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FOXOs, cancer and regulation of apoptosis

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Abstract

Forkhead box O (FOXO) transcription factors are involved in multiple signaling pathways and play critical roles in a number of physiological and pathological processes including cancer. The importance of FOXO factors ascribes them under multiple levels of regulation including phosphorylation, acetylation/deacetylation, ubiquitination and protein-protein interactions. As FOXO factors play a pivotal role in cell fate decision, mounting evidence suggests that FOXO factors function as tumor suppressors in a variety of cancers. FOXOs are actively involved in promoting apoptosis in a mitochondria-independent and -dependent manner by inducing the expression of death receptor ligands, including Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand, and Bcl-2 family members, such as Bim, bNIP3 and Bcl-X_L, respectively. An understanding of FOXO proteins and their biology will provide new opportunities for developing more effective therapeutic approaches to treat cancer.

Keywords

FOXO; cancer; apoptosis; Forkhead; death receptor ligand; Bcl-2 family members

Introduction

Forkhead box O (*FOXO*) genes are involved in multiple signaling pathways and play critical roles in a number of physiological and pathological processes. This review will focus on the role of these transcription factors in cancer as well as their regulation of programmed cell death.

The forkhead, or winged-helix family of transcription factors were named after the founding gene member in *Drosophila* (*forkhead* gene; for review, see Kaufmann and Knochel, 1996). The family members share a highly conserved 100 amino-acid DNA binding, or FOX domain encoding a winged-helix DNA-binding motif (Clark *et al.*, 1993). Based on the homology within this region, the forkhead genes are grouped into 19 subclasses of *FOX* genes, *FOXA*–*FOXS* (Kaestner *et al.*, 2000). While some are ubiquitously expressed in variety of tissue types, others are expressed in a restricted spatial-temporal manner (Clark *et al.*, 1993).

FOXO factors belong to the ‘O’ (‘other’) class of the FOX superfamily (Kaestner *et al.*, 2000). Although their FOXO forkhead box is clearly related to those found in other forkhead genes, they form the most divergent subfamily of Fox family due to a unique five amino-acid (GDSNS) insertion immediately prior to helix H3 within the forkhead domain that is directly involved in sequence-specific interaction with DNA-binding sites. FOXO proteins are

conserved from worm to human. To date, only one FOXO species has been identified in invertebrates (called dauer formation-16 in *Caenorhabditis elegans* (Lin *et al.*, 1997) and dFOXO in *Drosophila*). In mammals, four subfamily members have been identified: FOXO1 (previously known as FKHR; Galili *et al.*, 1993), FOXO3 (previously known as FKHRL1; Hillion *et al.*, 1997; Anderson *et al.*, 1998), FOXO4 (previously known as AFX; Corral *et al.*, 1993; Parry *et al.*, 1994; Borkhardt *et al.*, 1997) and FOXO6 (Jacobs *et al.*, 2003). FOXO1, FOXO3 and FOXO4 mRNAs are expressed ubiquitously in varying levels in mammals (Anderson *et al.*, 1998; Furuyama *et al.*, 2000; Biggs *et al.*, 2001). The *FOXO1* transcript is highly expressed in adipose tissues, *FOXO3* mRNA is abundant in the brain, heart, kidney and spleen, and the *FOXO4* transcript is expressed at higher levels in skeletal muscle. In contrast, *FOXO6* mRNA is predominantly present in the developing and adult brain, suggesting that FOXO6 may play an important role in the nervous system (Jacobs *et al.*, 2003).

While the DNA-binding domain (forkhead box) of FOXO factors is located in the N-terminal portion of these proteins, the transactivation domain is located in the C-terminal region of the molecule. FOXO proteins can act not only as transcriptional activators but also as transcriptional repressors (Ramaswamy *et al.*, 2002). Therefore, this family of transcription factors may exert positive and negative effects on gene expression depending on the promoter context and extracellular conditions. In addition, FOXO factors also contain a 'nuclear export sequence' and a 'nuclear localization signal' (Biggs *et al.*, 1999; Brownawell *et al.*, 2001), which allow nucleocytoplasmic shuttling.

FOXO proteins function predominantly as transcription factors in the nucleus and bind as monomers to their cognate DNA-targeting sequences. Selection of high-affinity DNA-binding sites from a pool of degenerate oligonucleotides has identified a consensus FOXO recognition element (FRE) as (G/C)(T/A)AA(C/T)AA (Biggs *et al.*, 1999; Furuyama *et al.*, 2000; Gilley *et al.*, 2003), which diverges from that of other forkhead proteins. Functional FRE sites that match this consensus sequence have been identified in the promoters of FOXO target genes encoding Fas ligand (FasL), insulin-like growth factor-binding protein 1, the apoptotic regulator Bcl-2 interacting mediator of cell death (Bim) and others (for reviews, see Accili and Arden, 2004; Greer and Brunet, 2005). By comparative genomic approaches, many additional putative FOXO-target genes and their potential *cis*-regulatory sites have been identified (Xuan *et al.*, 2005). Thus, FOXO transcription factors may preferentially interact with a distinct set of target sites in the genome and, thus, are involved in a number of signaling pathways and control diverse biochemical processes.

Several modes of action have been proposed by which nuclear FOXO proteins interact with DNA and partner proteins to regulate the transcription of specific target genes (for reviews, see Barthel *et al.*, 2005). (1) FOXO can bind directly to specific promoters and recruit transcriptional co-activators or co-DNA-binding transcription factors to activate transcription. (2) FOXO factors may repress transcription by competing with other transcription factors for a common binding site in a gene promoter. (3) FOXO factors may also cooperate with or titrate away specific transcription factors or cofactors, thereby regulating (activating or repressing) transcription through promoters that lack FOXO-binding sites.

As a convergence point of various signaling pathways, FOXO factors are subject to multiple levels of regulation. While regulation of subcellular FOXO localization is a major event, modulation of FOXO transactivation properties also provides additional control. Furthermore, FOXO factor abundance is regulated by degradation and/or through the control of *FOXO* gene expression.

Regulation of the subcellular localization and transcriptional activity of FOXO protein is achieved primarily by posttranslational modifications, such as phosphorylation and

acetylation. Two main classes of stimuli trigger FOXO phosphorylation with opposing effects on FOXO localization. In response to growth and survival factors such as insulin and insulin-like growth factor 1, FOXO factors become phosphorylated and localized to the cytoplasm. By contrast, phosphorylation of FOXO proteins in response to oxidative stress, for example, by Jun-N-terminal kinase or mammalian Ste 20-like kinase, results in retaining FOXO proteins in the nucleus even in the presence of growth factors.

Recent studies indicate that FOXO transcription factors are acetylated by p300 and CREB-binding protein, co-activators possessing acetyltransferase activity, at several conserved lysine residues, many of which are located in the DNA-binding domain (Fukuoka *et al.*, 2003; van der Horst *et al.*, 2004). Acetylation of FOXO factors appears to exert inhibitory effects on their transactivation activity, possibly by reducing their DNA-binding activity. Deacetylation of FOXO proteins has been shown to result from the activity of SIRT1, a nicotinamide adenine dinucleotide-dependent deacetylase (Brunet *et al.*, 2004; Motta *et al.*, 2004; van der Horst *et al.*, 2004). The effects of SIRT1 on FOXO function are complex and vary depending upon the FOXO target genes. It has been shown that SIRT1 promotes transcription of FOXO target genes involved in stress resistance, while decreasing transcription of genes involved in apoptosis (Greer and Brunet, 2005). Thus, SIRT1 appears to shift the FOXO-dependent response away from cell death toward stress resistance. This is in agreement with the concept that acetylation/deacetylation of FOXO protein may switch target specificity.

In addition, modulation of FOXO protein-protein interaction with accessory proteins or cofactors through covalent modifications (phosphorylation and acetylation) might alter their inherent transactivation potential as well as determine which target genes are regulated by specific FOXOs (Perrot and Rechler, 2003; Puigserver *et al.*, 2003). FOXO-binding partners can modulate FOXO transcription activity either positively, for example, β -catenin (Essers *et al.*, 2005) or negatively, for example, PPAR γ or the androgen receptor (Dowell *et al.*, 2003; Li *et al.*, 2003).

FOXO proteins are also regulated by the ubiquitin-proteasome system. Upon polyubiquitination, FOXO proteins are degraded by proteolysis through the proteasome pathway (Vogt *et al.*, 2005). Phosphorylation by Akt at serine 256 in FOXO1 creates a binding site for Skp2, the substrate-binding component of the Skp1/culin 1/F-box protein (SCF^{Skp2}) E3 ligase complex (Matsuzaki *et al.*, 2003; Huang *et al.*, 2005). For FOXO3, the critical kinase is I κ B kinase- β (IKK β) rather than Akt. It targets the residue serine 644 and interacts with an as yet unidentified ubiquitin ligase for ubiquitination-dependent proteasomal degradation (Hu *et al.*, 2004).

Furthermore, mRNA levels of the different FOXO gene subtypes vary from tissue to tissue in mammals (Furuyama *et al.*, 2000) and can be modulated under some circumstances (Furuyama *et al.*, 2000; Richards *et al.*, 2002). These findings suggest that the transcription of FOXO genes is subject to regulation. Altogether, multiple layers of regulation offer a large spectrum of options for the fine-tuning of FOXO function.

FOXO in cancer

FOXO factors play pivotal role in cell fate decisions. Their functions are regulated by multiple signaling pathways including Akt and SGK. Many of these pathways are known to be dysregulated in cancer, suggesting that FOXO factors may play a tumor suppressor role in a variety of cancer. A role of FOXO factors in tumorigenesis was initially suggested by the observation that three of the four known FOXO genes were found at chromosomal breakpoints in certain types of tumor (rhabdomyosarcomas for FOXO1, and acute myeloid leukemias for FOXO3 and FOXO4; Barr, 2001). These chromosomal translocations all result in a chimeric protein in which the DNA-binding domain of other transcriptional regulators (Pax3 or Pax7

for FOXO1 and mixed-lineage leukemia gene for FOXO 3 and FOXO4) are fused to the transactivation domain of FOXOs (Galili *et al.*, 1993; Parry *et al.*, 1994; Borkhardt *et al.*, 1997; Hillion *et al.*, 1997; Anderson *et al.*, 1998). Interestingly, these translocations occur at the identical position, immediately N terminus to the recognition helix (H3) of the FOXO family members and these fusions are no longer controlled by Akt and are constitutively retained in the nucleus (del Peso *et al.*, 1999).

Although the FOXO proteins are physically affected by chromosomal translocations, the mechanism by which these genetic alterations are related to their functions in tumorigenesis is unclear. Two models have been proposed to explain the role of FOXO1 in tumor formation of alveolar rhabdomyosarcoma (ARMS). One model is based upon the assumption that tumors arise from a gain of function of FOXO1. These fusions alter the transactivation properties of the respective proteins and are thereby thought to contribute to carcinogenesis. The main evidence supporting this notion is that the Pax3–FOXO1 fusion protein is a more potent transcription activator than normal Pax3 protein in increasing the transcription of Pax3 target gene (Xia *et al.*, 2002). The fusion protein is able to induce oncogenic transformation in cell culture (Scheidler *et al.*, 1996; Kempf and Vogt, 1999; Xia and Barr, 2004), suggesting that the fusion protein may function as an oncogenic transcription factor by enhancing activation of normal PAX3 target genes. However, in transgenic or knock-in murine models, expression of PAX3–FOXO1 fusion proteins fails to induce tumors (Anderson *et al.*, 2001; Lagutina *et al.*, 2002; Relaix *et al.*, 2003; Keller *et al.*, 2004b).

Therefore, a second model has been proposed in which, instead of an altered transcription of fusion gene, the disruption of one FOXO allele in ARMS results in a depletion of FOXO protein and subsequent loss of function is a critical event in tumorigenesis (Accili and Arden, 2004). As FOXO factors play a pivotal role in cell fate decisions by keeping cells in check by inhibiting cell proliferation and promoting cell death, disruption of their function leads to evasion of normal limitation on cell proliferation and transformation (Burgering and Kops, 2002). Indeed, immunoblots indicate that ARMS tumor and tumor cell lines harboring the PAX3–FOXO1 fusion gene do not express FOXO1 (Bois and Grosveld, 2003). Recent findings suggest that the two models are not mutually exclusive and both may contribute to tumorigenesis. Indeed, ectopic expression of the Pax3–FOXO1 fusion protein results in an elevated level of Skp2 (Zhang and Wang, 2003). Moreover, ARMS cell lines that bear the t(2;13) chromosomal translocation exhibit higher levels of Skp2 when compared to normal skeletal muscle cells (Zhang and Wang, 2003). Based on the observation that FOXO1 is degraded via ubiquitin-dependent proteasome pathway through interacting with Skp2 (Huang *et al.*, 2005) and that FOXO protein degradation often accompanies cell transformation (Hu *et al.*, 2004; Huang *et al.*, 2005), it is tempting to speculate that oncogenic Pax3–FOXO1 fusion protein not only transactivates expression of tumor-promoter genes, but also leads to a complete loss of the function of FOXOs via ubiquitin-mediated proteasomal degradation. Furthermore, mice with homozygous knock in of Pax3–FOXO1 develop ARMS in an Ink4a- orp53 -deficient background (Keller *et al.*, 2004a), further supporting this concept.

A role for FOXO inactivation in cellular transformation can also be inferred from the fact that FOXO negatively regulates cell survival and cell cycle progression in mammalian cells and that FOXO members are regulated by the PTEN tumor suppressor. Indeed, FOXO1 is cytosolic in PTEN-negative renal and prostate carcinoma cells (Nakamura *et al.*, 2000). Overproduction of FOXO proteins in PTEN-negative tumor-cells has the same effect on cell survival and cellular proliferation as the overexpression of PTEN in these cells. In addition, PTEN-null cells induce tumorigenesis in nude mice, which can be overridden by the expression of a constitutively active form of FOXO1 (Ramaswamy *et al.*, 2002). Furthermore, FOXO factors are dysregulated in several tumor types including breast cancer (Hu *et al.*, 2004), prostate cancer (Modur *et al.*, 2002), glioblastoma (Seoane *et al.*, 2004), rhabdomyosarcoma (Galili *et al.*,

1993) and leukemia (Parry *et al.*, 1994). Nuclear exclusion of FOXO3 correlates with expression of IKK β or phosphorylated Akt in many primary tumors and links with poor survival of the patients with breast tumors (Hu *et al.*, 2004). Cell proliferation and tumorigenicity in nude mice induced by IKK β expression can be overridden by FOXO3 (Hu *et al.*, 2004). Similarly, the expression of a constitutively active form of Foxo4 suppresses oncogene HER2-mediated tumorigenesis in nude mice (Yang *et al.*, 2005). Taken together, these observations suggest that FOXOs are mediators of tumor suppression.

FOXOs can associate with tumor suppressors or oncogenes, which suggests additional mechanisms by which FOXOs play a role in tumorigenesis. In response to stress stimuli or to nutrient deprivation, FOXO3 has been found to interact with the tumor suppressor p53 *in vitro* (Brunet *et al.*, 2004). Given that FOXOs share similar target genes including p21, GADD45, WIP1 and PA26 with p53, it suggests that these two proteins may coordinate tumor suppression. In addition, FOXO factors form a complex with SMAD transcription factors (which also act as tumor suppressors), resulting in transforming growth factor- β -dependent activation of *p21Cip1* and subsequent G₁ arrest (Seoane *et al.*, 2004). However, in glioblastomas FOXO factors are repressed by an overactive phosphoinositide 3-kinase (PI3K)–Akt pathway as well as by association with the oncogene FOXG, another FOX family member, to prevent FOXO from binding to the *p21Cip1* promoter, thereby promoting cancer cell proliferation (Seoane *et al.*, 2004). Furthermore, the oncogene β -catenin binds to FOXO factors (Essers *et al.*, 2005), thereby enhancing the ability of FOXO proteins to inhibit cell cycle progression (Essers *et al.*, 2005). Given that β -catenin associates with T-cell factor (TCF) and converts it from transcription repressor to activator, which has been implicated in cancer progression, in particular in colon cancer, it is possible that FOXO factors could counteract tumor progression by sequestering β -catenin away from TCF, thereby inhibiting cell cycle progression. The transcription factor and candidate tumor suppressor gene, RUNX3, which mediates apoptosis and cell growth inhibition in gastric epithelial cells, is frequently lost in gastric cancer cells. The physical interaction of RUNX3 and FOXO3 on the promoter of the proapoptotic factor Bim activates transcription of Bim, thereby inducing apoptosis in gastric cancer, suggesting that it may play an important role in tumor suppression in gastric cancer (Yamamura *et al.*, 2006).

FOXO in the regulation of apoptosis

Apoptosis plays a critical role in tumorigenesis. Factors regulating the expression levels of survival proteins are likely to play a crucial role in tumor formation. Overactivation of PI3K–AKT signaling is a hallmark of many human cancers. FOXOs have emerged as important effector arms of PI3K–AKT signaling.

In mammals, the PI3K–Akt signaling pathway is activated by insulin and growth factors. This regulates diverse cellular processes, such as cell proliferation and survival (Datta *et al.*, 1999; Cantley, 2002; Vivanco and Sawyers, 2002). Binding of insulin or growth factors to their receptors activates PI3K, resulting in the production of phosphatidylinositol 3,4,5-triphosphate, which creates a membrane-binding site for the serine-threonine kinase Akt, an important regulator of cell survival. The translocation of Akt to the plasma membrane leads to its activation via phosphorylation by 3'-phosphoinositide-dependent kinase 1 (Datta *et al.*, 1999; Cantley, 2002; Vivanco and Sawyers, 2002). Activated Akt then phosphorylates key regulatory proteins at serine and threonine residues that lie in (RXXR(S/T)) motif (Alessi *et al.*, 1996). FOXOs are phosphorylated by Akt at three consensus Akt sites, corresponding to Thr24, Ser256 and Ser319 of FOXO1 (Kops *et al.*, 1999; Nakae *et al.*, 1999; Rena *et al.*, 1999; Takaishi *et al.*, 1999; Tang *et al.*, 1999; Brunet *et al.*, 2004). This leads to the interaction of FOXOs with 14-3-3 proteins and the nuclear export of the FOXO-14-3-3 complex mediated by chromosomal region maintenance 1 (CRM1) and Ran GTPase. Translocation of FOXOs to

the cytoplasm results in inhibition of target gene transcription (Burgering and Kops, 2002). Growth factor withdrawal leads to the PI3K–Akt pathway inactivation, FOXO dephosphorylation at its Akt sites, nuclear translocation and target gene activation. Within the nucleus, FOXO triggers apoptosis by inducing the expression of death genes such as the *FasL* gene, and thereby participates actively in the process of apoptosis (Brunet *et al.*, 2004).

The tumor suppressor PTEN, a lipid 3'-phosphatase, functions as the key PI3K–Akt pathway antagonist through a negative effect on Akt. In PTEN-deficient tumor cell lines, FOXO1 proteins are constitutively phosphorylated and hence constitutively cytoplasmic (Nakamura *et al.*, 2000). In contrast, when PTEN is exogenously expressed, FOXO1 relocates to the nucleus and restores transcriptional activation (Nakamura *et al.*, 2000). In addition, a constitutively active form of FOXO1 that cannot be phosphorylated by Akt induces apoptosis in PTEN-null cells, which has the same effect upon PTEN reconstitution (Nakamura *et al.*, 2000). These data suggest that FOXOs are key mediators of tumor suppression downstream of PTEN and play a critical role in regulating apoptosis.

Besides Akt, CDK2 interacts with and phosphorylates FOXO1 at serine 249, leading to nuclear export and thereby inhibiting FOXO1 activity (Huang *et al.*, 2006). In response to DNA damage, the phosphorylation event was abolished through the CHK2-dependent cell cycle checkpoint pathway (Huang *et al.*, 2006). Thus, functional interaction between CDK2 and FOXO1 provides a mechanism that regulates programmed cell death after DNA strand breakage.

In addition, androgens provide an Akt-independent cell survival signal in prostate cancer through interaction with FOXO1. In prostate cancer, the androgen receptor and FOXO1 form a complex, which blocks the binding of FOXO1 to its DNA response element, thereby interfering with its ability to induce apoptosis and cell cycle arrest of prostate cancer cells (Li *et al.*, 2003).

Three consensus FRE sequences are found in the promoter of the proapoptotic gene *FasL*, which encodes a protein that activates the death receptor Fas/CD95/APO-1 and thereby promotes mitochondria-independent apoptosis (Brunet *et al.*, 1999). Binding of FOXO3 to those FREs leads to FOXO3-dependent transcription (Figure 1). In addition, a constitutively active form of FOXO3 induces the activity of the *FasL* native promoter (Brunet *et al.*, 1999). Furthermore, expression of a constitutively active form of FOXO3 triggers apoptosis in cerebellar granule neurons through the Fas signaling cascade (Brunet *et al.*, 1999).

In PTEN-deficient prostate carcinoma cell lines, FOXO1 and FOXO3 are cytoplasmically sequestered and inactive (Modur *et al.*, 2002). In addition, the expression level of tumor necrosis factor family member TRAIL (tumor necrosis factor-related apoptosis-inducing ligand; Modur *et al.*, 2002), a proapoptotic effector, is decreased (Modur *et al.*, 2002). Overexpression of FOXO1 and FOXO3 in the prostate cancer cell line results in apoptosis and increased expression of TRAIL. FREs of FOXO3 exist in the *TRAIL* promoter, indicating that TRAIL is a direct target of FOXO3 (Modur *et al.*, 2002). It is conceivable that the loss of PTEN contributes to tumor cell survival through decreased transcriptional activity of FOXOs followed by decreased TRAIL expression and apoptosis. Thus, FOXO proteins regulate cell survival by modulating the expression of death receptor ligands (for example, FasL and TRAIL) that function in autocrine and paracrine pathways (Figure 1).

In addition to the death receptor ligands, FOXO proteins have been shown to be involved in the transactivation of the Bcl-2 family, which has both pro- and antiapoptotic members and plays a critical role in regulating cell survival (Figure 1). One family member, the Bim, contains only a protein interaction motif known as the BH3 domain, which functions in the intrinsic, mitochondrial apoptotic pathway. FOXO proteins induce *Bim* expression in hematopoietic

cells deprived of growth factors (Dijkers *et al.*, 2000; Stahl *et al.*, 2002). In paclitaxel-sensitive breast cancer, upregulation of FoxO3a by paclitaxel results in increased levels of *Bim* mRNA and protein, leading to apoptosis in breast cancer cells and contributing to the tumor response to paclitaxel (Sunters *et al.*, 2003). Two functional FRE sites have been located in the *Bim* promoter (Gilley *et al.*, 2003). In addition, another proapoptotic member of the Bcl-2 family, bNIP3, a BH3-only protein, is a FOXO target gene (Tran *et al.*, 2002). Furthermore, FOXO4 indirectly suppresses the expression of the prosurvival Bcl-2 family member Bcl-X_L by regulating expression of the transcriptional repressor Bcl-6 (Tang *et al.*, 2002). Thus, FOXO factors can also trigger apoptosis by modulating the ratio of proapoptotic and prosurvival members of the Bcl-2 family. Taken together, FOXO transcription factors can induce cell death through mitochondria-dependent (Bcl-2 family members) and -independent (death cytokine) mechanisms.

Perspective

One distinguishing feature of FOXO family members is their overlapping but different patterns of expression, indicating that they may have redundant as well as distinct functions. In cell culture-based systems, FOXO1, FOXO3 and FOXO4 behave similarly. However, knockout murine models reveal unique roles for different FOXO proteins. Foxo1-null mice die on embryonic day 10.5 with defects in vascular development (Furuyama *et al.*, 2004; Hosaka *et al.*, 2004). Foxo3-null mice are viable, showing age-dependent infertility and have abnormal ovarian follicular development (Castrillon *et al.*, 2003). No histological abnormalities have been identified in Foxo4-null mice (Hosaka *et al.*, 2004). Interestingly, while individual disruption of each of the three Foxo genes has not revealed a direct role of FOXO family members in cancer, disruption of all three Foxos (Foxo1, Foxo3 and Foxo4) leads to tumorigenesis in mice in a context-dependent manner (Paik *et al.*, 2007; Tothova *et al.*, 2007), further supporting the notion that Foxos function as tumor suppressors and their isoforms display functional redundancy and diversification.

In addition, signaling downstream of FOXOs is both cell type specific and tissue-type specific within the same cell type. While activation of FOXOs suppresses growth in a variety of cell types, they induce apoptosis in cells of the immune system. For example, whereas FOXO3 upregulation of p27^{kip1} in A14 cells, mouse embryo fibroblasts, DLD-1 and 786-0 cells (Medema *et al.*, 2000; Nakamura *et al.*, 2000) results in a potent cell cycle arrest, it causes apoptosis in Ba/F3 cells (Dijkers *et al.*, 2000). However, the mechanism that confers cell-type specificity and determines why some cells become cell cycle arrested and others become apoptotic is still undetermined. One possible explanation is that activation of FOXOs results in cell type-specific gene regulation, which is regulated by interaction of FOXOs with other transcription factors or accessory proteins. Furthermore, cell types might respond differently to FOXO-mediated expression of the same gene product. Indeed, FOXOs control unique biological consequences in different cells or tissue contexts via modulation of distinct downstream targets.

FOXO transcription factors are emerging as master signaling regulators, which control a plethora of physiological and pathological processes, including cancer protection. Moreover, their functions are tightly regulated at multiple levels, which include phosphorylation, ubiquitylation, acetylation and protein-protein interactions. Therefore, FOXOs represent an interesting potential target to develop novel therapeutic approaches for cancer.

For instance, as inactivation of FOXOs appears to be a crucial step in tumorigenesis, restoring activity of these factors represents a potential effective therapeutic strategy. Mounting evidence indicates that constitutively active FOXO mutants, which restrain FOXOs in the nucleus, restore FOXO functions. Accordingly, developing chemical molecules that mimic FOXO mutants that act to restore the function of defective *FOXO* genes could be a potential choice

for cancer-related drug design. In addition, modulation of subcellular translocation could be another possibility. As FOXO is actively transported from the nucleus by a CRM1-dependent manner, nuclear export inhibitors including CRM1 inhibitors (for example, leptomycin B) could be considered. A high throughput, chemical genetic screen for inhibitors of FOXO1 nuclear export has been reported and other candidate molecules are under further investigation (Kau *et al.*, 2003). Recently, a bromotyrosine derivative, psammaplysene A, has shown to cause relocalization of FOXO1 to the nucleus in PTEN-deficient cells (Schroeder *et al.*, 2005), and could be another candidate. Furthermore, as Akt-dependent phosphorylation of FOXOs results in nuclear export and interaction with Skp2 and thereby consequent degradation, both PI3K–Akt pathway and Skp2 could be potential targets for cancer therapy.

Although substantial progress in understanding the function and regulation of FOXO proteins has been made, much remains to be discovered. Whether FOXO1, FOXO3, FOXO4 and FOXO6 have different subsets of target genes or share similar target genes is still not determined. In light of the observation that FOXOs rely heavily on cell type and tissue context to trigger different, even opposite, functions, it will be important to determine the mechanisms by which FOXO factors specify precise programs of gene expression and execute appropriate cellular functions accordingly. Thus, a detailed understanding of FOXO proteins and their biology will provide new opportunities for developing more effective therapeutic approaches to treat cancer.

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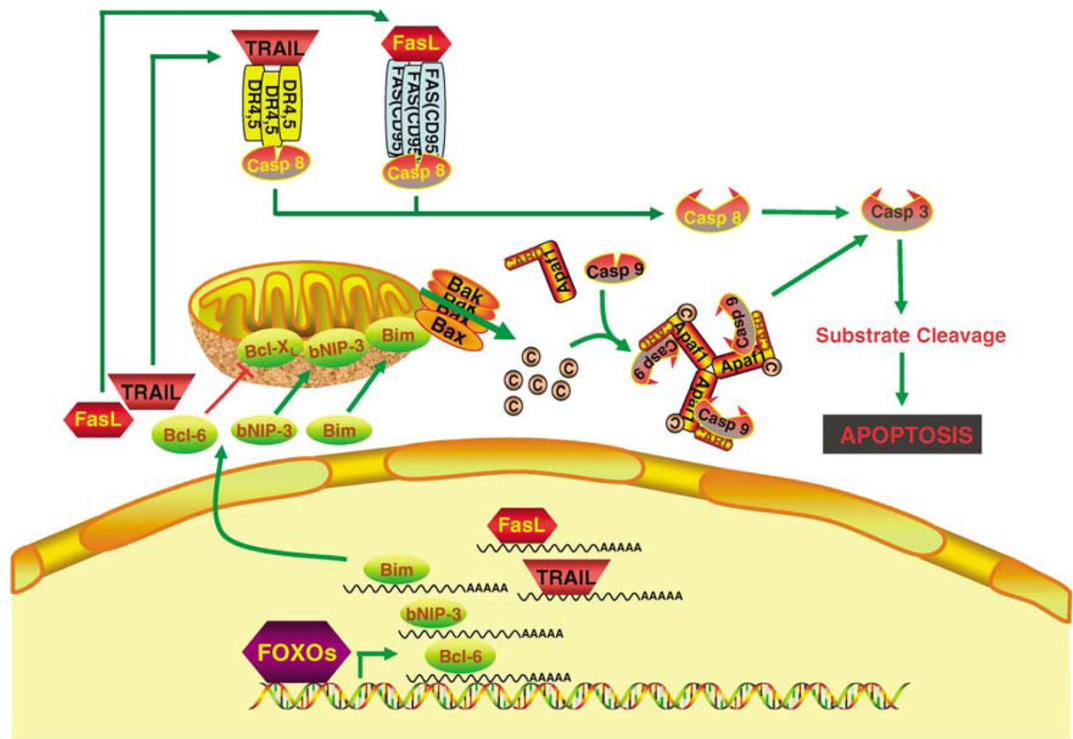


Figure 1.

Forkhead box O (FOXO) can induce apoptosis through mitochondria-dependent and -independent pathways. Upregulation of Fas ligand (FasL) or tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) results in cross-linking and activation of death receptors, which thereby activates caspase-8 and subsequent apoptosis. Direct transcriptional activation of the proapoptotic Bcl-2 family members, such as Bim and bNIP3, or indirectly suppressing the expression of the prosurvival Bcl-2 family member Bcl-X_L by regulating expression of the transcriptional repressor Bcl-6, leads to increase mitochondrial permeability and promote apoptosis. These pathways triggered by FOXOs may operate independently or cooperatively a cell- and/or tissue type-specific manner.