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FoxO transcription factors in the maintenance of cellular homeostasis during aging

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The FoxO family of Forkhead transcription factors functions at the interface of tumor suppression, energy metabolism, and organismal longevity. FoxO factors are key downstream targets of insulin, growth factor, nutrient, and oxidative stress stimuli that coordinate a wide range of cellular outputs. FoxO-dependent cellular responses include gluconeogenesis, neuropeptide secretion, atrophy, autophagy, apoptosis, cell cycle arrest, and stress resistance. This review will discuss the roles of the mammalian FoxO family in a variety of cell types, from stem cells to mature cells, in the context of the whole organism. Given the overwhelming evidence that the FoxO factors promote longevity in invertebrates, this review will also discuss the potential role of the FoxO factors in the aging of mammalian organisms.

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A traditional view of FoxO regulation and cellular function

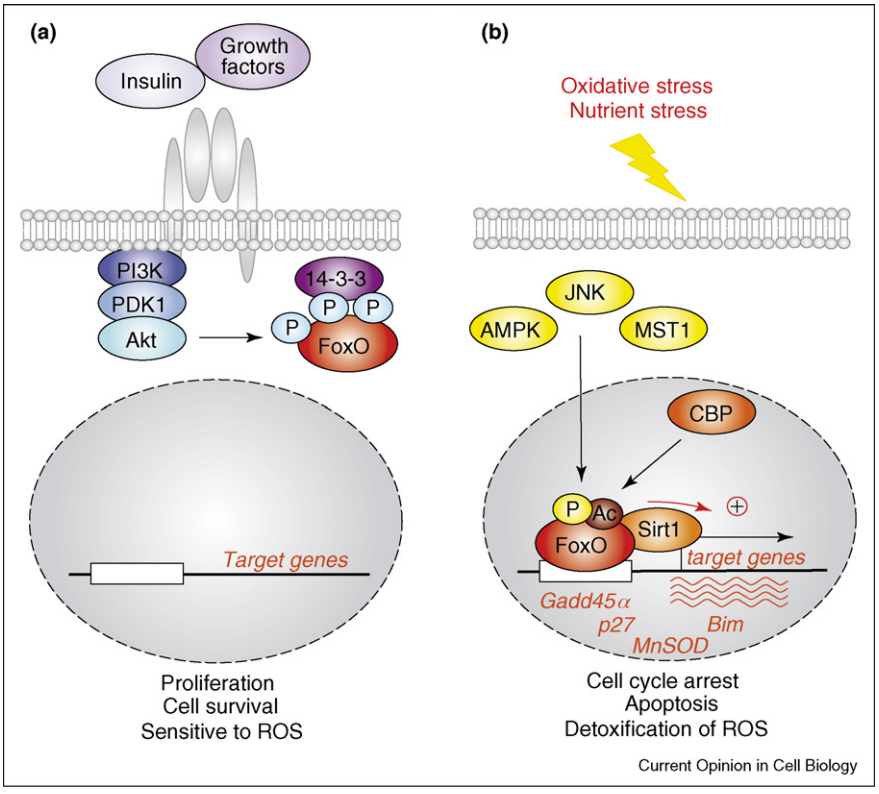
Mammals have four isoforms of the FoxO transcription factor family, FoxO1, FoxO3, FoxO4, and FoxO6. Three of the four FoxO isoforms, FoxO1, FoxO3, and FoxO4, are crucially regulated by Akt-dependent phosphorylation at three specific sites in response to growth factor and insulin stimulation (Thr32, Ser253, and Ser315 for human FoxO3) [1–4]. Akt-dependent phosphorylation of FoxO factors promotes FoxO export from the nucleus to the cytoplasm, thereby repressing FoxO transcriptional function (Figure 1A). FoxO6 lacks the C-terminal Akt-dependent site and is thus predominantly nuclear, though the phosphorylation of the two remaining Akt-dependent sites inhibits FoxO6 transcriptional activity [5,6]. FoxO factors have emerged as a convergence point of signaling in response to growth factor stimulation and oxidative stress (Figure 1) [1,7–12]. Insulin and growth factors inhibit FoxO factors through PI3K/Akt, while oxidative stress stimuli activate FoxO factors through a combination of modifications. In addition to the PI3K/Akt pathway, the other major signaling modules that directly regulate the activity of the FoxO factors include the stress-activated Jun-N-terminal kinase (JNK), the mammalian ortholog of the Ste20-like protein kinase (MST1), and the deacetylase Sirt1 (Figure 1) [9–11,13,14,15•]. The FoxO factors integrate these divergent signals through post-translational modifications, such as phosphorylation, acetylation, and mono/poly-ubiquitination, resulting in altered subcellular localization, protein stability, DNA-binding properties, and transcriptional activity [1,9,11,12,14,16]. FoxO-dependent transcription plays an important role in a wide variety of cellular outputs, including glucose metabolism, cell cycle arrest, differentiation, detoxification of reactive oxygen species (ROS), repair of damaged DNA, and apoptosis [17–26].

Accordingly, a myriad of target genes have been identified that mediate the role of FoxO factors in the various cellular processes (Table 1). The most well-defined FoxO-dependent target genes include the cell cycle inhibitors *p27* and *p21*, the stress response genes *manganese superoxide dismutase* (*MnSOD*), and *growth arrest- and DNA damage-inducible gene 45*α (*Gadd45*α), the proapoptotic factors *Bcl2-interacting mediator of cell death* (*Bim*) and *Fas ligand*, and the glycogenolytic gene *glucose-6-phosphatase* (*G6pc*) (Figure 1) [17,18,21,22,27–32]. Despite this enormous body of work, the mechanisms by which FoxO factors specify the different cellular responses, and the ensemble of cellular responses and gene targets that are FoxO-dependent have not been fully elucidated.

Recently discovered signaling pathways regulating FoxO activity

Recent studies are providing further insights into the complexity of FoxO regulatory pathways. FoxO factors have been shown to be regulated by a variety of additional stress stimuli, including DNA damage, nutrient deprivation, cytokines, and hypoxia [30,33•,34•,35•,36•,37•,38•]. For example, DNA damage affects FoxO activity via cyclin-dependent kinase 2 (CDK2) [35•]. CDK2 phosphorylates FoxO1 at Ser249, resulting in the sequestration of FoxO1 to the cytoplasm in the absence of DNA damage. In the presence of DNA damage, the repressive effect of CDK2 on FoxO1 sequestration is abolished and FoxO1 translocates to the nucleus to induce apoptosis [35•]. In addition, the energy sensor AMP-activated protein kinase (AMPK) has been shown to directly phosphorylate FoxO factors at six regulatory sites that are distinct from the Akt phosphorylation sites, resulting in FoxO activation

Figure 1



Negative regulation of FoxO transcription factors by growth factors and positive regulation of FoxO factors by oxidative and nutrient stress stimuli. **(a)** Upon stimulation with growth factors or insulin, Akt directly phosphorylates FoxO factors at three conserved residues, promoting their export from the nucleus by binding with the chaperone 14-3-3, and thus resulting in the inhibition of FoxO-dependent transcription. In the presence of growth factors or insulin FoxO-dependent transcription is inhibited promoting cellular proliferation and survival, but also rendering the cell sensitive to oxidative damage. **(b)** Oxidative and nutrient stress stimuli induce the phosphorylation, acetylation and monoubiquitination of FoxO factors at a number of regulatory sites by factors such as AMPK (AMP-dependent kinase), JNK (Jun-N-terminal kinase), MST1 (mammalian Ste20-like kinase) and CBP (CREB-binding protein). In response to oxidative stress, FoxO factors translocate to the nucleus and bind to the deacetylase Sirt1. Key modification signatures appear to recruit FoxO factors to specific genes involved in cell-cycle arrest and the response to stress. *p27*, cyclin-dependent kinase inhibitor; *MnSOD*, manganese superoxide dismutase; *Bim*, proapoptotic Bcl2-interacting mediator of cell death; *Gadd45α*, growth arrest- and DNA damage-inducible gene 45α.

[36•,37•]. Activation of FoxO factors by AMPK promotes the preferential expression of a gene expression program that enhances cellular stress resistance [36•,37•]. Although the regulation of FoxO factors is mostly conducted by post-translational modifications, a series of recent studies have highlighted how FoxO factors also integrate extracellular stimuli via alternate mechanisms. For example, the growth-inhibitory cytokine transforming growth factor-β (TGFβ) triggers the formation of a complex between FoxO, Smad, and C/EBPβ transcription factors at specific promoters, which results in the expression of cell cycle inhibitor genes, including *p15* and *p21* [30,33•,34•,39,40]. The synergy between FoxO and Smad transcription factors has been shown to be essential for the antiproliferative effects of TGFβ in several cell types, including epithelial and breast cancer cells [30,33•]. In addition, an intriguing new study shows that *FoxO3* transcription is induced by cellular hypoxia via direct binding of the hypoxia-inducible factor HIF1 to the *FoxO3* promoter [38•]. The increased expression of *FoxO3* resulted in enhanced cellular survival by attenuating HIF-induced apoptosis. These latest studies underscore the intricate regulation of the FoxO factors, by a wide range of diverse stimuli, including DNA damage, glucose availability, cytokines and hypoxia, that may serve to fine-tune FoxO activity in different cell types under different environmental contexts.

Functions of FoxO factors in the context of the whole organism: insights from invertebrates

Studies in invertebrates have shed light on the cellular role of FoxO factors in the context of the entire organism. In contrast to mammals, invertebrate model organisms possess just one isoform of the FoxO transcription factor family denoted DAF-16 for *Caenorhabditis elegans* and dFOXO for *Drosophila melanogaster*. The importance of DAF-16 in organismal metabolism and lifespan was revealed in a series of seminal studies on the insulin/FoxO pathway in worms. DAF-16 is necessary for the

Table 1

FoxO target genes and their functions

Cellular output	Gene	Function	Regulation in cells	Binding of promoter	Regulation of promoter	References
Metabolism	*G6pc*	Glycogenolysis	+	EMSA	+	[19,29,57•]
	Igfbp1	Regulates IGF activity	+	ChIP	+	[27,57•,58,59]
	Ppargc1α	Gluconeogenesis	+			[57•]
	Pck1	Gluconeogenesis	+	EMSA	+	[57•,58]
Food intake	*Agrp*	Orexigenic neuropeptide	+	ChIP; EMSA	+	[61••,62••]
	Npy	Orexigenic neuropeptide	+	ChIP; EMSA	+	[61••]
	Pomc	Anorexigenic neuropeptide	−	ChIP; EMSA	+	[61••,62••]
Atrophy	*Atrogin-1/MAFbx*	Muscle-specific ubiquitin ligase	+	EMSA	+	[66•,67•]
	MuRF1	Muscle-specific ubiquitin ligase	+			[66•]
Autophagy	*Bnip3*	Bcl2-related autophagy regulation	+	ChIP		[21,70••]
	Gabarapl1	Autophagosome formation	+	ChIP		[72••]
	LC3	Autophagosome formation	+	ChIP	+	[70••,72••]
	Atg12l	Autophagy-related gene	+	ChIP		[72••]
Apoptosis	*Bim*	Bcl2-interacting mediatior of cell death	+	ChIP	+	[15,28,75•]
	Hid	Proapoptotic	+	ChIP		[76]
	Fas ligand	Proapoptotic tumor necrosis factor ligand	+		+	[1]
Cell cycle arrest	$p27^{Kip1}$	Binds to and inhibits the cyclin E-CDK2 complex	+		+	[18,82••]
	$p21^{Cip1}$	Binds to and inhibits the cyclin E-CDK2 complex	+	ChIP	+	[30,34•]
	$p19^{INK4d}$ $p19^{Arf}$	Binds to and inhibits the cyclin D-CDK4/6 complexes	+	ChIP; EMSA	+	[32,83]
	$p15^{INK4b}$	Binds to and inhibits the cyclin D-CDK4/6 complexes	+	ChIP; EMSA	+	[32,33•,34•]
Angiogenesis	*Sprouty2*	Inhibitor of tyrosine kinase signaling	+	ChIP		[63••]
	eNOS	Endothelial function and neovascularization	−	ChIP	+	[64•]
	Ang2	Vascular remodeling factor	+			[64•]
	Cited2	CBP/p300-interacting transactivator	+	ChIP	+	[38•,63••]
Stress resistance	MnSOD	Manganese superoxide dismutase	+	ChIP	+	[20,82••]
	Gadd45α	Growth arrest-and DNA damage-inducible gene 45α	+		+	[21]

The list of target genes is not exhaustive.
Regulation in cells details whether FoxO factors promote (+) or suppress (−) target gene expression in cultured cells by the assessment of mRNA or protein levels.
Direct binding of FoxO factors to the promoter was assessed by chromatin immunoprecipitation (ChIP) or electromobility shift assay (EMSA).
Regulation of promoters was tested by the ability of FoxO factors to specifically drive luciferase expression.

increase in lifespan provided by the mutation of the insulin/insulin-like growth factor receptor *daf-2* [41–43]. DAF-16 is also necessary for a distinctive developmental arrest and diapause, called dauer, that is characterized by low metabolic activity and a long lifespan in response to starvation [43]. Interestingly, DAF-16 activity in the cells of specific tissues, particularly the intestine and nervous system, appears to be more pertinent for the promotion of long lifespan than DAF-16 activity in other tissues [44–46]. These results suggest that DAF-16/FoxO regulates the production of secondary signals or hormones that coordinate the metabolism and lifespan of a variety of tissues throughout the organism [47]. In flies, activating *dFOXO* in the cells of the fat body (the equivalent of mammalian white adipose and liver tissue) also extends lifespan [48,49]. Constitutive *dFOXO* overexpression and targeted overexpression in neurosecretory insulin-producing cells in flies increases glucose and lipid levels, though a functional dTOR signaling pathway is required to observe the metabolic effects mediated by dFOXO [50]. Thus, work from invertebrates has taught us that the insulin/FoxO pathway is crucial to coordinate metabolism and longevity, by acting in both a cell intrinsic and cell extrinsic manner via the production of secondary messengers that act systemically throughout the organism.

In mammals, FoxO isoforms display differential but overlapping expression throughout the organism

In mammals, the FoxO family have a complementary but overlapping expression pattern both during development and in a variety of adult tissues [5,51–53]. During mouse development, *FoxO1* is detected at highest levels in the adipose tissue, *FoxO3* is most expressed in the liver, *FoxO4*

in the skeletal muscle and *FoxO6* in the central nervous system. In adult mice, *FoxO1* is observed at the highest levels in adipose tissue, uterus, and ovaries, with lower levels in most other tissues including the skeletal muscle and spleen. The expression pattern of *FoxO3* is more ubiquitous but *FoxO3* is particularly highly expressed in the brain, spleen, heart, and ovaries. *FoxO4* is expressed most highly in skeletal muscle, cardiac muscle, and adipose tissue. Intriguingly, *FoxO6* is expressed almost exclusively in the adult brain. The expression of *FoxO1* and *FoxO3* in human tissues is similar to the murine expression profiles [54]. The expression pattern of the FoxO family in the adult mouse brain is particularly interesting and illustrates the differential expression of the FoxO isoforms. *FoxO1* is most abundant in the striatum, dentate gyrus and the ventral hippocampus, whereas *FoxO3* shows highest expression throughout the cortex, hippocampus and cerebellum, and *FoxO6* is expressed abundantly in the hippocampus, amygdala, and cingulate cortex [53]. The hippocampus, amygdala, and cerebellum coordinate distinct organismal behavioral responses, such as spatial learning, emotion, and motor coordination, respectively. The differential expression of the FoxO family in the adult murine brain may point to different cellular functions or potencies for the different FoxO isoforms in neurons. These observations suggest that during evolution, the FoxO pathway has been co-opted to play either: 1), related roles in response to different tissue-specific environment stimuli or 2), different tissue-specific roles in response to related environmental stimuli.

An integrative model for FoxO function?

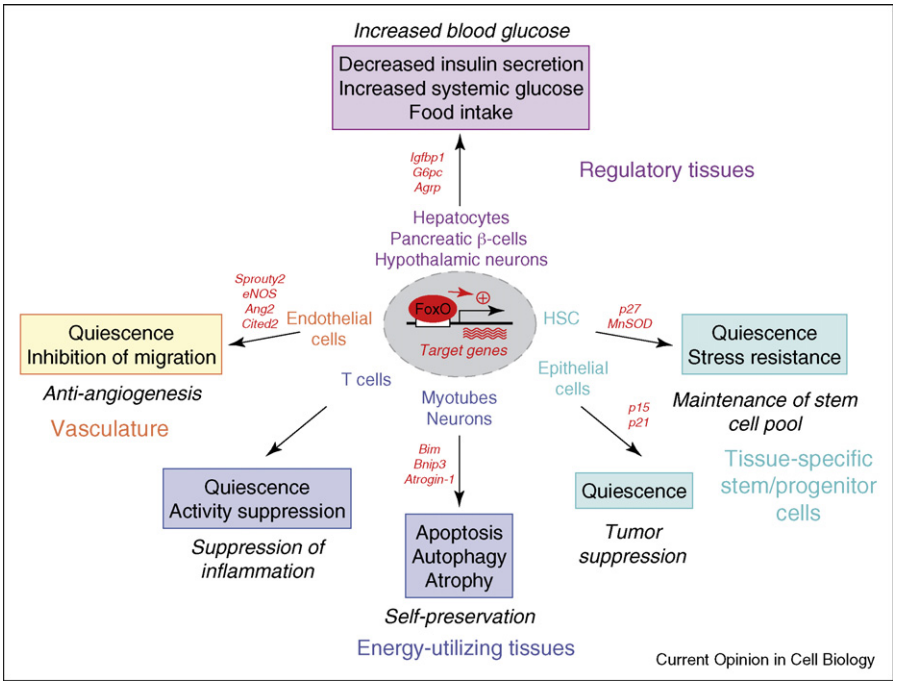
The extreme diversity of the cellular roles of the FoxO factors revealed from mammalian cell culture experiments has created a challenge to integrate these multiple roles into a unified model. In addition, many studies investigating FoxO regulation and function have been performed in immortalized or transformed cultured cell lines, which are out of the tissue context. Hence, it is difficult to extrapolate results to conditions *in vivo*, which are dependent on specialized cellular niches. Despite these limitations, it is becoming increasingly clear that the regulation of FoxO activity by specific stimuli triggers consistent cellular outputs within the same tissue. As cells and tissues differ with respect to the environmental stimuli that they sense, the remainder of this review will focus on the cellular roles of FoxO proteins in the different tissues of the body in response to tissue-specific stimuli. The classification of FoxO function and signaling by tissue provide an interesting framework to understand why the mammalian organism has evolved to utilize members of the FoxO family of factors to govern a variety of different cellular processes in response to different environmental contexts and aging.

For this review, we have divided mammalian tissues into three broad categories as a framework for our discussions: first, the 'regulatory' tissues, which include the liver, pancreas, and hypothalamus–pituitary axis; second, the vascular system that allows the connection between tissues; third, the 'energy-utilizing tissues' which include the skeletal muscle, nervous system, and hematopoietic system (Figure 2). In addition to regulating mature cells, the FoxO factors are also emerging as key regulators of stem cells in adult tissues (Figure 2). As an example, we will discuss the role of the FoxO family in the stem cells of the hematopoietic system. Given the role of FoxO factors in the longevity of invertebrates and mounting evidence for a role of the insulin signaling pathway in mammalian longevity [55,56], we will also speculate about the possible role of FoxO factors in these tissue categories to regulate mammalian lifespan.

FoxO factors increase organismal glucose levels and food intake by acting on regulatory cell types

Regulatory cells that control circulatory metabolites and hormones reside in the liver, pancreas, hypothalamus–pituitary axis, and adipose tissue. In these regulatory cells, FoxO appears to act at a number of different levels to systemically increase circulating glucose levels (Figure 2). For example, the ablation of *FoxO1* in hepatic cells reduces glucose levels in newborn and adult mice, supporting the notion that FoxO factors promote increased glucose levels in the circulation [57•]. FoxO1 regulates glucose levels by acting on target genes such as *glucose-6-phosphatase* (*G6pc*) (glycogenolytic), and *phosphoenolpyruvate carboxykinase 1* (*Pck1*), and *peroxisome proliferator-activated receptor gamma coactivator 1α* (*Ppargc1α*) (gluconeogenetic) (Table 1) [29,57•,58]. Interestingly, *FoxO1* deletion in the hepatocytes protects against excessive glucose production and diabetes in *insulin receptor* null mice [57•]. In cultured β-cells of the pancreas, modest expression of a constitutively nuclear form of FoxO1 leads to a 'metabolic diapause' status by repressing glycolysis, reducing insulin secretion, and increasing free fatty acid oxidation [59]. Expression of *FoxO1* in the β-cells of transgenic mice also prevents the compensatory proliferation of β-cells that occurs during insulin-resistance [60]. In contrast to the study performed *in vitro* [59], *FoxO1* expression *in vivo* does not decrease circulating insulin levels [60], suggesting that the FoxO factors can act at multiple levels to increase circulating glucose by either decreasing insulin secretion or by attenuating β-cell division. Thus, FoxO factors act in concert to increase systemic glucose by regulating hepatic gluconeogenesis and glycogenolysis and by reducing the net insulin production by the pancreas. These observations raise the intriguing possibility that FoxO factors would promote diabetes in mammals if they are constitutively active. It is possible that the FoxO factors have evolved to participate in the adaptation to low nutrient availability, and that the observed diabetic phenotype is an overshoot of this adaptative response when food is plentiful.

Figure 2



Role of FoxO transcription factors in tissue homeostasis. FoxO-dependent transcription serves to increase systemic glucose levels by regulating gluconeogenesis, insulin secretion, and food intake in the regulatory cells of the organism such as the hepatocytes, pancreatic β-cells, and hypothalamic neurons. In energy-utilizing cells, such as myocytes and neurons, the FoxO factors appear to be activated in response to harsh environment conditions such as starvation and oxidative stress to initiate atrophy, autophagy or apoptosis. In T cells, FoxO factors attenuate proliferation and hyperactivation. In the endothelial cells of the vascular system, the FoxO factors regulate genes to suppress proliferation and inhibit migration. FoxO factors also display a crucial role in the maintenance of hematopoietic stem cells (HSC) and epithelial progenitor cells by coordinating quiescence, stress resistance and/or terminal differentiation. Overall, FoxO factors appear to promote an organismal metabolic shutdown in response to harsh environmental conditions, such as starvation, to enable the organism to survive in anticipation of improved environmental conditions.

The hypothalamus regulates food intake and energy homeostasis. FoxO factors exert a further level of control of metabolite homeostasis by coordinating neuropeptide production in hypothalamic neurons [61••,62••]. Two independent reports have demonstrated that FoxO1 functions in the arcuate nucleus of the hypothalamus to increase food intake and bodyweight by inducing the expression of orexigenic hormones *agouti-related protein* (*Agrp*) and/or *neuropeptide Y* (*Npy*) (Figure 2). In addition, FoxO1 appears to have a suppressive effect on the expression of anorexigenic neuropeptides such as *pro-opiomelanocortin* (*Pomc*), by inhibiting the induction of this gene by the transcription factor Stat3. The anorexigenic hormones insulin and leptin decrease *FoxO1* expression in hypothalamic neurons. The suppressive effects of insulin and leptin on food intake are mediated in part by their ability to inhibit FoxO1 [61••]. These studies support the notion that in mammalian regulatory cells, the FoxO transcription factors shift metabolism away from glucose utilization toward a preservation status that is reminiscent of a 'metabolic diapause.' By acting at a number of different levels from gluconeogenesis to food intake, FoxO factors have evolved to help the organism adapt to nutrient deprivation. When food is available, FoxO transcription appears to increase organismal food intake and food seeking behaviors via hormonal control. In unfavorable nutrient conditions, FoxO factors alter organismal metabolism to enable the animal to continue functioning by maintaining systemic glucose and lipid levels.

FoxO factors restrict angiogenesis

The blood vessels of the circulatory system connect the 'regulatory' and 'energy-utilizing' tissues, and are composed of endothelial cells. Emerging evidence suggests that FoxO factors attenuate proliferation and migration of endothelial cells resulting in limited blood vessel formation (Figure 2). Acute deletion of *FoxO1*, *FoxO3*, and *FoxO4* in endothelial cells of mice using an inducible Mx-Cre transgene revealed an age-progressive overproliferation of endothelial cells that resulted in hemangiomas and premature death of the animal [63••]. Intriguingly, while *FoxO1*, *FoxO3*, and *FoxO4* deletion was achieved in endothelial cells throughout the body, the hemangiomas were observed in only a subset of tissues, particularly the uterus, liver and skeletal muscle, suggesting that specific

extracellular signals — perhaps vascular endothelial growth factor (VEGF) — also play an important role in tumor progression in the absence of FoxO factors. The factors initiating hemangioma progression in the absence of FoxO factors have not yet been characterized, but *Sprouty2*, a general receptor tyrosine kinase inhibitor, was identified as an important FoxO target gene involved in the suppression of proliferation and cellular survival [63••]. Although the ablation of three FoxO family members accentuated the vascular phenotype, *FoxO3* ablation in mice enhanced postnatal blood vessel formation in response to hind limb ischemia by abolishing the suppressive effect of FoxO3 on *eNOS* expression [64•]. FoxO1 and FoxO3, but not FoxO4, were demonstrated to bind directly to the *eNOS* promoter in cultured endothelial cells (Table 1). Silencing of *FoxO1* and *FoxO3* gene expression by siRNA in cultured endothelial cells enhanced vessel formation, migration and vessel sprouting in response to VEGF [64•]. These studies are interesting because they provide evidence of a redundant [34•,64•], but not completely overlapping role for the different isoforms of the FoxO family [63••,64•]. Indeed, while overexpression of constitutively active forms of *FoxO1* or *FoxO3* in endothelial cells inhibited vessel formation and migration, overexpression of a constitutively active form of *FoxO4* did not inhibit these processes [64•]. Furthermore, the program of genes regulated by FoxO1 and FoxO3 in endothelial cells was shown to have overlaps, but also differences [64•]. For example, both FoxO1 and FoxO3 regulated *eNOS*, but *angiopoietin 2* (*Ang2*) was exclusively regulated by FoxO1. These studies suggest that FoxO factors limit vascularization, and perhaps even restrict the supply of nutrients to tissues in response to harsh environmental stimuli, though this needs to be formally tested. The possibility that FoxO factors promote a metabolic shutdown is consistent with the observations that FoxO factors restrict vascularization in parallel to maintaining high glucose levels and encouraging food intake in the face of harsh environmental conditions. It is also intriguing that FoxO factors act both as cellular tumor suppressors and regulators of angiogenesis. This intersection provides two mechanisms whereby the FoxO family can limit the development of tumors; by a cell-intrinsic tight control of the cell cycle, as well as by extrinsically limiting the supply of the vasculature to developing tumors.

FoxO factors act to protect undamaged cells in energy-utilizing tissues in response to stress stimuli

The energy-utilizing cell types of the organism, such as those that reside in the skeletal muscle, nervous system, and immune system are responsive to the regulatory 'pacemaker' tissues such as the liver, pancreas, and hypothalamus (Figure 2). Although the entire set of stimuli that regulate FoxO in these energy-utilizing cells is not known yet, particularly for the immune system, it appears that the FoxO family becomes activated when the regulatory tissues and the vasculature are not functioning correctly or are themselves affected by the environment (e.g. during starvation). Under such unfavorable situations, these energy-utilizing cells appear to enter a self-preservation state.

Skeletal muscle

During nutrient deprivation or starvation, the skeletal muscle undergoes protein degradation induced by the activity of two highly conserved processes: first, ubiquitin-proteosomal atrophy and second, lysosomal autophagy. Autophagy is a protective cellular response to nutrient deprivation whereby lysosomal enzymes mediate the recycling of proteins, cytoplasm and cell organelles [65]. Recent studies have revealed that the FoxO factors are key mediators of both atrophy and autophagy in muscle in response to fasting, but also in response to denervation, glucocorticoids, and hind limb suspension [66•,67•,68,69,70••,71,72••]. During fasting, FoxO1 and FoxO3 induce the transcription of two muscle-specific E3 ubiquitin ligases, *atrogin-1/MAFbx* and *MuRF1*, and other components of the ubiquitin-proteasome system (e.g. *ZNF216*, a novel ubiquitin-binding protein containing a zinc-finger), resulting in skeletal muscle atrophy without apoptosis [66•,67•,68,69]. Although *atrogin-1* appears to be a direct FoxO target gene, it is not known if other components of the proteasome system, such as *ZNF216*, are direct FoxO target genes. Interestingly, the induction of *atrogin-1* initiates a positive feedback loop to attenuate cardiac hypertrophy by mediating polyubiquination of FoxO1 and FoxO3 to enhance FoxO transcriptional activity [73]. In parallel, FoxO3 has been shown to activate autophagy in response to fasting or denervation by directly controlling the transcription of autophagy-related genes including *LC3*, *Bnip3*, *Gabarapl1*, and *Atg12l* [70••,72••]. These autophagy-promoting genes were shown to be direct targets of FoxO factors in myotubes (Table 1). Interestingly, the induction of autophagy by FoxO factors does not seem to be restricted to mammalian muscle, as activating dFOXO in *Drosophila* in response to starvation also induces autophagy in the fat body [74]. The mammalian studies support the notion that FoxO factors promote an acute atrophy and autophagy in skeletal myotubes in response to starvation or inadequate trophic support by the regulatory 'pacemaker' tissues. It is not known if muscular atrophy and autophagy are beneficial to the organism, but atrophy and autophagy may be inducing an acute self-preservation state in anticipation of improved nutritional status. It is likely that prolonged stresses, such as starvation, limb immobilization, and chronic glucocorticoids, would negate the transient tissue-preservation provided by FoxO-dependent atrophy and autophagy, and perhaps lead to the induction of FoxO-dependent apoptosis or other FoxO-independent detrimental effects.

Nervous system

In the nervous system, the FoxO family appears to be activated in response to various stress stimuli, such as epileptic seizures and oxidative stress, and acts to eliminate damaged neurons by apoptosis. Epileptic brain injury in rats leads to FoxO1 and FoxO3 activation in hippocampal neurons and to the upregulation of the proapoptotic gene *Bim* leading to neuronal apoptosis [75•]. Similarly, ultraviolet damage in the *Drosophila* retinal nervous tissue induces apoptosis via dFOXO by inducing the proapoptotic gene *Hid* [76]. The induction of apoptosis by dFOXO in the fly retina requires activation of the JNK pathway, further supporting the idea that JNK activates FoxO factors [10,77]. Finally, oxidative stress induced by hydrogen peroxide treatment has been shown to promote apoptosis in rat primary cerebellar neurons by the activation of FoxO factors [15•]. The induction of neuronal apoptosis in response to oxidative stress is mediated at least in part by direct activation of FoxO factors by MST1 protein kinase, an important effector of cellular apoptosis [15•]. It is still unclear why apoptosis is the primarily cellular output to the activation of FoxO factors by environmental stressors in neurons, when FoxO factors clearly have the ability to protect other tissues, such as myotubes, by eliciting autophagy. It is possible that neurons may be more sensitive to apoptosis than other cell types. Although activation of the MST-FoxO pathway causes apoptosis in cultured neurons, the same pathway promotes longevity in *C. elegans* [15•]. Thus, the fact that FoxO factors promote apoptosis in the nervous system, and yet have the ability to elicit a long lifespan in invertebrates creates a conundrum, because apoptosis is a cellular output that is considered terminal and negative. Two explanations may account for this apparent discrepancy: first, limited apoptosis of damaged neurons may actually be beneficial to help preserve the function of the remaining nervous system or second, FoxO may need to be activated in the proper 'window' of time/magnitude, otherwise it is detrimental.

Immune system

In the immune system, FoxO transcription factors appear to act at multiple stages to limit the expansion and/or activation of the mature hematopoietic cells, though the specific stimuli that activate FoxO factors in these cells still remain to be established [78,79••]. The ablation of *FoxO3 in vivo* results in T cell proliferation and hyperactivity (e.g. elevated *IL-2* expression) because of NF-κB activation, leading to a multi-system inflammatory syndrome [80•]. This study indicates that FoxO3 normally suppresses inflammation in mammals. As the expression of certain genes associated with inflammation is increased during aging (reviewed by [81]), it is tempting to speculate that FoxO factors could also maintain tissue homeostasis by preventing uncontrolled inflammatory responses. However, more work is required to dissect the components of immune function that are beneficial during organismal maintenance versus inflammatory pathways that may accelerate aging.

FoxO factors limit the expansion and regulate the terminal differentiation of stem/precursor cells and proliferative/tumorigenic cells

In addition to their roles in mature cell types, the FoxO transcription factors also play a role to limit the expansion of stem/progenitor cells of tissues such as the hematopoietic system (Figure 2). Acute deletion of *FoxO1*, *FoxO3*, and *FoxO4* in adult murine bone marrow led to the expansion of both the myeloid and lymphoid lineages coupled with increased cell cycling of the long-term hematopoietic stem cells [79••], indicating that the FoxO family normally limits the proliferation of these stem cells. Ablation of either *FoxO3* alone, or in combination with *FoxO1* and *FoxO4* resulted in reduced maintenance of the hematopoietic stem cell pool [79••,82••], as a result of increased ROS and apoptosis, and reduced quiescence, which progressively worsened with age. Interestingly, the cell cycling and survival defects of FoxO-deficient hematopoietic stem cells could be significantly improved by treating mutant mice with the antioxidant *N*-acetyl-L-cysteine. This finding indicates that oxidative stress is a major factor regulating the proliferation and apoptosis of these stem cells, and also that chemical interventions can recover at least part of the cellular defect. A consequence of the ablation of FoxO factors in hematopoietic cells is increased cell cycle progression and/or resistance to apoptosis leading to lymphoma development via attenuation of *p27* and *p19* expression [63••,83]. In line with these studies, the loss of FoxO transcriptional activity renders progenitor cells or tumor cells resistant to the cytostatic effects of extracellular effectors such as TGFβ, which may contribute to tumor development (Figure 2) [30,33•,34•,84,85]. FoxO transcriptional activity is attenuated in progenitor and tumor cells by a variety of mechanisms [30,84,85], including: first, nuclear exclusion upon phosphorylation of FoxO factors by the PI3K/Akt signaling pathway; second, association of FoxO factors with the transcriptional repressor FoxG; third, ubiquitin-dependent degradation upon phosphorylation of FoxO factors by IκB kinase β (IKKβ) or extracellular signal-regulated kinase (ERK). Together, these studies frame the FoxO factors as pivotal suppressors of tumorigenesis by coordinating cellular quiescence in stem/progenitor cells. These findings also highlight the importance of the FoxO family in maintaining stem cell pools in adult tissues. Consistent with this possibility, ablation of *FoxO3* in female mice leads to the early depletion of the follicular pool and premature infertility [86]. The role of the FoxO family in other tissue-specific stem cells is not known yet, but an intriguing possibility is that the FoxO family may contribute to tissue maintenance and repair during aging by maintaining adult stem cell pools.

Conclusion

Although the regulation and roles of the FoxO family have been well studied, there is still a dearth of knowledge on the mechanisms that specify the decision between different cellular outputs in response to different environmental contexts for these promiscuous transcription factors. Similarly, it is not clear why four FoxO isoforms exist, though there is now evidence to suggest that their roles are not entirely overlapping [63••,64•]. A model that emerges is that different FoxO isoforms bind to the promoters of target genes with different affinities, perhaps because of differences in structure and post-translational modifications between isoforms resulting in opportunities for differential cellular outputs. Understanding if FoxO factors regulate other cellular responses in addition to those mentioned in this review and elucidating how FoxO factors specify at the molecular level their response to a multitude of environmental stimuli will provide important insights into their organismal function.

Given the evidence discussed here that the FoxO factors coordinate glucose homeostasis, angiogenesis, stem cell maintenance, immune, muscular, and neuronal functions, the implications for diabetes, cancer, autoimmune diseases, and neurodegeneration are apparent. Finally, there is overwhelming evidence to support a significant role for FoxO factors in the regulation of invertebrate lifespan, though further studies *in vivo* are required to make conclusions regarding the role of FoxO factors in mammalian longevity. Understanding how FoxO factor cellular functions are integrated into cohesive organismal responses will be significant for therapeutic interventions against age-dependent pathologies such as diabetes, cancer, autoimmune syndromes, and neurodegeneration.

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