The Complex Biology of FOXO

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Abstract: FOXO transcription factors control proliferation, apoptosis, differentiation and metabolic processes. Loss of FOXO function has been identified in several human cancers, and results in increased cellular survival and a predisposition to neoplasia, especially in epithelial cancer. FOXO factors are therefore bona fide tumor suppressors, and their potential use as therapeutic targets in cancer has been a matter of debate. Importantly, FOXO factors can also positively regulate cell survival through the activation of several detoxification genes, complicating its putative therapeutic potential. Targeting of FOXO factors has also been proposed for the treatment of metabolic dysfunctions such as diabetes mellitus, immunological disorders and neurodegeneration, as well as for the prevention of aging by maintaining the hematopoyetic stem cells niche. But again, data has accumulated that cautions against the potential use of the FOXO activators in these settings. Therefore, greater understanding of the regulation of FOXO target specificity is still needed to boost its use as a therapeutic target.

The four members of the FOXO family (FOXO1, FOXO3A, FOXO4 and FOXO6) have distinct but overlapping cellular functions, although they seem to bind a common set of DNA sites. This fact together with the observation that FOXOs are only partially dependent on their DNA binding activity to regulate their target genes highlights the fact that the interaction of the FOXOs with other transcription factors is crucial for the FOXO-mediated transcriptional programs.

In this review, we provide an overview of recent progress in the understanding of the modulation of FOXO activity and target specificity by transcription factors and coactivators.

Keywords: FOXO, insulin signaling, transcription factor, coactivator, metabolism, cell cycle, cell differentiation, cancer.

INTRODUCTION

The forkhead box (FOX) gene family of transcriptional regulators is named after de *Drosophila melanogaster* gene fork head (FKH), whose mutation causes defects in head fold involution during embryogenesis [1]. Over the past two decades, hundreds of FOX genes have been identified and classified into subfamilies such as FOXA, FOXP and FOXO. All FOX proteins contain a highly conserved ~100-residue DNA binding domain. The canonical FKH domain consists of three α-helices, three β-sheets and two wing regions that flank the β-sheet. Because of the butterfly-like winged structure adopted by the DNA-bound FOX proteins, the FKH domain has also been termed the winged-helix domain. Most FOX proteins bind to DNA as monomers, contacting their target sequences *via* the third α-helix and by flanking residues and the two wing regions [2].

The FOXO subfamily of forkhead transcription factors is conserved from *C. elegans* to mammals. *C. elegans* has one FOXO gene (DAF-16) whereas mammals have four FOXO family members: FOXO1 (FKHR), FOXO3A (FKHRL1), FOXO4 (AFX) and FOXO6. The genes encoding the first three proteins, which share high functional and sequence similarity, were identified in fusion genes from chromosomal translocations occurring in human rhabdomyosarcomas and acute myeloid leukemias. The more distantly related FOXO6 was identified by degenerate PCR screening [3-6].

FOXO proteins mainly act as potent transcriptional activators by binding to the conserved consensus core recognition motif TTGTTTAC [7, 8]. The three dimensional structure of the forkhead domain has been resolved by both X-ray crystallography and nuclear magnetic resonance, revealing small variations in the secondary structure content and topological arrangement among various forkhead domains [9-12]. Importantly, X-ray crystallography data show that the winged-helix DNA binding domain of FOXA3, and more recently FOXO1, has an overall structure similar to the globular domain of the linker histones H1 and H5 [13-15]. This structural similarity has been shown to endow certain forkhead proteins with the ability to bind their sites within condensed chromatin, a DNA context from which most other transcription factors are excluded. The chromatin binding and remodeling functions revealed for forkhead transcription factors could be crucial for initiating and dynamically modulating active chromatin states, enabling the diverse roles of FOXOs as gene regulatory factors [14, 16-18]. Apart from the well-folded and highly conserved FKH domain, other parts of FOXO proteins are predicted to be intrinsically disordered [19]. FOXO1, FOXO3A and FOXO4 have three conserved regions (CR1-3) located in these disordered regions. The CR3 is an acidic transactivation domain that mediates the interaction with the KIX domain of the coactivator CREB-binding protein (CBP) [20, 21], which in turn interacts with the transactivation domain of many transcription factors. The CR3 domain has been shown to

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partially overlap with the DNA-binding site on the FKH domain [11, 22-24].

Despite of having almost identical DNA binding motifs and sharing some downstream transcriptional targets [25, 26], FOXO factors have overlapping but distinct biological roles. The functional diversity of FOXO isoforms was revealed by targeted gene disruption in mice. FOXO1-*null* embryos die on embryonic day 10.5 as a consequence of incomplete vascular development. Moreover, differentiation assays showed a markedly different morphological response to vascular endothelial grow factor in endothelial cells derived from FOXO1-deficient embryonic stem cells compared with wild-type endothelial cells. FOXO1 thus plays a critical role in establishing a normal vasculature in the developing embryo [27, 28]. Both FOXO3A- and FOXO4-null mice survive to adulthood and are grossly indistinguishable from their littermate controls, indicating that these factors are dispensable for normal vascular development [27, 29]. FOXO3A-*null* female mice display aged-dependent infertility and abnormal ovarian follicular development [27, 29]. FOXO3A deficiency also leads to lymphoproliferation and widespread organ inflammation [30]. In contrast to the FOXO1- and FOXO3A-deficient mice, FOXO4-*null* mice display no apparent phenotype [27]. The differences in FOXO family members function exemplified by the distinct phenotype of FOXO1-, FOXO3A- and FOXO4-*null* mutant mice can be attributed at least in part to two important and related characteristics of the FOXO factors: their ability to activate or repress diverse target genes through the cooperative interaction with a various unrelated transcription factors, and their capacity to modulate transcriptional responses independently of direct DNA-binding [31]. Some of the observed differences may also be attributable to the differential expression patterns of FOXO factors, although FOXO isoforms are expressed in most mammal tissues to varying degrees [5, 8, 32]. FOXO1 is abundantly expressed in adipose tissues, FOXO3A is abundant in cardiac and neuronal tissues, and FOXO4 is highly expressed in skeletal and cardiac muscle. FOXO6 is predominantly expressed in the brain [33].

FOXO transcription factors promote cell-cycle arrest, DNA repair, detoxification of reactive oxygen species, apoptosis and autophagy by upregulating specific gene-expression programs [34-43]. FOXO-dependent cell cycle arrest and apoptosis may be critical for the tumor-suppressive effect of these transcription factors, and has boosted the research on FOXO factors as potential pharmacological targets [44]. Expression of active forms of these factors reduces tumorigenicity in nude mice [31, 45, 46] and FOXO factors have been found to interact with several tumor suppressors or oncogenes [47-49]. Moreover, FOXO factors are found at chromosomal translocations in human tumors

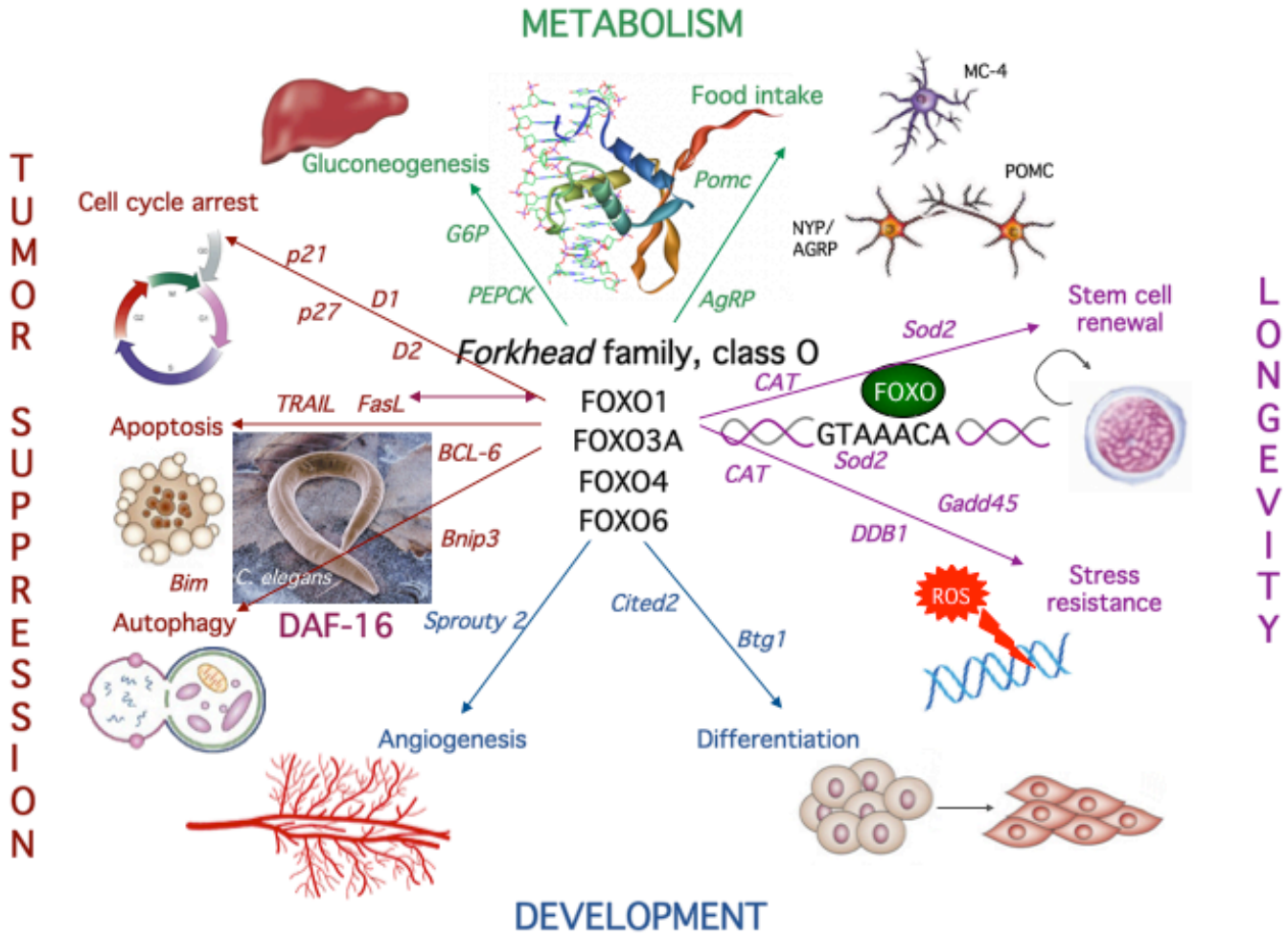

Fig. (1). The FOXOs are involved in a complex array of regulatory functions both tissue specific and systemic.

[3-5]. Because the ability to detoxify ROS and to repair damage is correlated with increased organism longevity [50], it has been proposed that these particular functions of FOXO transcription factors may be relevant to FOXO's ability to control longevity. It has been shown that FOXO transcription factors extend lifespan in invertebrates [51-55] and may also prolong mammalian lifespan [56, 57]. FOXO proteins also regulate cell differentiation in blood cells [58-60], vascular endothelial cells [61], smooth and skeletal muscle [62] and adipose tissue [63], which may contribute to their role in development. Finally, FOXO proteins control energy metabolism by promoting gluconeogenesis and by enhancing food intake [64-68] (Fig. **1**).

As FOXO cellular functions are diverse and in some cases antagonistic, the activity of these transcription factors must be tightly regulated by external stimuli. It is known that environmental signals, including insulin, growth factors, nutrients, cytokines and oxidative stress, control FOXO levels, subcellular localization and transcriptional activity. FOXO proteins are also regulated by a variety of post-translational modifications, mainly phosphorylation, acetylation, mono- and polyubiquitination [69].

The transcriptional regulatory functions of FOXO proteins require nuclear localization. This localization is favored in the absence of growth signals and is correlated with an attenuation of cell replication. Export from the nucleus is regulated by the phosphorylation of FOXO by the serine-threonine kinase AKT, and also by glucocorticoid-regulated kinase (SGK), casein kinase 1 (CK1), and DYRK1A (a member of the dual-specificity tyrosine-phosphorylated and regulated kinase group), which not only interferes with FOXO transcriptional activities, but also promotes its proteolytic degradation. Stress stimuli trigger the relocalization of FOXO proteins in the nucleus, even in the presence of growth factors. Indeed, in response to oxidative stress, the protein kinases MST1 (mammalian Ste20-like Kinase) and JNK (c-Jun kinase), trigger the relocalization of FOXO3A from the cytoplasm to the nucleus [69].

However, one of the main mechanisms by which precise regulation of FOXO is achieved is the interaction with binding protein partners. It has been described that FOXOs can associate with a variety of transcription factors, co-activators and co-repressors to regulate the expression of diverse target genes. These interactions seem to be critical in determining the activation of cell type-specific transcriptional programs. Furthermore, the activity of these transcription factors is differentially controlled in specific tissues in response to various types or intensities of external stimuli, and the specificity of the regulation is mediated by the interaction with specific factors and cofactors.

1. METABOLISM

1.1. Physiological Functions

1.1.1. Gluconeogenesis

FOXO1 is a direct transcriptional regulator of the gluconeogenic genes *glucose-6-phosphatase (G6Pase)* and *phosphoenolpyruvate carboxykinase (pepck)* [67, 70-72]. Insulin action inhibits liver gluconeogenesis at least in part *via* AKT inactivation of FOXO1 activity. In response to insulin, FOXO1 is phosphorylated by AKT and excluded from the nucleus [71]. A feed back regulatory loop has also been identified with changes in FOXO activity having a dose-responsive repressive effect on insulin signaling through inhibition of protein phosphatases, which leads to altered AKT activation, reduced insulin sensitivity, and impaired glucose metabolism [73]**.** Interest in this pathway has been boosted by the finding that induction of the PI3K/AKT signaling pathway shortens the lifespan of *C. elegans,* whereas caloric restriction and activation of DAF-16, the nematode FOXO orthologue, increase longevity [55, 74-76].

Hepatocyte Nuclear Factor 4 (HNF-4)

FOXO regulation of HNF-4 is a good an example of how FOXOs can work as activators in some situations and as repressors in others [31, 68, 77, 78]. HNF-4 is a transcription factor expressed mainly in the liver, kidney and intestine that binds to a specific DNA element (HNF-4 binding element (HBE)) as a homodimer and activates transcription of many genes that are involved in glucose, fatty acid, and cholesterol metabolism [79-83]. HNF-4 activates the expression of both glucokinase (GK) and G6Pase, which catalyze the first and last rate-limiting steps in glycolysis and gluconeogenesis, respectively. A number of studies have shown that GK is inhibited by fasting and activated by feeding, whereas G6Pase is activated by fasting and inhibited by feeding. Since HNF-4 can only work as a transcriptional activator, it was hypothesized that its opposite effects on GK and G6Pase gene transcription might arise from interaction with another transcription factor. In 2003 it was described that HNF-4 can interact with FOXO. The same group recently reported that in the absence of insulin, FOXO1 represses the HNF-4 potentiated expression of GK and simultaneously activates the HNF-4-dependent transcription of the G6Pase gene, presumably *via* interaction with CBP and the peroxisome proliferator activated receptor (PPAR) γ coactivator 1-α (PGC-1α). Both of these HNF-4-dependent effects are abrogated by treating cells with insulin, which promotes the translocation of FOXO1 to the cytosol [84], resulting in its dissociation from HNF-4 [85] and thus shifting the balance from gluconeogenesis to glycolysis in the fasted state. Although the molecular basis for the opposite outcomes of FOXO1 association with HNF-4 is not well understood, it must be noted that in the case of HNF-4 the interaction with FOXO1 leads to activation when FOXO1 is bound to an insulin response sequence (IRS) on the target promoter, but it is inhibitory in the absence of a functional IRS [84].

The Peroxisome Proliferator Activated Receptor (PPAR) γ Coactivator 1-α (PGC-1α) is a transcriptional coactivator identified as an upstream regulator of lipid catabolism, mitochondrial number and function (Fig. **2**). Consistent with its emerging role as a central regulator of energy metabolism, PGC-1α is abundantly expressed in tissues with high metabolic rates, such as heart, skeletal muscle, liver, brain and brown adipose tissue [86-90]. PGC-1α is a positive regulator of fasting-induced liver gluconeogenesis and this regulation is mediated through the interaction of PGC-1α with FOXO1 and HNF-4. HNF-4 is essential for expression of hepatic genes in the absence of exogenous ligands, while the action of FOXO1 and PGC-1α is attenuated by insulin [67, 91, 92]. Moreover, insulin regulation of PGC-1α action

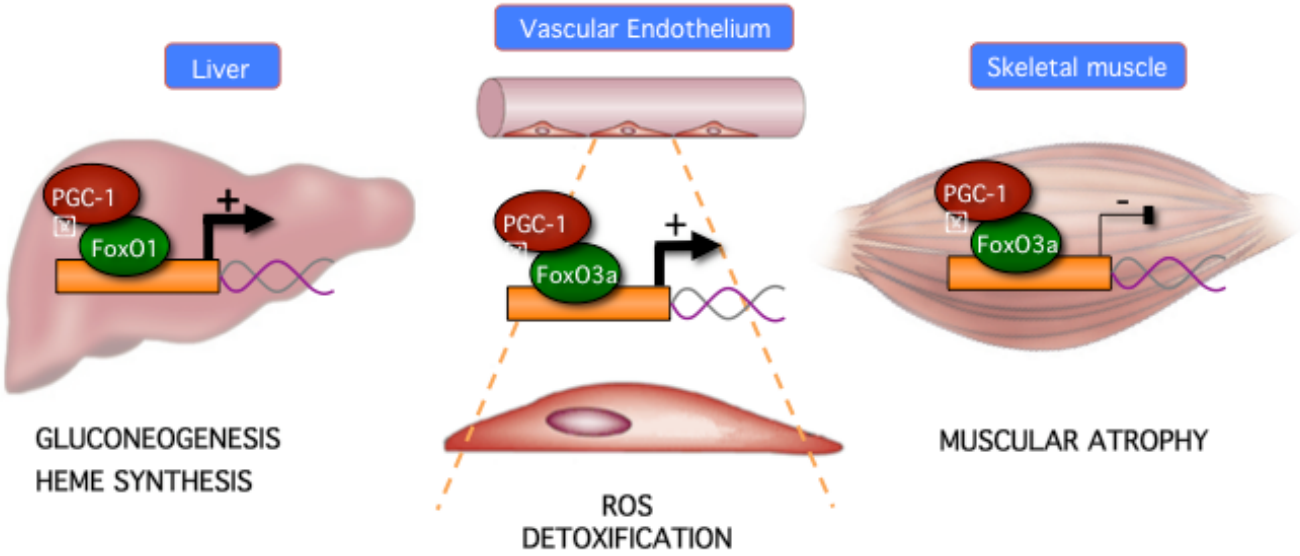

Fig. (2). The interaction of FOXO1 and FOXO3A with the coactivator PGC-1α illustrates FOXO's functional plasticity. FOXO1 and PGC-1α, cooperate to induce gluconeogenesis and heme synthesis in the liver, FOXO3A and PGC-1α co-regulate ROS detoxification genes in the vascular endothelium, while PGC-1α prevents FOXO3A induction by of muscle atrophy genes in skeletal muscle.

on gluconeogenesis depends on FOXO1 function in hepatic cells and mouse liver. FOXO1 acts as a transcriptional regulator of PGC-1α expression through direct binding to IRSs within the PGC-1α promoter [93]. Interestingly, it has been proposed that the formation of the FOXO1/PGC-1α complex is positively modulated by N-Acetyl glycosylation (*O*-GlcNAc) of both factors [94].

The central role played by FOXO1 and PGC-1α in the control of gluconeogenesis in response to fasting is supported by several reports showing that PGC-1α is regulated by metabolic sensors in a manner that closely resembles the regulation of FOXO1. PGC-1α, like FOXO factors, is negatively regulated by AKT [95], and activated by AMPK [96], and is also a target of the deacetylase SirT1 [97, 98], all of which are sensors of the cellular metabolic status. However, it has been noted that in conditions where insulin signaling is compromised, like in the insulin receptors knock out mice, low phosphorylation of FOXO1 by AKT does not result in an increased PGC-1α activity [99].

1.1.2. Oxidative Stress

As mentioned above, PGC-1α regulation of gluconeogenesis is mediated by both HNF-4 and FOXO1, and a direct interaction between FOXO1 and HNF-4 also mediates this regulation. However, so far it is unknown if a ternary PGC-1α/HNF-4/FOXO1 complex is formed on gluconeogenic gene promoters. The notion that such a ternary complex could exist is supported by the recent results regarding the regulation of selenoprotein P.

Selenoprotein P is a plasma protein produced in the liver that is responsible for the transport of the essential micronutrient selenium to various extra hepatic tissues [100, 101]. Selenoprotein P has been recently identified as a FOXO1 target gene [102]. It was also found that, like the gluconeogenic genes, Selenoprotein P is simultaneously regulated by HNF-4, FOXO1 and the coactivator of both PGC-1α, further stressing the functional link between these transcriptional regulators [103].

Importantly, Selenoprotein P is crucial for the activity of several reactive oxygen species (ROS) detoxification enzymes such as glutathione peroxidases and thioredoxin reductases [104, 105]. It has been known for some time that FOXO3A is a positive regulator of the key ROS detoxification enzymes Mn superoxide dismutase (MnSOD) and catalase [39, 41]. More recently roles for FOXO4 and FOXO1 in ROS detoxification have also been suggested [58, 106, 107].

Our own results [108] and those of others [109, 110] identify a key role of PGC-1α in ROS homeostasis in various cell types through the coordinated regulation of the antioxidant defense system, including the selenoproteins thioredoxin reductase 2 (TR2) [108] and glutathione peroxidase 1a (GPx1a) [111]. PGC-1α reduces ROS levels and prevents mitochondrial dysfunction and apoptotic cell death in response to oxidative stress conditions.

Further work by our group has shown that PGC-1α and FOXO3A cooperate in the transcriptional regulation of the mitochondrial oxidative stress protection system. We showed that FOXO3A-dependent induction of ROS detoxification genes requires PGC-1α, since this effect is severely curtailed in PGC-1α-deficient endothelial cells. PGC-1α action on these genes is equally dependent on the presence of FOXO3A. These factors can directly interact, as shown by co-immunoprecipitacion and *in vitro* interaction assays. Moreover, both proteins can localize on the same promoter regions in co-regulated genes and it was observed that a functional FOXO site is required for activation of the *sod2* promoter by PGC-1α. We also demonstrated that FOXO3A is a direct transcriptional regulator of PGC-1α, suggesting that an auto-regulatory cycle modulates FOXO3A/PGC-1α-mediated control of the oxidative stress response. These results support the notion that the FOXO3A/PGC-1α complex plays a key role in the oxidative stress protection, [112]. The concerted action of both PGC-1α and FOXO3A on oxidative stress protection is also highlighted by the observation that both factors are activated in response to

elevated cellular ROS levels [47, 110, 113]. A PGC-1α-FOXO1 interaction also seems to be important in *hepatic heme biosynthesis* [114]. It has been proposed that PGC-1α activation of the *ALAS-1* promoter (encoding the rate-limiting enzyme in hepatic heme biosynthesis) is mediated by PGC-1α coactivation of FOXO1, which directly binds to this promoter. The PGC-1α-FOXO1-mediated induction of the *ALAS-1* promoter is repressed by insulin, probably through the activation of AKT, which phosphorylates FOXO1 disrupting its binding to PGC-1α.

1.1.3. Glucose Oxidation: Pyruvate Dehydrogenase

PDK4

The pyruvate dehydrogenase complex (PDC) catalyzes the conversion of pyruvate to acetyl-CoA in mitochondria and is a key regulator of the oxidation of glucose to acetyl-CoA [115, 116]. Phosphorylation of PDC by the pyruvate dehydrogenase kinase 4 (PDK4) inhibits its activity, showing that PDK4 is a negative regulator of glucose oxidation. The expression of the PDK4 gene is increased by fasting and other conditions associated with the switch from the utilization of glucose to fatty acids as an energy source [117]. Given the central role of PDK4 in regulating PDC activity, it is important to understand the molecular mechanisms underlying the regulation of PDK4 expression by hormonal and nutritional factors. FOXO1, binding to IRS sites, is a positive regulator of PDK4 expression. PDK4 is also stimulated by glucocorticoids *via* two glucocorticoid receptor (GR) binding sites. Moreover, PDK4 is also regulated by the FOXO1 interacting factors HNF-4 and PGC-1α [118-121]. PGC-1α seems to be recruited to the promoter through its interaction with estrogen related receptor α (ERRα) [122, 123], a nuclear receptor involved in fatty acid transport, mitochondrial function and fatty acid oxidation [121]. Insulin acts as a key negative regulator of PDK4 by several interconnected mechanisms. It inhibits the PDK4 induction by both ERRs and ERRα/PGC-1α complexes in part by promoting the dissociation of FOXO1 and PGC-1α from the PDK4 promoter [122]. Furthermore, insulin also causes the dissociation of GR from the promoter. This result, together with the observation that mutations in IRSs sites reduce de ability of GR to stimulate PDK4 expression [118], suggests that GR and FOXO1 might interact and form a regulatory complex with EERα/PGC-1α on the PDK4 promoter, although PGC-1α does not appear to be necessary for the acute regulation of PDK4 by glucocorticoids or insulin [124].

1.1.4. Muscle Atrophy

Recent studies indicate that FOXO transcription factors play an important role in promoting muscle atrophy, induced in response to muscle denervation or chronic inflammation. This effect seems to depend in part on the ability of FOXO factors to upregulate genes associated with proteasome-mediated protein breakdown, including muscle atrophy F-box (MAFbx)/Atrogin-1 and muscle-specific RING finger protein 1 (MuRF-1) [125, 126]. The role of FOXO factors in muscle atrophy also likely involves the FOXO-mediated induction of apoptosis through the upregulation of cell death receptors and various propapoptotic signaling genes, such as the Bcl-2-interacting mediator of cell death (Bim) and the Bcl-2/adenovirus E1B 19-kDa-interacting protein 3 (BNip3) [38, 127-129]. A recent study [130] proposed that FOXO1-induced muscle atrophy and associated increases in the expression of proteolytic and apoptotic genes might occur *via* DNA-binding-dependent and -independent mechanisms This notion is supported by experiments done in cells expressing a DNA-binding-deficient form of FOXO1, which exhibited significant atrophy upon FOXO1 activation but no hallmark signs of apoptosis. Gene expression of MuRF-1 appeared to be independent of DNA binding, whereas expression of MAFbx/Atrogin-1 and Bim was significantly blunted in cells expressing DNA-binding-deficient FOXO1. BNip3 gene expression was significantly elevated in DNA-binding-deficient mutant cells.

When skeletal muscle is deprived of energy or treated with glucocorticoids, FOXO1 expression is increased resulting in gene activation, while refeeding suppresses FOXO transcription [131]. Similarly to the PDK4 promoter, the MuRF1 promoter contains a glucocorticoid response element (GRE) directly adjacent to an insulin response element (IRE), and is positively regulated by the binding of both GR and FOXO1 to these elements. Coexpression of GR and FOXO1 dramatically and synergistically increases reporter gene activity, whereas insulin-like growth factor 1 (IGF-1) inhibits dexamethasone-induced MuRF1 expression, suggesting that a GR/FOXO1 complex may also form in this promoter context [132]. Other transcription factors that are activated in muscle wasting include members of the CCAAT/enhancer binding protein (C/EBP) family [133]. C/EBPβ and δ are activated in muscle wasting by a glucocorticoid-dependent mechanism. Similarly, increased skeletal muscle expression and activity of C/EBPβ and δ during sepsis in rats is also dependent on GR [134], suggesting that GR may mediate C/EBPβ and δ induction. Furthermore, dexamethasone treatment increased C/EBPβ and δ levels and upregulated C/EBP-dependent gene activation in both cultured myotubes and in rats [135, 136]. Since FOXO1 is able to induce C/EBPβ expression [137] and regulates GR-dependent muscle atrophy, it would be interesting to know if FOXO1 cooperates with GR to induce C/EBPβ gene expression during atrophy. Interestingly, the pro-apoptotic activity of FOXO3A in muscular atrophy appears to be inhibited by PGC-1α. PGC-1α overexpression has been shown to prevent muscular atrophy associated with denervation and fasting, and to reduce the ability of FOXO3A to activate Atrogin-1 and MuRF-1. Thus, the rapid fall in PGC-1α levels during atrophy might enhance the FOXO-dependent loss of muscle mass [138].

FOXO3A-mediated upregulation of Atrogin-1 could also contribute to the inhibition of cardiac hypertrophy in the heart, where Atrogin-1 is reported to interact with and repress calcineurin (a pro-hypertrophic agent) by targeting it for ubiquitin-mediated proteolysis [139]. Interestingly, FOXO1 and FOXO3A are direct targets of Atrogin-1 dependent ubiquitination in the heart [140]. This ubiquitination does not target FOXO factors for proteasomal degradation, but rather en-hances their transcriptional activity, further supporting an important role for the FOXO/Atrogin-1 axis during postnatal heart growth. But induction of Atrogin-1 is not the only mechanism through which FOXO3A inhibits cardiac hypertrophy. It has been reported that overexpression of either wild-type or constitutively active FOXO1 or FOXO3A reduces calcineurin phosphatase activity and suppresses the

expression of the modulatory calcineurin interacting protein exon 4 isoform MCIP1.4, a direct target of the calcineurin/ nuclear factor of activated T cells (NFAT) cascade, leading to inhibition of cardiac hypertrophy in response to pathologic stimuli [141]. These findings are particularly interesting since they might indicate the existence of a putative FOXO/ NFAT regulatory complex.

1.2. Transcriptional Co-Factors Involved in Post-Translational Modification: Acetylation and Deacetylation (Fig. 3)

1.2.1. Sirtuins

1.2.1.1. SirT1

Yeast silent information regulator 2 (Sir2), is an NAD^+-dependent histone deacetylase and founding member of the family of enzymes known as sirtuins. Sir2 is involved in a wide range of cellular processes, including genomic stability, aging, ROS detoxification and metabolic sensing [142, 143]. In *C. elegans*, overexpression of Sir2 increases lifespan, which requires DAF-16, the yeast homologue of mammalian FOXO [144]. This observation has poised the question of whether a Sir2 type of activity plays a role in lifespan extension also in mammals and whether it is related to FOXO regulation. The *Sir2* family of genes is a highly conserved group of genes with seven human homologues, of which *SirT1* is the closest homologue of yeast and *C. elegans Sir2* [145], and hence its activity is the most thoroughly investigated.

In 2005, it was described that deacetylation of FOXO factors by SirT1 overrides the phosphorylation-dependent nuclear export induced by growth factors and renders FOXOs immobile within the nucleus in hepatocytes. The transcription of FOXO-dependent genes is accordingly increased, leading to activation of gluconeogenesis and increased glucose release from hepatocytes [146]. In the same year, it was reported that SirT1 controls hepatic glucose output through the positive modulation of PGC-1α activity on gluconeogenesis genes [97].

These results, together with previous data showing that gluconeogenesis is co-regulated by FOXO1, HNF-4 and PGC-1α, suggest the possibility that gluconeogenesis might be regulated by a FOXO1/SirT1/PGC-1α/HNF-4 complex. This idea is supported by a recent report showing that the SirT1 activator resveratrol inhibits GK expression in a FOXO1 dependent manner by increasing FOXO1 localization in the GK promoter and its interaction with HNF-4. This result suggests that SirT1 positively regulates the formation of a FOXO1/HNF-4 complex on this promoter [147].

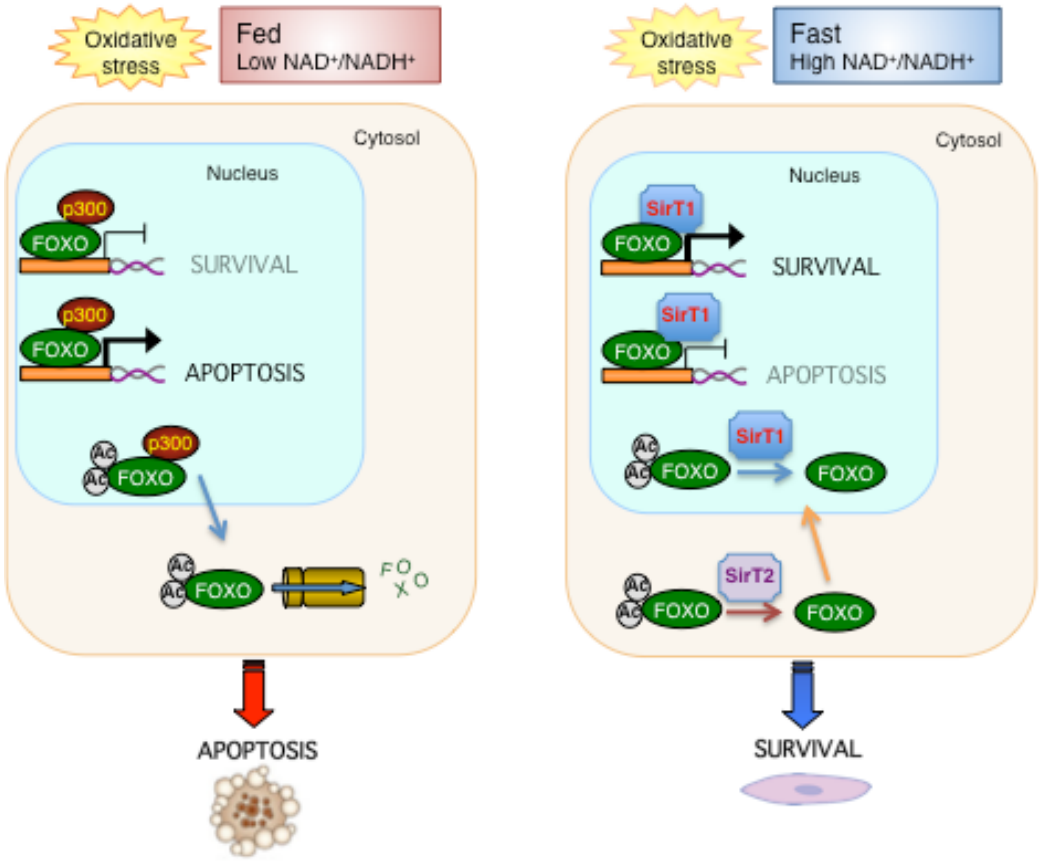

Fig. (3). The activation of FOXO factors can result in the induction of both apoptosis and survival genes. Interaction of FOXO with coregulators and posttranscriptional modification tip the balance. In response to increased oxidative stress, interaction with CBP/p300 reduces FOXO transcriptional activity on pro-survival genes while it serves as a coactivator on other FOXO target genes. CBP/p300 acetylates FOXO and triggers its nuclear exclusion and degradation by the proteasome. When the metabolic conditions are adequate, sirtuins like SirT1 and SirT2 deacetylate FOXO, facilitating its translocation to the nucleus. SirT1 coactivates FOXO on pro-survival genes while it reduces its activity on pro-apoptotic genes.

Regulation of FOXO activity by SirT1 also seems to be important for cell-cycle arrest and the upregulation of oxidative stress protection genes, including *p27* and *GADD45* [47, 148]. Moreover, SirT1 simultaneously inhibits FOXO3A activity on apoptotic genes such as *Bim* or *Fas-ligand*. SirT1 also stimulates the transcriptional activity of FOXO4 on *p27* and *sod2* gene expression [149]. More recently it has been shown that moderate overexpression of SirT1 protects the heart from paraquat-induced oxidative stress and apoptosis *via* a FOXO1-dependent mechanism [150]. This report also showed that overexpression of either SirT1 or constitutively-active FOXO1 stimulated the expression of the antioxidant enzyme catalase, whereas transduction of a dominant negative form of FOXO1 in cultured cardiac myocytes resulted in reduced catalase expression. Interestingly, SirT1 has also been shown to directly regulate FOXO3A and FOXO4 expression.

SirT1/FOXO interaction has also been described to regulate endothelial angiogenesis. The fundamental role of FOXO factors in the control of angiogenesis is well known. A recent study has shown that SirT1 regulates endothelial angiogenic functions during vascular growth by negatively regulating FOXO activity [151]. Vascular endothelial growth factor (VEGF) signaling has also been implicated in the regulation of FOXO transcriptional activity in endothelial cells [152]. A number of VEGF-responsive genes, such as VCAM-1, MMP-10, CITED-2 and MnSOD, have been identified to be upregulated by the synergistic actions of both VEGF and FOXO activities in the endothelium. These genes encode for proteins known to be involved in metabolism, cell signaling, cell adhesion, stress response, differentiation and other cellular functions [152]. These results contradict the documented ability of VEGF to induce the phosphorylation and nuclear exclusion of FOXO through activation of AKT signaling, suggesting that gene regulation by VEGF and FOXO is complex and may be modulated by different posttranslational modifications and the interaction of FOXO with other transcription factors such as the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [152].

1.2.1.2. Other Sirtuins

SirT2 and SirT3 have also been proposed to regulate FOXO activity. It has been shown that SirT2 is able to deacetylate FOXO3A in response to oxidative stress promoting its relocalization from the cytoplasm to the nucleus, thereby increasing its activity on ROS detoxification genes [153]. SirT3 is the only sirtuin whose increased expression has been linked to human longevity [154, 155]. Initially described as a mitochondrial specific deacetylase, it has been recently shown to be a nuclear protein that is translocated to the mitochondria upon oxidative stress [156, 157]. It has been described that FOXO3A and SirT3 directly interact in the mitochondria and that SirT3 activates FOXO3A-dependent gene expression, probably by increasing the binding of FOXO3A to the promoters of its target genes [158]. Moreover, it has been reported that SirT3 increases the FOXO3A activity on MnSOD and catalase genes in cardiomyocytes, thus preventing cardiac hypertrophy [159]. FOXO3A was previously shown to prevent cardiac hypertrophy by suppressing the calcium/calcineurin dependent activation of NFAT [141]. Therefore, NFAT inhibition might be indirect and mediated by a reduction in ROS levels.

1.2.2. CEBP/p300

Glucagon induces the activation of cAMP-dependent protein kinase (PKA) activation in response to fasting [160]. PKA then phosphorylates the cAMP response element (CRE)-binding protein (CREB), which in turn interacts with the acetylase p300. The acetylation by p300 boosts CREB transcriptional activity, resulting in the induction of the key gluconeogenesis genes *G6Pase* and *pepck*. Glucagon also stimulates the gluconeogenic program by triggering the dephosphorylation and nuclear translocation of the CREB regulated transcription coactivator 2 (CRTC2; also known as TORC2) [161]. The gluconeogenic program is also induced *via* the coactivation of CREB by PGC-1α in response to fasting [162]. Given the role of FOXO1 in regulating gluconeogenesis, these results raise the possibility of an interplay between the CREB and the FOXO1 pathways. Recently it has been shown that there is a fasting-inducible switch between p300 and SirT1 that determines which gluconeogenesis program is active at a time [163]. This study proposes that similarly to what happens in oxidative stress, early during fasting there is an activation of p300, which induces gluconeogenic gene expression *via* CRTC2. During late fasting, activation of SirT1 leads to CRTC2 downregulation and activation of FOXO1 dependent gluconeogenesis program.

Both p300 and CBP act as coactivators of FOXO proteins, enhancing gene transcription by recruiting basal transcriptional machinery or by remodeling chromatin structure through intrinsic histone acetyltransferase (HAT) activity [164]. It was first shown that DAF-16 recruits CBP to activate gene transcription in *C. elegans*; subsequently the physical interactions between human FOXO proteins and CBP were identified and mapped [21, 165]. As mentioned before, the CR3 acidic transactivation domain of FOXO proteins binds directly to the KIX domain of CBP [20, 21]. It has been recently reported that the intramolecular interaction between the FKH and the CR3 domains of FOXO proteins negatively regulates the association of CR3 with KIX [24]. However, upon binding to the forkhead response element (FRE) DNA, the FKH domain releases the CR3 domain, allowing it to interact with the KIX domain of CBP, which could enhance its transcriptional activity. p300 and CBP not only interact with FOXO factor, they also acetylate them. It was initially proposed that p300-mediated acetylation of FOXOs increased their transcriptional activity [166, 167]; however, a number of recent reports suggest that CBP/p300-mediated acetylation reduces FOXO transactivation activity on a number of key target genes, including those involved in oxidative stress protection. In 2003, it was described that CBP acetylates FOXO4 at three lysine residues and the CBP-induced acetylation of FOXO4 was proposed as a novel modification mechanism by which FOXO4 keeps the transcriptional activity mitigating in the nucleus [168]. More recently, FOXO4 was also found to be acetylated by p300 *in vivo* [169]. This acetylation negatively regulates the transactivation activity of FOXO4, although p300 also contributes to FOXO-mediated transactivation by recruiting the basal transcriptional complex. Interestingly, deacetylation of FOXO4 by SirT1 counteracts p300-mediated downregula-

tion of FOXO4 activity. Reversible acetylation also modulates the transactivation function of FOXO1. CBP binds and acetylates FOXO1 at three lysine residues, attenuating its transcriptional activity. This acetylation is counteracted by SirT1, which coactivates the transcriptional function of FOXO1 *via* its deacetylase function [170]. Acetylation reduces FOXO1 activity by attenuating its ability to bind the target DNA sequence and increasing its sensitivity to phosphorylation by the phosphatidylinositol 3-kinase (PI3K)/ AKT pathway [171, 172]. More recently, it has been reported that the decrease in DNA binding by FOXO1 is a result of the CBP/p300-mediated acetylation of the wing 2 region and it is not nearly as dramatic as the decrease in DNA affinity that is caused by MST1-mediated phosphorylation. In light of this, it is possible that the CBP/p300-mediated acetylation of FOXO1 might also change the transcriptional activity of FOXO1 by altering essential protein-protein interactions [12].

A molecular model has recently been proposed in which acetylation destabilizes FOXO1 binding to the nucleosome binding but not FOXO1-mediated stable nucleosome remodeling [174]. FOXO transcription factors stably bind target sites on nucleosomes and within linker histone-compacted chromatin arrays, perturbing histone, DNA contacts; this activity qualifies the FOXO proteins as ''pioneer factors" capable of initiating regulatory events in chromatin [14]. Stable nucleosome binding, which is essential for efficient FOXO chromatin remodeling, is dependent on the ''forkhead box" DNA binding domain. Recently it has been reported stable FOXO1 binding to nucleosome particles and efficient chromatin remodeling by acetylated FOXO1 or mutant forms containing amino acid substitutions mimicking acetylation. The authors propose that, while acetylation provides a first, essential step toward removal of FOXO factors from cellular chromatin, additional mechanisms, possibly in the form of FOXO co-activator/repressor protein partners, regulate the inherent capacity of FOXO factors to stably bind and remodel nuclear chromatin [173]. This scenario would help to explain the apparent paradox that, while acetylation curtails FOXO DNA and chromatin binding, in some instances acetylation of FOXO factors results in enhanced FOXO transcriptional activity [148, 174]. Interestingly, it has also been recently proposed that reactive oxygen species induce the formation of cysteine-thiol disulfide-dependent complexes of FOXO and p300/ CBP. Moreover, modulation of FOXO activity by p300/ CBP-mediated acetylation seems to be dependent on the formation of this redox-dependent complex [175].

2. CELL DIFFERENTIATION AND EM TRANSITION

2.1. Wnt Signaling

Glycogen synthase kinase (GSK) was discovered over 30 years ago as one of several kinases that phosphorylates and inactivates glycogen synthase [176] the final enzyme in glycogen biosynthesis. Molecular cloning identified two closely related isoforms, GSK3α and GSK3β [177, 178]. Some years later it was discovered that insulin inhibits GSK activity through AKT phosphorylation [179], while glucagon stimulates cAMP activated GSK [180, 181]. It was thus clear that GSK activity was inhibited when the cell had high glucose levels, and activated when glucose was limiting. GSK is also involved in the inhibition of protein synthesis, indicating that its role in the response to starvation also extends to protein metabolism [182, 183]. Research on GSK3 was once again on the front page when the wingless (Wnt) signaling pathway was elucidated. The Wnt pathway was first identified in *Drosophila* and *Xenopus,* where it regulates key developmental stages. Wnt inhibits GSK3, resulting in the dephosphorylation of β-catenin, which then translocates to the nucleus and activates the T cell transcription factor (TCF). TCF, originally identified as a T cell activating transcription factor [184-186], is required for a variety of developmental processes [187]. The importance of this pathway in adult organisms was soon apparent from studies showing that Wnt signaling is crucial in several types of cancer, particularly colon cancer, where colon epithelial cells undergo an epithelium to mesenchyme (EM) transition [188]. The intriguing position of GSK3 as a tumor suppressor and a starvation-induced factor might be related to the long standing observation called the Warburg effect, which describes how actively proliferating cells switch to glycolytic metabolism and suppress all pathways related to starvation, β-oxidation, or mitochondrial function, showing a fundamental connection between cell proliferation and the inactivation of GSK function [189].

In 2005, it was discovered that FOXO3A was activated by β-catenin following its activation by oxidative stress [49]. That was an unexpected result since β-catenin is a growth-promoting agent, that seemed to activate a cell arrest/pro-apoptotic protein. The physiological relevance of this apparent paradox was partially elucidated when in 2008 the same group reported that FOXO3A interaction with β-catenin resulted in the inhibition of the β-catenin/TCF) complex activity [190] and thus implying that FOXO3A can function as a negative regulator of Wnt signaling and as inhibitor of the EM transition. Modulation of β-catenin/ FOXO3A interaction by cellular stress is also significant in relation to cell proliferation since elevated H_2O_2 levels are characteristic of actively proliferating cells and particularly of cancer cells. Importantly, it has been shown in *C. elegans* that the β-catenin ortholog BAR-1 is required for the oxidative stress-induced expression of the DAF-16 target *sod-3* and for resistance to oxidative damage [49]. The connection between the Wnt/GSK3 pathway and the FOXO factors is further supported by the observation that GSK3 also protects against cardiac hypertrophy, a pathological condition characterized by the switching of the cardiomyocytes from oxidative metabolism to glycolysis [191].

2.2. Muscle

2.2.1. Smooth Muscle

2.2.1.1. Myocardin

FOXO proteins are involved in the differentiation of several cell types, but they seem to be particularly important in the differentiation of vascular endothelial cells and the vascular smooth muscle cells (SMCs) [192]. The role of FOXO factors in the developing vasculature has been highlighted by the observation that FOXO1-deficient mice die during embryogenesis and display malformations in major vessels of the embryo and yolk sac [27, 28]. In the

postnatal vasculature, the role of FOXO factors, has been further evidenced by the use of genetically engineered mice that allow the inducible ablation of *FOXO* genes. Accordingly, generalized deletion of all 3 *FOXO* genes (*foxO1*, *foxO3A*, and *foxO4*) results in the appearance of benign endothelial cell tumors termed hemangiomas [193].

Endothelial precursors (EPCs) can be differentiated to endothelial cells, SMCs and proliferative fibroblasts. SMCs and fibroblasts are closely related and can shift phenol-typically from one cell identity to the other quite easily. This shift is regulated by the interaction of the serum response factor (SRF) with its coactivator myocardin. FOXO4 inactivates the SRF/myocardin complex and thus appears to act as a brake on the differentiation of EPC into SMCs/ fibroblasts [194].

Differentiation of SMCs from embryonic stem cells (ESCs) during development is characterized by the appearance of proteins (such as smooth muscle [SM] α-actin and SM–myosin heavy chain [MHC]) whose expression is restricted to the SMC lineage. However, unlike skeletal and cardiac myocytes, which are terminally differentiated, SMCs in adult animals readily switch phenotypes in response to changes in local environmental cues, such as vascular injury or within atherosclerotic lesions. In these situations, SMCs decrease expression of SMC-specific contractile proteins and acquire a migratory and proliferative phenotype characterized by increased production of extracellular matrix components and matrix metalloproteases [195]. After resolution of the injury, SMCs resume transcription of SMC-specific genes and regain their fully differentiated phenotype.

SMC-restricted contractile protein genes contain evolutionarily conserved CArG box DNA sequences within their promoters, and these sequences are required for SMC gene transcription *in vivo*. Paradoxically, many other genes important for induction of SMC phenotypic switching within vascular lesions, including genes important for migration, proliferation, and extracellular matrix production, also contain CArG boxes within their promoters. CArG boxes serve as binding sites for SRF. SRF has the ability to simultaneously activate transcription of genes involved in opposing cellular processes, such as differentiation and proliferation. SRF itself is a weak transcriptional activator, and it was thought that SRF must bind to SMC gene promoters and subsequently recruit other muscle-specific promyogenic accessory factors with strong transcription activation domains (TADs). This idea was validated by the discovery of the SRF coactivator myocardin. Myocardin is exclusively expressed in SMCs and cardiomyocytes, possesses a powerful C-terminal TAD with the capability to selectively activate transcription of cardiac and SMC-specific contractile genes, and physically associates with SRF to form a ternary complex on CArG box DNA [196].

SRF cofactors can be divided into two families: the members of the ternary complex factor family of Ets domain proteins (Elk-1, Sap-1 and Net) and the myocardin-related transcription factors (MRTFs) represented by myocardin, MRTF-A (MAL, MKL) and MRTF-B [197]. Pathological remodeling of the vessel wall involves a switch in SMC phenotype, from the differentiated, contractile state to a proliferative, "synthetic" state [195]. During this switch, cytoplasmic signals activate Ets domain proteins, like Elk-1, which associate with SRF on a specific subset of CArG-boxes flanked by Ets-binding sites [198]. Elk-1 displaces myocardin from SRF, resulting in downregulation of a subset of smooth muscle genes [199, 200]. Although Elk-1 is a coactivator of SRF, it is substantially weaker than myocardin, such that the displacement of myocardin from SRF by Elk-1 results in an overall decrease in the expression of smooth muscle genes.

FOXO4 has been proposed to inhibit SMC differentiation under proliferating conditions in a DNA-binding independent manner. FOXO4 is being expressed most abundantly in myocyte-containing tissues [8]. FOXO4 interacts with both myocardin and SRF and inhibits their transcriptional activity while it is bound to the promoter of the target genes, since both FOXO4 and myocardin can both be isolated from the chromatin of smooth muscle genes. It is well established that stimulation of the PI3K/AKT signaling pathway stimulates SMC differentiation [201]. PI3K/AKT signaling promotes nuclear export of FOXO4, thereby releasing myocardin from its inhibitory influence and leading to SMC differentiation [202]. Therefore, the association of FOXO4 and myocardin appears to play a key role in the modulation of smooth muscle growth and differentiation. Several other studies support the notion that FOXO factors are antiproliferative in SMC. For example, FOXO3A has been shown to inhibit neointimal hyperplasia [203], while nuclear exclusion of FOXO3A is rapidly induced after carotid balloon injury [204].

This regulatory circuit also seems to be important in the differentiation of cardiac muscle, which also requires myocardin. Myocardin levels in the heart increase during cardiac hypertrophy [205], and myocardian overexpression induces an hypertrophic phenotype [206]; moreover, myocardin is inhibited by GSK3β [207], which as mentioned, inhibits hypertrophy. Importantly, myocardin expression in the heart is positively regulated by FOXO factors, although the specific FOXO factors involved have not been identified, and it is unknown if this regulation also occurs during SMC differentiation [208].

2.2.1.2. Sp1

Tumor necrosis factor α (TNF-α) is released by macrophages and SMCs and it enhances SMC migration and promotes the expression of cell adhesion molecules and matrix metalloproteinases (MMP). Recent data suggest that these effects are partially mediated by FOXO4, since FOXO4 genetic deficiency (knockout or knockdown) in cultured aortic smooth muscle cells impairs their ability to migrate in response to TNF-α, and TNF-α induction of MMP9 expression requires FOXO4. Detailed characterization of this regulatory circuit showed that it involved the formation of a complex between the transcription factor Sp1 and FOXO4 on the matrix metalloproteinase 9 (MMP9) promoter, and that FOXO4 DNA-binding activity was dispensable for this effect [209, 210].

2.2.2. Skeletal Muscle

2.2.2.1. Myocardin-Related Factors

FOXO1 has been shown to regulate myoblast fusion during the differentiation of skeletal muscle, a process in which myocardin-related factors play an important role. It has been proposed that MRTF-A, like myocardin, can toggle

between different protein complexes that redirect its activity toward different cellular functions during myogenic differentiation. MRTF-A and -B have been shown to cooperate with transforming growth factor β (TGFβ) signaling in this and other systems. MRTF-A induces EM transition *via* induction of *slug* gene in cooperation with Smads [211] and Smad3 has been identified as a general modulator of MRTF activity during early steps in cancer progression [212]. In proliferating myoblasts, myogenic differentiation is inhibited by transcriptional activation of the *Id3* gene by a MRTF-A/Smad1/4 complex, while FOXO1 is retained in the cytoplasm. In contrast, in differentiating myocytes, MRTF-A is downregulated, and FOXO1 translocates into the nucleus, where it induces the dissociation of the MRTF-A/Smad1/4 complex to dissociate from the *Id3* promoter, thereby suppressing the MRTF-A/Smad-dependent *Id3* expression. FOXO1 thus facilitates the final phase of myocyte differenttiation and the formation of myotubes [213] by interfering with TGFβ signaling. The TGFß downstream effector Smad3 also binds to myocardin to activate transcription from a Smad binding element (SBE) in the promoter of the *SM22α* gene [214, 215].

2.2.2.2. Notch

After ligand-induced cleavage, the intracellular domain of the Notch receptor translocates to the nucleus where it interacts with the DNA-binding protein Cs1 to generate an active transcriptional complex. In mammalian cells, Notch activation is generally thought to maintain stem cell potential and inhibit differentiation, thereby promoting carcinogenesis [216-220]. However, the Notch pathway plays a critical role in muscle differentiation during embryogenesis, as evidenced by Notch1$^{-/-}$ animals, which show defects in myoblast differentiation [221, 222]. Also, active Notch signaling or Notch1 receptor gain of function inhibits differentiation of C2C12 and 10T/2 myoblasts by suppressing transcription of the myogenic determination gene (*MyoD*) [223, 224].

FOXO transcription factors, as we have shown, regulate SMC myogenesis, and they are also implicated in skeletal muscle differentiation and maintenance [36, 62, 225-227]. MyoD is the predominant myogenic factor in fast (glycolytic) fibers while myogenin is the predominant factor in slow (β-oxidative) fibers. Targeted knock out of FOXO1 in skeletal muscle results in higher numbers of fast/MyoD-containing myofibers and reduced numbers of slow, Mef2c-containing fibers [228].

A functional connection between FOXO1 and Noch1 was initially appreciated based on the observation that FOXO gain-of function and Notch1 activation have similar effects on myoblast differentiation, and vascular morphogenesis [226]. In C2C12 cells, growth factor withdrawal results in myogenic conversion, and ectopic expression of a constitutively active FOXO1 mutant blocked this effect in a DNA-binding independent manner [227]. Constitutively active Notch1 also blocks myoblast differentiation, and FOXO siRNA rescued this inhibition, supporting the hypothesis that FOXO1 and Notch1 are functionally connected. The same study also showed that FOXO1 directly interacts with Cs1 and this interaction is required for Notch1-mediated induction of the transcriptional target Hes1. Hes1 has been proposed to suppress myoblast differentiation by inhibiting MyoD (fast fibers) without affecting Myf5 (slow fibers). The authors proposed that FOXO1 works by aiding the displacement of Cs1-associated corepressors (NcoR/Smrt) and allowing association of coactivators (Maml1) [228]. These findings provide a molecular mechanism by which two distinct signaling modu-les, PI3K and Notch, can coordinately and synergistically regulate muscle differentiation, but they also suggest that Notch/FOXO1 might functionally interact in other cellular contexts likely to regulate progenitor cell maintenance and differentiation, particularly since the FOXO factors have been also shown to be necessary for the maintenance of hematopoyetic stem cells [58].

In some cell types such as keratinocytes, increased Notch activity causes exit from the cell cycle and commitment to differentiation, whereas down-modulation or loss of Notch1 function promotes carcinogenesis [229-232]. Recent evidence has established that Notch1 expression in keratinocytes is induced by p53 upon UVB exposure [233] and is an important p53 target gene in tumor suppression. In this cellular context it has been proposed that Notch downregulates FOXO3A expression and therefore prevents the induction of apoptotic cell dead upon UVB exposure [234].

2.2.2.3. Pax

Paired and homeodomain containing proteins (Pax) are essential for regulating embryonic organogenesis and differentiation in metazoans [235]. The closely related Pax3 and Pax7 are specifically expressed in the central nervous system as well as in skeletal muscle. During embryonic myogenesis, Pax3 and Pax7 are expressed exclusively in skeletal muscle progenitor cells [236] and play important roles in regulating expression of myogenic transcription factors [237]. Genetic studies suggest that Pax3 and Pax7 are potential upstream regulators of MyoD during both embryonic and postnatal myogenesis [238, 239]. The importance of a possible link between FOXOs and Pax3/7 was initially underscored by the finding of a naturally occurring chromosomal translocation between FOXO1 and Pax3/7 that results in a Pax3/7-FOXO1 fusion protein in human alveolar rhabdomyosarcomas [240]. FOXO3A has been proposed to play an important role in skeletal muscle regeneration, since FOXO3A deficiency markedly reduces the myotube-forming potential of satellite cells, thus revealing a muscle regeneration defect after injury. The underlying molecular mechanism might involve the formation of a FOXO3A/Pax3/7 transcriptional activating complex on the *myod* promoter [241].

2.3. TGF-β

2.3.1. Smad

The TGF-β family members are multifunctional proteins that regulate various biological processes, including cell growth, differentiation, apoptosis, motility, and extracellular matrix production, and thus play essential roles in embryonic development and the pathogenesis of various diseases [242]. TGF-β transduces signals through heteromeric complexes of serine/threonine kinase receptors and intracellular Smad proteins [243]. Phosphorylated Smad2 and Smad3 (Smad2/3) proteins form oligomers, that might or might not include Smad4, and translocate to the nucleus, where they regulate the transcription of target genes. TGF-β-induced

gene expression is frequently modulated by other transcription factors and cofactors that confer target specificity on Smad complexes. TGF-β regulates the EM transition, having a cytostatic effect on the epithelium and a proliferative effect on the mesenchyme. The TGF-β/Smad signaling pathway thus functions in cancer development and progression as a double-edged sword, acting as a tumor suppressor in early tumorigenesis and as a tumor enhancer at later stages. TGFβ1 is also a potent profibrogenic factor; for example, in the damaged liver it stimulates transdifferentiation and proliferation of hepatic stellate cells, increased expression of extracellular matrix components, hepatocyte apoptosis and liver fibrosis [244].

Several studies have shown that the FOXOs regulate TGFβ/Smad signaling both positively and negatively, depending on the cellular context. As already mentioned, FOXO1 facilitates the final phase of myocyte differentiation and the formation of myotubes [213] by forming an inhibitory complex with Smad1/4 and therefore interfering with TGFβ signaling. Another example of a negative regulatory circuit is the regulation of plasminogen activator inhibitor-1 (PAI-1) expression. PAI-1 is elevated in pathological conditions associated with hyperinsulinemia, including atherosclerosis and hepatic and renal fibrosis [245, 246]. It is expressed in many cell types under the control of a variety of signals [247], being the most important TGF-β and insulin [248, 249]. A recent study has shown that FOXO1 prevents insulin-stimulated PAI-1 gene expression, at least in part through the inhibition of TGF-β signaling. The authors also propose that FOXO1 activity likely depends on its repressive interaction with Smad3 on the PAI-1 promoter [250].

Several studies have shown that FOXOs and Smads can cooperate in the regulation of metabolism and cell cycle control genes. The first clue of such a connection came from the finding that TGF-β signaling, like DAF-16, is involved in the control of *C. elegans* metabolism and development [251, 252]. Ablation of DAF-2, the *C. elegans* ortholog of the mammalian insulin receptor, exhibited genetic synergy with the nematode DAF-7/DAF-3 (TGF-β/Smad) pathway, suggesting that DAF-16 can cooperate with nematode Smad proteins in regulating the transcription of key metabolic and developmental control genes [55]. This work also showed that DAF-16 and DAF-3 could form heterodimers that repress the expression of genes regulating metabolism and development.

Mammalian TGF-β delivers cytostatic signals to epithelial, neuronal, and immune cells, and loss of TGF-β contributes to tumor development [242]. The TGF-β cytostatic program involves transcriptional activation of the cyclin-dependent kinase inhibitors *p21Cip1* and *p15Ink4b* and repression of the growth-promoting transcription factors c-*myc* and *Id1-Id3* [253, 254]. In 2004 FOXO proteins were identified as key partners of Smad3 and Smad4 in TGFβ-dependent formation of a *p21Cip1* transactivation complex [48]. TGF-β induces the binding of Smad2/3 and Smad4 to the promoter and the simultaneous removal of c-Myc. TGF-β response requires the binding of FOXO factors to a functional FOXO binding site (IRE) adjacent to the Smad binding site (SBS), and is mediated by the direct interaction of FOXO factors with Smad3/Smad4. Whole genome analysis later showed that a complete set of genes are corregulated by FOXO/Smad complexes, and indicated that the transcription factor C/EBPβ was likely to be part of the regulatory network in a significant number of the corregulated promoters [255-257].

2.3.2. C/EBPs

C/EBPs are executors of lineage commitment and terminal differentiation programs, and have more recently emerged as important negative regulators of cell proliferation. However, C/EBPβ has also been shown to promote tumorigenesis in the skin, and progenitor expansion before terminal differentiation [258-261]. A subset of FOXO/Smad-dependent TGFβ gene responses additionally require C/EBPβ [256, 257]. For example, in human epithelial cells C/EBPβ is essential for TGFβ induction of the cell-cycle inhibitor p15INK4b by a FOXO-Smad complex and for repression of c-Myc by an E2F4/5-Smad complex. The molecular mechanisms underlying C/EBPβ-mediated effects have not been completely resolved, but diverse configurations of FOXO and Smad-binding elements in the promoters of target genes were identified. Several reports have also indicated that TGFβ signaling can also induce Smad3/4-dependent inhibition of C/EBPβ function [262-264].

2.3.3. FOXG1

The transcription factor FOXG1 is a determinant of forebrain size in vertebrates and has been associated with cancer development [2, 265]. FOXG1 functions as a transcriptional repressor that protects neuroepithelial progenitor cells from cytostatic signals [266]. Its mechanism of action seems to involve at least in some cases the inhibition of FOXO activity through direct interaction with FOXO factors. Interestingly, FOXG1 also inhibits *p21Cip* induction by TGF-β [48], and this is likely to be important in tumor progression [267]. The relevance of this regulation in cell differentiation *in vivo* has been further supported by a recent work showing that FOXO3A is required for TGF-β-dependent generation of Cajal–Retzius (CR) neurons in FOXG1 deficient zones through the induction of *p21* [268].

2.3.4. Human Kruppel-Like Factor 5 (KLF5)

Human Kruppel-like factor 5 (KLF5) is a zinc finger transcription factor belonging to the Sp/Kruppel-like family. KLF5 is pro-proliferative in some cell types, including immortalized but non-tumorigenic epithelial cells, but anti-proliferative in cancer cells [269-271]. The pro-proliferative KLF5 becomes anti-proliferative when TGF-β signaling is activated in epithelial cells, playing an essential role in TGF-β induced *p15* expression. KLF5 inhibits *p15* transcription in the absence of TGF-β but it induces it when TGF-β is activated, when KLF5 interacts with Smad on the *p15* promoter. Acetylation of KLF5 by the coactivator p300 is responsible for this reversal of KLF5 function [272-274]. The acetylase p300 is a well established co-activator of the TGF-β/Smad pathway in epithelial cells [275]. It has been recently proposed that FOXO3A also positively regulates p15 expression and interacts with both Smad4 and KLF5 on the *p15* promoter, with this interaction being also dependent on the acetylation of KLF5 by p300 [273].

2.3.5. HNF4α

Hepatic bile acids are highly cytotoxic and their synthesis is tightly controlled. During cholestatic liver injury (BDL), hepatic cells release proinflammatory cytokines (IL-1β, TNF-α) and growth factors (hepatic growth factor, TGF-β). Numerous studies have shown that TGF-β1, TNF-α, and insulin inhibit transcription of cholesterol 7a-hydroxylase (*Cyp7a1*) gene and bile acid synthesis in human hepatocytes. However, it has been recently reported that Smad3, FOXO1, and HNF4α can synergistically stimulate rat *Cyp7a1* gene transcription forming a complex on the *Cyp7a1* promoter, while both insulin and TNFα prevent TGF-β activation of *Cyp7a1* [276].

*2.*4. Hepatocytes

C/EBPs are a family of six proteins, the most widely expressed and most well studied being the C/EBPα and C/EBPβ isoforms [277]. These transcription factors control the differentiation of a range of cell types, and play key roles in regulating cellular proliferation through interaction with cell cycle proteins. More recently, C/EBPs have been described as both tumor promoters and tumor suppressors. The ability of C/EBPα to direct cellular fate in a context-specific manner is thought to depend on the presence of specific collaborating transcription factors [278].

The specification of hepatocytes during normal development occurs in the absence of C/EBPα, but recently it has been proposed that C/EBPα can direct hepatoblasts towards the hepatocyte lineage in the setting of transplantation. C/EBPα-deficient bipotent embryonic hepatoblasts give rise almost exclusively to biliary epithelial cells when transplanted, whereas wild-type cells develop into hepatocytes under these conditions [279]. This notion is supported by the observation that C/EBPα null mouse hepatocytes display traits normally associated with biliary epithelial cells [280].

C/EBPβ has been known for some time to contribute to insulin-regulated gene expression through interaction with an IRS in the promoter of the gluconeogenic gene *pepck* and *insulin-like growth factor binding protein-1* (*Igfbp1*), where C/EBPβ interacts with FOXO1 [281]. FOXO1 binds *pepck* in a C/EBPα-dependent manner *in vivo*, while insulin inhibits the C/EBPα-dependent transcription of *pepck* [282]. The relevance of this regulation is highlighted in the perinatal stage. In prenatal stage nutrients are provided from mother *via* the placenta, but nutritional supplies from mother stop abruptly after birth. Thus, newborns have to survive by their own metabolic functions immediately after birth; C/EBPα is indispensable for the onset of gluconeogenesis in perinatal liver [283, 284]. However, C/EBPα is already expressed in fetal liver, indicating that additional factors are required. Importantly, FOXO1 expression is strongly increased in the perinatal liver and promotes C/EBPα-dependent transcription of *pepck*. These results not only indicate that FOXO1 regulates gluconeogenesis cooperatively with C/EBPs, but also establish a link between metabolism and FOXO factors in liver development [282, 285].

Multidrug Resistance

Another regulatory circuit likely to be corregulated by FOXOs and C/EBPs is multidrug resistance. The development of multidrug resistance 1 (MDR1) can be mediated by a number of different mechanisms, but basically involves the induction of detoxification systems. Elevated gene expression of MDR1 has often been a major cause of chemoresistance in many cancer cells. Investigation of the transcriptional control of MDR1 revealed that the proximal promoter region of the human MDR1 gene contains a FOXO-binding site, which partially overlapped with a C/EBPβ binding region. FOXO1 was shown to regulate MDR1 expression, but cooperativity with C/EBPβ, although likely to occur, has not yet been tested [286].

*2.*5. Decidualization of Endometrial Stromal Cells

2.5.1. C/EBPβ

C/EBPß and FOXO1 also cooperate in the transcription of the human decidual prolactin (dPRL) promoter in differentiating human endometrial stromal (ES) cells [287]. During the menstrual cycle, ovarian estradiol and progesterone stimulate the ordered growth and differentiation of endometrial tissue compartments. In humans, this includes decidualization of ES cells [288]. The decidual process requires elevated intracellular cAMP levels and sustained activation of the PKA pathway [289]. Expression of the tissue specific dPRL promoter is a biochemical marker of this process [290]. Previous studies have shown that C/EBPβ is induced during ES cell differentiation [291] and participates in the formation of a nucleoprotein complex that binds the proximal dPRL promoter region upon PKA activation. Differentiation of human ES cells into decidual cells is also associated with the induction of FOXO1, that was identified as a cAMP inducible gene in differentiating human ES cells, that interacts and cooperates with C/EBPβ. The complex binds to a composite FOXO-C/EBPβ response unit in the proximal promoter region or dPRL [287]. FOXO1 has also been shown to regulate the expression of C/EBPα [292] and C/EBPβ [137].

2.5.2. HoxA

C/EBPβ is not the only transcription factor that cooperates with FOXO in endometrial decidualization. Homeobox transcription factors (Hox) contain a highly conserved DNA binding domain termed the homeodomain [293]. Hox proteins are developmentally regulated transcription factors that are important for spatial identity and differentiation of tissues in the developing embryo. The Hox factors play crucial roles in the modulation of vascular function although little is known about their downstream target genes. HoxA5 is expressed in quiescent endothelial cells but it becomes down-regulated upon endothelial cell activation by angiogenic stimuli. Moreover, increased HoxA5 expression blocked angiogenesis *in vivo* and cell migration *in vitro* [294]. Therefore, both the expression pattern and activity of HoxA5 resemble those of FOXO1 [295]. HoxA10 also has a similar pattern of expression that FOXO1 during different stages of the baboon menstrual cycle and pregnancy [296], and HoxA10 null mutant mice exhibit infertility due to compromised endometrial decidualization during blastocyst implantation [297].

The FOXO1 transcriptional target IGFBP-1 is mainly produced by hepatocytes and decidualized endometrium. IGFBP-1 inhibits IGF-dependent cellular growth and

differentiation both *in vitro* [298] and *in vivo* [299, 300] and is thought to play a role during blastocyst implantation [295]. Transgenic mice overexpressing HoxA5 show impaired postnatal growth that correlates with a strong upregulation of IGFBP-1 in the liver [301]. HoxA5 upregulation of IGFBP-1 is probably dependent on its interaction with FOXO1 since these factors cooperatively activate the *Igfbp1* promoter in a fibroblast cell line. However, the same authors proposed that Hoxa5 can also repress FOXO1 activity in another cellular system. FOXO1 also associates directly with HoxA10 *in vitro,* and this complex also cooperatively transactivates the *Igfbp1* promoter [296].

2.5.2. Progesterone Receptor (PR)

The postovulatory rise in progesterone levels in preparation for pregnancy induces extensive remodeling of the endometrium, associated with morphological and biochemical differentiation of stromal cells into decidual endometrium. As mentioned, differentiation of human ES cells into decidual cells is associated with the induction of FOXO1 and its transcriptional targets. Knockdown of the progesterone receptor (PR) in ES cells disrupts the regulation of FOXO1 target genes involved in differentiation (*Igfbp1*, *PRL*, and *WNT4*) and cell cycle regulation (*CDKN1*, *CCNB2* and *CDC2*) [302]. This suggested a functional link between PR and FOXO1 in the endometrium [302]. This idea is supported by a recent report demonstrating that many type I endometrial cancers in which PTEN is inactivated (ultimately leading to a constitutively activation of AKT and inhibition of FOXO1), expression of a constitutively active FOXO1 mutant induces cell cycle arrest and apoptosis in a PR-dependent manner [303]. This functional link is probably dependent on a PR/FOXO1 interaction, as already shown in the case of *Igfbp1* [304]. Liganded PR is recruited together with FOXO1 to the *Igfbp1* promoter to induce its expression [305]. Similarly, FOXO3A has been shown to interact and induce hormone dependent activation of PR-A [306].

2.6. Adipocytes

An essential role for FOXO1 has recently been proposed in adipocyte differentiation. Downregulation of FOXO1 decreases the expression of the adipogenic transcription factors PPAR-γ and C/EBPα [292], and FOXO1 has been associated with pro-inflammatory gene expression in obese subjects. Obesity is associated with a low-grade inflammation in adipose tissue resulting from increased production of pro-inflammatory cytokines and which can subsequently contribute to the development of insulin resistance. However, the mechanisms underlying the transcriptional regulation of pro-inflammatory genes are still unclear. In TNFα-treated adipocytes, AKT-dependent phosphorylation of FOXO1 is reduced, enhancing its transcriptional activity. It has been proposed that FOXO1 could increase pro-inflammatory gene expression by inducing C/EBPβ through direct binding to its promoter [137].

Adiponectin is an adipocyte-derived hormone that plays an important role in energy metabolism. It enhances insulin sensitivity and improves fatty acid oxidation in skeletal muscle. Although adiponectin is predominantly produced by adipose tissues, plasma adiponectin concentration and adiponectin gene expression are inversely correlated with adiposity [307]. Adiponectin gene expression is turned on during adipocyte differentiation after clonal expansion [308], coinciding with the initiation of FOXO1 activity. FOXO1 is inhibited during clonal expansion (days 1 and 2), through phosphorylation and exclusion from the nucleus. By day 3 of differentiation, phosphorylation of FOXO1 is decreased, and it is located in the nucleus [64]. FOXO1 has been proposed to positively regulate the expression of adiponectin by binding to its promoter. This activation involves interaction of FOXO1 with C/EBPα, that serves as a FOXO coactivator, and this interaction is enhanced by the deacetylase SirT1 [309].

2.7. Hematopoiesis

Given the importance of FOXO/C/EBPs cooperative interaction in other systems, it would be interesting to investigate whether these factors are functionally linked in the control of hematopoiesis and hepatopoyetic stem cells (HSCs). The role of FOXO transcription factors in the regulation of hematopoiesis has recently been investigated using mice harboring the interferon-inducible transgene *Mx-Cre* in a *FOXO1/3/4LoxP/LoxP* background, enabling conditional deletion of FOXO1, 3 and 4 in the adult hematopoietic system. FOXO deficient mice exhibit increased levels of myeloid cells and aberrant development of the B and T lymphoid compartments, including a decrease in peripheral blood lymphocytes. The FOXO deficient animals eventually develop leukocytosis characterized by a relative neutrophilia and lymphopenia. Deletion of FOXO1, 3 and 4 not only induced HSC proliferation, but also increased the level of apoptosis in HSCs and myeloid progenitors. This might explain the reduction of HSC numbers that followed the initial expansion, and was reflected in a defective long-term repopulating capacity of bone marrow cells [58]. Interestingly, a critical role in regulation of both myelopoiesis and self-renewal of HSCs is played by C/EBPα [310]. It was recently demonstrated that the inhibitory phosphorylation of GSK-3 by AKT results in dephosphorylation and subsequent activation of C/EBPα in hematopoietic progenitors. Moreover, active GSK-3 induces eosinophil differentiation and inhibits neutrophil development, whereas dephosphorylation of C/EBPα induces neutrophil differentiation [311]. Therefore, inhibition of GSK-3 activity affects lineage development, at least in part, through regulation of C/EBPα transcriptional activity, suggesting that pharmacological modulation of this signaling module could provide a means of clinically modulating bone marrow activity.

A recent report has evidenced an unexpected role of FOXO3A in Chronic myeloid leukaemia (CML) that is closely link to the capacity of FOXO3A to maintain the renewal capacity of hematopoietic stem cells. Chronic myeloid leukaemia (CML) is caused by a genetic abnormality that generates BCR-ABL, a constitutively active tyrosine kinase [312]. The tyrosine kinase inhibitor imatinib is a breakthrough for CML therapy, however, imatinib does not deplete the leukaemia initiating cells (LICs) that drive the recurrence of CML [313]. FOXO3A was found to play an essential role in the maintenance of CML LICs. The authors also showed that in this setting TGFβ activity is fundamental to control FOXO3A nuclear localization [314].

3. NUCLEAR HORMONE RECEPTORS: AT THE CROSSROADS OF METABOLISM AND CELL PROLIFERATION CONTROL

Interaction between FOXOs and nuclear hormone receptors (NHR) was first detected in *C. elegans,* where the NHR DAF-12, which had been implicated in insulin-like signaling, was shown to interact with the forkhead factor DAF-16 [315]. FOXO factors have since been shown to interact with numerous NHRs (androgen receptor (AR), estrogen receptor α (ERα), PR, GR, constitutive androstane receptor (CAR), retinoic acid receptor (RAR), pregnane X receptor (PXR), PPARγ, thyroid hormone receptor (TR), and HNF-4 [77, 78, 316, 317]. It has been suggested that FOXOs might interact with nuclear receptors through an LxxLL motif located C-terminal of the forkhead DNA-binding domain [318].

3.1. PPARs

3.1.1. PPARγ

The PPAR family of ligand-activated transcription factors includes three isoforms (α, β/δ, γ) that differ in their tissue distribution and ligand specificity: PPAR-β/δ are ubiquitously expressed in many tissues; PPARα is predominantly found in hepatocytes, cardiomyocytes and enterocytes, where it regulates lipid catabolism; and PPARγ is mainly expressed in insulin-responsive tissues, where it plays a pivotal role in adipocyte differentiation and the expression of adipose-specific genes [319]. It was originally reported that FOXO1 prevents the differentiation of preadipocytes [63] and negatively regulates PPARγ expression in primary adipocytes [320], although this effect did not seem to involve the direct binding of FOXO1 to the promoter, since a DNA-binding mutant was competent in this regulation. The transcription factor responsible for the direct DNA binding activity is still unknown. However, a later report suggested that FOXO could positively regulate adipocyte differentiation, since knockdown of FOXO1 downregulates key adipogenic transcription factors such as C/EBPα and PPARγ [292].

In contrast to the previously discussed FOXO/C/EBPβ cooperativity, FOXO1 interaction with PPARγ results in a negative regulation of its transcriptional activity by preventing PPARγ/RXR interaction with DNA. Through its binding to PPARγ, FOXO1 is recruited to PPAR response elements (PPRE) on PPARγ target genes, where it interferes with promoter occupancy of the receptor. The FOXO1 transrepressional function, which is independent of the transactivation effects, does not require a functional FOXO1 DNA binding domain, but does require an evolutionally conserved 31 amino-acid domain containing the L*XX*LL motif [318]. SirT2 deacetylation of FOXO1 has been shown to result in its increased repressive interaction with PPARγ, which has been proposed to mediate Sirt2 dependent suppression of adipocyte differentiation [321]. Conversely, it has also been proposed that PPARγ can inactivate FOXO1 [315].

An antagonistic action of FOXO1 on PPARγ activity is further supported by functional correlations such as the observation that FOXO1 down-regulation, like PPARγ activation, improves peripheral insulin sensitivity in diabetic mice [322]. FOXO1-induced increases in insulin resistance are normally attributed to its gluconeogenic activity. Other more recent reports show that FOXO1 haploinsufficiency protects mice against obesity-related insulin resistance and results in increased PPARγ levels and activity [323]. This effect is attributed to the fact that FOXO works as a negative regulator of PPARγ activity [318]. Furthermore, adipocytes from insulin resistant mice show reduced phosphorylation and increased nuclear accumulation of FOXO1, coupled to lowered expression of endogenous PPARγ target genes. Thus, it seems that the innate FOXO1 transrepression function enables insulin to augment PPARγ activity, which in turn leads to insulin sensitization, and this feed-forward cycle represents positive reinforcing connections between insulin and PPARγ signaling.

GLUT4: a Case Study in FOXO-Mediated Transrepression of PPARγ

Glucose uptake in eukaryotic cells is mediated by the GLUT family of glucose transport proteins. GLUT4 is referred to as 'the insulin-responsive isoform' because it is mainly expressed in insulin-responsive tissues, where it mediates glucose uptake in response to acute insulin stimulation [324]. PPARγ, in its unliganded form, binds to cis-elements on the GLUT4 promoter, keeping it in a repressed state. Binding of the ligand thiazolidinedione to PPARγ detaches PPARγ from the GLUT4 promoter, resulting in increased GLUT4 expression and, subsequently, enhanced insulin responsiveness [325]. In 2002 it was shown that the oncogenic fusion protein Pax3/FOXO1 activates GLUT4 gene expression both *in vivo* and *in vitro* [326]. In fact, FOXO1 can either repress or activate transcription of the GLUT4 gene, depending on the cell context. The proposed model is that FOXO1, following insulin stimulation, is excluded from the nucleus, which is followed by a partial derepression of PPARγ activity on the GLUT4 promoter.

3.1.2. PPARα

3.1.2.1. Myocytes

Accumulated evidence suggests that FOXO1 works as a coactivator of PPARα in myocytes. FOXO1 was initially found to enhance the expression of lipoprotein lipase (LPL), a PPARα target gene [327]. LPL regulates lipid usage in muscle cells by hydrolyzing plasma triglycerides to fatty acids, and is upregulated during fasting. It was observed that FOXO1-induced LPL levels increased even further in the presence of PPARα ligand, suggesting a cooperative interaction between PPARα and FOXO1. This idea is supported by functional correlations; for example FOXO1 activation in muscle cells increases CD36 (a PPARα target gene) in plasma membrane and sarcolema, and increases fatty acid uptake and oxidation [328-330]. However, it was later proposed that PPARα can inhibit FOXO1 transcriptional activity by decreasing its DNA binding capacity [331].

3.1.2.2. Hepatocytes

Hypertriglyceridemia is characterized by increased production of very-low-density lipoprotein (VLDL) and/or decreased clearance of triglyceride (TG)-rich particles. An important factor in plasma VLDL-TG metabolism is apolipoprotein C-III (apoC-III). ApoC-III is a VLDL that it is thought to inhibit hepatic uptake of TG-rich particles. It

functions as an inhibitor of lipoprotein lipase and hepatic lipase, and plays a pivotal role in the hydrolysis and clearance of TG-rich particles such as VLDL-TG and chylomicrons. Elevated plasma apoC-III levels are associated with impaired clearance of TG-rich particles, leading to the accumulation of TG-rich lipoprotein remnants in plasma and the development of hypertriglyceridemia [332]. FOXO1 stimulates hepatic apoC-III expression, which is counteracted by insulin [333]. PPARα has been shown to interact with and antagonize FOXO1-induced hepatic upregulation of apoC-III expression [331].

The pseudokinase tribble 3 (Trb3) has been proposed to regulate insulin sensitivity *via* AKT inhibition [334]. Fasting and diabetes promote Trb3 expression through PGC-1α coactivation of PPARα on the Trb3 promoter [335]. As we have already seen, PGC-1α is also a FOXO1 coactivator, and FOXO1 might therefore have been predicted to increase Trb3 expression. However, FOXO1 inhibits Trb3 expression while insulin induces it, and it has been proposed that part of FOXO1 activity on Trb3 may be mediated by its transrepression of PPARα, although the *cis*-acting elements in the Trb3 promoter required for FOXO1 repression are distinct from those utilized by PGC-1α/PPARα [65].

3.1.3. PPARδ

There is evidence of significant redundancy in the regulatory effects of PPARα and PPARδ. PPARδ is the major PPAR isoform in muscle, and is thus likely to be more important in muscle bioenergetics. An important PPARα/δ target gene, PDK4 [336], is almost unaltered in PPARα null mice [337], but its expression is almost blunted in PPARδ knockous [338]. As already noted, PDK4 is also a FOXO1 target gene [120]. Activation of PPARδ in muscle induces a fasting-like phenotype characterized by increased fatty acid oxidation and suppressed glucose oxidation [339]. This is similar to the phenotype induced by FOXO1, suggesting that some PPARδ effects in muscle might be mediated *via* FOXO1. In fact, PPARδ has been suggested to induce FOXO1 gene expression through direct binding to its promoter [340]. The notion that FOXO/PPAR complexes may regulate fatty acid use is further supported by the findings that both factors induce muscle fiber type switch [228, 341], and that both have been reported to initiate a muscle atrophy program [125, 342].

3.2. The Liver X Receptors (LXR)

Liver X Receptors (LXR) α and β are central regulators of cholesterol metabolism in mammals [343]. LXR activation in rodent liver upregulates Cyp7a1, a member of the cytochrome P450 family that is crucial for bile acid synthesis [344]. In the intestine, LXR controls the reabsorption of cholesterol *via* the expression of ABCG5 and ABCG8 [345]. Pharmacologic activation of these receptors *in vivo* results in increased high-density lipoproteins (HDL) levels, net whole body cholesterol loss, and reduced atherosclerosis [346]. Sterol regulatory element binding protein 1c (SREBP1c) is a master regulator of lipogenic gene expression in liver and adipose tissue, where its expression is regulated by a heterodimer of retinoid X receptor (RXR) and LXR. Expression of FOXO1 negatively correlates with that of SREBP1c in skeletal muscle during nutritional change, and it has been described that FOXO1 suppresses RXR/LXR-mediated SREBP1c promoter activity *in vitro* and *in vivo*. These findings provide *in vitro* evidence that RXR/LXR upregulates SREBP1c gene expression and that FOXO1 antagonizes this effect of RXR/LXR in skeletal muscle [347], suggesting a FOXO1/LXR interaction, but this has not been confirmed.

3.3. PXR and CAR

PXR and CAR were originally identified as xenosensors that regulate the expression of Phase I and Phase II drug-metabolizing enzymes and transporters. Recent results suggest that PXR and CAR also have important endobiotic roles in energy metabolism by affecting the metabolism of fatty acids, lipids and glucose [348, 349]. Expression of the major gluconeogenic enzymes *pepck* and *g6pase* is dramatically suppressed in PXR transgenic mice [350]. Consistently, activation of PXR cancels cAMP/ CREB dependent induction of *g6pase* and this effect was found to be mediated by PXR direct interaction with CREB [351]. Further investigation showed that activated PXR and CAR act as corepressors of FOXO1, thus identifying another positive regulator of gluconeogenesis, resulting in the suppression of FOXO1-mediated activation of gluconeogenic gene expression [352]. These results further support the notion that interplay between the CREB and FOXO1 pathways is important in the control of gluconeogenesis [163], mediated at least in part by FOXO1 itself, acting as a negative regulator of the CREB pathway. The biological effect of the FOXO1–PXR complex seems to be gene specific, since FOXO1 can also act as a co-activator in PXR and CAR mediated xenobiotic responses [352]. Positive cooperation among FOXO1, HNF-4, PGC-1α and PRX/CAR has also been reported in the regulation of ALAS1, a key regulator of heme synthesis, and hence the response to toxic agents that require P450 protein-dependent detoxification [353].

3.4. Steroid Receptors

Steroid hormone receptors such as AR, ER, and GR are ligand-dependent transcription factors that belong to the nuclear receptor superfamily [354]. Steroid receptors have been implicated in the development of several types of cancer such as prostate cancer, breast cancer and ovarian cancer. FOXO transcription factors play a significant role in the prevention of tumorigenesis [355, 356]. Association of FOXOs with steroid receptors can either inhibit or enhance their transcriptional activity and these interactions could play a role in the development of steroid-dependent cancers.

3.4.1. AR

AR is responsible for male sexual differentiation and male pubertal changes. AR signaling is also necessary for the development and maintenance of prostate cancer, and antagonists are currently used for therapy [357], although the molecular mechanisms are poorly understood. It was initially observed that loss of PTEN was an important event during human prostatic tumorigenesis. PTEN is a tumor suppressor and a negative regulator of the PI3K/AKT pathway. It was subsequently noted that inhibition of PI3K drastically reduced the transcriptional activity of AR, resulting in decreased androgen-induced proliferation [358, 359]. Further investigation demonstrated that expression of FOXO1 in

prostate cancer cells is lower than in healthy prostate tissue, and forced expression of FOXO1 suppressed AR-dependent gene expression in a manner independent of the FOXO1 transcriptional activating function, suggesting that FOXO1 may inhibit AR activity [360]. In fact FOXO1 interacts directly with the C terminus of AR in a ligand-dependent manner and disrupts ligand-induced AR subnuclear compartmentalization. Similar to other steroid receptors, the AR is composed of an N-terminal domain (NTD) containing a major activation function, a DNA-binding domain, a hinge region, and a C-terminal ligand-binding domain (LBD), which contains a weak activation function. Androgens induce an interaction between the N- and C-terminal regions, an event that is critical for the biological actions of the receptor [361, 362], and it is this interaction that is disrupted by FOXO1. FOXO1 also cancels the binding of p160 steroid receptor co-activator (SRC) to the AR NTD. Moreover, the AR N/C interaction is inhibited by PTEN, which also inhibits AIB1 (a member of the SCR-1 family) recruitment to AR NTD [363]. FOXO1 inhibition of the AR has recently been found to be partially dependent on its interaction with the histone deacetylase HDAC3. *In vitro,* FOXO1 reduces the promoter activity of the AR target gene prostate specific antigen (PSA) [316, 364]. Furthermore, the AR has also been reported to repress the expression of both FOXO1 and FOXO3A, which is also normally expressed in prostate tissue [316].

Evidence that FOXO3A is also a negative regulator of the AR comes from the identification of a FOXO3A binding site in the promoter of the antiapoptotic FAD D-like interleukin-1β-converting enzyme (FLICE)-like inhibitory protein (FLIP). Treatment with androgens in the absence of PI3K/AKT signaling increases FLIP expression, while it is downregulated by expression of either PTEN or FOXO3A. A FOXO3A binding site was identified in the FLIP promoter and was shown to be necessary for the combined effects of androgens and FOXO3A on FLIP transcription. FOXO3A was also shown to interact with AR, suggesting that FOXO3A, like FOXO1, can work as a negative modulator of AR activity [365].

Beyond prostate cancer, it has been noted that some other physiological actions of the growth hormone (GH)-IGF-1 system and androgens are very similar and overlap. For example, many studies have shown that both hormones act to stimulate muscle strength, increase bone mineral density, and decrease abdominal fat accumulation [366].

3.4.2. ER

Another steroid receptor implicated in the proliferation of prostate cancer cells is ERβ. ERα and ERβ interaction with FOXO1 has been described. ER is mainly expressed in mammary gland tissue, ovaries and uterus. Binding of estrogen leads to homodimerization and transcription of estrogen-responsive genes, which stimulate cell proliferation, invasion, metastasis and angiogenesis while inhibiting apoptosis [367]. Breast cancer is the most common cancer diagnosed in women worldwide, and is the second leading cause of cancer death. Approximately 70% of human breast cancers express ERα, and it is frequently overexpressed in breast cancer cells. Moreover, the cumulative exposure of breast epithelium to estrogen has been associated with the development of breast cancer. Many ERα-positive tumor cells undergo apoptotic cell death when they are deprived of estrogen, and ablation of the ERα gene delays the onset of tumor development in mouse models, indicating that ER-mediated signaling plays an important role in breast cancer [368]. Apart from ER-mediated effect of estrogen, membrane initiated actions are diverse, including activation of PI3K/AKT. Importantly, constitutive activation of the PI3K/AKT signally pathway has also been associated with development of breast cancer and overexpression of PI3K is sufficient to confer a malignant phenotype. Activation of the PI3K pathway serves to repress FOXOs-mediated growth arrest and apoptosis [369]. One study has shown that upregulation of FOXO3A in breast cancer cells enhances expression of the proapoptotic (and FOXO target) *Bim* and induces apoptosis [370]. Another study showed that the estradiol-induced increased survival of breast cancer cells depends on inactivation of FOXO1 [371].

FOXO1 interacts with ERα in a ligand-dependent manner. However, there are conflicting reports as to the effect of this interaction on ER transcriptional activity. It has been reported that FOXO1 can both activate [317] and repress [77] ERα. Importantly, the cell cycle arrest induced by FOXO1 in mammary cancer cells is abrogated by estradiol [77]. More recently, FOXO3A was found to interact with both ERα and ERβ and to inhibit their transcriptional activities. Gene expression profiling by DNA microarray suggested that FOXO3A has a global inhibitory effect on the expression of ER target genes. This report also demonstrated that FOXO3A suppresses proliferation of breast cancer cells by inducing the expression of key CDK inhibitors and reducing the expression of cyclin D1. Moreover, FOXO3A suppresses estradiol-induced tumorigenesis in an animal model of breast cancer, suggesting that FOXO3A interaction with ERs is critical for its tumor-suppression activity in estrogen-dependent breast tumors [372].

3.4.2. TR and RAR

TR and RAR. FOXO1 has been proposed to interact and stimulate both RAR and TR mediated transactivation in a ligand-independent manner [77].

4. OTHER TRANSCRIPTION FACTORS

4.1. p53

The transcription factors p53 and FOXO are both activated in response to stresses that lead to events such as cell survival or apoptosis. Several studies show striking similarities between p53 and FOXO [373], such as post-translational modifications, common signaling pathways, common target genes, similar stress responses, and similar mutual interactions with various proteins. For example, both are associated with cell cycle arrest, apoptosis, tumor suppression and aging [374], and they share several target genes like those involved in the regulation of metabolism and apoptosis. Recently it has been shown that FOXO3A can activate transcription *via* p53 sites. FOXO3A and p53 interact directly and cooperatively to regulate tumor suppression and metabolic control in a nutritional status sensitive manner [375, 376]. It has been further noted that FOXO3A decreases the DNA binding activity of p53 and

promotes its cytoplasmic translocation [375], where is directed to the mitochondria and suppresses the antiapoptotic function of Bcl-2 and Bcl-xL [377]. The structure of the FOXO3A/p53 complex has been elucidated revealing that p53 destabilizes an intramolecular interaction between the forkhead domain and the CR3 activation domain, while interacting with both domains simultaneously [23]. A recent report proposes that FOXO3A and p53 can have opposing actions in relation to apoptosis. Oxidative stress induces interaction of FOXO3A and p53, and results in the inhibition of FOXO3A trasncriptional activity while p53 activity is unaffected in COS-7 cells. p53 prevents FOXO3A induction of the pro-apoptotic *Bim* and *Bcl6*, but expression of *p27* and *cyclinG2* is not [378].

4.2. c-myc

Proapoptotic Arf/p53 signaling is the main Myc-induced tumor-suppressing pathway [379]. Additionally, Myc cooperates with constitutive AKT signaling to accelerate B-cell lymphomagenesis [380]. AKT-mediated phosphorylation of FOXO proteins is the critical PI3K signaling component that substitutes for oncogenic Ras in Myc-induced proliferation and focus formation *in vitro* [381, 382]. The tumor-suppressive potential of FOXO factors during Myc-driven lymphomagenesis is mediated through the induction of *Arf*. FOXO proteins bind to a specific site within the *Ink4a/Arf* locus and activate *Arf* expression. Moreover, expression of the p53 upstream regulator p19Arf is virtually undetectable in most FOXO negative Myc-driven lymphomas. These data, while providing further evidence for a close link between the FOXO and p53 tumor suppressor pathways, also indicate that the observed collaboration between p53 and FOXO is also consistent with the action of an unidentified FOXO target upstream of p53 [383].

Microarray analysis of a diverse group of human cancers has shown almost mirror image expression patterns for FOXO factors and Myc, supporting the notion of a negative cross-talk between the two signaling pathways. Recently, a direct interaction between Myc and FOXO3A has been demonstrated. FOXO1, FOXO3A, and FOXO4 have been found to transactivate *p27* and to control cell cycle progression and apoptosis in various cell types [40, 42]. Activation of WEHI 231 B lymphoma cells with anti-IgM induces FOXO3A and hence increases *p27* levels [384]. On the other hand, ectopic expression of Myc reduces the induction of *p27* associated with anti-IgM treatment [385]. Recent evidence shows that activation of *p27* by FOXO3A is repressed by Myc, and that Myc directly inhibits FOXO3A-mediated activation of *p27* promoter [386].

4.3. STAT3

The signal transducer and activator of transcription 3 (STAT3) participates in various critical cellular processes [387], including the differentiation of neural stem cells into astrocytes [388]. In addition, STAT3 supports the renewal capacity of neural stem cells [389]. These activities have been connected to the observation that deregulation of STAT3 signaling can contribute to glial cell transformation [390]. STAT3 has in fact been assigned a pro-oncogenic function in a subset of glioblastomas [391] and in several cell types outside the nervous system [392]. It has been shown that STAT3 activation promotes the survival of certain glioblastoma cell lines *in vitro* [393]. That STAT3 has also a tumor-suppressive function, intimately linked to PTEN function, was evidenced in knockout studies. PTEN deficiency, and consequent AKT activation, tightly correlates with inactivation of the LIFRβ–STAT3 signaling pathway in astrocytes. The cytokine receptor LIFRβ has been identified as a direct target of FOXO3A in human glioblastomas, providing an explanation of how the PTEN–AKT–FOXO cascade modulates both glial developmental and the LIFRβ–STAT3 signaling pathway. The intersection of these two signaling networks allows the loss of PTEN to down-regulate LIFRβ expression and inhibit STAT3 activity, thereby relieving STAT3's suppression of glial cell proliferation, invasiveness, and transformation. Strikingly, in contrast to STAT3's tumor-suppressive function in the PTEN pathway, STAT3 in its pro-oncogenic mode associates with the oncoprotein epidermal growth factor receptor type III variant (EGFRvIII) in the nucleus thereby inducing glial transformation [390].

Direct inhibitory interaction between FOXO3A and STAT3 has recently been demonstrated in the context of the leptin signaling pathway. Leptin is a hormone secreted by adipose tissue that regulates food intake and energy expenditure. Leptin levels are often higher in obese subjects. However, in these subjects leptin fails to be effective because its signaling pathway is impaired, a phenomenon known as leptin resistance. Leptin actions are mediated by binding and activation of the long form leptin receptor (OBRb) [394]. Activated OBRb turns on the JAK2-STAT3 pathway, inducing STAT3 phosphorylation and translocation into the nucleus, where it regulates its target genes such as the neuropeptide pro-opiomelanocortin (POMC) [395, 396]. A recent study has shown that STAT3 activates POMC promoter in response to leptin through a mechanism that requires an specificity protein 1 (SP1)-binding site. FOXO1 binds to STAT3 and prevents the formation of a STAT3/SP1 on the POMC. FOXO3A inhibition of STAT3-mediated leptin action thus suggests a potential mechanism of leptin resistance [210].

The negative interplay between the STAT3 and FOXO pathways is also implicated in the regulation of liver gluconeogenesis, where STAT3 suppresses the expression of gluconeogenic genes [397]. The deacetylase SirT1, which as we have seen induces gluconeogenesis in response to fasting at least in part through activation of FOXO1 and PGC-1α, has recently been shown to deacetylate and inactivate STAT3, hence suppressing its inhibitory action on gluconeogenesis [398].

4.4. RUNX

RUNX transcription factors are α subunits of the polyomavirus enhancer-binding protein 2 (PEBP2)2/core-binding factor (CBF), which consists of α and β subunits. Three RUNX transcription factors, RUNX1, RUNX2, and RUNX3, have been identified in mammals. RUNX1 is essential for definitive hematopoiesis, RUNX2 plays critical roles in osteoblast maturation and osteogenesis, and RUNX3 is ubiquitously expressed and involved in a variety of biological activities including the development of the gastrointestinal tract, neurogenesis, and lineage specification

of thymocytes. RUNX3 is an important downstream effector of TGFβ signaling in gastric epithelium. It activates Smads through direct interaction [399]. RUNX3-deficient mice exhibit hyperplasias in gastric mucosa due to reduced apoptosis and increased proliferation of gastric epithelial cells. RUNX3-deficient gastric epithelial cells are less sensitive to the proapoptotic and growth inhibitory effects of TGFβ [400]. Investigation of RUNX3's tumor suppressor activity revealed that RUNX3 interacts with FOXO3A in gastric cancer cell lines, leading to activation of Bim and subsequent induction of apoptosis. The same interaction is also observed in mouse embryonic fibroblasts, suggesting that RUNX3 is involved in apoptosis mediated by Bim, which is transcriptionally regulated by FOXO3A in a variety of cell types [401]. In this regard is notable that TGFβ induces the expression of Bim through a Smad-dependent mechanism [402].

Another recent report linking TGFβ to RUNX and FOXOs, showed that TGFβ specifically induces the expression of RUNX1 in two hepatocyte cell lines that undergo apoptosis upon TGFβ treatment. RUNX1 regulates Bim *via* its direct interaction with FOXO3A on the identified IRS promoter element. Consistently, knockdown of RUNX1 or FOXO3A decreased TGFβ-induced Bim expression [403]**.** Since Smads and FOXOs are known to cooperate in the induction of cell cycle arrest genes, it could be that a Smad/ FOXO/RUNX complex is formed on the Bim promoter to induce apoptosis.

4.5. NF-κB

NF-κB and c-Jun are known as the two major transcription factors mediating TNF receptor (TNFR) signaling [404]. TNF is a potent cytokine that has pleiotropic functions in inflammation, cell proliferation, and apoptosis [405]. In the TNFR cascade, NF-κB plays a dominant role mediating the inflammatory response while blocking apoptosis *via* inhibition of the c-Jun-N-terminal kinase (JNK) [406]. Microarray analysis of FOXO3A upregulated genes in endothelial cells identified several genes of the TNF signaling pathway, including TNF-α, TANK (TRAF-associated NF-κB activator), TTRAP (TRAF and TNF receptor-associated protein), and κB-Ras1 (IκB-interacting Ras-like protein-1), which might be responsible for the activation of JNK and suppression of NF-κB, suggesting a link between FOXO3A and the TNF receptor signaling [407]. A more recent report has showed a direct inhibitory interaction between FOXO4 and NFκB. FOXO4 transcriptional activity is transiently repressed in colitis induced by trinitrobenzene sulfonic acid (TNBS), consistent with a role of FOXO4 in intestinal mucosal immunity and inflammatory bowel disease (IBD). FOXO4-null mice are more susceptible to TNBS injury and show increased transcriptional activity of NF-κB *in vivo*, suggesting that FOXO4 suppresses the inflammatory response to TNBS through the inhibition of NF-κB transcriptional activity. This report further showed that FOXO4 inhibits the *in vitro* activity of NF-κB on several target genes through direct interaction [408].

4.6. Four and a Half LIM 2 (FHL2)

Four and a Half LIM 2 (FHL2) is differentially expressed in normal human myoblasts and their malignant counterparts [409]. FHL2 has been proposed to control the hypertrophic response to stress in cardiomyocytes [410] and to interact with FOXO1 both *in vitro* and in prostate cancer cells. Binding by FHL2 inhibits the transcriptional activity of FOXO1 and facilitates its deacetylation by SirT1. These findings raise the interesting possibility that interaction of FHL2 with FOXO1 and SirT1 might promote prostate tumorigenesis in response to increased stress during aging [174].

4.7. Hipoxia Induced Factor 1α (HIF-1α)

The transcription factor HIF-1 directs the induction of glycolysis genes in response to hypoxia. HIF-1 has two subunits, α and β. HIF-1α is a labile protein that is stabilized under hypoxic conditions [411-413]. Cells that lack PTEN (particularly prostate tumors and glyoblastomas) have increased HIF-1 activity. FOXO3A is a direct inhibitor of HIF-1α and prevents its activation by p300 on the *glut-1* promoter, a HIF-1 target gene. Since HIF is linked to tumor angiogenesis *via* VEGF induction, it has been suggested that FOXO may inhibit tumor vasculogenesis at least in part through the inhibition of HIF-1 [414].

4.8. ΔEF1

Mature lymphocytes are maintained in the quiescent state until recognition of antigen (Ag). Several observations suggest that FOXO transcription factors help to maintain lymphocyte quiescence while activation of PI3K is required for lymphocyte cell cycle entry [415, 416]. FOXO1 and FOXO3A are expressed in resting T and B cells, and are rapidly phosphorylated and deactivated upon Ag-receptor activation in a PI3K-dependent manner [417, 418]. Activation of mouse B cells is accompanied by PI3K-dependent down-regulation of *Ccng2* (encoding the protein cyclin G2) and *Rbl2* (encoding the retinoblastoma-like protein p130/Rb2) [419], both of which are FOXO target genes previously implicated in FOXO-dependent quiescence in fibroblasts [420, 421]. The promoter sequences of *Ccng2* and *Rbl2* contain several binding sites for the transcription factor ΔEF1, a member of the ZEB family of zinc finger factors [422]. ΔEF1 can be either a repressor or an activator of transcription in different systems [423]. This protein is involved in T cell development and represses IL-2 transcription in T cells [424, 425], but its function in B cells had not been investigated. It was found that ΔEF1 binds to *Ccng2* and *Rbl2* promoters and activates their expression, synergizing strongly with FOXO dependent activation. This cooperation does not require direct FOXO DNA-binding and enhances cell cycle arrest in B lymphoma cells, thus establishing a novel functional cooperation in B cells between FOXO transcription factors and ΔEF1 [419].

5. NON-TRANSCRIPTION FACTOR FOXO INTERACTING PROTEINS

5.1. TRIB2

TRIB2, a human ortholog of the *Drosophila* gene *tribbles*, known to regulate furrow formation [426], has been recently found to be highly expressed in human melanomas where it drives nuclear exclusion of FOXO3A [427].

Although the mechanism involved remains to be elucidated, this regulation could play an important role in the maintenance of the malignant phenotype of melanoma cells.

5.2. Follicle Stimulating Hormone Receptor (FSHR)

Follicle stimulating hormone (FSH) is required for fertility in females, where it binds to FSHR on granulosa cells in the ovary. In males, FSHR is present on Sertoli cells in the testes, where FSH is necessary for high quality sperm production and normal testicular volume. FSH stimulates the PI3K/AKT pathway [428] and forms a membrane-bound complex that appears to include AKT2 [429], and FOXO1 [430]. This observation may be related to previous data showing that FSH induces HIF in a PI3K/AKT dependent manner [431].

5.3. Pin1

Pin1 is a peptidyl-prolyl isomerase that specifically recognizes phosphorylated serines and threonines flanked by a COOH-terminal proline residue [432]. Pin1-mediated isomerization modulates the activity of its substrates by inducing conformational changes in the peptide backbone. It is involved in numerous processes, including the regulation of cell proliferation and death. Moreover, Pin1 is over-expressed in many human cancers and is linked to tumorigenesis [433]. In response to cellular stress, FOXOs are phosphorylated and recognized by Pin1. Pin1 negatively regulates FOXO monoubiquitination at the level of deubiquitination through HAUSP/USP7 (Herpes virus Associated USP). This inhibits nuclear FOXO translocation in response to hydrogen peroxide–induced stress and ultimately leads to decreased transcription and expression of FOXO4 transcriptional targets, including p27kip1. Notably, an inverse correlation between low p27kip1 levels and Pin1 expression was found in a panel of primary human breast cancers [434].

5.4. Poly(ADP-Ribose)Polymerase-1 (PARP-1)

PARP-1 is an abundant and ubiquitous nuclear enzyme that catalyzes the nicotinamide adenine dinucleotide (NAD)-dependent addition of ADP-ribose polymers on a variety of target proteins [435]. PARP-1 has been implicated in diverse biological processes, including transcriptional regulation, chromatin remodeling, DNA repair, cell proliferation, and apoptosis [436, 437]. These roles include actions of PARP-1 as a transcriptional coregulator (either a coactivator or a corepressor) of a variety of transcription factors. In some cases, enzymatic activity of PARP-1 is required for this coregulatory function [438], while in others it is not (for example, NF-κB, B-Myb, and RAR) [439]. PARP-1 can function as a negative regulator of FOXO1, preventing FOXO1-mediated induction of the cell cycle inhibitor p27Kip1. Knockdown of PARP-1 decreases cell proliferation in a manner dependent on FOXO1 function. PARP-1 is recruited to the *p27Kip1* gene promoter through binding to FOXO1, where it poly(ADP-ribosyl)ates the FOXO1 protein. However, the enzymatic activity of PARP-1 is not required for its repression of FOXO1 function. These results suggest that PARP-1 acts as a corepressor for FOXO1, which could play an important role in the regulation of cell proliferation by modulating p27Kip1 gene expression [440].

CONCLUDING REMARKS

The FOXO transcription factors orchestrate their main biological functions trough a complex network of interactions with different transcription factors and cofactors. They show remarkable flexibility in their modes of action, they can rely or not on specific binding sites on the target promoters, the may work as activators or repressors, and they can engage in multiple simultaneous complex interaction, each one of them responding to a specific signaling specificity. Beyond their crucially relevant biological functions they are teaching us how molecular networks are orchestrated, and it seems that there is much more to come in the near future.

Although there are not yet any clinical trials that target FOXO factors, its crucial role as tumor suppressor and regulator of cell metabolism make the FOXO factors potentially useful as pharmacological targets. We believe, that given the multitude of biological functions regulated by FOXOs it is likely that pharmacological modulation of specific interactions like those that mediate FOXO regulation by sirtuins and CBP/p300 would provide the necessary target specificity that will make modulation of FOXOs activity clinically relevant [427].

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