OutFOXOing disease and disability: the therapeutic potential of targeting FoxO proteins

Kenneth Maiese1,2,3,4,5, Zhao Zhong Chong1, and Yan Chen Shang1
1 Division of Cellular and Molecular Cerebral Ischemia, Wayne State University School of Medicine, Detroit, MI 48201, USA
2 Departments of Neurology and Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201, USA
3 Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, MI 48201, USA
4 Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201, USA
5 Institute of Environmental Health Sciences, Wayne State University School of Medicine, Detroit, MI 48201, USA

Abstract

Forkhead transcription factors have a ‘winged helix’ domain and regulate processes that range from cell longevity to cell death. Of the mammalian forkhead family members in the O class, FoxO1, FoxO3a and FoxO4 can fill a crucial void for the treatment of disorders that include aging, cancer, diabetes, infertility, neurodegeneration and immune system dysfunction. Yet, observations that forkhead family members also can compromise clinical utility have fueled controversy and highlight the necessity to further outline the integrated cellular pathways governed by these transcription factors. Here we discuss recent advances that have elucidated the unique cellular pathways and clinical potential of targeting FoxO proteins to develop novel therapeutic strategies and avert potential pitfalls that might be closely intertwined with its benefits for patient care.

Introduction

Since the initial discovery of the fly Drosophila melanogaster gene forkhead, more than 100 forkhead genes and 19 human subgroups are known to exist that range from FOXA to FOXS with a current nomenclature replacing prior terms [1,2] (Box 1). Mammalian FoxO proteins are assigned to the O class of the forkhead transcription super-family and consist of the members FoxO1, FoxO3, FoxO4 and FoxO6. FoxO6 is the most recently cloned FoxO family member with demonstrated expression in the mouse brain [3]. Yet, focus upon FoxO1, FoxO3 and FoxO4 has revealed that these proteins are involved in processes ranging from cell death to cell longevity. Interestingly, FoxO proteins are homologous to the transcription factor abnormal DAuer Formation-16 (DAF-16) in the worm Caenorhabditis elegans that can determine metabolic insulin signaling and lead to lifespan extension [2,4-6]. Subsequent studies have shown that metabolic signaling with FoxO proteins is conserved among multiple species including Caenorhabditis elegans, Drosophila melanogaster and mammals, which suggests the broad impact FoxO proteins can impart upon mammalian cell function. In this
paper, we will discuss the role of the mammalian FoxO family members for several disease entities, including aging, cancer, diabetes, infertility, neurodegeneration and immune system dysfunction, and highlight the potential development of new therapeutic avenues through the targeting of FoxO proteins.

The structure and expression of FoxO proteins

As transcription factors, FoxO proteins must bind to DNA to either activate or repress target gene expression. The forkhead box (FOX) family of genes is characterized by a conserved forkhead domain termed the ‘forkhead box’ or a ‘winged helix’ as a result of the butterfly-like appearance on X-ray crystallography and nuclear magnetic resonance [2]. Although not all winged helix domains can be identified as Fox proteins, the forkhead domain in FoxO proteins consists of three α-helices, three β-sheets and two loops that are referred to as the wings [2, 7]. Variations in this structure exist that consist of absent β-sheets, absent loops, or additional α-helices, but high sequence homology is present in the α-helices and β-sheets. For FoxO transcription factors, fourteen protein–DNA contacts occur in the forkhead domain with the primary recognition site located at α-helix H3. Both the first and second loops make contact with DNA, but it is the second loop that can enhance the specificity and stability of the binding. Furthermore, FoxO proteins preferentially bind DNA to the FoxO-recognized element with the consensus sequence T/C-G/A-A-A-A-C-A-A [8,9]. However, it should be noted that the mechanisms that lead to DNA binding with FoxO proteins are not completely defined and might depend upon other factors, such as variations in the N-terminal region of the recognition helix, changes in electrostatic distribution and the abilities of FoxO proteins, which can be controlled by the C-terminal region of the forkhead domain to be shuttled to the cell nucleus [10]. Thus, the forkhead domain and its ultimate structure can determine the ability of specific FoxO proteins to increase or repress target gene expression.

FoxO proteins are expressed in a variety of tissues that exemplify the robust ability of these proteins to influence normal physiology as well as during progressive disease. Initially, FOXO1, FOXO3a and FOXO4 were identified in fusion genes from chromosomal translocations in human soft-tissue tumors and leukemias. FOXO1, also termed forkhead in rhabdomyosarcoma (FKHR), and FOXO3a, also known as forkhead in rhabdomyosarcoma like protein 1 (FKHRL1), and their genes were identified through chromosomal translocations in alveolar rhabdomyosarcoma tumors [2]. The FOXO4 gene, also known as acute leukemia fusion gene located in chromosome X (AFX) was identified as a gene that fused to mixed-lineage leukemia (MLL) transcription factor as a result of the t(X;11) chromosomal translocation in acute lymphoblastic leukemia [4]. In addition, a fusion between FoxO2 and MLL occurs in some cases of acute myeloid leukemia that encodes a protein that is similar to the forkhead family members FoxO1 and FoxO4 [4].

Box 1

**Nomenclature**

- A current nomenclature has replaced prior terms, such as forkhead in rhabdomyosarcoma (FKHR), the *Drosophila* gene forkhead (fkh) and forkhead related activator (FREAC)-1 and -2.
- Within the subclasses of the Fox proteins that are each designated by a letter, an Arabic number is provided such that the actual name of a Fox protein would follow the designation of ‘Fox’ then a subclass or subgroup ‘Letter’ is provided and, finally, the member ‘Number’ is listed.
In relation to human Fox proteins, all letters are capitalized (i.e. FOXO3a), otherwise only the first and subgroup letters are listed as uppercase (i.e FoxO3a).

With subsequent studies, FoxO protein expression has been described in the ovary, prostate, skeletal muscle, brain, heart, lung, liver, pancreas, spleen, thymus and testis [2] (Table 1). For example, in mouse embryos and adults, mRNA expression of FoxO1, FoxO3a and FoxO4 were considered to be complementary and highest in muscle, adipose tissue and liver, but FoxO3a had a greater distribution in the heart, brain and kidney than FoxO1 [11]. Other studies also illustrate that FoxO proteins can be varied in their distribution in the mouse brain, which suggests independent physiological functions for various FoxO proteins. FoxO3 was expressed throughout the brain including cognitive and motor regions, such as the hippocampus, cortex and cerebellum. By contrast, FoxO1 was localized to pathways that can determine motor function in the striatum and subregions of the hippocampus. FoxO6 also was found in the hippocampus, but extended further to regions that involve the amygdala and nucleus accumbens, regions of the brain that might have a role in emotions that involve pleasure, fear and addiction [3]. As a result, knowledge of the tissue expression for mammalian FoxO proteins can provide further insight into their function throughout the body during both homeostatic function and disease processes.

The role of FoxO proteins in stem cells and functional development

In many respects, our knowledge of the FoxO protein gene targets is in its infancy and continues to grow as new work demonstrates that mammalian forkhead transcription factors have both independent and redundant roles in development. On the surface, FoxO3a−/− and FoxO4−/− mice develop without incidence and are largely indistinguishable from control littermates with similar weight gain [12]. However, mice deficient in FoxO1 die by embryonic day 10 or 11 and lack vascular system development [12]. Upon closer inspection, FoxO3a−/− null animals experience several developmental abnormalities that were not present in mice deficient for FoxO4. FoxO3a−/− null mice become infertile and experience follicular activation to the extent that ovarian follicles are depleted with the subsequent death of oocytes [13], which suggests a specific role for FoxO3a in relation to oocyte and follicular development (Table 2). Additional work using a mouse model of FoxO3a overexpression in oocytes further suggests that FoxO3a retards oocyte growth and follicular development and leads to anovulation and luteinization of unruptured follicles [14].

FoxO proteins appear to function as crucial components for the development of the immune and hematopoietic systems. In mice deficient for FoxO3a, lymphoproliferation, organ inflammation of the salivary glands, lung and kidney and increased activity of helper T cells results, further supporting an important role for at least FoxO3a in preventing T-cell hyperactivity [15]. FoxO3a also appears to be necessary for neutrophil activity, because FoxO3a−/− null mice are resistant to models of neutrophilic inflammation that involve immune complex-mediated inflammatory arthritis and thioglycollate-induced peritonitis [16].

In regards to progenitor cell expansion and differentiation, FoxO proteins are involved in the maintenance of erythroid, neuronal and hematopoietic stem cells. Simultaneous deletion of FoxO1, FoxO3a and FoxO4 in mice led to the defective repopulation of hematopoietic stem cells with resultant apoptosis [17]. However, other studies suggest that FoxO3a alone can play a role in maintaining hematopoietic stem cells, because these cells are significantly decreased in aged FoxO3−/− mice compared with the littermate controls [18]. Several cellular factors might determine whether FoxO proteins function in concert or independently to progenitor cell growth. For example, the ability of the growth factor and cytokine erythropoietin (EPO) to foster erythroid progenitor cell development is dependent upon the inhibition of FoxO3a activity [19,20], but also might require regulation of specific gene expression through an EPO–FoxO3a

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association to promote erythropoiesis in cultured cells [21]. In addition, rat enteric nervous system precursor development that occurs in the presence of the growth factor glial cell line-derived neurotrophic factor seems to require the inactivation of FoxO1 and FoxO3a [22]. These studies highlight the ability of FoxO proteins to regulate functional development and stem cell proliferation. More importantly, these studies point to the need for further understanding of the potential functional overlap of FoxO proteins that could determine whether FoxO proteins will function independently or in concert to exert their biological effects.

The multitasks of FoxO proteins in cellular metabolism, function and disease

**FoxO, diabetes and cellular metabolism**

FoxO proteins are involved in several pathways responsible for cell metabolism, onset of diabetes mellitus (DM) and diabetic complications (Table 2). DM is a significant health concern and affects ~16 million individuals in the United States and more than 165 million individuals worldwide [23,24]. Experimental investigations have suggested that FoxO3a might be beneficial during elevated glucose exposure and DM. Interferon-gamma driven expression of tryptophan catabolism by cytotoxic T lymphocyte antigen 4 might activate FoxO3a to protect dendritic cells from injury in nonobese diabetic mice [25]. In addition, loss of FoxO1 in the liver of mice leads to impaired glycogenolysis and gluconeogenesis, which suggests an important role for FoxO1 to regulate glucose production [26]. Yet, the role of forkhead transcription factors can vary among different cell types and tissues. For example, additional investigations have associated diabetic nephropathy to post-translational changes in FoxO3a by demonstrating that phosphorylation of FoxO3a increases in rat and mouse renal cortical tissues two weeks after the induction of diabetes by streptozotocin [27]. Furthermore, elevated glucose levels can be cytotoxic to cells [28] and enteric neurons can be protected from hyperglycemia by glial cell line-derived neurotrophic factor that can affect protein kinase B (Akt) signaling and prevent FoxO3a activation [29]. Interestingly, the ability of Akt to also inhibit pyruvate dehydrogenase kinase-4 expression, a protein that conserves gluconeogenic substrates during DM, requires the inhibition of FoxO3a activity [30]. In addition, the human immunodeficiency virus (HIV)-1 accessory protein Vpr has been reported in human hepatoma cells to contribute to insulin resistance by interfering with FoxO3a signaling with protein 14–3-3 [31].

FoxO proteins also are closely linked to the prevention of diabetic complications through the preservation of cellular energy reserves and mitochondrial integrity [32]. In caloric restricted mice that experience decreased energy reserves, mRNA expression was progressively increased for FoxO1, FoxO3a and FoxO4 over two years with either a 30% reduction in diet or reduced feeding schedule [33]. This work is complementary to subsequent studies that demonstrate in *Drosophila* and mammalian cells that upregulation of FoxO1 expression leads to increased insulin signaling to regulate cellular metabolism [34]. However, the role of FoxO proteins to maintain cellular energy reserves is not entirely clear, because additional studies such as with FoxO1 have shown that overexpression of this transcription factor in skeletal muscles of mice can lead to reduced skeletal muscle mass and poor glycemic control [35]. Therefore, FoxO proteins can play a vital role during disorders of cellular metabolism, but it is clear that several parameters such as cell and tissue type or duration of activity can ultimately determine whether specific FoxO proteins preserve or impair metabolic regulation.

**FoxO, oxidative stress, apoptosis and longevity**

FoxO proteins interface with several pathways that regulate cellular apoptosis, senescence and lifespan during oxidative stress. The release of reactive oxygen species (ROS) during oxidative stress can contribute to a variety of disease states such as diabetes, ischemia, Alzheimer’s disease and stroke [1]. In regards to chronic neurodegenerative disorders such as Alzheimer’s
disease, amyloid is toxic in cell culture [36,37] and is associated with the phosphorylation of FoxO1 and FoxO3a that could be blocked with ROS scavengers [38]. Interestingly, a common denominator in the pathways linked to amyloid toxicity might be β-catenin. β-catenin might regulate FoxO protein function [39] and has been demonstrated to be necessary for protection against amyloid toxicity in neuronal cells [37]. Furthermore, in some cellular populations such as mouse hematopoietic stem cells, the conditional deletion of FoxO1, FoxO3a and FoxO4 can lead to an increase in ROS [17]. However, it appears that FoxO proteins, such as FoxO1 and FoxO3a, must be present for oxidative stress to result in apoptotic cell injury [40]. This observation correlates well with other cell culture and animal studies of oxidative stress that demonstrate that protein inhibition or gene knockdown of FoxO1 or FoxO3a results in stroke reduction by estradiol [41], mediates the protective effects of metabotropic glutamate receptors [42], enhances neuronal survival through NAD+ precursors [43] and provides trophic factor protection with EPO [44] and neurotrophins [45].

As exemplified by FoxO3a, FoxO proteins are intimately involved with apoptotic cellular injury. FoxO3a in conjunction with c-Jun N-terminal kinase (JNK) has been shown to modulate an apoptotic ligand activating a Fas-mediated death pathway in cultured motoneurons [46], to lead to apoptosis through tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and BH3-only proteins Noxa and Bim in neuroblastoma cells [47] and to promote proapoptotic activity of p53 [48]. These pathways for FoxO3a can result in cytochrome c release and caspase-induced apoptotic death [42-44,47] as well as offer a unique regulatory mechanism for the FoxO3a protein [42-44].

The subsequent induction of apoptotic cell injury by FoxO proteins can initially require the negative regulation of cell cycle progression. During periods of oxidative stress, FoxO3a and FoxO4 promote cell cycle arrest in mouse myoblastic cell lines through modulation of growth-arrest and DNA-damage-response protein 45 (Gadd45) [49]. FoxO3a activation in a colon carcinoma cell line also prevents cellular proliferation through Myc target genes that involve the Mad/Mxd family of transcriptional repressors [50]. In addition, treatment of chronic myelogenous leukemia cell lines with the Bcr-Abl tyrosine kinase inhibitor imatinib requires FoxO3a activation to antagonize cell proliferation and promote apoptotic cell death through increased TRAIL production [51]. By contrast, loss of FoxO3a activity in association with c-Myc, p27 and nuclear factor-κB (NF-κB) can result in cell cycle induction and malignant transformation of mouse cells in the presence of oncogene activation [52]. However, the transition from cell cycle arrest to apoptotic cell injury is not well defined and requires further investigation. Under some conditions, FoxO3a activation in association with the silent information regulator 2 (Sir2) homolog, SIRT1, can lead to cell cycle arrest, but not result in apoptotic death during oxidative stress [53].

Inflammatory cell modulation also has a significant impact upon cellular apoptosis [54,55] and studies of immune mediated diseases have elucidated several cellular targets for Fox proteins in this regard [15]. Prevention of inflammatory activation and apoptosis in the nervous system such as in systemic lupus erythematosus in animal models might require the upregulation of FoxJ1 and FoxO3a that can block NF-κB activation and interferon-gamma secretion [56]. Furthermore, animal studies using experimental autoimmune encephalomyelitis to mimic multiple sclerosis and myelin injury have shown that osteopontin, a protein expressed in multiple sclerosis lesions, leads to the prolonged survival of myelin-reactive T cells and disease progression through a combination of events that involve FoxO3a inhibition, NF-κB activation and the expression of the proapoptotic proteins Bim, Bak and Bax [57].

FoxO proteins interact with several pathways that regulate cellular lifespan and aging as demonstrated by early studies linking DAF-16 in Caenorhabditis elgans to increased longevity [58,59]. Increased cellular lifespan in yeast is dependent upon the Sir2 protein. Prior work has
shown that stimulation of the NAD+-dependent deacetylase SIRT1, a mammalian ortholog of Sir2, in mammalian cells during starvation is dependent upon FoxO3a as well as p53 [60]. Yet, the relationship between SIRT1 and FoxO proteins is not entirely clear at present. Additional work has shown in cell culture that SIRT1 might repress the activity of FoxO1, FoxO3a and FoxO4, which suggests that cellular longevity could benefit from reduction in FoxO protein generated apoptosis [61]. Other experimental studies provide alternative views to illustrate that SIRT1 binds to FoxO proteins, such as FoxO4, to catalyze its deacetylation and enhance FoxO4 activity whereas acetylation of FoxO4 by cyclic-AMP responsive element binding (CREB)-binding protein serves to inhibit FoxO4 transcriptional activity [62,63]. As an extension of these studies, FoxO proteins also have been linked to cell aging and senescence. In cultured human dermal fibroblasts, gene silencing of FoxO3a protein resulted in cell morphology consistent with senescence, cell population doubling times and the generation of ROS, which suggests that FoxO protein activity could be required to extend cell longevity [64]. In fact, recent work in animal models of aging demonstrates a reduction in SIRT1 activity in the heart, but no significant change in FoxO3a expression with advanced age. However, during exercise training, an upregulation of FoxO3a and SIRT1 activity was observed in the heart [65], which suggests a broader impact for FoxO3a during normal physiology and during disabilities that are associated with aging in the nervous and cardiovascular systems. In addition, FoxO proteins might be protective during aging, because loss of FoxO3a activity in explanted vascular smooth muscle of aged animals might limit tissue antioxidant properties through decreased manganese-superoxide dismutase (MnSOD) and lead to enhanced cell injury with aging [66]. Furthermore, extension of cellular lifespan at least in primary human cultured vascular cells might require the negative regulation of Akt to allow for FoxO3a activity and block cellular senescence [67]. Thus, these studies serve to illustrate not only the complex nature of FoxO proteins during conditions such as oxidative stress to function as either positive or negative modulators of cellular function, but also the fine cellular balance that exists between cellular apoptosis and cellular longevity.

**FoxO and cancer**

The antiproliferative and proapoptotic effects of FoxO proteins make these transcription factors almost ideal therapeutic targets to control unchecked neoplastic growth (Table 2). For example, somatic deletion of FoxO1, FoxO3a and FoxO4 in mice resulted in the growth of thymic lymphomas and hemangiomas, which illustrates the potential of FoxO proteins to function as repressors of tumor growth [68]. Studies with breast cancer parallel this work and show that increased activity of FoxO3a in association with JNK in breast cancer cell lines [69] or in association with cyclin-dependent kinase inhibitor p27 in isolated human breast cancer cells can suppress breast cancer progression [70].

Early studies with prostate cancer observed that the tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome ten) was mutated in almost eighty percent of tumors with the loss of FOXO1 and FOXO3a activity. These observations were followed by subsequent work to show that overexpression of FoxO1 and FoxO3a in prostate tumor cell lines could result in apoptosis, which suggests that FoxO1 and FoxO3a were necessary for the control of prostate cell tumor growth [71]. Complementary studies have further supported the tumor repressive role of FoxO proteins in prostate cancer. Suppression of FoxO3a activity can result in enhanced prostate tumor cell growth [72], whereas agents that increase FoxO3a activity in both androgen sensitive and androgen insensitive prostate cell lines prevent prostate cancer cell progression [73]. However, it has been shown in prostate cell lines that FoxO3a is a positive regulator of androgen receptor expression and therefore might play a complex role in prostate cancer cell proliferation and growth inhibition [74].
FoxO proteins also might play a significant role in other cancers such as ovarian, colonic and hematopoietic cancers. FoxO3a activation can inhibit cell cycle progression and proliferation of tumor growth in colon carcinoma cell lines [50]. Several studies also suggest that the loss of FoxO3a activity in primary leukemic cells might participate in oncogenic transformation in B-chronic lymphocytic leukemia [75], in the progression of chronic myelogenous leukemia cell lines [51] and in the transformation of cells into Kaposi's sarcoma [76]. Interestingly, loss of functional FoxO3a in human ovarian cancer cell lines can limit the sensitivity of ovarian cancer cells to chemotherapy such as cisplatin [77], which suggests that FoxO proteins might be responsible for altered treatment outcomes in the presence of combined therapeutic approaches.

**FoxO and cellular mechanisms of regulation**

Post-translational modification of FoxO proteins becomes essential to modulate these processes and relies upon biochemical pathways associated with phosphorylation, acetylation and ubiquitylation [2,4-6] (Figure 1). In relation to the inhibition of FoxO protein activity, the serine-threonine kinase Akt is a primary mediator of phosphorylation of FoxO1, FoxO3a and FoxO4 and involved in pathways of cytoprotection [78,79]. Cellular apoptosis can be prevented during the phosphorylation of FoxO proteins by Akt to sequester FoxO transcription factors in the cytoplasm by association with 14–3-3 proteins [20,44] and prevent the transcription of proapoptotic target genes. The exception to this rule involves FoxO6, which under most conditions resides in the nucleus of cells and is phosphorylated by Akt in the nucleus [10].

Other post-translational mechanisms exist that might or might not be independent from Akt to regulate FoxO proteins. Akt phosphorylation of FoxO proteins not only controls intracellular trafficking with retention in the cytoplasm, but also leads to ubiquitination and degradation through the 26S proteosome [2,4-6]. In the absence of Akt, IκB (inhibitor of NF-κB) kinase (IKK) also can directly phosphorylate and block the activity of FoxO3a. This leads to the proteolysis of FoxO3a by way of the ubiquitin-dependent proteasome pathway [80]. Given that FoxO3a has been shown to be a substrate for caspase 3-like proteases, modulation of caspase 3 activity also might offer a unique regulatory mechanism that blocks the proteolytic degradation of FoxO3a that can yield proapoptotic N-terminal fragments [42,43]. As a close homolog to Akt, the serum- and glucocorticoid-inducible protein kinase (Sgk) also can phosphorylate and retain FoxO3a in the cytoplasm [81]. Knowledge that Sgk and Akt preferentially phosphorylate FoxO3a at different sites might offer new opportunities to target FoxO3a and enhance clinical efficacy. However, phosphorylation of FoxO proteins does not always lead to negative regulation. The protein kinase mammalian sterile 20-like kinase-1 (MST1) can phosphorylate FoxO proteins directly and lead to their activation [82]. As an additional post-translational modification, mammalian FoxO proteins are acetylated by histone acetyltransferases that include p300, the CREB-binding protein (CBP) and the CBP-associated factor and are deacetylated by histone deacetylases, such as SIRT1 [2,4-6]. As a result, multiple cellular pathways can converge upon FoxO proteins and provide another dimension to further explore for the post-translational regulation of these transcription factors during normal physiology and disease.

**Progress and potential of targeting FoxO proteins**

Given the integral ability of FoxO proteins to control cell proliferation, cell metabolism and cell survival, it is evident that mammalian forkhead family members are considered to be an extremely attractive therapeutic target for multiple disorders. For example, in a small percentage of women who suffer from premature ovarian failure (POF), mutations in FOXO3a and FOXO1a have been observed [83]. Although the mutations identified are presently considered to represent one of multiple factors responsible for POF, further analysis in larger populations of patients with POF combined with functional studies could increase our
understanding of the role of FoxO proteins in disorders of human fertility. In relation to immune system function, recent work has demonstrated in synovial biopsy tissue of patients with rheumatoid arthritis and osteoarthritis the phosphorylation of FOXO1 in macrophages, FOXO3a in T lymphocytes and FOXO4 in macrophages, which suggests that inhibitory post-translational phosphorylation of these FOXP family members might lead to inflammatory cell activation [84]. Other complementary clinical studies have shown that FOXO1 gene transcript levels were downregulated in peripheral blood mononuclear cell of patients with systemic lupus erythematosus and rheumatoid arthritis [85], which illustrates a potential etiology for these disorders and possibly providing a biomarker of disease activity.

Clinical studies also suggest that FoxO proteins play a significant role in DM, oxidative stress, immune system function and cancer (Table 2). In a study of 734 individuals, the c.-343–1582C>T polymorphism of FOXO3a displayed a significant association with body mass index such that the highest body mass index was present in individuals homozygous for this allele [86]. Analysis of the genetic variance in FOXO1a and FOXO3a on metabolic profiles, age-related diseases, fertility, fecundity and mortality have observed higher HbA1c levels and increased mortality risk associated with specific haplotypes of FOXO1a. In addition, there was an increased risk of stroke in two haplotypes of FOXO3a block-A, which suggests an association with cerebral oxidative stress disorders such as diabetes and stroke with FOXO1a and FOXO3a [87]. Clinical studies also suggest a relationship between the regulation of immune system activity and the induction of apoptotic pathways that are dependent upon FoxO proteins. Although it was noted that further studies are required to better define the role of FOXO3a, this FoxO protein might work in concert with Fas signaling to clear activated T cells following a decrease in cytokine stimulation in patients with autoimmune lymphoproliferative syndromes, which suggests that specific FoxO proteins might be targeted for treatment of autoimmune disorders [88]. One of the most interesting therapeutic applications for FOXP proteins involves strategies directed against human cancer. Early studies of breast cancer in relation to FOXO3a suggested that activation of FOXO3a was associated with lymph nodal metastasis and a poor prognosis [89]. However, other studies reported that FOXO3a was confined to the cytoplasm of human tumor cells, inactivated by IKK and was associated with a poor prognosis in breast cancer [80], which suggests that FOXO3a subcellular localization and pathways that enhance its activity could be used not only as prognostic assays but also as therapeutic targets. In fact, use of a triple mutant FoxO3a in which three phosphorylation sites have been altered to prevent phosphorylation of this protein has been proposed as a potential therapeutic target against melanoma tumors [90]. Yet, the causal relationship between FOXO proteins and DM, oxidative stress, immune system function and cancer are not entirely clear and can vary among cell and tissue types. Further studies are warranted to assess variations in FOXO protein activity and the functional significance of FOXO proteins during these disorders (Table 3).

Perspectives, considerations and concluding remarks for FoxO proteins

The range of applicable disease processes that can be affected by aberrant FoxO protein function appears to be limitless and might arise from the observation that FoxO proteins can both enhance as well as repress gene function in processes that involve cell apoptosis, cell cycle regulation and cell senescence. As a result, it must be recognized that simplistic recipes to modulate FoxO proteins can become a double-edge sword to yield both beneficial and detrimental biological outcomes (Table 3). For example, under some conditions, FoxO proteins might prevent cell cycle progression in cells without leading to apoptotic injury. Although such an outcome might be considered beneficial to block degenerative disorders, in the setting of cancer, such results would severely limit clinical utility. Furthermore, FoxO transcription factors can foster apoptosis in prostate cancer cells, but also might contribute to androgen receptor expression and potentially diminish any clinical benefits.
Given the considerations to target FoxO proteins in the development of novel therapeutic strategies, it becomes essential to further both basic as well as clinical research to overcome the present challenges of existing or developing therapies. Although the current knowledge of FoxO proteins continues to expand at an almost exponential rate, several lines of investigation could be especially fruitful to consider for the development of therapeutic strategies (Box 2). Further cellular, animal and clinical studies are required to determine the links between tissue expression and biological function of FoxO proteins. For example, FoxO proteins are expressed throughout the brain and particularly in sensitive cognitive and motor regions, but it is unclear how FoxO transcription factors can influence neuronal plasticity, cerebral vascular function and glial cell function and whether changes in cellular function are tied to previously unrealized gene targets for FoxO proteins. Studies also are required to determine whether the function of FoxO proteins change from development to maturation of an organism, because current animal studies and clinical studies usually offer a ‘snap-shot’ approach of FoxO protein expression that can limit the ability to form robust conclusions. Continued studies are required to elucidate unrecognized signal transduction pathways that can directly influence FoxO protein intracellular trafficking and gene transcription. Such studies might be particularly important in relation to disorders that rely upon the application of several adjuvant or combinational therapies. FoxO protein transcriptional activity might sometimes assist the treatment of neoplastic growth, but the involvement of FoxO proteins in drug resistance is not entirely clear and might rely upon currently unknown factors, such as the modulation of FoxO post-translational activity, interaction among various FoxO members and the ability of FoxO proteins to influence endocrine pathways, that could limit therapeutic potential. Prediction of biological effects of FoxO proteins also is difficult and might be influenced by several factors that include tissue localization, age of the organism and metabolic state, advocating the broader analysis of FoxO proteins during both homeostatic conditions and exposure to cellular stress. As the pursuit of new investigations continue to divulge the advantages of FoxO proteins, fertile knowledge for the clinical application of these mammalian forkhead family members should unfold at a surprisingly fast pace.

**Box 2**

**Outstanding questions**

- FoxO proteins are expressed in multiple tissues and organs that include the brain in cognitive and motor regions involving the hippocampus, cortex and cerebellum. What is the functional significance of FoxO protein expression in tissues and how is function altered in stages from development to maturation?
- What are the cellular pathways that determine whether FoxO proteins will have functional overlap during normal physiology and disease?
- Which cellular conditions and environmental stimuli can trigger FoxO proteins to either enhance cellular longevity or impair metabolic regulation?
- Independent of the currently known mechanisms to impart post-translation regulation upon FoxO proteins such as Akt, acetylation, ubiquitination and intracellular trafficking, what are the alternate modulatory pathways that can directly alter FoxO protein activity and potentially become a therapeutic target?
- How can FoxO proteins be targeted to not only enhance treatment efficacy with chemotherapeutic agents in cancer, but also reduce or eliminate drug insensitivity?
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References


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Figure 1.
FoxO proteins are integrated in multiple signal transduction pathways that govern cell longevity, metabolism, survival and tumorigenesis. During stimuli such as oxidative stress, FoxO can be linked to several pathways that directly modulate FoxO activity and its phosphorylation status (p-FoxO), such as by protein kinase B (Akt), or that require FoxO to ultimately determine several cellular processes such as cell cycle regulation, apoptotic injury and malignancy. These interconnected pathways involve IκB kinase (IKK), IκB, protein 14–3-3, growth-arrest and DNA-damage-response protein 45 (Gadd45), Fas ligand (Fas L), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), the BH3-only proteins Noxa and Bim, p53, c-myc, p27, mitochondrial membrane potential (Mito), cytochrome c (Cyto-c) and caspases.
### Table 1

Examples of FoxO protein expression

<table>
<thead>
<tr>
<th>Organ</th>
<th>Location within organ</th>
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<tbody>
<tr>
<td>Brain</td>
<td>Cerebellum, cortex, hippocampus</td>
</tr>
<tr>
<td>Immune and hematopoietic systems</td>
<td>Intestinal mucosa, megakaryocytes, progenitor cells, reticulocytes, thymus, spleen</td>
</tr>
<tr>
<td>Peripheral organs</td>
<td>Adipose, cardiac tissue, embryonic liver, kidney, pancreas, skeletal muscle</td>
</tr>
<tr>
<td>Reproductive tissue</td>
<td>Ovaries, prostate, testis</td>
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Table 2

<table>
<thead>
<tr>
<th>Disease modalities</th>
<th>Biological effects of FoxO proteins</th>
<th>Refs</th>
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</thead>
<tbody>
<tr>
<td>Infertility</td>
<td>Foxo3a depletion leads to oocyte death and infertility</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>Overexpression of Foxo3a leads to oocyte growth retardation, follicular failure and anovulation</td>
<td>[14]</td>
</tr>
<tr>
<td>Inflammatory and immune disease</td>
<td>Mutations in FOXO1a and FOXO3a lead to clinical premature ovarian failure</td>
<td>[83]</td>
</tr>
<tr>
<td></td>
<td>Absence of Foxo3a leads to lymphoproliferation, organ inflammation, enhanced helper T-cell activity</td>
<td>[15]</td>
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<td></td>
<td>Increased FOXO3a levels lead to changes in T cell response and activity</td>
<td>[88]</td>
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<tr>
<td></td>
<td>Foxo3a is necessary for neutrophil activation</td>
<td>[16]</td>
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<tr>
<td></td>
<td>Rheumatoid arthritis leads to increased post-translational phosphorylation of FOXO1, FOXO3a and FOXO4 in synovial tissue biopsy</td>
<td>[84]</td>
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<td>Progenitor cells in the hematopoietic and nervous systems</td>
<td>Erythropoietin leads to the inhibition of Foxo3a activity</td>
<td>[19,20,44]</td>
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<td></td>
<td>Erythropoietin–Foxo3a association promotes erythropoiesis</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>Inactivation of FoxO1 and FoxO3a promotes enteric nervous system development</td>
<td>[22]</td>
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<td></td>
<td>Foxo3a maintains the hematopoietic stem cell pool</td>
<td>[18]</td>
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<tr>
<td>Obesity and diabetes</td>
<td>Single nucleotide polymorphism in FOXO3a linked to high body mass index</td>
<td>[86]</td>
</tr>
<tr>
<td></td>
<td>Insulin resistance associated with Foxo3a signaling</td>
<td>[31]</td>
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<tr>
<td></td>
<td>Streptozotocin induced diabetes leads to phosphorylation of Foxo3a</td>
<td>[27]</td>
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<tr>
<td></td>
<td>NAD⁺ precursors reduce diabetic complications and inhibit Foxo3a activity</td>
<td>[23,32,43,58]</td>
</tr>
<tr>
<td>Aging, longevity and oxidative stress</td>
<td>Cellular lifespan and tolerance to oxidative stress influenced by interactions of FoxO1, FoxO3a and FoxO4 with SIRT1</td>
<td>[53,60-63]</td>
</tr>
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<td></td>
<td>Foxo3a expression and SIRT1 activity increased in cardiac tissue during exercise training</td>
<td>[65]</td>
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<td></td>
<td>Loss of Foxo3a in vascular cells leads to cell senescence</td>
<td>[67]</td>
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<tr>
<td></td>
<td>Phosphorylation of Foxo3a can prevent cell injury during oxidative stress</td>
<td>[41-45]</td>
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<tr>
<td>Tumorigenesis</td>
<td>Loss of FoxO1 and FoxO3a promotes breast and prostate cancer growth whereas activity of FoxO1 and FoxO3a blocks tumor progression</td>
<td>[69-72]</td>
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<td></td>
<td>Foxo3a activation leads to inhibition of cell cycle progression and prevents proliferation of human colon cancer cells</td>
<td>[50]</td>
</tr>
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<td></td>
<td>Loss of FoxO3a activity leads to oncogenic transformation in hematopoietic derived cancers and Kaposi's sarcoma</td>
<td>[51,75,76]</td>
</tr>
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<td></td>
<td>Changes in FoxO3a activity can alter the sensitivity of tumor cells to chemotherapeutic agents</td>
<td>[77]</td>
</tr>
</tbody>
</table>

Nomenclature: ‘FoxO’ pertains to cell culture, tissue or animal studies; ‘FOXO’ pertains to human studies.
Table 3
Balancing the role of FoxO proteins between normal physiology and disease

<table>
<thead>
<tr>
<th>Clinical or biological presentation</th>
<th>Normal physiology</th>
<th>FoxO chromosomal aberrations or protein deregulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell growth and development</td>
<td>Maintenance of neuronal, erythroid and hematopoietic stem cells; promotion of cell cycle arrest to monitor and check neoplastic growth</td>
<td>Loss of stem cell pools, failure to repopulate progenitor cells; loss of cell cycle regulation that leads to tumorigenesis</td>
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<td>Immune system activation</td>
<td>Modulation of T cell activity and neutrophil inflammation; potential protection for neurons and myelin in systemic lupus erythematosus and multiple sclerosis</td>
<td>Inflammatory cell activation, neuronal degeneration, myelin destruction; cartilage loss in disorders that include systemic lupus erythematosus, multiple sclerosis and rheumatoid arthritis Depletion of ovarian follicles, retardation in follicular development</td>
</tr>
<tr>
<td>Fertility</td>
<td>Regulation of follicular activation and oocyte development</td>
<td>Inflammatory cell activation, neuronal degeneration, myelin destruction; cartilage loss in disorders that include systemic lupus erythematosus, multiple sclerosis and rheumatoid arthritis Depletion of ovarian follicles, retardation in follicular development</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Control of glycogenolysis and gluconeogenesis</td>
<td>Depletion of ovarian follicles, retardation in follicular development</td>
</tr>
<tr>
<td>Cell survival</td>
<td>Resistance to oxidative stress through the reduction in reactive oxygen species</td>
<td>Inflammatory cell activation, neuronal degeneration, myelin destruction; cartilage loss in disorders that include systemic lupus erythematosus, multiple sclerosis and rheumatoid arthritis Depletion of ovarian follicles, retardation in follicular development</td>
</tr>
<tr>
<td>Cell longevity</td>
<td>Possible enhancement of SIRT1 activity during starvation; potential increase in cardiovascular tolerance with exercise</td>
<td>Potential inhibition of SIRT1 activity; induction of cell senescence; increased release of reactive oxygen species</td>
</tr>
</tbody>
</table>