FOXO transcription factors: key regulators of cell fate

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Abstract
FOXO (forkhead box O) transcription factors are crucial regulators of cell fate. This function of FOXO proteins relies on their ability to control diverse and at times, opposing cellular functions, such as proliferation, differentiation, DNA repair, defence against oxidative stress damage and apoptosis, in response to hormones, growth factors and other environmental cues. This review discusses our current understanding of the regulation and role of FOXO transcription factors in determining cell fate and highlights their relevance to tumorigenesis and drug resistance.

PI3K (phosphoinositide 3-kinase), FOXO (forkhead box O; forkhead members of the O subclass) and cancer
The PI3K signal transduction pathway critically regulates cell proliferation, differentiation and apoptosis [1]. Perturbation in the PI3K signalling pathway is strongly implicated in the pathogenesis of many diseases, including heart and neural diseases, autoimmune/inflammatory disorders, cancer and the development of chemo- and endocrine-resistance in tumour cells. Constitutive activation of the PI3K pathway, a hallmark of many cancers, is commonly a consequence of enhanced expression of genes that encode either class I PI3K subunits (e.g. 110α) or PKB (protein kinase B) or is a result of genetic mutations that inhibit negative regulators of the pathway. For example, somatic deletions or mutations of PTEN (phosphatase and tensin homologue deleted on chromosome 10), an antagonist of the PI3K pathway, have been identified in a large proportion (12–60%) of human tumours of different tissue origins [2].

The mammalian FOXO family of transcription factors FOXO1, FOXO3a, FOXO4 and FOXO6 are major substrates of the protein kinases PKB and SGK (serum- and glucocorticoid-induced protein kinase), which relay PI3K signals to target genes [3–5]. The newly identified FOXO member, FOXO6, lacks the C-terminal PKB consensus phosphorylation motif and is constitutively located in the nucleus [6]. FOXO proteins function as transcriptional factors that interact with the core consensus DNA sequence GTAAA(C/T)A to modulate target gene expression [3].

Diverse functions of FOXO transcription factors in cell proliferation, death and differentiation
In mammals, the ability of FOXO factors to mediate cell-cycle arrest, DNA repair and apoptosis makes them attractive candidates as tumour suppressors (Figure 1) [3]. Loss of FOXO function can lead to uncontrolled cell proliferation. Furthermore, reduced ability to repair damaged DNA due to impaired FOXO activity may also result in genomic instability and carcinogenesis. Finally, a deficiency in FOXO proteins in abnormal and damaged cells that would normally undergo programmed cell death may result in tumour development and expansion. FOXO transcription factors control cell proliferation and survival by regulating the expression of genes involved in cell-cycle progression [e.g. p27kip1, p130(RB2), cyclin D1/2 and Bcl-6 (B-cell lymphocytic-leukaemia proto-oncogene 6)] and apoptosis [e.g. Bim, Fas ligand, TRAIL (tumour-necrosis-factor-related apoptosis-inducing ligand) and Bcl-X+] (Table 1) [3,7–14]. Thus one way by which PKB and the related SGK promote cell survival is by phosphorylating FOXOs, which results in their sequestration in the cytoplasm away from cell death-inducing genes. PKB phosphorylation also reduces the DNA-binding ability of FOXO and enhances its degradation. Common FOXO target genes that mediate apoptosis include bNIP3 and BCL2L11, which encode the pro-apoptotic Bcl-2 family members, bNIP3 and Bim [7–9]. Furthermore, FOXOs also indirectly down-regulate the expression of the pro-survival Bcl-2 family member Bcl-XL by inducing the expression of the transcriptional repressor Bcl-6 [10]. In neurons, FOXO3a triggers cell death circuitously by inducing the expression of Fas Ligand, which triggers programmed cell death through the death receptor pathway [3].
Growth factor (GF) stimulation leads to activation of PI3K, which results in Akt/PKB activation. Akt/PKB phosphorylates and inactivates FOXO proteins. FOXO activity towards different target genes and subsequent cell fate is influenced by SIRT- and JNK-dependent modifications. PDK, phosphoinositide-dependent kinase.

Mutations in PTEN occur in 60–80% of prostate cancers, leading to a constitutive activation of the PI3K pathway and a resultant loss of FOXO3a and FOXO1 activity [11]. As a consequence, the expression of the FOXO-regulated gene TRAIL, a pro-apoptotic member of the tumour necrosis factor family, is decreased in human metastatic prostate tumour cells, culminating in increased survival and disease progression. FOXO factors mediate G1 phase cell-cycle arrest by inducing the expression of negative cell-cycle regulators, such as the CKI (cyclin-dependent kinase inhibitor) p27Kip1 and the retinoblastoma-related protein p130 [12–15]. In response to transforming growth factor β, FOXO proteins have also been shown to bind to and activate the promoter of another CKI, p21Waf1/Cip1 [16]. At the same time, activation of FOXOs represses the expression of the G1 cyclins D1 and D2, either directly or indirectly by increasing Bcl-6 expression [10,17]. Finally, FOXO proteins are also implicated in regulating the expression of genes, such as cyclin B1, cyclin G2 and Cdc25B (cell division cycle 25B), important for G2/M cell-cycle phase transition [3]. However, this remains controversial as another FOX (forkhead box) subfamily member, FOXM1 (forkhead box M), has also been shown to regulate the expression of G2/M regulators, such as cyclin B, Cdc25B phosphatase, Aurora B kinase and Polo-like kinase [18,19]. The jury is still out as to whether FOXO or FOXM proteins are the predominant regulators of these G2/M genes. To add an extra level of complexity, recent results also suggest the possibility that FOXM1 and FOXO3a cooperate to regulate gene transcription [20].

Besides cell-cycle arrest and apoptosis, FOXO proteins can promote cellular differentiation. For example, FOXO3a
facilitates erythroid differentiation by inducing the expression of BTG1 (B-cell translocation gene 1) [21]. BTG1 in turn modulates protein arginine methylation activity necessary for erythroid differentiation. Furthermore, recent evidence demonstrated that FOXO3a directly binds and represses the transcription of the Id1 (inhibitor of differentiation 1) gene, a suppressor of erythroid differentiation, through the recruitment of an HDAC1 (histone deacetylase 1)–mSin3a complex (P. Coffer and E.W.-F. Lam, unpublished work).

**Role of FOXO in longevity and stress responses**

Aside of their roles as tumour suppressors, FOXO proteins also play a part in protection of cells against genotoxic and environmental stresses. Recent studies have identified a number of FOXO-regulated genes involved in a variety of cellular stress responses, ranging from defence against oxidative and caloric stresses to DNA repair [3]. In particular, FOXO3a enhances the expression of the antioxidant enzymes mitochondrial MnSOD (manganese superoxide dismutase) and catalase, which are scavengers of oxygen-free radicals [22]. Cells lacking MnSOD have greatly reduced oxidant damage protection, demonstrating the critical role of this mitochondrial antioxidant in the oxidative stress defence pathway. Consistent with this, enhanced expression of FOXO3a increases both hydrogen peroxide scavenging and oxidative stress resistance. The physiological role of FOXOs in protecting cells against oxidative stress is perhaps best highlighted during human pregnancy, when the human endometrial stromal cell compartment is exposed to marked fluctuations in oxygen tension. Through a process called decidualization, human endometrial stromal cells become resistant to oxidative stress-induced apoptosis [23]. This differentiation process is critical for embryo implantation and dependent upon the induction of FOXL, which in turn increases the expression of MnSOD.

In keeping with its role in mediating stress response, we recently found that FOXO3a serves as an important sensor for cellular stresses, in particular for cytotoxic stress induced by chemotherapeutic agents such as taxol and doxorubicin [24,25]. Although activation of FOXO3a by anticancer drugs may induce cell-cycle arrest and programmed cell death, chronic FOXO3a activation may render some cancer cells resistant to drug therapy by inducing the expression of multidrug resistance transporter genes such as MDR1 (ABCB1) (R. Hui and E.W.-F. Lam, unpublished work). Paradoxically, activated FOXO3a further promotes cell survival of cancer cells by enhancing PI3K/PKB activity through an as yet undefined mechanism (R. Hui and E.W.-F. Lam, unpublished work). Although FOXO proteins are promising therapeutic targets, a better understanding is required of how these transcription factors regulate two seemingly opposing cellular responses to cytotoxic stress: apoptosis and drug resistance.

**Regulation of FOXO by phosphorylation**

The transcriptional activity of FOXO proteins is carefully controlled at multiple levels, including initiation of gene expression, subcellular localization, protein degradation and most prominently, signal-induced post-translational modifications [6]. In addition to phosphorylation by PKB and other kinases, FOXO factors are further modified by acetylation. Phosphorylation of FOXO proteins by PKB or SGK prevents their nuclear translocation, thereby inhibiting their transcriptional competence. Other kinases such as CK1 (casein kinase 1) and DYRK1A (dual-specificity tyrosine phosphorylated and regulated kinase 1A) have also been demonstrated to phosphorylate FOXO proteins and inhibit their activity [3].

PKB-dependent phosphorylation disables FOXO proteins by inhibiting the FOXO nuclear translocation signal and exporting these transcription factors from the nucleus in a CRM1 transporter-dependent manner [26]. Phosphorylated FOXO proteins are retained in the cytoplasm by the 14-3-3 chaperone proteins. Another consequence of PKB phosphorylation is that phosphorylated FOXO proteins will be targeted for proteosomal degradation through interaction with specific ubiquitin E3 ligases such as Skp2 [27]. Loss of function of FOXO transcription factors due to PI3K/PKB-dependent phosphorylation and proteosomal degradation has emerged as an important event in the malignant transformation of certain cells [27,28]. Skp2, an oncogenic subunit of the Skp1–Cul1–F-box protein ubiquitin complex, promotes ubiquitination and degradation of FOXL. This effect of Skp2 requires PKB-specific phosphorylation of FOXL at Ser380. Expression of the FOXL protein is lost in a mouse lymphoma model, which overexpresses Skp2. Conversely, it has been reported that phosphorylation of cytoplasmic FOXO by the stress-activated JNK (c-Jun N-terminal kinase) microtubule-associated protein reverses the nuclear–cytoplasmic shuttling in response to PI3K/PKB activation. For instance, reactive oxygen species generated as a consequence of oxidative stress induces the activation of the small GTPase Ral, which in turn activates JNK. JNK-dependent phosphorylation of FOXO proteins results in nuclear accumulation and enhanced transcriptional activity, leading to increased expression of antioxidants such as MnSOD [29]. In Drosophila and Caenorhabditis elegans, the JNK pathway increases life span, which is mediated by orthologues of the mammalian FOXO proteins [30,31]. JNK has also been reported to phosphorylate 14–3–3 proteins, resulting in the release of bound FOXO transcription factors [32]. However, the ability of JNK to relocate FOXO to the nucleus appears to be dependent on simultaneous repression of the PKB activity, at least in breast cancer cells. In primary neurons, another protein kinase, MST1, has been shown to mediate oxidative cell death by activating FOXO transcription factors [33]. Like JNK, MST1 has been shown to phosphorylate FOXO proteins, to disrupt their interaction with 14–3–3 proteins and to promote nuclear accumulation.

**Regulation of FOXO by acetylation**

So what determines whether FOXO will promote apoptosis or longevity? The mechanisms that allow FOXO proteins to
Regulation of FOXO by other transcription cofactors

Other transcription factors also influence the ability of FOXO proteins to modulate target genes. For instance, β-catenin, a transcription factor that plays a major role in development and tissue self-renewal, binds directly to FOXO proteins and enhances their transcriptional activity. Furthermore, the ability of β-catenin to interact with FOXO has been reported to increase in response to oxidative stress. Additionally, loss of the β-catenin orthologue BAR-1 in C. elegans reduces the ability of DAF-16 to regulate the expression of sod-3, the equivalent of MnSOD in mammalian cells [42]. A recently identified C. elegans protein, SMK-1, has also been shown to be important for DAF-16-dependent longevity and oxidative stress responses, but not for other DAF-16 functions. Moreover, SMK-1 has been shown to be essential for the induction of oxidative but not heat-stress-responsive genes [43]. Other transcription factors, such as Myc, NF-κB (nuclear factor κB), Smad and p53, have also been implicated in regulating FOXO activity and target gene specificity to mediate cell fate decisions [3].

Concluding remarks

In summary, although the FOXO transcription factors function directly downstream of the PI3K/PKB signalling pathway, they are also targets of a number of other kinase cascades. FOXO proteins integrate the metabolic, mitogenic and stress signals with the transcription machinery to mediate diverse cellular functions and responses, such as proliferation, differentiation, DNA repair, defence against oxidative stress damage and apoptosis. FOXO activity is modulated by targeted phosphorylation, acetylation and interaction with other transcription factors, which accounts for their extraordinary ability to regulate seemingly opposing gene transcription programmes.

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References

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