fichtlscherer S et al. FOXO-dependent expression of the proapoptotic protein Bim: pivotal role for apoptosis signaling in endothelial progenitor cells. FASEB J 2005; 19:974-6.
20. Tran H, Brunet A, Grenier JM et al. DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science 2002; 296:530-4.
21. Stahl M, Dijkers PF, Kops GJ et al. The forkhead transcription factor FoxO regulates transcription of p27Kip1 and Bim in response to IL-2. J Immunol 2002; 168:5024-31.
22. Kuiperij HB, van der Horst A, Raaijmakers J et al. Activation of FoxO transcription factors contributes to the antiproliferative effect of cAMP. Oncogene 2005; 24:2087-95.
23. Kops GJ, Medema RH, Glassford J et al. Control of cell cycle exit and entry by protein kinase B-regulated forkhead transcription factors. Mol Cell Biol 2002; 22:2025-36.
24. Barthel A, Schmoll D, Unterman TG. FoxO proteins in insulin action and metabolism. Trend Endocrinol Metab 2005; 16:183-189.
25. Nakae J, Oki M, Cao YH. The FoxO transcription factors and metabolic regulation. FEBS Lett 2008; 582:54-67.
26. Gross DN, van den Heuvel APJ, Birnbaum MJ. The role of FoxO in the regulation of metabolism. Oncogene 2008; 27:2320-2336.
27. Zhang WW, Patil S, Chauhan B et al. FoxO1 regulates multiple metabolic pathways in the liver—Effects on gluconeogenic, glycolytic and lipogenic gene expression. J Biol Chem 2006; 281:10105-10117.
28. Tang ED, Nunez G, Barr FG et al. Negative regulation of the forkhead transcription factor FKHR by Akt. J Biol Chem 1999; 274:16741-6.
29. Chan TO, Rittenhouse SE, Tsichlis PN. AKT/PKB and other D3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation. Annu Rev Biochem 1999; 68:965-1014.
30. Brunet A, Bonni A, Zigmond MJ et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 1999; 96:857-68.
31. Nakae J, Barr V, Accili D. Differential regulation of gene expression by insulin and IGF-1 receptors correlates with phosphorylation of a single amino acid residue in the forkhead transcription factor FKHR. EMBO J 2000; 19:989-96.
32. Zhang X, Gan L, Pan H et al. Phosphorylation of serine 256 suppresses transactivation by FKHR (FOXO1) by multiple mechanisms. Direct and indirect effects on nuclear/cytoplasmic shuttling and DNA binding. J Biol Chem 2002; 277:45276-84.
33. Obsil T, Ghirlando R, Anderson DE et al. Two 14-3-3 binding motifs are required for stable association of forkhead transcription factor FOXO4 with 14-3-3 proteins and inhibition of DNA binding. Biochemistry 2003; 42:15264-15272.
34. Pombo CM, Bonventre JV, Molnar A et al. Activation of a human Ste20-like kinase by oxidant stress defines a novel stress response pathway. EMBO J 1996; 15:4537-46.
35. Feig LA, Buchsbaum RJ. Cell signaling: life or death decisions of ras proteins. Curr Biol 2002; 12:R259-61.
36. Komatsu D, Kato M, Nakayama J et al. NADPH oxidase 1 plays a critical mediating role in oncogenic Ras-induced vascular endothelial growth factor expression. Oncogene 2008; 27:4724-32.
37. Song JJ, Lee YJ. Differential cleavage of Mst1 by caspase-7/-3 is responsible for TRAIL-induced activation of the MAPK superfamily. Cell Signal 2008; 20:892-906.
38. Anand R, Kim AY, Brent M et al. Biochemical analysis of MST1 kinase: Elucidation of a C-terminal regulatory region. Biochemistry 2008; 47:6719-6726.
39. Brent MM, Anand R, Marmorstein R. Structural basis for DNA recognition by FoxO1 and its regulation by posttranslational modification. Structure 2008; 16:1407-16.
40. Matsuzaki H, Daitoku H, Hatta M et al. Insulin-induced phosphorylation of FKHR (Foxo1) targets to proteasomal degradation. Proc Natl Acad Sci USA 2003; 100:11285-90.
41. Dehan E, Pagano M. Skp2, the FoxO1 hunter. Cancer Cell 2005; 7:209-210.

42. Li HH, Willis MS, Lockyer P et al. Atrogin-1 inhibits Akt-dependent cardiac hypertrophy in mice via ubiquitin-dependent coactivation of Forkhead proteins. J Clin Invest 2007; 117:3211-3223.
43. van der Horst A, de Vries-Smits AM, Brenkman AB et al. FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. Nat Cell Biol 2006; 8:1064-73.
44. Matsuzaki H, Daitoku H, Hatta M et al. Acetylation of Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. Proc Natl Acad Sci USA 2005; 102:11278-11283.
45. Yang YH, Hou HY, Haller EM et al. Suppression of FOXO1 activity by FHL2 through SIRT1-mediated deacetylation. EMBO Journal 2005; 24:1021-1032.
46. Motta MC, Divecha N, Lemieux M et al. Mammalian SIRT1 represses forkhead transcription factors. Cell 2004; 116:551-63.
47. Gomis RR, Alarcon C, He W et al. A FoxO-Smad synexpression group in human keratinocytes. Proc Natl Acad Sci USA 2006; 103:12747-52.
48. Liu ZP, Wang Z, Yanagisawa H et al. Phenotypic modulation of smooth muscle cells through interaction of Foxo4 and myocardin. Dev Cell 2005; 9:261-70.
49. Essers MAG, de Vries-Smits LMM, Barker N et al. Functional interaction between β-catenin and FOXO in oxidative stress signaling. Science 2005; 308:1181-1184.
50. Yue TL, Bao W, Jucker BM et al. Activation of peroxisome proliferator-activated receptor-α protects the heart from ischemia/reperfusion injury. Circulation 2003; 108:2393-2399.
51. Cao Z, Ye P, Long C et al. Effect of pioglitazone, a peroxisome proliferator-activated receptor γ agonist, on ischemia-reperfusion injury in rats. Pharmacology 2007; 79:184-92.
52. Puigserver P, Rhee J, Donovan J et al. Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1α interaction. Nature 2003; 423:550-5.
53. Cen B, Selvaraj A, Prywes R. Myocardin/MKL family of SRF coactivators: Key regulators of immediate early and muscle specific gene expression. J Cell Biochem 2004; 93:74-82.
54. Arden KC. FoxO: linking new signaling pathways. Mol Cell 2004; 14:416-8.
55. Arce L, Yokoyama NN, Waterman ML. Diversity of LEF/TCF action in development and disease. Oncogene 2006; 25:7492-504.
56. Lanzafame AA, Turnbull L, Amiramahdi F et al. Inositol phospholipids localized to caveolae in rat heart are regulated by α_1-adrenergic receptors and by ischemia-reperfusion. Am J Physiol 2006; 290:H2059-65.
57. Woodcock E, Lambert K, Phan T et al. Inositol phosphate metabolism during myocardial ischemia. J Mol Cell Cardiol 1997; 29:449-460.
58. Amirahmadi F, Turnbull L, Du XJ et al. Heightened α_{1A}-adrenergic receptor activity suppresses ischaemia/reperfusion-induced Ins(1,4,5)P_3 generation in the mouse heart: a comparison with ischaemic preconditioning. Clin Sci (Lond) 2008; 114:157-64.
59. Thandroyen F, McCarthy J, Burton K et al. Ryanodine and caffeine prevent ventricular arrhythmias during acute myocardial ischemia and reperfusion in rat heart. Circ Res 1988; 62:306-314.
60. Heidbuchel H, Tack J, Vanneste L et al. Significance of arrhythmias during the first 24 hours of acute myocardial infarction treated with alteplase and effect of early administration of a β-blocker or a bradycardiac agent on their incidence. Circulation 1994; 89:1051-1059.
61. Kopecky SL, Aviles RJ, Bell MR et al. A randomized, double-blinded, placebo-controlled, dose-ranging study measuring the effect of an adenosine agonist on infarct size reduction in patients undergoing primary percutaneous transluminal coronary angioplasty: the ADMIRE (AmP579 Delivery for Myocardial Infarction REduction) study. Am Heart J 2003; 146:146-52.
62. Gottlieb RA, Burleson KO, Kloner RA et al. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J Clin Invest 1994; 94:1621-1628.
63. Elsasser A, Suzuki K, Schaper J. Unresolved issues regarding the role of apoptosis in the pathogenesis of ischemic injury and heart failure. J Mol Cell Cardiol 2000; 32:711-24.
64. Fujio Y, Nguyen T, Wencker D et al. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. Circulation 2000; 101:660-667.
65. Matsui T, Tao JZ, delMonte F et al. Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo. Circulation 2001; 104:330-335.
66. Matsui T, Rosenzweig A. Convergent signal transduction pathways controlling cardiomyocyte survival and function: the role of PI 3-kinase and Akt. J Mol Cell Cardiol 2005; 38:63-71.
67. Modur V, Nagarajan R, Evers BM et al. FOXO proteins regulate tumor necrosis factor-related apoptosis inducing ligand expression. Implications for PTEN mutation in prostate cancer. J Biol Chem 2002; 277:47928-37.
68. Ciechomska I, Pyrzynska B, Kazmierczak P et al. Inhibition of Akt kinase signalling and activation of Forkhead are indispensable for upregulation of FasL expression in apoptosis of glioma cells. Oncogene 2003; 22:7617-7627.

69. Sunters A, de Mattos SF, Stahl M et al. FoxO3a transcriptional regulation of bim controls apoptosis in paclitaxel-treated breast cancer cell lines. J Biol Chem 2003; 278:49795-49805.
70. Kitsis RN, Mann DL. Apoptosis and the heart: a decade of progress. J Mol Cell Cardiol 2005; 38:1-2.
71. Bahi N, Zhang J, Llovera M et al. Switch from caspase-dependent to caspase-independent death during heart development: essential role of endonuclease G in ischemia-induced DNA processing of differentiated cardiomyocytes. J Biol Chem 2006; 281:22943-52.
72. Tanaka M, Ito H, Akimoto S et al. Hypoxia induces apoptosis with enhanced expression of Fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. Circ Res 1994; 75:426-433.
73. Stephanou A, Scarabelli TM, Brar BK et al. Induction of apoptosis and Fas receptor/Fas ligand expression by ischemia/reperfusion in cardiac myocytes requires serine 727 of the STAT-1 transcription factor but not tyrosine 701. J Biol Chem 2001; 276:28340-28347.
74. Adams JW, Pagel AL, Means CK et al. Cardiomyocyte apoptosis induced by Gαq signaling is mediated by permeability transition pore formation and activation of the mitochondrial death pathway. Circ Res 2000; 87:1180-1187.
75. Yamamoto S, Yang G, Zablocki D et al. Activation of Mst1 causes dilated cardiomyopathy by stimulating apoptosis without compensatory ventricular myocyte hypertrophy. J Clin Invest 2003; 111:1463-74.
76. Odashima M, Usui S, Takagi H et al. Inhibition of endogenous Mst1 prevents apoptosis and cardiac dysfunction without affecting cardiac hypertrophy after myocardial infarction. Circ Res 2007;May 11;100(9):1344-52.
77. Skurk C, Izumiya Y, Maatz H et al. The FOXO3a transcription factor regulates cardiac myocyte size downstream of AKT signaling. J Biol Chem 2005; May 27;280(21):20814-23
78. Rokudai S, Fujita N, Kitahara O et al. Involvement of FKHR-dependent TRADD expression in chemotherapeutic drug-induced apoptosis. Mol Cell Biol 2002; 22:8695-708.
79. Zhao J, Brault JJ, Schild A et al. FoxO3 coordinately activates protein degradation by the autophagic/lysosomal and proteasomal pathways in atrophying muscle cells. Cell Metab 2007; 6:472-483.
80. Salih DAM, Brunet A. FoxO transcription factors in the maintenance of cellular homeostasis during aging. Curr Opin Cell Biol 2008; 20:126-136.
81. Fukuoka M, Daitoku H, Hatta M et al. Negative regulation of forkhead transcription factor AFX (Foxo4) by CBP-induced acetylation. Int J Mol Med 2003; 12:503-8.
82. Daitoku H, Hatta M, Matsuzaki H et al. Silent information regulator 2 potentiates Foxo1-mediated transcription through its deacetylase activity. Proc Natl Acad Sci USA 2004; 101:10042-7.
83. Alcendor RR, Kirshenbaum LA, Imai S et al. Silent information regulator 2α, a longevity factor and class III histone deacetylase, is an essential endogenous apoptosis inhibitor in cardiac myocytes. Circ Res 2004; 95:971-80.
84. Brunet A, Sweeney LB, Sturgill JF et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004; 303:2011-5.
85. Giannakou ME, Partridge L. The interaction between FOXO and SIRT1: tipping the balance towards survival. Trend Cell Biol 2004; 14:408-412.
86. Toth A, Nickson P, Qin LL et al. Differential regulation of cardiomyocyte survival and hypertrophy by MDM2, an E3 ubiquitin ligase. J Biol Chem 2006; 281:3679-89.
87. Hahn JY, Cho HJ, Bae JW et al. β-Catenin overexpression reduces myocardial infarct size through differential effects on cardiomyocytes and cardiac fibroblasts. J Biol Chem 2006; 281:30979-89.
88. Baurand A, Zelarayan L, Betney R et al. β-Catenin downregulation is required for adaptive cardiac remodeling. Circ Res 2007; May 11;100(9):1353-62.
89. Almeida M, Han L, Martin-Millan M et al. Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting β-catenin from T-cell factor- to forkhead box O-mediated transcription. J Biol Chem 2007; 282:27298-305.
90. McMullen JR, Shioi T, Zhang L et al. Phosphoinositide 3-kinase(p110α) plays a critical role for the induction of physiological, but not pathological, cardiac hypertrophy. Proc Natl Acad Sci USA 2003; 100:12355-12360.
91. Lorell B. Transition from hypertrophy to failure. Circulation 1997; 96:3824-3827.
92. Tardiff JC. Cardiac hypertrophy: stressing out the heart. J Clin Invest 2006; 116:1467-1470.
93. Ni YG, Berenji K, Wang N et al. Foxo transcription factors blunt cardiac hypertrophy by inhibiting calcineurin signaling. Circulation 2006; 114:1159-68.
94. Fang CX, Dong F, Thomas DP et al. Hypertrophic cardiomyopathy in high-fat diet-induced obesity: role of suppression of forkhead transcription factor and atrophy gene transcription. Am J Physiol 2008; 295:H1206-H1215.
95. McMullen JR, Amirahmadi F, Woodcock EA et al. Protective effects of exercise and phosphoinositide 3-kinase(p110α) signaling in dilated and hypertrophic cardiomyopathy. Proc Natl Acad Sci USA 2007; 104:612-617.

96. Hauck L, Harms C, Grothe D et al. Critical role for FoxO3a-dependent regulation of p21(CIP1)/(WAF1) in response to statin signaling in cardiac myocytes. Circ Res 2007; 100:50-60.
97. Xing W, Zhang TC, Cao D et al. Myocardin induces cardiomyocyte hypertrophy. Circ Res 2006; Apr 28;98(8):1089-97.
98. Evans-Anderson HJ, Alfieri CM, Yutzey KE. Regulation of cardiomyocyte proliferation and myocardial growth during development by FOXO transcription factors. Circ Res 2008; Mar 28;102(6):686-94.
99. Hosaka T, Biggs WH, Tieu D et al. Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. Proc Natl Acad Sci USA 2004; 101:2975-2980.
100. Small EM, Warkman AS, Wang DZ et al. Myocardin is sufficient and necessary for cardiac gene expression in Xenopus. Development 2005; 132:987-997.
101. Creemers EE, Sutherland LB, McAnally J et al. Myocardin is a direct transcriptional target of Mef2, Tead and Foxo proteins during cardiovascular development. Development 2006; 133:4245-4256.