Drug Design, Development and Therapy

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REVIEW

Forkhead Box Protein O1: Functional Diversity and Post-Translational Modification, a New Therapeutic Target?

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Abstract: Forkhead box protein O1 (FoXO1) is a transcription factor involved in the regulation of a wide variety of physiological process including glucose metabolism, lipogenesis, bone mass, apoptosis, and autophagy. FoXO1 dysfunction is involved in the pathophysiology of various diseases including metabolic diseases, atherosclerosis, and tumors. FoXO1 activity is regulated in response to different physiological or pathogenic conditions by changes in protein expression and post-translational modifications. Various modifications cooperate to regulate FoXO1 activity and FoXO1 target gene transcription. In this review, we summarize how different post-translational modifications regulate FoXO1 physiological function, which may provide new insights for drug design and development.

Keywords: forkhead box protein O1, post-translational modification, transcription

Introduction

The forkhead box (FoX) family mainly function as transcription factors characterized by a conserved DNA-binding domain (the Forkhead box).[1] The human FoX O class (FoXOs) belong to FoX family and plays a crucial role in energy metabolism, differentiation, apoptosis, cellular proliferation, stress.[2,3] FoXO subfamily includes four members: FoXO1, FoXO3, FoXO4, and FoXO6, which are encoded by four different genes and represent functional diversity in regulation of cellular processes.[1,3] FoXO1 is a transcription factor involved in the regulation of a wide variety of physiological process including glucose metabolism, lipogenesis, apoptosis, and autophagy.[4–7] FoXO1 dysfunction is involved in the pathophysiology of various diseases including metabolic diseases, atherosclerosis, and tumors.[2,8–10] The Human *FoXO1* gene is located at chromosomal location 13q14.11 and encodes a 655 amino acid (aa) protein.[11–13] FoXO1 tissue expression is ubiquitous and within the cell FoXO1 distribution is cytoplasmic or nuclear. The cellular distribution of FoXO1 depends on the internal environment and homeostasis. Nuclear FoXO1 binds to cis-acting response elements of downstream target genes to mediate its transcription-regulatory function.[12,14–16]

The FoXO1 protein comprises several functional domains, or motifs, including a conserved NH2-terminal DNA binding domain (DBD, residues 158–237), a nuclear localization signal motif (NLS, residues 251–253), a nuclear export sequence motif (NES, residues 374–401), and a COOH-terminal transcription activation domain (TAD, residues 596–655).[1] DBD of FoXO1 is to recognize

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and bind to the consensus sequence of target gene promoter to activate transcription. NLS motif is responsible for transportation into the nucleus for transactivation, conversely, NES motif is to mediate transportation into the cytoplasm interacting with nuclear exportin-1 and 14-3-3 protein.[17] The FoXO1 TAD can bind to other regulatory proteins to co-repress or coactivate its function.[18] FoXO1 secondary structure includes four α-helices (α1, α2, α3, and α4), four β-strands (β1, β2, β3, and β4), one β turn, and two wing regions (W1 and W2). The domain or motif order in the protein is β1-α1-β2-α2-α4-α3-β3-β turn-wing 1-β4-wing 2, located at the N-terminus (residues 156–249) (Figure 1).[1,15,18] Mutations or covalent alterations of residues may significantly affect FoXO1 conformation and flexibility, resulting in a functional change.[19]

FoXO1 function is regulated in response to different physiological or pathogenic conditions at transcription and post-translational levels.[20–27] Modulators of FoXO1 activity have presented promising therapeutic value in the treatment of diabetes and obesity.[28–31] In this brief review, to best understand the molecular regulatory mechanism modulating FoXO1 activity, we summarize how different post transcriptional modifications (PTMs) regulate FoXO1 physiological function. This review may provide new insights for drug design and development.

PTM of FoXO1 and the Effects on Protein Activity

PTM is an essential way to regulating protein function and control fundamental physiological processes. A range of modifications are involved in regulating FoXO1 activity, including phosphorylation, acetylation, methylation, ubiquitination, glcNAcylation, and glutathionylation. These modifications modulate FoXO1 activity by affecting its subcellular distribution, DNA binding affinity, or gradation.

Phosphorylation

Phosphorylation modification is the most common type of covalent PTM involved in regulating protein function. Phosphorylation can modulate protein localization, conformation, turnover, DNA binding, and protein-protein interactions.[32] Phosphorylation site(s) are found within domains or motifs that are phosphorylated by specific protein kinases and can be associated with specific cellular processes.

Phosphoinositide 3-Kinase/Protein Kinase B (PI3K/PKB)

FoXO1 is a critical mediator of the insulin signaling pathway regulation of metabolic homeostasis in the liver and pancreatic β-cells in response to glucose alterations or stress.[5,14,33] Phosphoinositide 3-kinase/protein kinase B (PI3K/PKB or Akt) is the main upstream kinase in FoXO1 signaling transduction pathway regulation. PKB is a negative regulator that phosphorylates FoXO1 at specific residues, including Threonine 24 (Thr24), Serine 256 (Ser256), and Serine 319 (Ser319).[14,22,33–47] The Thr24/Ser256 residue is within the peripheral region of the DBD, adjacent to the NLS, indicating that phosphorylation may affect FoXO1 subcellular localization or NLS function.[43,48] The FoXO1 Ser319 residue is between the NLS and NES and its phosphorylation potentially promotes nuclear export.[40,48]

FoXO1 is cytoplasmic in unstimulated β-cells, and translocates to the nucleus when exposed to H_2O_2 or hyperglycemia.[33,49] In the liver, or other tissue, under basal conditions, FoXO1 is largely nuclear and transports into the cytoplasm with insulin treatment. However,

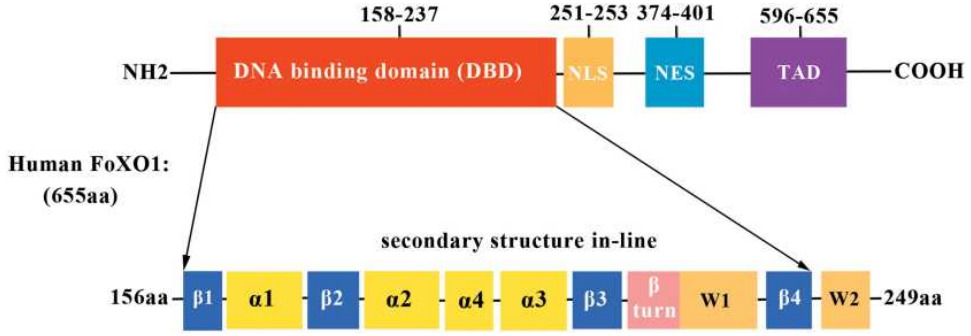

Figure 1 Schematic diagram of human FoXO1 domain alignment and secondary structure. The residue numbers are shown for each FoXO1 domain.
Abbreviations: NLS, nuclear localization signal motif; NES, nuclear export sequence; TAD, transcription activation domain.

FoXO1 insulin-stimulated translocation is inhibited by mutations in Thr24, Ser256, and Ser319.[14,44,47,50] Insulin inhibits gluconeogenesis in the liver in a PKB-dependent manner. This leads to decreased expression of the key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P). Interestingly, nuclear FoXO1 can bind the insulin response element (IRE) in the PEPCK and G6P promoters.[14,43]

PKB-induced FoXO1 phosphorylation promotes cytoplasmic localization 14-3-3 protein binding, leading to reduced nuclear FoXO1 protein levels and FoXO1-mediated gene transcription in insulin/IGF-1 (insulin-like growth factor-1) signaling pathways.[14,35,43,51,52] The 14-3-3 protein is a scaffold protein that sterically binds FoXO1. The FoXO1 binding region, residue 250–262, of FoXO1 is important for 14-3-3 protein binding and FoXO1 DNA binding ability decreases when FoXO1 is 14-3-3 bound, conformational change is possibly involved in the process.[22,43,48,51,53] The PTM may result in protein conformational change and affect functional activity through influencing molecular weight, hydrogen bond, water solubility or flexibility.[54] Saline et al report that the phosphorylated Ser22 and Thr24 unchanged the local secondary structure of the FoXO1, therefore, further study is needed to investigate the effect of phosphorylation at other residues on FoXO1 conformation.

Furthermore, in endothelial cells, dephosphorylation FoXO1 at Ser256 by peptidyl-prolyl isomerase (PPIase) leads to FoXO1 accumulation in the nucleus and increased transcription of genes involved in chemotaxis and apoptosis. These observations suggest that FoXO1 may play an important role in cardiovascular diseases.[55] Additionally, insulin-PKB-induced FoXO1 phosphorylation and cytoplasmic accumulation are necessary for subsequent polyubiquitination and proteasome-mediated degradation.[56]

AMP-Activated Protein Kinase (AMPK)

AMP-activated protein kinase (AMPK) is a key regulator of energy homeostasis and is involved in regulating FoXO1 functions including in oxidative stress, glucose metabolism, tumorigenesis, and lifespan extension.[57–61] AMPK can directly phosphorylate FoXO1 on residues Thr166, Ser202, Ser314, Ser321, Thr463, and Ser466. In *C. elegans*, AMPK-mediated FoXO1 phosphorylation enhances FoxO-dependent transcriptional activity and has beneficial effects of lifespan extension.[61] The effect of individual phosphorylation sites requires additional investigation. In the hypoxic environment of pulmonary artery smooth muscle, AMPK is activated to increase FoXO1 nuclear localization and catalase expression.[57] A study examining the regulation of hepatic gluconeogenesis reported that transforming growth factor β-induced activation of protein phosphatase 2A could dephosphorylate AMPK phosphorylated FoXO1 to promote FoXO1 nuclear translocation, but the phosphorylated residues involved were not addressed.[60]

Yun et al reported that AMPK phosphorylated the FoXO1 TAD domain located Thr649 residue to increase FoXO1 nuclear localization and transcriptional activity to combat oxidative stress in CHANG liver cells.[58] Recent evidence shows that AMPK-mediated phosphorylation of Ser22 in the FoXO1 NH2-terminal domain reduces PKB-induced Thr24 phosphorylation. This results in inhibition of chaperone protein (14-3-3) binding, activating FoXO1-dependent transcriptional activity.[22]

MST1

The mammalian sterile 20-like kinase 1 (MST1) is involved in the regulation of cell death in response to oxidative stress. Early studies have demonstrated that MST1 phosphorylates the Ser207 of FoXO3, equivalent to FoXO1 Ser212 in the DBD, leading to FoXO3 nuclear accumulation.[62] Consistent with this study, a more recent study reports that MST1 induces Ser212 FoXO1 phosphorylation and promotes FoXO1 nuclear accumulation through inhibiting the interaction with 14-3-3 proteins.[63] MST1-induced phosphorylation of FoXO1 has a protective effect against ischemia or reperfusion in cardiomyocytes.[64,65]

In regulatory T cells (Treg), phosphorylation of FoXO1 Ser212 by MST1 facilitates the FoXO1 stability and promotes Treg cell development and function.[66] Recent studies demonstrate that MST1 mediated endothelial angiogenesis occurring through FoXO1 Ser212 phosphorylation enhances FoXO1 nuclear localization.[67] However, these findings were not consistent with those presented by Brent et al, who demonstrated that the phosphorylated serines could block FoXO1 binding to cis-acting elements of target genes.[15]

CDK1/2, CDK4

The cyclin-dependent kinase 1 (CDK1) phosphorylates FoXO1 at Ser249 in the W2 region and promotes nuclear translocation, leading to stimulation of FoXO1 transcription in postmitotic neurons without affecting its DNA binding ability.[15,68] Nevertheless, both CDK1 and CDK2 can phosphorylate FoXO1 Ser249 and induce cytoplasmic localization resulting in inhibition of FoXO1 transcription in LNCaP and

DU145 cells, showing a regulatory role in apoptosis and DNA damage.[69,70] These differential effects indicate that CDK1/2 play different roles in various biological and pathogenetic processes. However, CDK4 phosphorylates FoXO1 Ser329 and inhibits its activity resulting in a decrease of FoXO1-induced recombination activating protein (Rag) expression to play a major role in B cell differentiation and genomic instability.[71,72]

Many other phosphorylation sites are also involved in the FoXO1 subcellular localization and transcriptional activity regulation. Protein kinase A (PKA) also phosphorylates FoXO1 at Ser276 (between NLS and NES), increasing its nuclear localization and stability and increasing gluconeogenesis.[45] The casein kinase 1 (CK1) phosphorylates FoXO1 at the proximal Ser322 and Ser325 residues in the region between NLS and NES and promotes FoXO1 cytoplasmic localization and nuclear export through interaction with the chromosomal region maintenance protein-1 (CRM1).[40] FoXO1 Ser329 is phosphorylated by the dual specificity tyrosine phosphorylated regulated kinase 1A (DYRK1A). This promotes cytoplasmic FoXO1 distribution in HEK-293 cells, which has an inhibitory effect on FoXO1 activity.[73] Nemo-like Kinase (NLK) is kinase the functions upstream of FoXO1 and can phosphorylate mouse FoXO1 at Ser284, Ser295, Ser326, Ser380, Ser391, Thr399, Ser413, and Ser415 in TIGK cells. NLK phosphorylates human FoXO1 at Ser329 and decreases FoXO1 nuclear localization. NLK-mediated FoXO1 phosphorylation also inhibits the FoXO1-mediated expression of zinc finger E-box-binding homeobox 2 (ZEB2), a transcriptional inhibitor that regulates nervous system development and inflammatory responses.[74–76]

Mitogen-activated protein kinase (MAPK) is also involved in controlling FoXO1 activity. Extracellular signal-regulated protein kinase (Erk) and p38 MAPK (p38) directly phosphorylate FoXO1 on Ser246, Ser284, Ser295, Ser326, Ser413, Ser415, Ser429, Ser467 Ser475, and Ser284, Ser295, Ser326, Ser467, Ser475, respectively, and regulate FoXO1-induced angiogenic genes.[77]

Overall, there are numerous studies describing the phosphorylation of FoXO1 residues by upstream kinases and the corresponding effects on FoXO1 function. However, the physiological implication of other phosphorylated residues remains to be determined.[76,77]

Acetylation

The transcriptional regulation of FoXO1 is also involves modification by protein acetylation. The cAMP response element-binding protein-binding protein (CBP) and its homolog, p300 (CBP/p300) are histone acetyl transferases involved in the regulation of multiple pathophysiological processes such as oxidative stress.[78,79] CBP binds and acetylates FoXO1 at lysine245 (Lys245), Lys248, and Lys262, near to the DBD in the wing 2 region (residues 244–249), and negatively regulates FoXO1 transcriptional activity in mammalian cells.[80] These three acetylated lysines, as well as Lys265 acetylated by CBP/p300, induce charge changes in the protein and decrease the affinity of FoXO1 binding to target gene cis-acting elements, including G6P.[15,81] Inconsistent with prior studies, Perrot et al shows that p300 directly acetylates FoXO1 and enhances the nuclear localization and transactivation of FoXO1 under basal conditions, and that this effect is reversed by insulin stimulation.[82] Similar results have shown that p300 acetylates FoXO3a and enhances its transcriptional activity.[83] The specific p300 acetylation site(s) were not identified in the study, and the divergent effects of CBP/p300 on FoXO1 function need further exploration.[82]

FoXO1 acetylation at Lys245, Lys248, Lys262, Lys265, and Lys274 upregulates c-Myc expression and promotes apoptosis in GBM cells. This effect is suppressed by Class IIa histone deacetylase (HDAC), and is not dependent on Akt-induced phosphorylation.[84] By contrast, HDAC9, a HDAC subtype, deacetylates FoXO1 and enhances its transcriptional activity by upregulating gluconeogenic enzymes in the liver.[24]

Single PTMs can synergistically combine with other PTMs to coactivate, co-repress, or antagonize each other. Acetylated FoXO1 can facilitate FoXO1 phosphorylation through PI3K/PKB by the cascade amplification effect. This leads to increased cytoplasmic translocation, which indirectly promotes FoXO1 degradation and represents an overlying inhibitory effect on FoXO1 activity.[81,85] Conversely, acetylated FoXO1 can be deacetylated by silent information regulator 1 (SirT1) and SirT2, which promote its nuclear localization and enhance transcriptional activity.[23,80,86–89] SirT1 deacetylates FoXO1 and increases its activity and degradation through the ubiquitin-proteasomal degradation pathway in βTC-3 cells under hyperglycemia or oxidative stress conditions.[49]

When cytosolic FoXO1 is acetylated at Lys262, Lys265, and Lys274 it disassociates from SirT2 and interacts with an E1-like protein (atg7) to modulate autophagic process. These may have implications for human colon cancer therapies.[4] FoXO1 CoRepressor (FCoR) is a novel FoXO1-binding protein expressed in mouse adipocytes. FCoR directly acetylates mouse FoXO1 Lys259, Lys 262, Lys 271, and Lys 291 (Lys262, Lys 265, Lys 274, and Lys 294 of human FoXO1) in vitro through disrupting the FoXO1 and Sirt1 interaction,

resulting in decreased FoXO1-dependent gene expression and adiposity.[90] The beneficial effect of FCoR on insulin sensitivity and adiposity is helpful for exploring therapeutic targets for the prevention of human obesity and type 2 diabetes.

Accumulating evidence demonstrate that acetylation participates in the regulation of FoXO1 function. Further research could explore how different acetyltransferases function on the same lysine residues, how the different modifications interact, and the functional roles of some acetylated lysines, such as the Lys597 in the FoXO1 TAD, newly identified through mass spectrometry.

Methylation

Protein arginine methyltransferases 1 (PRMT1) methylates the arginyl residue guanidino nitrogens of multiple proteins involved in the regulation of cellular processes including glucose metabolism and stress tolerance.[91–93] In mice, FoXO1 is methylated by PRMT1 at Arg248 (Arg248) and Arg250 (equivalent to Arg251 and Arg253 in the human FoXO1 NLS). These residues are near Ser253 (Ser256 in human), and their methylation has an inhibitory effect on the Akt-mediated phosphorylation of FoXO1 Ser253. This leads to increased FoXO1 nuclear retention and transcriptional activity in response to oxidative stress-induced apoptosis.[91,93,94]

A recent study demonstrated that the Lys273 residue of FoXO1 is also methylated by euchromatic histone lysine methyltransferase 2 (EHMT2), which is a histone methyltransferase to regulate apoptotic process and cell differentiation.[27,95] This methylation decreases FoXO1 stability through S-phase kinase-associated protein 2 (SKP2) mediated- ubiquitination degradation in colon cancer.[27] The differential effect of methylation of different FoXO1 residues on protein function suggests biological regulatory complexity.

Ubiquitination

FoXO1 activity is controlled by the ubiquitination process. FoXO1 is degraded via the 26S ubiquitin-proteasome pathway in HepG2 and INS-1 cells, and polyubiquitination-mediated degradation is promoted by insulin through PI3K/PKB-induced phosphorylation, which leads to cytoplasmic translocation.[56,96] SKP2, an E3 ubiquitin ligase, is involved in ubiquitination-mediated degradation and inhibition of FoXO1 activity through interacting with PKB-induced phosphorylation at Ser256.[97] The E3 ubiquitin-protein ligase Mdm2 is also involved in FoXO1 ubiquitination-mediated degradation requiring the PKB-mediated phosphorylation.[98]

Intriguingly, ubiquitin-specific protease 7 (USP7) directly deubiquitinates monoubiquitinated FoXO1 and has an inhibitory effect on FoXO1 transcriptional activity, resulting in decreased expression of downstream genes including G6Pase and PEPCK.[99] USP7 does not affect FoXO1 protein levels and subcellular localization, but decreases FoXO1 DNA binding affinity.[99]

GlcNAcylation

GlcNAcylation as a Ser/Thr residue-targeted post-translational protein modification involved in the regulation of glucose metabolism, oxidative stress, and tumorigenesis.[100–102] FoXO1 GlcNAcylation by O-GlcNAc transferase (OGT) increases FoXO1-induced G6Pase expression, and this effect is not associated with FoXO1 protein expression, PKB-mediated phosphorylation, or subcellular distribution.[103] Hyperglycemia increases FoXO1 GlcNAcylation and FoXO1 transcriptional activity, suggesting that FoXO1 GlcNAcylation plays an important role in diabetic pathophysiology.[103–106] Reports indicate that the Thr317, Ser318, Ser550, Thr648, and Ser654 residues are the FoXO1 GlcNAcylated sites and that GlcNAcylated Thr317 affects the transcriptional activity of human FoXO1.[104] However, Fardini et al revealed that this effect is not obvious in mouse FoXO1.[107]

Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1 α) is not only a GlcNAcylated protein but also a coactivator that interacts with OGT to enhance FoXO1 GlcNAcylation and subsequent transcriptional activity.[108] Further work is needed to investigate the molecular mechanisms involved in GlcNAcylation and the regulatory functions through which GlcNAcylation may present as potential therapeutic target for drug development.[109]

Glutathionylation

Cysteine S-glutathionylation is post-translational protein modification that plays an important role in the physiological reaction to oxidative stress.[110,111] FoXO1 glutathionylation mainly enhances FoXO1 DNA-binding capacity and subsequent transcriptional activity in response to oxidative environments, and does not affect FoXO1 phosphorylation status and subcellular location.[112] However, which of the specific cysteine residues contribute to the effect and how remain unknown.

Perspectives

FoXO1 as a key transcription factor that participates in physiological processes including glucose metabolism, apoptosis,

Table 1 Human FoXO1 Posttranslational Modifications

Post-Translational Modification	Site of Residues	Upstream Kinase	Biological Function	Effect on FoXO1 Function	References
Phosphorylation	Thr24, Ser256, and Ser319	PI3K/PKB	Gluconeogenesis	Inhibition	[14,22,33–47]
	Ser22, and Thr649	AMPK	Oxidative stress	Activation	[22,57]
	Ser212, Ser218, Ser234, and Ser235	MST1	Oxidative stress, Treg cell development	Activation	[63,66,67]
	Ser249	CDK1/2	Apoptosis, proliferation	Activation or inhibition	[68–70]
	Ser276	PKA	Gluconeogenesis	Activation	[45]
	Ser322, Ser325	CK1		Nuclear export	[40]
	Ser329	CDK4	Differentiation	Inhibition	[71,72]
		DYRK1A		Cytoplasmic localization	[73]
		NLK	Inflammation	Inhibition	[74]
	Ser246, Ser284, Ser295, Ser326, Ser413, Ser415, Ser429, Ser467, and Ser475	MAPK	Angiogenesis	Activation	[77]
Acetylation	Lys245, Lys248, Lys262, and Lys265	CBP/p300	Oxidative stress, gluconeogenesis	Inhibition	[15,80]
	Lys262, Lys265 and Lys274	?	Apoptosis, autophagy	Inhibition	[4]
	Lys262, Lys 265, Lys 274, and Lys 294	mFCoR	Differentiation	Inhibition	[90]
Methylation	Arg251, Arg253	PRMT1	Gluconeogenesis, oxidative stress, apoptosis	Activation	[91,94]
	Lys273	EHMT2	Proliferation, apoptosis	Inhibition	[27]
Ubiquitination		SKP2	Proliferation	Inhibition	[97]
		Mdm2	Apoptosis	Inhibition	[98]
GlcNAcylation	Thr317, Ser318, Ser550, Thr648, and Ser654	O-GlcNAc transferase (OGT)	Gluconeogenesis	Activation	[104]
S-glutathionylation	Not identified		Oxidative stress	Activation	[112]

Abbreviations: PI3K/PKB, phosphoinositide 3-kinase/protein kinase B; AMPK, AMP-activated protein kinase; MST1, mammalian sterile 20-like kinase 1; CDK1/2, Cyclin-dependent kinase 1/2; PKA, protein kinase A; CK1, casein kinase 1; DYRK1A, tyrosine phosphorylated regulated kinase 1A; NLK, nemo-like kinase; CBP/p300, cAMP response element-binding protein-binding protein (CBP)/p300; mFCoR, mouse FoXO1 CoRepressor; PRMT1, protein arginine methyltransferases 1; EHMT2, euchromatic histone lysine methyltransferase 2; SKP2, S-phase kinase-associated protein 2; Mdm2, E3 ubiquitin-protein ligase Mdm2; OGT, O-GlcNAc transferase.

adipocyte differentiation, and oxidative stress. FoXO1 dysfunction contributes to a wide variety of physiopathological processes including tumor progression, Type 2 diabetes mellitus, obesity, and atherosclerosis.[5,7,9,33,113–116] FoXO1 activity is closely linked with protein expression and is regulated by PTMs, including phosphorylation, acetylation, ubiquitination, methylation, glcNAcylation, and glutathionylation (Table 1). A growing body of evidence indicates that various modifications interact with each other or other coactivators (corepressors) to regulate FoXO1 activity and the transcription of FoXO1-mediated target genes (Figure 2).[81,97,108] However, the regulatory role of modified FoXO1 in various diseases and cellular processes remain incompletely understood. Considerably more work will need to be done to fully

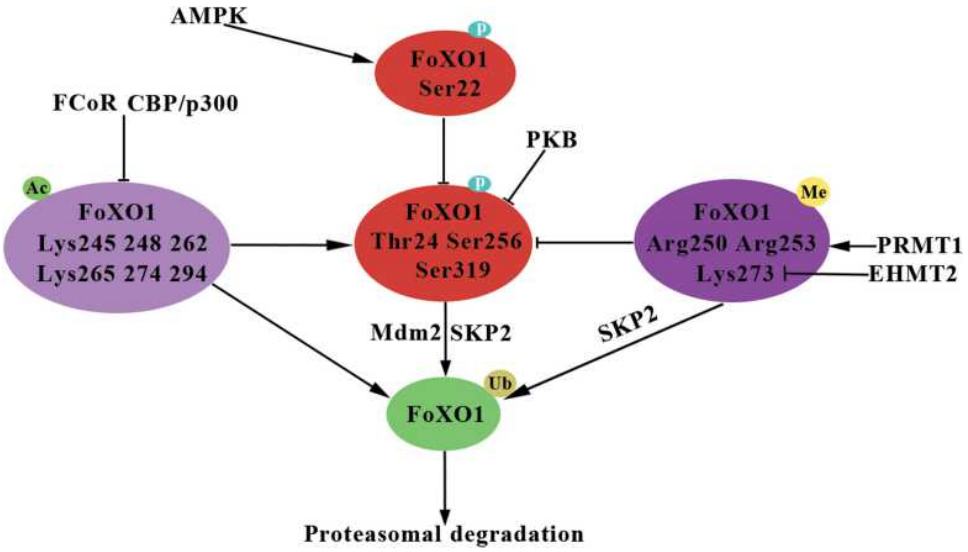

Figure 2 Overview of interaction of FoXO1 post-translational modifications.
Notes: Arrows denote activation. Black lines with perpendicular bars indicate inhibition.
Abbreviations: P, phosphorylation; Ub, ubiquitination; Ac, acetylation; Me, methylation; PKB, protein kinase B; AMPK, AMP-activated protein kinase; CBP/p300, cAMP response element-binding protein-binding protein (CBP)/p300; FCoR, FoXO1 CoRepressor; PRMT1, protein arginine methyltransferases 1; EHMT2, euchromatic histone lysine methyltransferase 2; SKP2, S-phase kinase-associated protein 2; Mdm2, E3 ubiquitin-protein ligase Mdm2.

understand the underlying molecular mechanisms of FoXO1 function, and the results of such studies will provide further insight into drug development, such as FCoR agonists, for human diseases.[90,117–119]

Disclosure

The authors report no conflicts of interest in this work.

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