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Forum Review

FOXO Transcription Factors in Cell-Cycle Regulation and the Response to Oxidative Stress

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ABSTRACT

Mammalian forkhead members of the class O (FOXO) transcription factors, including FOXO1, FOXO3a, and FOXO4, are implicated in the regulation of a variety of cellular processes, including the cell cycle, apoptosis, DNA repair, stress resistance, and metabolism. FOXO proteins are negatively regulated by the phosphatidylinositol 3-kinase–Akt signaling pathway, which is activated by growth factors and cytokines. Recent studies indicate that the activities of FOXO proteins are also regulated by oxidative stress, which induces their phosphorylation, translocation to the nucleus, and acetylation–deacetylation. Similar to the tumor suppressor p53, FOXO is activated by stress and induces the expression of genes that contribute to cell-cycle arrest, suggesting that it also functions as a tumor suppressor. *Antioxid. Redox Signal.* 7, 752–760.

INTRODUCTION

THE GENOME OF CELLS is continually damaged by environmental insults, such as ultraviolet light (UV) and ionizing radiation; by oxidative stress, such as that attributable to reactive oxygen species derived from oxidative metabolism; and, in dividing cells, by errors in DNA replication and mitosis. Organisms have evolved mechanisms that maintain genomic integrity by inducing cell-cycle arrest in response to DNA damage. Cell-cycle checkpoints at the G_1-S and G_2-M transitions are thus responsive to DNA damage and constitute a major mechanism for genomic surveillance (43, 75). These checkpoints allow the cell time to repair DNA damage before resumption of cell-cycle progression, or, if the damage is too extensive, they trigger cellular senescence or apoptosis. Incomplete repair of DNA damage as a result of defective checkpoint operation leads to damage accumulation over time and, eventually, to age-related conditions such as cancer.

The FOXO (forkhead member of the class O) family of forkhead transcription factors comprises three functionally related proteins—FOXO1 (also known as forkhead in rhabdomyosarcoma, or FKHR) (22), FOXO3a (FKHR-like 1, or FKHRL1) (1, 26), and FOXO4 (acute lymphocytic leukemia–1 fused gene from chromosome X, or AFX) (4)—that are vertebrate orthologues of the *Caenorhabditis elegans* (*C.elegans*) transcription factor DAF-16 (38, 49). The genes for the three mammalian proteins of this family were initially identified as sites of chromosomal breakpoints in human cancers (1, 4, 22, 26). The DNA-binding domains of the transcription factors PAX3 or PAX7, which are important in neuromuscular differentiation, are thus fused to the transactivation domain of FOXO1 in some rhabdomyosarcomas (22). Similarly, the DNA-binding domain of MLL (mixed-lineage leukemia), which functions as a positive regulator of Hox genes during embryonic development, is fused to the transactivation domains of FOXO3a or FOXO4 in certain leukemias (1, 4). These fusions alter the transactivation properties of the respective proteins and are thereby thought to contribute to carcinogenesis. FOXO proteins participate in regulation of the cell cycle, apoptosis, DNA repair, and detoxification by either inducing or suppressing the expression of target genes. Furthermore, the intracellular localization of these proteins is regulated by oxidative stress, as well

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as by a phosphatidylinositol 3-kinase (PI3K)–mediated signaling pathway. In this review, we focus on the role of FOXO proteins in regulation of the cell cycle and in the cellular response to oxidative stress.

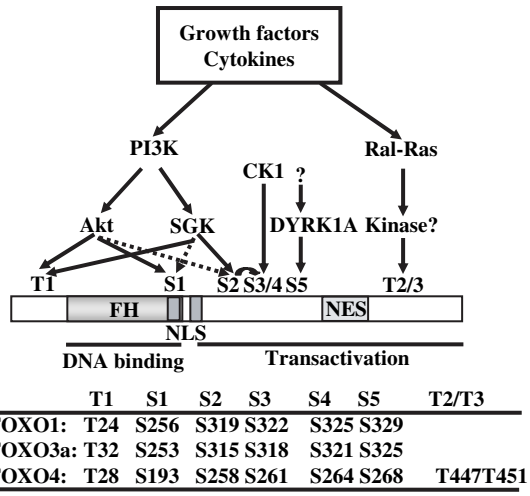
SIGNALING PATHWAYS THAT REGULATE FOXO PROTEINS

Regulation of the subcellular localization and transactivation activity of FOXO proteins is achieved primarily by post-translational modifications, such as phosphorylation and acetylation. Genetic studies in *C. elegans* have indicated that the forkhead transcription factor DAF-16 functions downstream of Akt (also known as protein kinase B, or PKB) in a PI3K signaling pathway (50). Certain single-gene mutations, such as *daf-2* [which affects the gene for the insulin and insulin-like growth factor–1 (IGF-1) receptor] and *age-1* (catalytic subunit of PI3K), increase adult life span and promote constitutive dauer formation in *C. elegans* (18, 30). Such mutants also exhibit an increased resistance to a variety of environmental insults, including oxidative stressors and UV (27, 37, 39, 45, 69), and these longevity and stress resistance phenotypes are dependent on DAF-16 (30, 65). DAF-16 possesses four consensus sequences (RXRXXS/T) for phosphorylation by Akt (50), three of which are conserved in the mammalian FOXO proteins, suggesting that mammalian FOXO family members are also regulated by the PI3K-Akt signaling pathway.

In mammals, the PI3K-Akt signaling pathway is activated by a variety of cytokines and growth factors, including insulin and IGF-1, and it regulates various cellular processes, such as cell proliferation and survival (11, 14, 70). The constitutive activation of this signaling pathway leads to the development of tumors both through deregulation of cell-cycle progression and through an increase in cellular resistance to proapoptotic signals. The activation of PI3K triggered by the binding of growth factors or cytokines to their receptors results in the production of phosphatidylinositol 3,4,5-trisphosphate (PIP^3), which provides a membrane binding site for the serine-threonine kinase Akt. The translocation of Akt to the plasma membrane leads to its activation through phosphorylation by 3'-phosphoinositide–dependent kinase 1 (PDK1) (11, 14, 70). The tumor suppressor PTEN (phosphatase and tensin homologue on chromosome 10), which functions as a lipid 3'-phosphatase, reduces the amount of PIP_3, with the result that PI3K signaling is turned off (12).

The three highly conserved Akt recognition sequences in FOXO1, FOXO3a, and FOXO4 are designated T1, S1, and S2 (Fig. 1). Akt indeed phosphorylates FOXO proteins, and these three sites become phosphorylated in a PI3K-dependent manner in a variety of mammalian cells in response to stimulation with cytokines or growth factors such as insulin and IGF-1 (3, 6, 34, 53, 62, 64).

The serum- and glucocorticoid-induced kinase (SGK), which is structurally related to Akt, also phosphorylates FOXO proteins (7). SGK is also activated by PI3K- and PDK1-mediated signaling in response to extracellular stimuli and has been suggested to play a role in cell-cycle progression, although it is not recruited to the plasma membrane by PIP_3 (7, 10, 32, 51). Although Akt and SGK are able to phosphorylate the same sites (T1, S1, S2) of FOXO, these kinases differ in their efficacies in this regard (7). Although T1 is similarly phosphorylated by both kinases, Akt preferentially targets S1, whereas SGK preferentially phosphorylates S2. The biological consequences of this difference between Akt and SGK remain to be determined in mammals. Although both Akt and SGK are able to phosphorylate DAF-16 in *C. elegans*, SGK appears to play the predominant role in the control of development, stress resistance, and longevity, whereas Akt is more important in the regulation of dauer formation (25). It is thus possible that the difference in FOXO phosphorylation site preferences between Akt and SGK results in the activation of distinct cellular responses by these kinases in mammals.



	T1	S1	S2	S3	S4	S5	T2/T3
FOXO1:	T24	S256	S319	S322	S325	S329	
FOXO3a:	T32	S253	S315	S318	S321	S325	
FOXO4:	T28	S193	S258	S261	S264	S268	T447T451

FIG. 1. Phosphorylation of FOXO proteins. The phosphorylation of FOXO proteins is regulated by growth factor–activated signaling pathways, including those mediated by PI3K-Akt and by Ras-Ral. The general phosphorylation sites, T1 to T3 and S1 to S5, that are phosphorylated by various kinases are indicated. The specific amino acid positions corresponding to these sites are also shown for human FOXO1, FOXO3a, and FOXO4. The S1 site is located within the nuclear localization sequence (NLS) and its phosphorylation might inhibit the nuclear import of FOXO. The positions of the forkhead (FH) domain, nuclear export sequence (NES), and DNA binding and transactivation domains are also indicated.

Activation of the PI3K-Akt pathway also triggers indirectly the phosphorylation of FOXO proteins on sites in addition to T1, S1, and S2. Both Ser^{322} (S3) and Ser^{325} (S4) of human FOXO1 thus become phosphorylated in IGF-1–stimulated cells as a result of phosphorylation of the S2 site (Ser^{319}) by Akt (54). Phosphorylation of Ser^{319} generates a consensus sequence for phosphorylation of Ser^{322} by casein kinase 1 (CK1). In turn, phosphorylation of Ser^{322} generates a consensus site for phosphorylation of Ser^{325} by CK1. In contrast, phosphorylation of Ser^{329} (S5) of FOXO1 is unaffected by IGF-1 signaling. Instead, this residue appears to be phosphorylated constitutively by DYRK1A (dual-specificity tyrosine-phosphorylated and regulated kinase 1A) (72). Inhibition of the PI3K-Akt signaling pathway or mutation (to alanine) of the phosphorylation sites of FOXO targeted by Akt or CK1 thus does not influence the phosphorylation status of S5.

The phosphorylation of FOXO is also affected by the Ras-Ral signaling pathway, which targets Thr^{447} (T2) and Thr^{451} (T3) in the COOH-terminal region of human FOXO4 (17). A mutant protein in which both of these threonine residues are replaced by alanines still translocates from the nucleus to the cytoplasm in response to Akt signaling, but its transactivation activity is greatly impaired. Although normal activation of endogenous Ral increases the transactivation activity of FOXO, activation of Ral by oncogenic Ras appears to enhance the inhibition of FOXO activity by Akt. It remains to be determined whether this regulation characterized for FOXO4 is also operative for FOXO1 and FOXO3a. The region surrounding the T2 and T3 sites of FOXO4 is not well conserved in the other FOXO family members, however.

REGULATION OF FOXO LOCALIZATION BY THE PI3K-AKT SIGNALING PATHWAY

Although each newly synthesized FOXO protein is imported into the nucleus through interaction of its nuclear localization sequence (NLS) with the nuclear import machinery (5), stimulation of cells with growth factors such as IGF-1 or expression of constitutively active forms of PI3K or Akt results in nuclear exclusion of FOXO (3, 6, 34, 53, 62, 64). In contrast, serum deprivation or inhibition of PI3K or of Akt results in the relocation of FOXO from the cytoplasm to the nucleus. The PI3K-Akt signaling pathway thus regulates nucleocytoplasmic shuttling of FOXO. Mutational analysis has shown that the phosphorylation of FOXO proteins on the three sites (T1, S1, S2) targeted by Akt promotes their exclusion from the nucleus and consequent inhibition of target gene transcription (3, 6, 34, 53, 62, 64).

Nuclear export is a highly regulated process that involves various accessory proteins such as Crm1, which binds to the nuclear export sequence (NES) of the target protein. All members of the FOXO family contain a consensus NES. Crm1 was shown to be required for the nuclear export of FOXO by the observation that treatment of cells with leptomycin B, an inhibitor of Crm1, blocks this process irrespective of the status of the phosphorylation sites targeted by Akt (3, 5). Activation of Akt at the plasma membrane of growth factor–stimulated cells is followed by translocation of the kinase into the nucleus, where it phosphorylates FOXO on the T1, S1, and S2 sites (5, 8). Phosphorylation of FOXO proteins by Akt generates consensus sequences [RSXp(S/T)XP] for the binding of 14-3-3 (73) and induces their association with 14-3-3. 14-3-3 proteins regulate the cell cycle and prevent apoptosis by controlling the nucleocytoplasmic distribution of signaling molecules with which they interact. They recognize and bind both of the phosphorylated sites surrounding Thr^{32} (T1) and Ser^{253} (S1) of human FOXO3a (8). Interaction of 14-3-3 with FOXO is not sufficient to trigger the nuclear export of FOXO, however. Deletion of the putative NES motif in FOXO3a thus inhibits its nuclear export in spite of the presence of intact Akt phosphorylation sites and the binding of 14-3-3 (8). Conversely, an intact NES also is not sufficient for nuclear export, given that mutation (to alanine) of the Akt phosphorylation sites of FOXO and consequent prevention of 14-3-3 binding result in retention of FOXO within the nucleus even of cells that have been stimulated with IGF-1 (8). Instead, subsequent phosphorylation of S3 and S4 by CK1 facilitates the interaction of FOXO that has already been phosphorylated by Akt with the nuclear export machinery, including the Ran GTPase and Crm1. Thus, mutation (to alanine) of the CK1 phosphorylation sites of FOXO1 prevented its binding to Ran (54). Moreover, the binding of 14-3-3 to phosphorylated FOXO3a appears to prevent the latter from reentering the nucleus, given that Ser^{253} (S1) is located within the NLS (8). The DYRK1A phosphorylation site (S5) is adjacent to the S4 site phosphorylated by CK1. Although S5 is constitutively phosphorylated, its phosphorylation appears to contribute to the subcellular localization of FOXO because its mutation (to alanine) in FOXO1 increases the transactivation activity and nuclear localization of this protein in nonstimulated cells (72).

Taken together, these various observations indicate that newly synthesized FOXO accumulates in the nucleus as a result of interaction of its NLS with the nuclear import machinery, binds to its cognate consensus sequence in the promoters of target genes, and modulates gene expression in cells not exposed to growth factors or cytokines. In response to cell stimulation, however, activated Akt (or SGK) phosphorylates three sites (T1, S1, S2) within FOXO, which results in the phosphorylation of two additional sites (S3, S4) by CK1. The phosphorylated protein binds to 14-3-3 and the nuclear export machinery (Crm1, Ran) and thereby exits the nucleus. The transactivation activity of FOXO is thus regulated by its nucleocytoplasmic shuttling, which is in turn controlled by FOXO phosphorylation (Fig. 2).

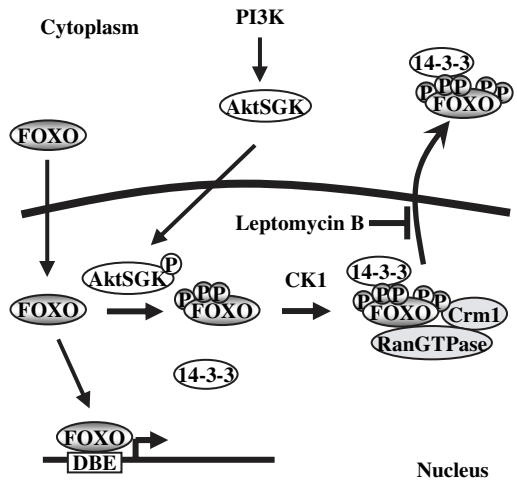


FIG. 2. Regulation of nucleocytoplasmic shuttling of FOXO by the PI3K-Akt signaling pathway. Newly synthesized FOXO molecules are imported into the nucleus, where they interact with the FOXO binding motif (DBE) present in the promoters of target genes and thereby regulate gene expression. Growth factor stimulation triggers the activation of PI3K and Akt (or SGK) and the subsequent translocation of activated Akt to the nucleus. Phosphorylation of FOXO by activated Akt induces its interaction with 14-3-3, as well as its further phosphorylation by CK1, resulting in the nuclear export of the FOXO–14-3-3 complex mediated by Crm1 and the Ran GTPase in a manner sensitive to leptomycin B.

FOXO TARGET GENES INVOLVED IN CELL-CYCLE REGULATION

Progression of the cell cycle is tightly controlled by intracellular and extracellular signals. In normal dividing cells, cell-cycle progression is regulated by the balance between the amounts and activities of cyclin–CDK (cyclin-dependent kinase) complexes, such as cyclin D–CDK4, cyclin E–CDK2, and cyclin B–CDK1, and those of their inhibitors (CKIs) such as $p21^{Cip1}$ and $p27^{Kip1}$ (29, 57–59). Growth factors trigger various early events that promote the G_0-G_1 transition of the cell cycle; these events include up-regulation of cyclin D expression and $p27^{Kip1}$ degradation, which result in consecutive activation of the cyclin D–CDK4 and cyclin E–CDK2 complexes and progression into S phase. Activation of the PI3K-Akt signaling pathway appears to be required for the entry of quiescent cells into the cell cycle. Activation of this pathway was shown to be sufficient to induce DNA synthesis in serum-deprived fibroblasts (31). Negative regulation of FOXO by the PI3K-Akt signaling pathway was thus also implicated in cell-cycle regulation. Indeed, expression of a constitutively active form of FOXO that is resistant to phosphorylation by Akt led to cell-cycle arrest or apoptosis as a result of induction or suppression of the transcription of genes involved in cell-cycle regulation (Fig. 3). A combination of comprehensive microarray analysis of gene expression and the determination of the canonical DNA consensus sequence for FOXO binding (TTGTTTAC) has facilitated the identification of FOXO target genes (21).

Ectopic expression of FOXO4, FOXO3a, or FOXO1 blocks cell-cycle progression at G_1 phase by inducing the expression of $p27^{Kip1}$(17, 42, 47, 61, 63). This CKI binds to the cyclin E–CDK2 complex and inhibits its activity, thereby preventing entry of cells into S phase (55). FOXO directly activates transcription of the $p27^{Kip1}$ gene, whose promoter contains multiple consensus FOXO binding motifs (42, 47). FOXO also increases the expression of a reporter gene controlled by the $p27^{Kip1}$ gene promoter (42, 47). In addition, FOXO has been shown to increase the stability of the $p27^{Kip1}$ protein (47).

FOXO also contributes to regulation of the exit of cells from the cell cycle into a state of quiescence (G_0) (36). The abundance of p130, a member of the retinoblastoma protein family, is low in cycling cells, but increases as cells exit the cell cycle (23). In G_0 cells, p130 is hypophosphorylated and binds to the transcription factor E2F-4, and this complex is thought to repress the expression of genes required for reentry of cells into the cell cycle, thereby maintaining the quiescent state (60). FOXO binds directly to consensus binding motifs in the promoter of the p130 gene and activates its transcription, resulting in the accumulation of p130 protein (36). The PI3K-Akt signaling pathway thus controls the abundance of p130 during the cell cycle via regulation of FOXO. FOXO also binds and activates the promoter of the cyclin G2 gene and thereby increases the amount of cyclin G2 protein (41). Cyclin G2 is an unconventional cyclin that is highly expressed in quiescent cells, but undergoes marked down-regulation in a manner dependent on activation of the PI3K-Akt signaling pathway as cells enter the cell cycle (2, 28).

FOXO has been implicated in a $p27^{Kip1}$-independent mechanism of inhibition of cell-cycle progression by the observation that forced expression or conditional activation of FOXO leads to reduced expression of cyclins D1 and D2 and consequent cell-cycle arrest even in the absence of $p27^{Kip1}$ (52, 56). Chromatin immunoprecipitation assays have revealed that FOXO1 binds to the promoter of the cyclin D1 gene (52). A mutant form of FOXO1 that was not able to recognize the canonical FOXO binding motif or to induce $p27^{Kip1}$ gene expression was still able to suppress the expression of cyclins D1 and D2 (56), suggesting that FOXO might bind to the promoters of the cyclin D1 and D2 genes indirectly by interacting with other transcription factors and modulating their activities. Alternatively, the regulation of cyclin D gene expression by FOXO might be mediated by the direct binding of FOXO to a promoter element other than the classical FOXO binding motif. Indeed, DNA microarray analysis in *C. elegans* has suggested the existence of an additional consensus sequence that might be recognized by FOXO (46).

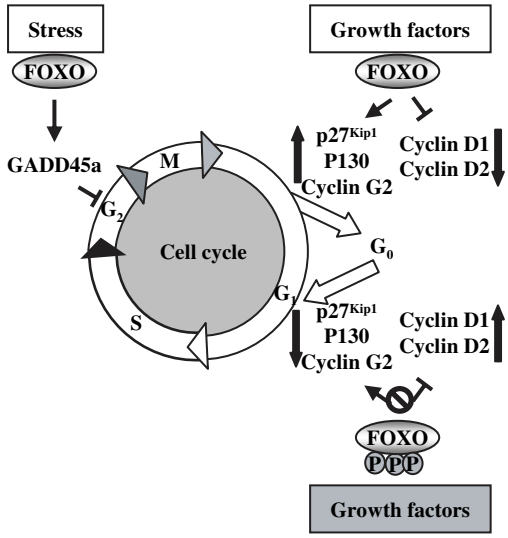


FIG. 3. Cell-cycle regulation by FOXO-mediated activation or suppression of gene expression. In the absence of growth factors, activated FOXO both induces expression of the genes for $p27^{Kip1}$, p130, and cyclin G2 (all of which inhibit cell-cycle progression) and suppresses that of the genes for cyclins D1 and D2 (both of which promote cell-cycle progression), thereby rendering cells quiescent (G_0 phase). In the presence of growth factors, activation of the PI3K-Akt signaling pathway induces the nuclear exclusion of FOXO and thereby prevents its effects on these target genes. The expression of cyclins D1 and D2 is thus up-regulated and that of $p27^{Kip1}$, p130, and cyclin G2 is down-regulated, resulting in the reentry of cells into the cell cycle. In response to oxidative stress, FOXO is activated and induces the expression of genes, such as that for GADD45a, that inhibit G_2-M progression, thereby triggering cell-cycle arrest at G_2 phase.

In summary, in the absence of growth factor–induced PI3K-Akt signaling, the sustained activity of FOXO results in up-regulation of the expression of $p27^{Kip1}$, p130, and cyclin G2, as well as in inhibition of the expression of cyclins D1 and D2, thereby ensuring maintenance of the quiescent state. Stimulation of cells with growth factors and the consequent activation of the PI3K-Akt signaling pathway result in the phosphoryla-

tion, nuclear exclusion, and inhibition of FOXO and in reentry of cells into the cell cycle as a consequence of the loss of the effects of FOXO on the expression of these target genes.

FOXO also plays a role in G_2 phase of the cell cycle. Ectopic expression or conditional expression of a constitutively active form of FOXO induces G_2 delay or G_2 arrest in addition to G_1 arrest (20, 66). DNA microarray analysis has implicated GADD45a (growth arrest– and DNA damage–inducible protein a of 45 kDa) as a potential mediator of G_2 arrest induced by FOXO activation (20, 66). A role for GADD45a in G_2-M checkpoint control (71) has also been suggested by the observation that purified recombinant GADD45a inhibits the histone H1 kinase activity associated with the cyclin B–Cdc2 complex by inducing the dissociation of this complex, which is required for G_2 transition (74). A central region of human GADD45a (amino acids 65–84) has been shown to mediate its interaction with Cdc2 (also known as CDK1) and to be required for inhibition of the kinase activity of Cdc2. FOXO directly binds and activates the promoter of the GADD45a gene and increases the amount of GADD45a protein (20, 66). Further evidence that FOXO-induced G_2 arrest is mediated by GADD45a was provided by the observation that such arrest is partially compromised in GADD45a-deficient mouse embryonic fibroblasts (66). GADD45a is also a stress-inducible protein, and its induction by FOXO is implicated in the cellular stress response (see below).

OXIDATIVE STRESS AND FOXO

An absence of Akt signaling in *C. elegans* results in activation of the FOXO homologue DAF-16 and in dauer formation, a phenotype that is characterized by increased resistance to oxidative stress (27, 49, 50). The stress resistance phenotype is dependent on DAF-16, implicating this transcription factor in the regulation of genes related to stress resistance (30, 65). Similarly, mammalian FOXO plays a role in cellular resistance to oxidative stress. In quiescent cells that lack Akt activity, FOXO localizes to the nucleus and induces the expression of manganese superoxide dismutase, an antioxidant enzyme that confers resistance to oxidative stress (35). FOXO3a also upregulates the expression of sterol carrier protein x (SCPx) and SCP2 (13). SCPx is a thiolase that contributes to the breakdown of branched chain fatty acids and to the biosynthesis of bile acids, whereas SCP2 has been shown to protect bound fatty acids against oxidative damage induced by the combination of hydrogen peroxide (H_2O_2) and Cu^{2+} (13).

REGULATION OF FOXO IN RESPONSE TO OXIDATIVE STRESS

Among various genes that are regulated by FOXO, that for GADD45a is induced by a variety of stressors, including ionizing radiation, UV, and reactive oxygen species such as H_2O_2, suggesting that FOXO might also be activated by cellular stress. DAF-16 translocates from the cytoplasm to the nucleus in response to certain types of oxidative stress in *C. elegans* (24). Similarly, oxidative stress caused by H_2O_2, menadione, or heat shock triggers the relocalization of FOXO from the cytoplasm to the nucleus in mammalian fibroblasts exposed to growth factors (9, 33) (Fig. 4). The apparent nuclear accumulation of FOXO in the nucleus of cells not exposed to growth factors is not further increased by stimulation of these cells with H_2O_2. FOXO3a is phosphorylated on eight serine or threonine residues, not including those residues targeted by Akt, in response to oxidative stress, although the kinases responsible for such phosphorylation remain to be identified (9). It is possible that some of these phosphorylation events triggered by stress interfere with the interaction between FOXO and 14-3-3, thereby inducing nuclear accumulation of FOXO. Alternatively, in this regard, c-Jun NH_2-terminal kinase (JNK) phosphorylates 14-3-3 and thereby induces dissociation of its target protein Bax (67). JNK is a member of a group of mitogen-activated protein kinases, known as stress-activated protein kinases, that are activated by oxidative stress (15). It is therefore possible that 14-3-3 is phosphorylated by JNK that has been activated by oxidative stress and that its phosphorylation promotes its dissociation from FOXO, thereby allowing translocation of FOXO to the nucleus.

In addition to inducing its nuclear translocation, oxidative stress promotes the interaction of FOXO with protein acetylases, including p300 [or the related protein CREB-binding protein (CBP)] and p300/CBP–associated factor (PCAF), and its consequent acetylation at several lysine residues (9, 19, 33, 40, 44, 48, 68). Human FOXO4 is acetylated by CBP at lysines

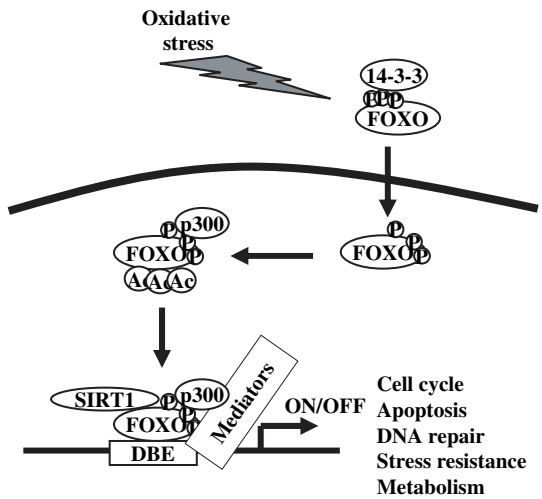


FIG. 4. Relocalization and activation of FOXO in response to oxidative stress. Oxidative stress induces the phosphorylation of FOXO by unidentified kinases and its consequent translocation to the nucleus. The transcriptional coactivator p300 (or CBP) interacts with FOXO and catalyzes its acetylation on several lysine residues, resulting in inhibition of its transactivation activity. The deacetylase SIRT1 also binds to and deacetylates FOXO, thereby reversing the inhibitory effect of p300 on FOXO activity. The effects of acetylation and deacetylation of FOXO, however, appear to be promoter-specific; whereas SIRT1 enhances the FOXO-mediated expression of genes that contribute to cell-cycle regulation or the stress response, it suppresses that of genes whose products participate in apoptosis.

186, 189, and 408 *in vitro* (19). An additional lysine residue of FOXO4, lysine 237, is also acetylated by p300 and CBP *in vivo* (33). The acetylation of FOXO4 by p300 (or CBP) inhibited its activation of a promoter containing multiple copies of the FOXO response element linked to a reporter gene, as well as of $p27^{Kip1}$ expression, indicating that acetylation inhibits the transactivation activity of FOXO4 directly (19, 33). Human FOXO3a is also acetylated on five lysine residues (lysines 242, 259, 271, 290, and 569) in response to oxidative stress (9).

A physiological interaction of FOXO with SIRT1, an NAD^+-dependent deacetylase and the closest homologue of yeast SIR2 among the seven members of the mammalian sirtuin family (9, 33, 44, 68), has also been demonstrated. The binding of SIRT1 to FOXO is accompanied by deacetylation of the latter in an NAD^+-dependent manner. Deacetylation of transcriptional complexes including transcription factors and histones has been traditionally associated with repression of target gene expression. However, deacetylation of histones and unidentified molecules by the yeast histone deacetylase Rpd3 was recently shown to be required for gene expression in response to stress, including oxidative stress (16). The deacetylation of FOXO by SIRT1 also appears to reverse the inhibitory effect of acetylation on its transactivation activity. Depletion of endogenous SIRT1 in SaoS2 cells (which lack p53) by RNA interference thus resulted in impairment of FOXO-mediated GADD45a expression induced by oxidative stress (33). However, the effect of FOXO acetylation–deacetylation on the transcription of target genes is not quite so straightforward. SIRT1 has thus been shown to suppress the FOXO-mediated expression of genes, such as those for Bim and Fas ligand, that contribute to the apoptotic response, suggesting that the regulation of FOXO activity by acetylation–deacetylation is promoter-specific (9).

CONCLUSION

The FOXO family of proteins regulates various biological activities, including cell-cycle progression, the cellular response to oxidative stress, DNA repair, and apoptosis. The activity of FOXO proteins is regulated by phosphorylation by multiple protein kinases, as well as by acetylation and deacetylation. Phosphorylation of three highly conserved residues of FOXO by the PI3K-Akt signaling pathway results in the nuclear exclusion of FOXO mediated by interactions with 14-3-3, the Ran GTPase, and Crm1 and in the consequent inhibition of target gene transcription. Deregulation of the PI3K-Akt signaling pathway is implicated in tumorigenesis. The tumor suppressor PTEN regulates FOXO through the PI3K-Akt signaling pathway (47). In addition, like the tumor suppressor p53, FOXO is activated by oxidative stress and other stressors and induces the expression of genes that contribute to cell-cycle arrest, suggesting that FOXO also functions as a tumor suppressor.

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ABBREVIATIONS

CBP, CREB-binding protein; CDK, cyclin-dependent kinase; CK1, casein kinase 1; CKI, CDK inhibitor; DYRK1A, dual-specificity tyrosine-phosphorylated and regulated kinase 1A; FOXO, forkhead member of the class O; GADD45a, growth arrest– and DNA damage–inducible protein a of 45 kDa; H_2O_2, hydrogen peroxide; IGF-1, insulin-like growth factor–1; JNK, c-Jun NH_2-terminal kinase; NES, nuclear export sequence; NLS, nuclear localization sequence; PDK1, 3'-phosphoinositide–dependent kinase 1; PI3K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homologue on chromosome 10; SCP, sterol carrier protein; SGK, serum- and glucocorticoid-induced kinase; UV, ultraviolet light.

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