Review

Applications of post-translational modifications of FoxO family proteins in biological functions

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The functions of the FoxO family proteins, in particular their transcriptional activities, are modulated by post-translational modifications (PTMs), including phosphorylation, acetylation, ubiquitination, methylation and glycosylation. These PTMs occur in response to different cellular stresses, which in turn regulate the subcellular localization of FoxO family proteins, as well as their half-life, DNA binding, transcriptional activity and ability to interact with other cellular proteins. In this review, we summarize the role of PTMs of FoxO family proteins in linking their biological and functional relevance with various diseases.

Keywords: FoxO, post-translational modification, cancer

Introduction

FoxO family proteins are a subgroup of the forkhead family of transcription factors. This family is characterized by a conserved DNA-binding domain, ‘forkhead box (Fox)’, and comprises more than 100 members in humans, classified from ‘A’ to ‘R’ on the basis of sequence similarity (Myatt and Lam, 2007). Mammalian members of class ‘O’ transcription factors, including FoxO1, FoxO3a, FoxO4 and FoxO6, are homologs of the Caenorhabditis elegans abnormal dauer formation-16 (DAF-16) and Drosophila dFoxO (Kenyon et al., 1993; Lin et al., 1997; Lee et al., 2003; Greer and Brunet, 2005).

FoxO family proteins are involved in multiple important biological processes, such as cell-cycle arrest, DNA repair, apoptosis, glucose metabolism, aging and autophagy (Greer and Brunet, 2005; Zhao et al., 2007). Because FoxO family proteins have a broad impact on mammalian cell function, FoxO activity is associated with aging, cancer, diabetes, infertility, neurodegeneration and immune system dysfunction (Maiese et al., 2008). For example, abnormal FoxO activity leads to the development of diabetes in a mouse model, as a result of decreased cellular insulin sensitivity (Nakae et al., 2002). In regard to cancer research, FoxO is considered to be a tumor-suppressor. Conditional deletion of FoxO1, FoxO3 and FoxO4 simultaneously results in the development of thymic lymphomas and hemangiommas (Paik et al., 2007). In a mouse model with a PTEN (phosphatase and tensin homolog) deletion, FoxO inactivation is essential for the survival of leukemia-initiating cells (Yilmaz et al., 2006).

To better identify the role of FoxO family proteins in disease, it is important to understand the molecular mechanism by which FoxO activity is regulated under various physiological or pathological conditions. In general, FoxO activity is regulated by numerous post-translational modifications (PTMs), including phosphorylation, acetylation, ubiquitination, methylation and glycosylation (Figure 1). These PTMs are catalyzed by enzymes with various mechanisms. The different PTMs allow FoxO molecules to function in various cellular activities by changing their subcellular location, molecular half-life or DNA-binding activity. Therefore, this review focuses on how the PTMs of FoxO family proteins regulate their molecular and cellular functions in physiological or pathological conditions.

Phosphorylation of FoxO proteins

FoxOs are targeted for phosphorylation by a plethora of protein kinases. So far, several enzymes have been identified as kinases of FoxOs. As shown in Table 1, each kinase modifies different sites on FoxOs and results in different changes in FoxO functions.

**AKT/protein kinase B**

Phosphorylation modification is a critical mechanism to regulate the nuclear–cytoplasmic shuttling of FoxOs and their transcriptional activity. The serine–threonine kinase AKT/protein kinase B (PKB) is an important downstream component of phosphoinositide 3-kinase (PI3K) signaling (Franke et al., 1997; Stecca and Ruiz i Altaba, 2010). It has been reported that FoxO3 phosphorylation induced by AKT leads to the retention of FoxO3 in the cytoplasm. AKT inhibition increases the nuclear translocation of FoxO3 and the activation of its target genes, which induces apoptosis (Brunet et al., 1999)
glucocorticoid-inducible kinases (SGKs) are also activated by the PI3K pathway (Pearce, 2003). Like AKT, SGKs phosphorylate and inactivate FoxO3. However, SGKs display a marked preference for phosphorylating Ser^315 of FoxO3, whereas AKT favors phosphorylating Ser^253 of FoxO3 (Brunet et al., 2001).

Although AKT/PKB and SGKs are enzymes that are well known for regulating the cytoplasmic retention of FoxOs, the exact mechanism by which FoxOs stay in the cytoplasm is still unknown. The chaperone protein 14-3-3 has been identified as an important protein involved in the nuclear–cytoplasmic shuttling of FoxOs. 14-3-3 binds to FoxO3 in the nucleus immediately before FoxO3 relocates to the cytoplasm (Brunet et al., 2002). Also, binding to 14-3-3 significantly changes the structure of the FoxO nuclear localization signal (NLS) and reduces its flexibility (Obsilova et al., 2005). It seems that binding to 14-3-3 enhances the nuclear export of FoxOs and decreases their nuclear entry. Several research groups have pointed out that the association of FoxOs and 14-3-3 is regulated by the phosphorylation of FoxOs. It has been shown that either AKT or SGK phosphorylates FoxO3 first. FoxO3, in turn, associates with 14-3-3 (Brunet et al., 1999; Obsilova et al., 2005). Subsequently, dephosphorylation of Thr^2^/Ser^313 in FoxO3, mediated by the phosphatase PP2A, is required for the dissociation of FoxO3 from 14-3-3 (Singh et al., 2010). It has also been reported that phosphorylation of FoxO1 at Thr^2^ by PKB is necessary and sufficient for its binding to 14-3-3. In the case of insulin-like growth factor 1 (IGF-1)-stimulated exclusion of FoxO1 from the nucleus, the interaction of FoxO1 with 14-3-3 is not required. This finding indicates that at least one other mechanism, distinct from 14-3-3 binding, may contribute to FoxO nuclear exclusion (Rena et al., 2001).

Although the decreased transcripational activity may result from a reduced abundance of FoxO proteins in the nucleus, FoxO transcriptional activity may be reduced by other mechanisms that do not depend on altering the subcellular distribution of the FoxO transcription factors. For example, activation of AKT/PKB by insulin is still able to inhibit transcription stimulated by FoxO1 with a mutation in the nuclear export signal (NES), despite the retention of the FoxO1 NES mutant in the nucleus (Tsai et al., 2003). Furthermore, FoxO1 phosphorylation at Ser^256 alters its in vitro binding activity by changing positive charges to negative charges in the DNA-binding domain of FoxO1 (Zhang et al., 2002). In the case of FoxO4, gel filtration and sedimentation equilibrium experiments showed that its binding to target DNA can be completely inhibited by the phosphorylation of Thr^28^ and Ser^192^, the 14-3-3 binding sites, by AKT/PKB (Obsil et al., 2003; Obsilova et al., 2005).

In addition to inducing the retention of FoxOs in the cytoplasm and leading to a decreased DNA-binding activity, AKT/PKB also induces the degradation of FoxOs by the proteasomal pathway in response to insulin and growth factors (Matsuzaki et al., 2003; Plas and Thompson, 2003; Aoki et al., 2004; Huang et al., 2005). The phosphorylation of FoxOs by AKT leads to cytoplasmic localization and subsequent degradation via the ubiquitin-proteasome pathway. In addition, the E3 ligase Skp2 promotes the degradation of FoxO1 following its phosphorylation by AKT at Ser^256^ (Huang et al., 2005). By contrast, chronic PKB activation leads to a dramatic increase in FoxO3 at both the transcriptional and protein levels (van Gorp et al., 2006).

**Mammalian Ste20-like kinase and Jun-N-terminal kinase**

Although AKT/PKB negatively regulates FoxO transcriptional factor function, some kinases promote FoxO activity by increasing the nuclear translocation of FoxO molecules. For example, under oxidative stress, mammalian Ste20-like kinase (MST1) phosphorylates FoxO proteins at a conserved site within the forhead domain (FHD) that disrupts their interaction with 14-3-3, promoting FoxO nuclear translocation and thereby inducing cell death in neurons (Lehtinen et al., 2006). Oxidative stress also induces FoxO4 phosphorylation in a Jun-N-terminal kinase (JNK)-dependent pathway. Following phosphorylation at Thr^467^ and Thr^551^, both the nuclear translocation and transcriptional activity of FoxO4 increase (Essers et al., 2004). In Drosophila,
JNK-induced FoxO activation increases life span (Wang et al., 2005). The JNK-induced activation of FoxOs not only depends on its phosphorylation, but also depends on other interacting proteins or pathways. For example, JNK phosphorylates 14-3-3, releasing FoxO3 from 14-3-3 and thereby antagonizing the effects of AKT signaling (Sunayama et al., 2005). The JNK pathway also decreases the activity of AKT in HIT cells, leading to decreased phosphorylation of FoxO1 following nuclear localization (Kawamori et al., 2006).

**Extracellular signal-regulated kinase/p38**

Mitogen-activated protein kinases (MAPKs) can be divided into three well-characterized subfamilies, extracellular signal-regulated kinase (ERK), JNK, and p38. Although JNK plays an important role in regulating FoxO activity under oxidative stress, FoxO1 is phosphorylated by ERK and p38 (Asada et al., 2007). ERK directly phosphorylates FoxO3, and the phosphorylated FoxO3 can be degraded via an MDM2-mediated ubiquitin-proteasome pathway (Yang et al., 2008). The simultaneous blockade of MEK and MDM2 signaling by AZD6244 and Nutlin-3a up-regulates levels of the BH3-only proteins Puma and Bim, in part through the transcriptional up-regulation of FoxO3 (Zhang et al., 2010).

In addition to direct regulation, ERK also decreases FoxO activity by phosphorylating other proteins. Several studies have suggested a link between ERK, p66shcA, and FoxO3. In p66shcA-deficient MEFs, H2O2-induced phosphorylation/inactivation of FoxO3 is inhibited, and this is accompanied by an increased activity of FoxO3 and the up-regulation of genes involved in free radical scavenging and oxidative stress resistance. Furthermore, the phosphorylation of Ser399 on p66shcA was found to be critical for FoxO3a phosphorylation (Nemoto and Finkel, 2002). In addition, activation of ERK is necessary and sufficient for p66shcA Ser179 phosphorylation (Hu et al., 2005). Therefore, ERK-induced p66shcA phosphorylation may play an important role in regulating FoxO3 activity. SGK is another bridge between MAPK and FoxOs. Some stress stimuli induce SGK protein expression through a p38/MAPK-dependent pathway, resulting in the phosphorylation and negative regulation of FoxO3 (Leong et al., 2003). The DNA damage-induced inhibition of FoxO3 transcriptional activity occurs through ERK1/2-mediated SGK1 post-translational regulation in a p53-dependent pathway (You et al., 2004). Furthermore, ERK and AKT can regulate each other under certain specific stimuli. For example, under oxidative stress, inhibition of PI3K modulates the intracellular distribution of phospho-ERK1/2, whereas MEK inhibition affects phospho-Akt (Thr308) and phospho-Akt (Ser473) (Kodira et al., 2009).

**Cyclin-dependent kinase**

The initiation of the cell cycle depends on the activity of complexes of cyclin and cyclin-dependent kinases (CDKs) (Pines, 1994). CDK2 specifically phosphorylates FoxO1 at Ser249 in vitro and in vivo. The phosphorylation of Ser249 results in cytoplasmic localization and inhibition of FoxO1 (Huang et al., 2006). In addition to CDK2, CDK1 can also phosphorylate FoxO1 at Ser249 in vitro and in vivo. The phosphorylation of FoxO1 at Ser249 disrupts FoxO1 binding to 14-3-3 and, in turn, promotes the nuclear accumulation of FoxO1 as well as FoxO1-dependent transcription, thereby leading to cell death in neurons (Yuan et al., 2008). However, the mechanisms by which both CDK1 and CDK2 phosphorylate FoxO1 at Ser249, but with opposite function on FoxO1, have not been clearly elucidated.

**AMP-activated protein kinase**

AMP-activated protein kinase (AMPK) is a central regulator of energy homeostasis in mammals (Hardie et al., 1994). AMPK phosphorylates human FoxO3 at six regulatory sites (Thr179, Ser399, Ser433, Ser355, Ser588 and Ser626) and activates FoxO3 transcriptional activity without affecting the subcellular localization of FoxO3 (Greer et al., 2007). Prolonged inactivation of p38 signals nuclear localization of FoxO3a in an AMPK-dependent manner, leading to sequential activation of its target genes, which induces autophagy, cell-cycle arrest and cell death (Chiacchiera et al., 2009). Similarly, the activation of AMPK by 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) activates FoxO3 by promoting its nuclear translocation and significantly reducing ROS levels (Li et al., 2009b).

In addition to direct phosphorylation, AMPK also regulates FoxO activity by modulating other proteins. For example, AMPK enhances the activity of Sirt1, a histone deacetylase, by increasing cellular NAD+ level, resulting in the deacetylation and activation of FoxO1 and FoxO3 (Canto et al., 2009).

**IkappaB kinase**

IκB kinase (IκK), a central regulator of NF-κB, plays an important role in controlling cell proliferation, survival, the prevention of apoptosis and tumorigenesis. IκK physically interacts with phosphorylases, and inhibits FoxO3a independently of AKT and causes proteolysis of FoxO3a via the ubiquitin-dependent proteasome pathway (Hu et al., 2004). The inhibition of IκK activity results in an increase in the total protein level of FoxO3 and up-regulation of TNF-related apoptosis-inducing ligand (TRAIL) (Wilson et al., 2010). The phosphorylation of FoxO by IκK not only decreases its stability, but also changes its subcellular localization. In both primary AML samples and an MV4-11/FoxO3-GFP cell line, FoxO3 is in a constitutively inactive state owing to its cytoplasmic localization. In this case, PI3K/Akt or ERK-specific inhibition does not result in FoxO3 nuclear translocation. In contrast, the anti-Nemo peptide, a specific inhibitor of IκK activity, was found to induce FoxO3 nuclear localization in leukemia cells (Chapuis et al., 2010).

Generally, phosphorylation acts not only as a veritable on and off switch for the transcriptional activity of FoxOs, but also as a precise mechanism for regulating the cellular localization and stability of FoxOs. Although multiple phosphorylation sites and enzymes associated with FoxOs have been discovered by scientists, there are still many questions that remained to be answered. For instance, some phosphorylation sites of FoxOs can be modified by more than one enzyme; however, different enzymes can elicit opposite effects by phosphorylating the same site. Therefore, a complete understanding of the phosphorylation of FoxOs remains a fascinating challenge for future investigation.

**Acetylation of FoxO proteins**

Like phosphorylation, acetylation also regulates different FoxO protein functions. Multiple deacetylases and acetylases have...
been identified to modify FoxOs to change their DNA-binding activity, stability and interaction with other proteins.

**Deacetylases**

FoxOs can be deacetylated by sirtuins and HDACs; however, the exact effect of FOXO deacetylation is still not clear. Sirt1, an NAD-dependent class III HDAC, deacetylates FOXO3 and forms a complex with FOXO3 in response to oxidative stress (Brunet et al., 2004). The coactivator-interacting LXXLL motif (aa 459–463) of FOXO1 was identified as the Sirt1 binding motif (Nakae et al., 2006). Sirt1 has a dual effect on the regulation of FOXOs: Sirt1 increases the ability of FOXOs to induce cell-cycle arrest but inhibits the ability of FOXOs to induce cell death (Brunet et al., 2004; Motta et al., 2004; van der Horst et al., 2004; Chen et al., 2009; Erion et al., 2009). It seems that Sirt1 increases or decreases the DNA-binding activity of FOXOs according to the target gene's promoter. In addition to DNA-binding activity, Sirt1 also regulates the nuclear–cytoplasmic shuttling of FOXOs. Resveratrol, a Sirt1 activator, is thought to promote FOXO1 deacetylation and nuclear retention, thus increasing the activity of FOXO1 (Qiang et al., 2010).

Several reports have shown that some proteins are also involved in binding with the FOXO–Sirt1 complex. The interaction of FOXO1 and Sirt1 is enhanced by four-and-a-half LIM domain protein 2 (FHL2). In prostate cancer cells, FHL2 inhibits FOXO1 activity by promoting the deacetylation of FOXO1 by Sirt1 (Yang et al., 2005). In pancreatic β cells, FOXO1 forms a complex with the promyelocytic leukemia protein (Pml) and Sirt1, activating expression of NeuroD and MafA which act against oxidative stress (Kitamura et al., 2005).

Recently, several reports showed that other members of the class III HDACs, Sirt2 and Sirt3, also play an important role in FOXO deacetylation. Sirt2 deacetylates FOXO3, increases the DNA-binding activity of FOXO3 and elevates the expression of FOXO target genes, p27kip1, manganese superoxide dismutase (MnSOD) and Bim (Wang et al., 2007). In 3T3-L1 adipocytes, Sirt2 deacetylates FOXO1 and induces FOXO1 binding to the promoter of PPARγ and subsequently represses PPARγ activity (Wang and Tong, 2009). Reduced interaction between Sirt2 and FOXO1 enhances insulin-stimulated phosphorylation of FOXO1 by AKT/PKB, which in turn increases FOXO1 cytosolic localization (Jing et al., 2007). In response to stress or starvation, FOXO1 is acetylated by dissociation from Sirt2, and bound to Atg7 to influence the autophagic process leading to cell death. This FOXO1-modulated cell death is associated with tumor-suppressor activity in human colon tumors and a mouse xenograft model (Zhao et al., 2010). Sirt3 is a mitochondria-localized sirtuin and can also increase MnSOD and catalase expression by activating FOXO3, which decreases the cellular levels of ROS and blocks cardiac hypertrophy (Sundaresan et al., 2009).

**Acetylases**

DAF-16 in *C. elegans* and mammalian homologs of FOXOs can interact with both the KIX and E1A/SRC interaction domains of the histone acetyltransferases (HATs) p300/CBP. The binding of CBP/p300 to FOXO factors is essential for the transactivation of the target of FOXOs (Nasrin et al., 2000; Perrot and Rechler, 2005; Yang et al., 2009). However, the acetylation of FOXOs attenuates FOXO-mediated transcriptional activity. For example, peroxide stress induces the binding of CBP to FOXO4 and acetylates FOXO4, leading to the inhibition of FOXO4 transcriptional activity (van der Horst et al., 2004). The HDAC inhibitor trichostatin A (TSA) suppresses the expression of p27kip1 that is induced by FOXO4 in HEK293T cells, whereas three lysine residue mutations, K186/189/408R, enhance the transcriptional activity of FOXO4 (Fukuoka et al., 2003). The mechanism by which acetylation decreases the transactivation activity of FOXOs may be that acetylated FOXOs have an attenuated DNA-binding activity (Matsuzaki et al., 2005; van der Heide and Smidt, 2005). Also, acetylated FOXO is more likely to localize to the cytoplasm. Mutations mimicking the acetylated state exclude FOXO1 from the nucleus because it is more sensitive to AKT-mediated phosphorylation (Qiang et al., 2010). In *in vitro* kinase reactions, mutated FOXO1 proteins, which mimic constitutively acetylated states, are more easily phosphorylated by AKT/PKB than wild-type FOXO1 (Matsuzaki et al., 2005). Reversely, AKT/PKB-induced FOXO1 phosphorylation stimulated by insulin is also required for insulin-induced acetylation of FOXO1 (Perrot and Rechler, 2005). Hence, cross-talk occurs between AKT/PKB-induced FOXO phosphorylation and the acetylation of its neighboring amino acids. However, whether there is cross-talk between other kinases inducing FOXO phosphorylation and acetylation is unknown.

There is increasing evidence that the acetylation of transcription factors can regulate their transcriptional activity by altering their DNA-binding activity and their interaction with transcription-regulatory proteins, as well as their stability and subcellular localization. Although the acetylation of FOXOs attenuates their transcriptional activity, stress-induced acetylation of FOXO1 appears to increase its stability and prevents its ubiquitin-dependent degradation in pancreatic β cells (Kitamura et al., 2005). However, the acetylation of FOXO1 by p300 does not alter its stability. Both wild-type p300 and the acetyltransferase-defective mutant p300-DY increase the half-life of FOXO1 protein in cells, indicating that acetylation itself is not critical for stabilization of the FOXO protein (Perrot and Rechler, 2005).

Although many lines of evidence indicate that acetylation plays an important role in regulating FOXO function, many questions remain regarding the regulation of FOXOs by acetylation: what is the exact mechanism of synergy or antagonism among different enzymes mediating phosphorylation and acetylation of FOXOs? Is there a specific cytoplasmic family of acetylases and deacetylases for FOXOs? Although acetylation may not exactly parallel phosphorylation, both phosphorylation and acetylation can cooperate to regulate FOXO proteins in response to different stimuli.

**Ubiquitination of FOXO proteins**

Ubiquitination involves the attachment of ubiquitin to lysine residues on substrate proteins or itself, which can result in protein mono-ubiquitination or poly-ubiquitination. It has been shown that FOXO proteins are also subject to poly- and mono-ubiquitination.
Poly-ubiquitination

The degradation of FoxO proteins is mediated by the ubiquitin-proteasome pathway (Matsuzaki et al., 2003; Plas and Thompson, 2003; Aoki et al., 2004). Several ubiquitin E3 ligases are necessary for the ubiquitination of FoxOs, as shown by Skp2, which associates with the Skp1/Cul1/F-box protein ubiquitin complex, ubiquitinates and promotes the degradation of FoxO, which is phosphorylated at Ser.

Huang et al., 2005

Oxidative stress MST

2003

I

Thompson,

2003

Serum starvation AKT

/shortdownarrow

Stimulus Enzyme Modification of FoxO Output

Serum starvation AKT ↓ Phosphorylation ↓ Nuclear import ↑

SGK ↓ Poly-ubiquitination ↓ Stability ↓

AMPK ↑ Phosphorylation ↓ Nuclear import ↑

Sirt2–FoxO1 binding ↓ Phosphorylation ↑ (cytoplasm) Autophagy ↑

Oxidative stress MST1 ↑ Phosphorylation ↑ Nuclear import ↑

JNK ↑ Phosphorylation ↑ Nuclear import ↑

Sirt1–FoxO1 binding ↑ Acetylation ↓ Nuclear import/translation activity ↑

Sirt2–FoxO1 binding ↑ Acetylation ↓ Nuclear import/translation activity ↑

HAUSP ↓ Mono-ubiquitination ↓ Transcription activity ↑

CBP–FoxO1 binding ↑ Acetylation ↑ Transcription activity ↓

Table 2 The modification types of FoxO family proteins and the enzymes in response to serum starvation or oxidative stress.

Mono-ubiquitination

It has been demonstrated that FoxO4 becomes mono-ubiquitinated in response to increased cellular oxidative stress (van der Horst et al., 2006). Herpes-virus-associated ubiquitin-specific protease (HAUSP, also known as USP7), a deubiquitinating enzyme, is shown to specifically interact with FoxO4 and therefore deubiquitinates it in response to oxidative stress. Knockdown of HAUSP increases FoxO4 mono-ubiquitination, its nuclear targeting and FoxO4-dependent transcriptional activity. Although MDM2 is considered to be an E3 ligase that promotes FoxO1 and FoxO3 poly-ubiquitination and degradation, MDM2 can also induce FoxO4 mono-ubiquitination, which promotes FoxO4 nuclear localization and increases transcriptional activity under oxidative stress (Brenkman et al., 2008). However, the exact mechanism of mono-ubiquitination-induced FoxO4 nuclear localization is still unknown.

Methylation and glycosylation of FoxO proteins

It has also been shown that FoxO1 is methylated at several arginine residues by protein arginine methyltransferase (PRMT1), which directly blocks AKT-mediated phosphorylation of FoxO1 at Ser in vitro and in vivo (Yamagata et al., 2008). PRMT1 knockdown enhances nuclear exclusion, poly-ubiquitination and proteasomal degradation of FoxO1. Methylated FoxO1 was retained in the nucleus, where it activated the expression of Bim and other FoxO target genes.

Glycosylation is a highly complex process that attaches glycans to proteins, lipids, or other organic molecules. There are two kinds of glycosylation including N-linked glycosylation and O-linked glycosylation. FoxO1 is a target for O-glycosylation (Kuo et al., 2008). O-glycosylation of FoxO1 does not affect the nuclear/cytoplasmic distribution of FoxO1, but results in the up-regulation of G6Pase expression (Kuo et al., 2008) and other gluconeogenic genes (Housley et al., 2008), indicating that glycosylation is an important PTM that regulates FoxO1 activation. FoxO3 is also a target of O-glycosylation. The phosphorylation of FoxO1 and FoxO3 by AKT is not directly related to GlcNAcylation (Housley et al., 2008).

PTMs of FoxO proteins under starvation and oxidative stress

It is unlikely that one modification on FoxO proteins dominates other modifications on FoxO. As we mentioned above, different stimuli induce PTMs of FoxO proteins with various mechanisms. For example, in the case of starvation and oxidative stress, several PTMs occur in the same FoxO proteins (Table 2). The regulation of FoxO in such conditions seems to be largely dependent on a combined effect of several PTMs. Interestingly, a same stress may induce an opposite modification on FoxO proteins, so that they are not only acetylated but also deacetylated in response to oxidative stress (Brunet et al., 2004; van der Horst et al., 2004; Kitamura et al., 2005). The difference of these PTMs of FoxO proteins in response to certain stimuli may result from the differences in used cell lines.
Conclusion
Above, we have summarized recent findings regarding the PTMs of FoxO proteins. In most cases, however, the functional roles of these FoxO modifications are far from well understood. Additionally, more in vivo experiments, such as the use of 'knock-in' technologies, are needed to verify the biological significance of each modification of FoxOs. Once we gain a more complete understanding of the whole picture of FoxO function and its modifications, we will be able to develop more highly effective strategies for treating FoxO-related diseases, including cancer and aging.

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