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Review

Post-translational regulation of FOXO

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The Forkhead box O (FOXO) family transcription factors play critical roles in a series of cellular processes, including the cell cycle, cell death, metabolism, and oxidative stress resistance. FOXO proteins are subject to several post-translational modifications, which are closely related to their activity. In this paper, we review the post-translational modifications of FOXOs and their biological functions.

Keywords FOXO; phosphorylation; acetylation; ubiquitination; methylation

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Introduction

The Forkhead box O (FOXO) is the O type subfamily of the forkhead transcription factor superfamily. It is highly evolutionarily conserved among species. In mammalian species, FOXO mainly includes FOXO1, FOXO3, FOXO4, and FOXO6, while its homolog genes, DAF16 and dFOXO, exist in lower organisms, such as *Caenorhabditis elegans* and *Drosophila*, respectively [1,2]. FOXO members share conserved DNA-binding domains, forming helix-turn-helix structures, which specifically bind to conserved DNA sequence 5′-TTGTTTAC-3′ [3]. FOXO transcriptionally activates or inhibits a series of downstream targets, thereby involved in the regulation of various biological processes, including the cell cycle, cell apoptosis, resistance to oxidative stress, and metabolism [4,5]. The activity of FOXO is dynamically regulated in response to various types or intensities of external stimuli. To further understand the molecular mechanism of how the activity of FOXO is regulated, many groups focus on the post-translational modifications (PTMs) by which FOXO is dynamically controlled. In this review, we will discuss the multi-type post-translational regulations of FOXO, including phosphorylation, acetylation, ubiquitination, and methylation, as well as the biological functions of these PTMs.

Phosphorylation of FOXO

FOXOs are subject to phosphorylation by a panel of protein kinases at different sites leading to the alteration of their subcellular location, protein stability, and DNA-binding activity.

Akt/SGK protein kinases

The phosphoinositide 3 kinase (PI3/K) pathway is a major regulator of FOXOs activity. Both the serine–threonine kinases protein kinase B (also named as Akt) and serum/glucocorticoid inducible kinase (SGK) are important downstream components of PI3/K signaling [6]. These two kinases recognize the same substrate phosphorylation motif, RXRXXS/T (R stands for arginine, X stands for any amino acid, and S/T means serine/threonine), and have been identified as the major enzymes for the phosphorylation of FOXO3 at Thr32, Ser253, and Ser315 [7–10]. The phosphorylation of these key sites increases the association with 14-3-3 proteins, which results in the translocation of FOXO proteins from the nucleus to cytoplasm leading to their transcriptional inactivation [7,9,11]. Furthermore, phosphorylation of FOXO1 at Ser256 (counterpart of FOXO3 at Ser253) alters its DNA-binding activity *in vitro* [12]. Akt is more responsible for the phosphorylation of Ser253, while SGK preferentially phosphorylates Ser315. Moreover, both kinases efficiently phosphorylate the N-terminal Thr32 residue of FOXO3 [8]. It has been reported that the phosphorylation of Ser315 by SGK is required for casein kinase 1-mediated phosphorylation at adjacent sites Ser318 and Ser321, which in turn enhances the nuclear export rate of FOXO proteins [13].

Hippo/MST kinases

Mammalian sterile 20-like kinase (MST), which shares a high degree of homology with the *Drosophila* ortholog Hippo, plays an important role in the regulation of cell size control and apoptosis [14,15]. Upon oxidative stress, MST1 binds to FOXO3 and phosphorylates Ser207 within its Forkhead domain. MST1-mediated phosphorylation of

FOXO3 disrupts its binding to 14-3-3 proteins, promotes its nuclear accumulation and therefore induces the expression of downstream pro-apoptotic genes that induce neuronal cell death [16]. It has been reported that MST1 also regulates FOXO1 through a similar mechanism [17]. Furthermore, the MST1–FOXO pathway also plays an important role in drug treatment-induced cancer cell death [18].

Cyclin-dependent kinases

Cyclin-dependent kinase 1 (Cdk1) can phosphorylate FOXO1 at Ser249 and block FOXO1's interaction with 14-3-3 proteins, driving FOXO1 into the nucleus to activate a cell death program in neurons [19,20]. Cdk2 also specifically phosphorylates FOXO1 at the same site but results in cytoplasmic localization and inhibition of FOXO1 [21,22]. The mechanism of the opposite biological outputs of FOXO1's phosphorylation at Ser249 needs to be further investigated.

AMP-activated protein kinase

The AMP-activated protein kinase (AMPK) plays a critical role in the regulation of energy homeostasis in cells [23]. AMPK has been shown to phosphorylate FOXO3 at six sites. Phosphorylation by AMPK leads to the activation of FOXO3 activity without altering its subcellular localization [24]. The AMPK–FOXO pathway is also conserved in *C. elegans*. It has been reported AMPK phosphorylates DAF-16 (FOXO ortholog in worm) at multiple sites and activates DAF-16-dependent transcription [25]. The molecular mechanism by which AMPK activates FOXO remains unclear.

ERK and IKK protein kinases

ERK has been shown to phosphorylate FOXO3 at Ser294, Ser344, and Ser425, which results in its nuclear exclusion. More importantly, phosphorylation of FOXO3 at these sites renders it unstable due to the increased interaction with the ubiquitin E3-ligase, MDM2 [26]. Similarly, activation of IKK has been proved to induce phosphorylation of Ser644 on FOXO3. Phosphorylation at this residue leads to both nuclear exclusion and degradation of FOXO3 proteins [27]. Both ERK and IKK are identified as oncogenes in tumorigenesis [28,29], which supports the hypothesis of FOXO proteins as tumor suppressors.

Acetylation of FOXOs

Similar to the phosphorylation, acetylation has been shown to regulate the transcriptional activity and mediate different biological functions of FOXOs. The effect of acetylation on FOXOs is controlled by the histone acetyltransferase and histone deacetylases (HDACs).

Histone acetyltransferase

The CBP/P300 are the essential enzymes for the acetylation of FOXOs [30]. Among the numerous reported sites, lysine242 and lysine245 of FOXO3 (lysine245, lysine248 of FOXO1) are of great importance: their acetylation severely diminished FOXO's DNA-binding capacity [31]. Furthermore, acetylated FOXO is more likely to localize to the cytoplasm [32]. On the other hand, FOXOs can also recruit CBP/P300 to the promoter of target genes. Acetylation of histone leads to the transactivation of the targets [33,34]. The dual effect of CBP/P300 on FOXO regulation still needs to be further understood.

HDACs

Sirt1 is the first member of the Sir2 family in mammals that has been demonstrated to play a critical role in longevity through its deacetylase activity [35]. FOXOs have been characterized as the essential substrate of Sirt1. Sirt1 and FOXO3 formed a complex in cells in response to oxidative stress. Sirt1 has a dual effect on the regulation of FOXOs: Sirt1 increases the ability of FOXOs to induce cell-cycle arrest and resistance to oxidative stress but inhibits the ability to induce cell apoptosis [36,37]. Other members of Sir2 family like Sirt2 and Sirt3 can also interact and deacetylate FOXO [38–40].

Recently, HDAC3 has been reported as a novel deacetylase of FOXO. HDAC4/5 recruits HDAC3 to FOXO, which results in the acute transcriptional induction of downstream genes via deacetylation and activation of FOXO [41].

Ubiquitination of FOXOs

Polyubiquitination

The degradation of FOXOs is determined by the ubiquitin-proteasome pathway. Several ubiquitin E3 ligases are proved to be necessary for the ubiquitination of FOXOs. For example, the E3 ligase MDM2 binds to FOXO3 to promote its degradation. Protein kinase ERK is required for this process [26]. Skp2, another E3 ligase, can recognize the Ser256 phosphorylated FOXO1 and degrade it by polyubiquitination [42]. The C-terminus of Hsc70-interacting protein (CHIP) promotes the ubiquitination and degradation of FOXO1 in smooth muscle cells [43]. A ring-finger E3 ligase COP1 has also been reported to mediate FOXO1's protein stability in Fao hepatoma cells [44].

Monoubiquitination

FOXO4 has been shown to be monoubiquitinated by MDM2 upon oxidative stress [45]. Monoubiquitination of FOXO induces its nuclear localization and enhances FOXO-dependent transcriptional activity. A deubiquitinating enzyme named USP7 can inhibit FOXO4 activity

through removing the monoubiquitin of it [46]. It has also been shown that monoubiquitination has little effect on the protein half-life of FOXO4 [45]. It is interesting that the E3 ligase MDM2 is also reported to promote FOXO3's degradation through polyubiquitination. The dual effects of MDM2 on different FOXO members need to be further studied.

Methylation of FOXOs

Arginine methylation

It has been reported that FOXO1, like many other proteins, can also be methylated by the protein arginine methyltransferase PRMT1. PRMT1 methylates FOXO1 at the conserved Arg248 and Arg250, which directly blocks Akt-mediated phosphorylation of FOXO1 at Ser253, thereby resulting in its long-lasting retention in the nucleus, leading to oxidative-stress-induced apoptosis [47]. Recently, the conserved PRMT1–FOXO signaling has also been demonstrated in the worms [48].

Lysine methylation

Methylation of lysines by different methyltransferases has been demonstrated to play important roles in regulation of both histone proteins and non-histone proteins [49]. Recently, our lab demonstrated that the methyltransferase Set9 methylates FOXO3 at lysine 270. The FOXO3 methylation leads to the inhibition of its DNA-binding activity and transactivation [50]. Accordingly, the lysine methylation reduces oxidative stress-induced and FOXO3-mediated Bim expression and neuronal apoptosis (**Fig. 1**).

Glycosylation FOXOs

Glycosylation is the enzymatic process that attaches glycans to proteins, lipids, or other organic molecules. Proteins can be glycosylated on different amino acid side chains, and these modifications are designated as *N*-glycosylation and *O*-glycosylation. It has been reported that FOXO1 can be *O*-glycosylated and lead to the up-regulation glucose-6-phosphatase and other gluconeogenic genes expression [51,52].

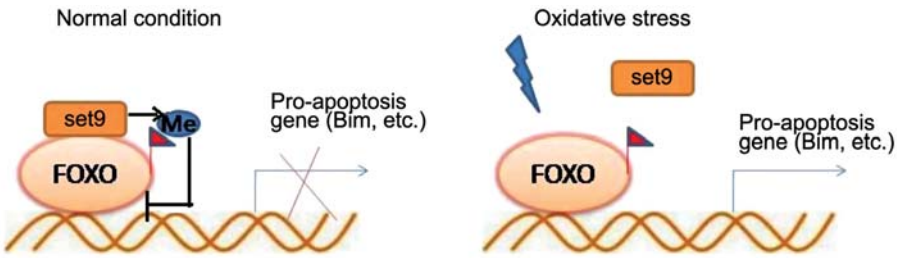

Figure 1 Model for Set9-regulated FOXO function

Table 1 Summary of the post-translational modifications of FOXO proteins

Modification	Enzyme	Sites	Effect on FOXO's activity
Phosphorylation	Akt/SGK	T32, S253, S315 (FOXO3)	↓
Phosphorylation	Casein kinase 1	S318, S321 (FOXO3)	↓
Phosphorylation	MST1	S207 (FOXO3)	↑
Phosphorylation	Cyclin-dependent kinase 1	S249 (FOXO1)	↑
Phosphorylation	Cdk2	S249 (FOXO1)	↓
Phosphorylation	ERK	S294, S344, S425(FOXO3)	↓
Phosphorylation	IKK	S644 (FOXO3)	↓
Phosphorylation	AMPK	T179, S399, S413, S555, S588, S626 (FOXO3)	↑
Acetylation	P300/CBP	K245, K248, K262 (FOXO1)	↓
Polyubiquitination	MDM2, COP1, Skp2, CHIP	ND	↓
Monoubiquitination	MDM2		↑
Arginine methylation	PRMT1	R248, R250 (FOXO1)	↑
Lysine methylation	Set9	K270 (FOXO3)	↓
Glycosylation	ND	ND	↑

↑ means upregulated;↓ means downregulated.
ND, not determined.

Summary and Prospective

We have reviewed recent findings of the PTMs of FOXO proteins (**Table 1**). Further work is still needed to elucidate the whole cross-talk map of FOXO PTMs. Additionally, the *in vivo* roles of PTMs also requires identification by transgene or knock-in animal models. A full understanding of PTMs will help us to treat FOXO-related diseases, including cancer and aging.

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References

1 Kenyon C, Chang J, Gensch E, Rudner A and Tabtiang R. A *C. elegans* mutant that lives twice as long as wild type. Nature 1993, 366: 461–464.
2 Weigel D, Jürgens G, Küttner F, Seifert E and Jäckle H. The homeotic gene fork head encodes a nuclear protein and is expressed in the terminal regions of the *Drosophila* embryo. Cell 1989, 57: 645–658.
3 Furuyama T, Nakazawa T, Nakano I and Mori N. Identification of the differential distribution patterns of mRNAs and consensus binding sequences for mouse DAF-16 homologues. Biochem J 2000, 349: 629–634.
4 Calnan DR and Brunet A. The FoxO code. Oncogene 2008, 27: 2276–2288.
5 van der Horst A and Burgering BM. Stressing the role of FoxO proteins in lifespan and disease. Nat Rev Mol Cell Biol 2007, 8: 440–450.
6 Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 2009, 9: 550–562.
7 Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS and Anderson MJ, *et al*. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 1999, 96: 857–868.
8 Brunet A, Park J, Tran H, Hu LS, Hemmings BA and Greenberg ME. Protein kinase SGK mediates survival signals by phosphorylating the forkhead transcription factor FKHRL1 (FOXO3a). Mol Cell Biol 2001, 21: 952–965.
9 Zheng WH, Kar S and Quirion R. Insulin-like growth factor-1-induced phosphorylation of the forkhead family transcription factor FKHRL1 is mediated by Akt kinase in PC12 cells. J Biol Chem 2000, 275: 39152–39158.
10 Kashii Y, Uchida M, Kirito K, Tanaka M, Nishijima K, Toshima M and Ando T, *et al*. A member of Forkhead family transcription factor, FKHRL1, is one of the downstream molecules of phosphatidylinositol 3-kinase-Akt activation pathway in erythropoietin signal transduction. Blood 2000, 96: 941–949.
11 Tang ED, Nuñez G, Barr FG and Guan KL. Negative regulation of the forkhead transcription factor FKHR by Akt. J Biol Chem 1999, 274: 16741–16746.
12 Zhang X, Gan L, Pan H, Guo S, He X, Olson ST and Mesecar A, *et al*. Phosphorylation of serine 256 suppresses transactivation by FKHR (FOXO1) by multiple mechanisms. Direct and indirect effects on nuclear/cytoplasmic shuttling and DNA binding. J Biol Chem 2002, 277: 45276–45284.
13 Rena G, Woods YL, Prescott AR, Peggie M, Unterman TG, Williams MR and Cohen P. Two novel phosphorylation sites on FKHR that are critical for its nuclear exclusion. EMBO J 2002, 21: 2263–2271.
14 Xiao L and Yuan Z. Redemystifying MST1/hippo signaling. Protein Cell, 2010, 1: 706–708.
15 Zeng Q and Hong W. The emerging role of the hippo pathway in cell contact inhibition, organ size control, and cancer development in mammals. Cancer Cell 2008, 13: 188–192.
16 Lehtinen MK, Yuan Z, Boag PR, Yang Y, Villén J, Becker EB and DiBacco S, *et al*. A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. Cell 2006, 125: 987–1001.
17 Yuan Z, Lehtinen MK, Merlo P, Villén J, Gygi S and Bonni A. Regulation of neuronal cell death by MST1-FOXO1 signaling. J Biol Chem 2009, 284: 11285–11292.
18 Valis K, Prochazka L, Boura E, Chladova J, Obsil T, Rohlena J and Truksa J, *et al*. Hippo/Mst1 stimulates transcription of the proapoptotic mediator NOXA in a FoxO1-dependent manner. Cancer Res 2011, 71: 946–954.
19 Kim AH and Bonni A. Cdk1-FOXO1: a mitotic signal takes center stage in post-mitotic neurons. Cell Cycle 2008, 7: 3819–3822.
20 Yuan Z, Becker EB, Merlo P, Yamada T, DiBacco S, Konishi Y and Schaefer EM, *et al*. Activation of FOXO1 by Cdk1 in cycling cells and postmitotic neurons. Science 2008, 319: 1665–1668.
21 Huang H and Tindall DJ. CDK2 and FOXO1: a fork in the road for cell fate decisions. Cell Cycle 2007, 6: 902–906.
22 Huang H, Regan KM, Lou Z, Chen J and Tindall DJ. CDK2-dependent phosphorylation of FOXO1 as an apoptotic response to DNA damage. Science 2006, 314: 294–297.
23 Williams T and Brenman JE. LKB1 and AMPK in cell polarity and division. Trends Cell Biol 2008, 18: 193–198.
24 Greer EL, Oskoui PR, Banko MR, Maniar JM, Gygi MP, Gygi SP and Brunet A. The energy sensor AMP-activated protein kinase directly regulates the mammalian FOXO3 transcription factor. J Biol Chem 2007, 282: 30107–30119.
25 Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D and Gygi SP, *et al*. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in *C. elegans*. Curr Biol 2007, 17: 1646–1656.
26 Yang JY, Zong CS, Xia W, Yamaguchi H, Ding Q, Xie X and Lang JY, *et al*. ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. Nat Cell Biol 2008, 10: 138–148.
27 Hu MC, Lee DF, Xia W, Golfman LS, Ou-Yang F, Yang JY and Zou Y, *et al*. IκB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. Cell 2004, 117: 225–237.
28 Karin M, Cao Y, Greten FR and Li ZW. NF-κB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2002, 2: 301–310.
29 Thompson N and Lyons J. Recent progress in targeting the Raf/MEK/ERK pathway with inhibitors in cancer drug discovery. Curr Opin Pharmacol 2005, 5: 350–356.
30 van der Heide LP and Smidt MP. Regulation of FoxO activity by CBP/p300-mediated acetylation. Trends Biochem Sci 2005, 30: 81–86.
31 Matsuzaki H, Daitoku H, Hatta M, Aoyama H, Yoshimochi K and Fukamizu A. Acetylation of Foxo1 alters its DNA-binding ability and sensitivity to phosphorylation. Proc Natl Acad Sci USA 2005, 102: 11278–11283.
32 Qiang L, Banks AS and Accili D. Uncoupling of acetylation from phosphorylation regulates FoxO1 function independent of its subcellular localization. J Biol Chem 2010, 285: 27396–27401.
33 Nasrin N, Ogg S, Cahill CM, Biggs W, Nui S, Dore J and Calvo D, *et al*. DAF-16 recruits the CREB-binding protein coactivator complex to the

insulin-like growth factor binding protein 1 promoter in HepG2 cells. Proc Natl Acad Sci U S A 2000, 97: 10412–10417.
34 Zhao Y, Liao W, Yang J, Wu L, Zheng Z, Yu Y and Zhou W, *et al*. Acetylation of FoxO1 activates Bim expression to induce apoptosis in response to histone deacetylase inhibitor depsipeptide treatment. Neoplasia 2009, 11: 313–324.
35 Bordone L and Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. Nat Rev Mol Cell Biol. 2005, 6: 298–305.
36 Kobayashi Y, Furukawa-Hibi Y, Chen C, Horio Y, Isobe K, Ikeda K and Motoyama N. SIRT1 is critical regulator of FOXO-mediated transcription in response to oxidative stress. Int J Mol Med 2005, 16: 237–243.
37 Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y and Tran H, *et al*. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004, 303: 2011–2015.
38 Wang F, Chan CH, Chen K, Guan X, Lin HK and Tong Q. Deacetylation of FOXO3 by SIRT1 or SIRT2 leads to Skp2-mediated FOXO3 ubiquitination and degradation. Oncogene 2012, 31: 1546–1557.
39 Sundaresan NR, Gupta M, Kim G, Rajamohan SB, Isbatan A and Gupta MP. Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. J Clin Invest 2009, 119: 2758–2771.
40 Jacobs KM, Pennington JD, Bisht KS, Aykin-Burns N, Kim HS, Mishra M and Sun L, *et al*. SIRT3 interacts with the daf-16 homolog FOXO3a in the mitochondria, as well as increases FOXO3a dependent gene expression. Int J Biol Sci 2008, 4: 291–299.
41 Mihaylova MM, Vasquez DS, Ravnskjaer K, Denechaud PD, Yu RT, Alvarez JG and Downes M, *et al*. Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. Cell 2011, 145: 607–621.
42 Huang H, Regan KM, Wang F, Wang D, Smith DI, van Deursen JM and Tindall DJ. Skp2 inhibits FOXO1 in tumor suppression through ubiquitin-mediated degradation. Proc Natl Acad Sci U S A 2005, 102: 1649–1654.
43 Li F, Xie P, Fan Y, Zhang H, Zheng L, Gu D and Patterson C, *et al*. C terminus of Hsc70-interacting protein promotes smooth muscle cell proliferation and survival through ubiquitin-mediated degradation of FoxO1. J Biol Chem 2009, 284: 20090–20098.
44 Kato S, Ding J, Pisck E, Jhala US and Du K. COP1 functions as a FoxO1 ubiquitin E3 ligase to regulate FoxO1-mediated gene expression. J Biol Chem 2008, 283: 35464–35473.
45 Brenkman AB, de Keizer PL, van den Broek NJ, Jochemsen AG and Burgering BM. Mdm2 induces mono-ubiquitination of FOXO4. PLoS One 2008, 3: e2819.
46 van der Horst A, de Vries-Smits AM, Brenkman AB, van Triest MH, van den Broek N, Colland F and Maurice MM, *et al*. FOXO4 transcriptional activity is regulated by monoubiquitination and USP7/HAUSP. Nat Cell Biol 2006, 8: 1064–1073.
47 Yamagata K, Daitoku H, Takahashi Y, Namiki K, Hisatake K, Kako K and Mukai H, *et al*. Arginine methylation of FOXO transcription factors inhibits their phosphorylation by Akt. Mol Cell 2008, 32: 221–231.
48 Takahashi Y, Daitoku H, Hirota K, Tamiya H, Yokoyama A, Kako K and Nagashima Y, *et al*. Asymmetric arginine dimethylation determines life span in *C. elegans* by regulating forkhead transcription factor DAF-16. Cell Metab 2011, 13: 505–516.
49 Huang J and Berger SL. The emerging field of dynamic lysine methylation of non-histone proteins. Curr Opin Genet Dev 2008, 18: 152–158.
50 Xie Q, Hao Y, Tao L, Peng S, Rao C, Chen H and You H, *et al*. Lysine methylation of FOXO3 regulates oxidative stress-induced neuronal cell death. EMBO Rep 2012, 13: 371–377.
51 Kuo M, Zilberfarb V, Gangneux N, Christeff N and Issad T. O-glycosylation of FoxO1 increases its transcriptional activity towards the glucose 6-phosphatase gene. FEBS Lett 2008, 582: 829–834.
52 Housley MP, Rodgers JT, Udeshi ND, Kelly TJ, Shabanowitz J and Hunt DF, *et al*. O-GlcNAc regulates FoxO activation in response to glucose. J Biol Chem 2008, 283: 16283–16292.