FoxO3 hastens autophagy and shrinks the heart but does not curtail pathological hypertrophy in adult mice

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The FoxO subfamily of Forkhead box transcription factors regulate many target genes that are involved in diverse cellular processes. FoxO proteins show partial functional redundancy due to the conserved DNA-binding domain, but also exhibit isoform specificity with regard to cellular functions. Three of the four FoxO isoforms—FoxO1, FoxO3, and FoxO4, but not FoxO6—are expressed in the heart. The biological functions of FoxO proteins in the heart have been mainly revealed by studies on FoxO1 and FoxO3 using either cultured cardiomyocytes or loss-of-function animal models. Combined loss of FoxO1 and FoxO3 in the heart does not affect cardiac development, suggesting functional redundancy of FoxO proteins in regulating cardiac development. Indeed, loss of all three FoxO isoforms is required for an oncogenic phenotype in mice. So far, a viable animal model of FoxO gain-of-function is lacking, hindering a better understanding on the pathophysiological significance of FoxO proteins in adult hearts.

Schips et al., report the first mouse model of cardiac-specific and temporally controllable overexpression of a constitutively active form of FoxO3 (caFoxO3). Using this model, they demonstrate for the first time in intact adult mice that activation of FoxO3 is sufficient to activate autophagy and induce reversible cardiac atrophy under baseline conditions. However, overexpression of caFoxO3 fails to suppress cardiac hypertrophy induced by transverse aortic constriction.

The nuclear localization and transactivation of FoxO proteins are regulated by post-translational modifications such as phosphorylation, glycosylation, acetylation, and ubiquitination. The phosphorylation of FoxO proteins in different cell types by various kinases such as Akt, IKKβ, and AMP-activated kinase generally leads to translocation of FoxO proteins to the cytoplasm and triggers the degradation of FoxO proteins by the ubiquitin–proteasome system, thereby inhibiting FoxO. Notably, the non-canonical K63-linked ubiquitination of FoxO proteins by their target gene Atrogin-1, a ubiquitin E3 ligase, can in turn increase the transcriptional activity of FoxO proteins, forming a feed-forward mechanism in cardiomyocytes. Mutating the Akt phosphorylation sites on FoxO proteins disables Akt-mediated inhibition and results in exclusive FoxO nuclear localization. Overexpression of FoxO1 or FoxO3 in embryonic hearts is embryonically lethal, likely due to defective cell cycle regulation. To circumvent this problem, Schips et al. employed a tetracycline-controllable binary transgenic system to achieve caFoxO3 overexpression in adult hearts. Mounting evidence primarily from cell culture studies suggests a major role of FoxO in regulating cardiac growth. FoxO proteins appear to coordinate two major pathways that can regulate cardiac growth: IGF–Akt and calcineurin–NFAT cascades. IGF–Akt signalling increases ribosome biogenesis and protein translation and, in parallel, inhibits FoxO activities by Akt-mediated phosphorylation and cytoplasmic sequestration of FoxO proteins, resulting in cardiac hypertrophy. In the absence of IGF/Akt signalling, FoxO activates the expression of Atrogin-1. Atrogin-1 can target calcineurin for proteasomal degradation, suppressing the calcineurin–NFAT pathway and consequently preventing calcineurin-dependent cardiac hypertrophy. Additionally, sustained activation of FoxO proteins attenuates the Akt-associated PP2A phosphatase activity and thereby increases Akt activities, forming a negative feedback loop. However, the effect of FoxO gain-of-function to counteract physiological cardiac growth has not been directly tested at the organ level until this report by Schips et al. They revealed that activation of FoxO3 reduces cardiomyocyte size and heart weight, which is accompanied by reactivation of the foetal gene program, but also that cardiac contractility is preserved. Moreover, the atrophic phenotype is reversible when caFoxO3 transgene expression is shut off. These findings are consistent with the increased heart size in FoxO3-deficient mice.

Recent studies suggest that FoxO proteins are capable of regulating both the ubiquitin–proteasome system and autophagy in cardiac muscle cells. Ubiquitin ligases Atrogin-1 and MuRF1 are bona fide target genes of FoxO proteins in myocytes, including cardiac myocytes. Surprisingly, neither Atrogin-1 nor MuRF1 expression was altered in the baseline conditions. However, overexpression of caFoxO3 fails to suppress cardiac hypertrophy induced by transverse aortic constriction.

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caFoxO3-expressing hearts, suggesting that caFoxO3-induced atrophy is unlikely to be caused by enhancing the ubiquitin–proteasome system-mediated degradation of sarcomeric proteins. In contrast, autophagy appears to be activated in caFoxO3-expressing hearts, as evidenced by increased expression of several autophagy-related genes, increased autophagosomes, and hastened autophagy flux. These in vivo data confirm the findings of previous cardiomyocyte culture studies. Since FoxO3-mediated autophagy activation contributes to skeletal muscle atrophy, the activation of autophagy in caFoxO3-overexpressing hearts may mediate the accompanied cardiac atrophy, but this has not been established by this study.

Expression of caFoxO3a was previously shown to suppress agonist- and stretch-induced pathological hypertrophy in cultured cardiomyocytes. Interestingly, the in vitro anti-hypertrophic effects of FoxO proteins are not confirmed in the caFoxO3-expressing mouse heart. In response to transverse aortic constriction-induced pressure overload, the caFoxO3-expressing heart displayed a comparable increase in heart mass compared with controls. The impact on cardiac function in this pathological setting remains to be examined.

Inadequate protein quality control due to proteasome functional insufficiency is increasingly implicated in cardiac pathogenesis, and autophagic activation may play a compensatory role in cardiac protein quality control. To this end, creation of the inducible caFoxO3 transgenic mice provides an important tool for a better understanding of the role of autophagy in cardiac remodelling and failure.

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