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REVIEW

Current perspective on the regulation of FOXO4 and its role in disease progression

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Abstract

Forkhead box O4 (FOXO4) is a member of the FOXO family that regulates a number of genes involved in metabolism, cell cycle, apoptosis, and cellular homeostasis via transcriptional activity. It also mediates cell responses to oxidative stress and treatment with antitumor agents. The expression of FOXO4 is repressed by microRNAs in multiple cancer cells, while FOXO4 function is regulated by post-translational modifications and interaction with other proteins. The deregulation of FOXO4 is closely linked to the progression of several types of cancer, senescence, and other diseases. In this review, we present recent findings on the regulation of FOXO4 in physiological and pathological conditions and provide an overview of the complex role of FOXO4 in disease development and response to therapy.

Keywords FOXO4 · Cell proliferation · Apoptosis · Cancer · Drug response

Abbreviations

FOX	Forkhead box
FOXO	Forkhead box O
PI3K	Phosphatidylinositol-3-kinase
JNK	c-Jun N-terminal kinase
MLL	Mixed-lineage leukemia
FHD	Forkhead winged-helix DNA-binding domain
NLS	Nuclear localization sequence
NES	Nuclear export sequence
TAD	Transactivation domain
CRM1	Chromosomal maintenance 1
TNF-α	Tumor necrosis factor alpha
MST1	Mammalian ste20-like kinase
AMPK	AMP-activated protein kinase
ERK	Extracellular signal-regulated kinase
NLK	Nemo-like kinase
CK1	Casein kinase 1
DYRK1A	Dual-specificity tyrosine-phosphorylated regulated kinase 1A
CBP	CREB-binding protein
SIRT1	Silent information regulator 1
HDAC	Histone deacetylase
MDM2	Murine double minute 2
SKP2	S-phase kinase-associated protein 2
USP7	Ubiquitin-specific protease 7
TNPO1	Transportin 1
TCF	T cell factor
NF-κB	Nuclear factor κB
SOD2	Superoxide dismutase 2
ROS	Reactive oxidative species
GADD45	Growth-arrest and DNA damage-response protein 45
Bcl-xl	B cell lymphoma extra-large
Bcl-6	B-cell lymphoma 6
Bim	Bcl-2 interacting mediator
Bax	Bcl-2 associated X protein
SMC	Smooth muscle cell
SRF	Serum response factor
HIF1α	Hypoxia-inducible factor 1α protein
VEGF	Vascular endothelial growth factor
MAFbx	Muscle atrophy F-box
MuRF1	Muscle ring finger 1
TNF	Tumor necrosis factor
WNK	With-no-lysine kinase
AGE	Advanced glycation end product
TNM	Tumor node metastasis

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Introduction

The forkhead box (FOX) family consists of 19 subfamilies of transcription factors that are exclusively present in organisms ranging from fungi to humans. Within this family, the class O of forkhead box (FOXO) was named after the *Drosophila* FOXO gene that shares a highly conserved forkhead winged-helix domain. This common structural motif has 110 amino acids and is mainly responsible for DNA binding [1, 2]. The mammalian FOXOs play important biological roles in the regulation of cell development, energy homeostasis, and oxidative stress resistance in many tissues and also act as sensors and effectors of treatment measures [3]. Additionally, FOXOs have been implicated in carcinogenesis [4], which has recently drawn increasing attention.

In invertebrates, there is only one FOXO gene, while four FOXO genes are found in mammals, namely, FOXO1, FOXO3, FOXO4, and FOXO6 [5, 6]. The expression of these four FOXO genes is ubiquitous, including FOXO6, which was initially misinterpreted to be present only in the brain but is actually distributed in tissues of both central and peripheral organs [7]. The activities of FOXOs are negatively regulated by the phosphatidylinositol-3-kinase (PI3K)/AKT pathway and positively regulated by the c-Jun N-terminal kinase (JNK) pathway, which are critical in tumorigenesis and cancer progression [8, 9]. Like other members of FOXOs, FOXO4 has recently been revealed to be a crucial transcription factor that is involved in the regulation of several cellular processes. The expression of FOXO4 is tightly controlled by noncoding RNAs, particularly microRNAs, and its subcellular localization is dependent on phosphorylation modification, which is important for it to exert its functions. In addition, FOXO4 was extensively identified as a key tumor suppressor by regulating its target genes associated with antioxidative stress, cell cycle arrest, and apoptosis [10]. Thus, it has been thought that FOXO4 dysregulation may lead to a variety of cancers. Increasing evidence has also shown that antitumor drugs target FOXO4 activity in a clinical context, implicating FOXO4 as a potential biomarker for cancer [11, 12].

Here, we present the latest findings and important progress in the study of FOXO4 regulation and functions, as well as its target molecules in physiological and pathological conditions. We then provide an overview of the involvement of FOXO4 in the development of diseases including cancers, and the cellular response of FOXO4 with respect to the sensitivity and resistance to antitumor drugs, thereby suggesting perspectives on the targeting of FOXO4 in cancer treatment.

Structural domains of FOXO4

FOXO4, also known as acute leukemia fusion gene from chromosome X (AFX), was initially demonstrated as a gene that fused with the mixed-lineage leukemia (MLL) zinc finger transcription factor owing to a t(X;11) chromosomal translocation in acute leukemia [13]. Mammalian FOXO4 was found to be expressed in various tissues, including the brain, kidney, lung, skin, prostate, and muscles. Of these, the skeletal muscle is considered to be the site with the most abundant expression [14]. FOXO4 encodes a protein of 505 amino acids, and its structure is conserved across different species. It comprises four domains, including a highly conserved forkhead winged-helix DNA-binding domain (FHD), a nuclear localization sequence (NLS), a nuclear export sequence (NES), and a C-terminal transactivation domain (TAD) (Fig. 1). Like other FOXO transcription factors, the conserved FHD in FOXO4 is responsible for binding to the consensus core

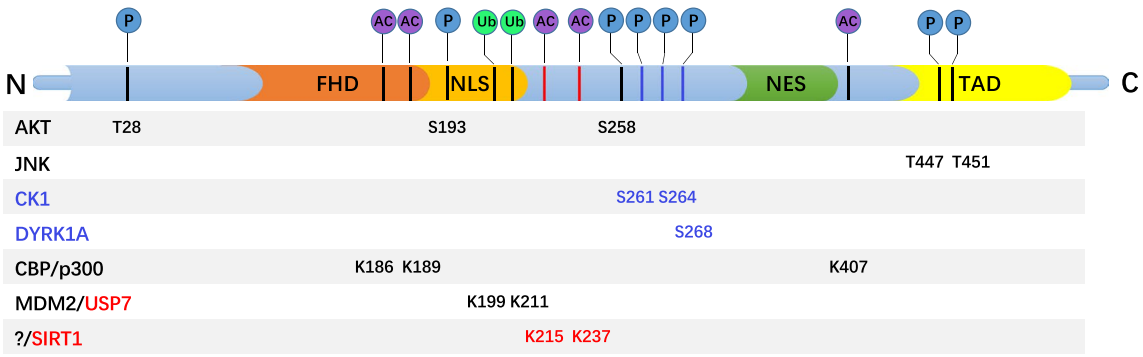

Fig. 1 Structural domains of FOXO4 protein and the major post-translational modification sites. *FHD* forkhead winged-helix DNA-binding domain, *NLS* nuclear localization sequence, *NES* nuclear export sequence, *TAD* transactivation domain, *P* phosphorylation, *AC* acetylation, *Ub* ubiquitination. The post-translational modification has been verified (black); expected site based on sequence alignment with other FOXO family members (blue); removal of the corresponding modifications (red)

recognition motif TTGTTTAC in the nucleus of cells, and this activity is inhibited by the insulin and IGF-1 signaling pathway [15]. The NLS domain is required for the translocation of FOXO4 into the nucleus through interaction with the nuclear import machinery, while nuclear export is achieved by exportins such as chromosomal maintenance 1 (CRM1) [16], which binds to the NES. In addition, there is substantial evidence that PKB-mediated phosphorylation of FOXO4 inactivating the NLS results in the nuclear exclusion of FOXO4 [17]. FHD is primarily responsible for the direct interaction between FOXO4 and DNA, which can mediate its binding with other proteins, such as p53 [18, 19]. However, the TAD in the C-terminal of FOXO3 could also bind with the DNA-binding domain of p53 [20]. Because the TAD is conserved among the FOXOs, this raises the possibility that the TAD in FOXO4 might also interact with several co-regulators, including p53. Therefore, future studies on the molecular details of the interaction of TAD in FOXO4 with other regulators should help to understand its role in mediating the transactivation of FOXO4 target genes.

Regulation of FOXO4 expression and activity

Transcriptional regulation of FOXO4

As a transcription factor, FOXO4, which regulates the mRNA expression of several genes, has been extensively studied, whereas not much is known about the transcriptional regulation of FOXO4 itself. The transcription factor E2F-1 was shown to induce FOXO1 and FOXO3 expression [21], and FOXC1 up-regulates FOXO1 in the eye [22]. Intriguingly, one study by Essaghir et al. showed that the mRNA expression of FOXO1 and FOXO4 can be induced by FOXO3 activation, implying the presence of a positive feedback network controlling FOXO gene expression [23]. FOXO4 is also transcriptionally regulated by PI3K/AKT signaling since the stimulation of thyrocytes with growth factors and fibroblasts with insulin resulted in a decrease in FOXO4 mRNA levels [23, 24]. Inhibition of the PI3K/AKT cascade by LY294002 increased the transcription of FOXO4, which might be caused by impaired phosphorylation of FOXO3 and thus resulted in elevated FOXO3 activity [24]. Furthermore, acute starvation or caloric restriction has been shown to trigger the upregulation of FOXO4 mRNA levels in skeletal muscles [25, 26]. In addition, the transcription of FOXO4 is also altered upon exposure to some natural substances, such as matrine [27] and puerarin [28]. In future studies, it will be necessary to address which transcription factors and signaling pathways directly regulate FOXO4 mRNA levels.

MicroRNAs (miRNAs) regulate post-transcriptional FOXO4 gene expression

miRNAs are short noncoding RNA molecules that function in the post-transcriptional regulation of gene expression and silencing RNA through base-pairing with complementary sequences of the target mRNAs [29]. In humans, more than 3000 mature miRNAs have been annotated [30]. Many of these are evolutionarily conserved and appear to regulate about 60% of the mRNAs in humans and other mammals [31]. Previous studies found that many miRNAs target the 3′-untranslated region (3′UTR) of FOXO4 mRNA to repress its transcription under pathological conditions. In diabetic retinopathy, the expression of miRNA-29a and miRNA-29b targets FOXO4 and alleviates the pathological process by high-glucose stimulation [32]. In nasopharyngeal carcinoma, FOXO4 is directly regulated by miRNA-421. It was shown that the upregulation of miRNA-421 induced cell proliferation and apoptosis resistance via targeting of the 3′UTR of FOXO4 and inhibiting its transcriptional activity [33]. In contrast, the silencing of miRNA-421 increased FOXO4 mRNA levels and its downstream target genes [33]. Moreover, decreased expression of FOXO4 correlated with increased miRNA-499-5p levels was detected in invasive colorectal cancer cell lines and lymph node-positive specimens, resulting in enhanced tumor cell migration and invasion [34]. FOXO4 is also regulated by miRNA-1274a, which was found to be highly expressed in gastric cancer tissues and cultured cells. The overexpression of miRNA-1274a activated the PI3K/AKT pathway and promoted gastric cancer cell migration and proliferation in vivo [35]. In osteosarcoma, miRNA-664 upregulation enhanced human osteosarcoma cell growth by suppressing the expression of FOXO4 [36]. Likewise, miRNA-150 directly reduced FOXO4 mRNA levels, which consequently decreased the expression of FOXO4 targets, such as p27, FASL, and BIM, and increased the expression of cyclin D1 in cervical cancer [37]. Consistent results of miRNA-150 targeting FOXO4 3′UTR were also obtained in a study on human non-small cell lung cancer cells [38]. More recently, more miRNAs were identified to reduce FOXO4 mRNA levels, such as miR-148a-3p in coronary atherosclerosis patients [39], rno-miR-466c-5p in congenital scoliosis [40], miR-214 and miR-23b in vascular smooth muscle cells [41, 42], miR-6785-5p in gastric carcinoma [43], and miR-532-3p in colorectal cancer [44]. These lines of evidence highlight that the improvement of FOXO4 expression by administering anti-microRNA is a potential strategy for treating diseases with high mortality. Therefore, future studies are needed to explore the contribution of the comprehensive miRNA network that influences the expression of FOXO4 not only via direct binding but also indirectly.

Post-translational regulation of FOXO4 activity

After translation, FOXO4 proteins usually undergo several post-translational modifications, mainly involving phosphorylation, acetylation, and ubiquitination, which control their subcellular localization and alter their DNA-binding affinity. This, in turn, affects the transcriptional activity of FOXO4 on its target genes. Among the post-translational modifications, the phosphorylation of FOXO4 by many kinases has been well characterized. A major mechanism of FOXO4 phosphorylation at two residues (Thr447 and Thr451) is mediated by the activated JNK signaling pathway in the presence of H_2O_2-induced oxidative stress or tumor necrosis factor alpha (TNF-α) in response to inflammation, leading to the nuclear translocation of FOXO4 and CDKN1A/p21 expression [45–47]. Furthermore, oxidative stress activates mammalian ste20-like kinase (Mst1), and nutrient deprivation activates AMP-activated protein kinase (AMPK) [48], which trigger the phosphorylation and nuclear translocation of FOXOs. FOXO proteins only perform transcriptional activity inside the nucleus. However, FOXO4 transcriptional activity is repressed by PI3K/AKT-mediated phosphorylation at three residues (Fig. 1) under suitable stimulation with insulin or growth factor. These phosphorylations allow the binding of 14-3-3 protein, which causes the nuclear exclusion of FOXO4 and, in turn, inhibit FOXO4-dependent gene transcription. In addition to PI3K/AKT, other kinases may also be negative regulators of FOXO4, such as extracellular signal-regulated kinase (ERK) [49] and nemo-like kinase (NLK) [50]. Evidence has shown that NLK-inhibited FOXO4 activity co-occurs with the inhibition of FOXO4 monoubiquitination under oxidative stress [50]. Besides, although it has not been investigated yet, the potential phosphorylation of FOXO4 by casein kinase 1 (CK1) and dual-specificity tyrosine-phosphorylated regulated kinase 1A (DYRK1A) are marked due to the conserved residues surrounding the phosphorylation sites between FOXO4 and FOXO1 [51, 52].

In addition to phosphorylation, acetylation also impacts on FOXO4 DNA binding, subcellular localization, and transcriptional activity [53]. In the context of a cellular redox state, FOXO4 was found to be acetylated by CREB-binding protein (CBP) and its homolog p300 in vitro and in vivo [54, 55]. It was reported that CBP acetylates FOXO4 at three lysine residues and reduces its transcriptional activity on a number of key targets in the nucleus. Analogously, FOXO4 acetylation can be regulated by the cellular redox state through the cysteine residue-mediated formation of cysteine-thiol disulfide-dependent complexes of FOXO4 with p300 acetyltransferase which decreases FOXO4-triggered cell cycle arrest and apoptosis [56]. Acetylation inhibits FOXO4 activity, whereas deacetylation improves it. It was described that NAD-dependent deacetylase silent information regulator 1 (SIRT1) binds FOXO4 to catalyze its deacetylation and enhances DNA binding at specific target genes [57]. FOXO4 can also be deacetylated by histone deacetylases (HDAC) at three lysine residues [54], where the sites are acetylated by CBP, indicating that HDAC counteracts the CBP-mediated downregulation of FOXO4 DNA-binding activity.

The ubiquitin–proteasome system plays an essential role in protein degradation. Like the case for many other proteins, FOXO degradation occurs through ubiquitination reactions that are mediated by various enzymes, such as ubiquitin E3 ligase [58]. Polyubiquitylation of FOXO1 and FOXO3a results in their inactivation and degradation, whereas FOXO4 is monoubiquitylated in response to oxidative stress, leading to nuclear localization and an increase in subsequent transcriptional activation [59]. Previous studies described that the monoubiquitination of FOXO4 is mediated by E3 ubiquitin ligase murine double minute 2 (MDM2) [60], and the polyubiquitylation of FOXO4 may be mediated by S-phase kinase-associated protein 2 (Skp2) [58, 61], facilitating its degradation. The monoubiquitination of FOXO4 promotes its nuclear localization, which was demonstrated to respond to cellular redox state in cultured cells [59, 62]. Moreover, monoubiquitinated FOXO4 can be deubiquitinated by deubiquitinating enzymes. Ubiquitin-specific protease 7 (USP7) is one such enzyme that binds to the C-terminal of the FHD domain of FOXO4 and removes ubiquitin from the proteins, resulting in the relocalization of FOXO4 from the nucleus to the cytoplasm and inhibition of its transcriptional activity [59] (Fig. 2).

FOXO4 activity is regulated by interaction with other proteins

In addition to the post-translational modifications, the activity of FOXO4 can also be affected by interaction with other co-activators or co-repressors. In a study on the FOXO4–p300 interaction, it was reported that FOXO4 potentially formed intermolecular disulfides with the nuclear import factor transportin 1 (TNPO1), which aids the nuclear translocation of FOXO4 and thereby promotes its transcriptional activity [63]. B-catenin is a multifunctional protein that mediates Wnt signaling by binding to transcriptional repressors of the T cell factor (TCF) family. Oxidative stress-induced expression of FOXO4 leads to the competition of FOXO4 with TCF for binding to β-catenin and enhances FOXO4 activity [64]. In many cancer cells, overexpressed pin1 has been shown to interact with FOXO4 and inhibit p27kip1 expression through stimulation of the activity of USP7 on FOXO4 deubiquitination [65]. Furthermore, Ku70, a protein that is critical for the repair of DNA damage in oxidative stress, binds to FOXO4 and negatively regulates FOXO4-mediated cell cycle arrest and p27kip1 expression

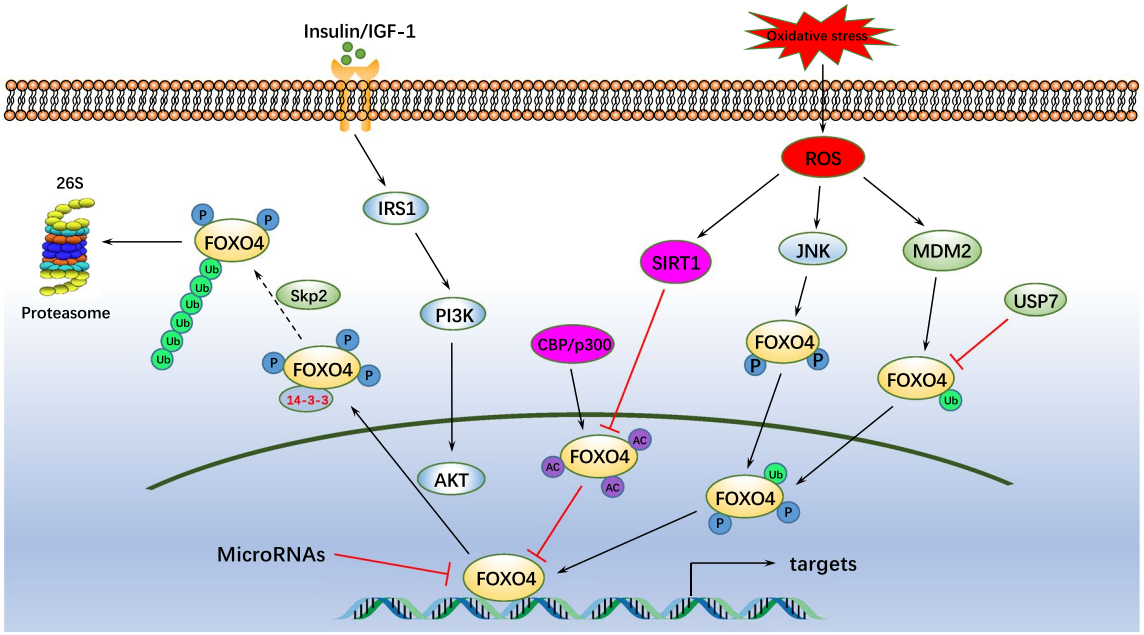

Fig. 2 Regulation of FOXO4 by post-translational modifications. Activation of JNK in response to oxidative stress directly phosphorylated FOXO4 and enhanced its translocation into the nucleus. However, the stimulation of cells by insulin and growth factors results in the activation of PI3K/AKT signaling, which in turn leads to FOXO4 phosphorylation and combination with 14-3-3. This phosphorylation facilitates its cytoplasmic localization and proteasome degradation through Skp2-mediated polyubiquitination of FOXO4. CBP/p300-mediated acetylation inhibited FOXO4 transcriptional activity, while this inhibition could be eliminated by SIRT1-induced deacetylation of FOXO4. The monoubiquitination of FOXO4 by MEM2 promotes its translocation to the nucleus, which is reversed by USP7-mediated deubiquitination

[66, 67]. As transcription factors, FOXO4 and p53 share many common features in cell proliferation, apoptosis, and tumor suppression. In fact, evidence illustrated that FOXO4 directly binds to p53 at the site of DNA damage [68], and disturbance of their specific interaction by a competing peptide, such as FOXO4-D-Retro-Inverso [18], promotes the apoptosis of senescent cells. Besides, a direct inhibitory interaction between FOXO4 and nuclear factor-kappa B (NF-κB) was identified under stress conditions such as acute colitis [69].

Functions of FOXO4

In the last two decades, a cohort study has investigated the biological functions of FOXO4 and confirmed its potential role in regulating various cellular processes, such as antioxidant state, apoptosis, cell cycle arrest, autophagy, and cellular homeostasis, by inducing the transcription of many genes (for a comprehensive list of FOXO4 targets, see Fig. 3). Generally, the deletion of FOXOs can provide insight into their biological roles. In contrast to the knockout of other members of the family, FOXO4-knockout mice do not present any obvious abnormalities [70], whereas conditional triple deletions of FOXO1, FOXO3a, and FOXO4 result in a modest tumor phenotype of thymic lymphomas and hemangiomas [71]. Moreover, FOXO4 deficiency exacerbates colitis in response to inflammatory stimuli [69].

Antioxidative stress

Consistent with other members of FOXO, one of the most important functions of FOXO4 is the role in cellular responses to oxidative stress. On the one hand, FOXO4 mediates the cellular oxidative state by promoting detoxification through transcriptional activation of antioxidative enzymes, such as superoxide dismutase 2 (SOD2) and catalase [72]; on the other hand, reactive oxidative species (ROS) regulate FOXO4 activity either via activating the upstream FOXO4 regulatory pathways or detection of cellular redox potential [63]. As already mentioned above, appropriate oxidative stress can specifically activate post-translational modifications of FOXO4, resulting in its nuclear translocation, for instance, JNK-mediated phosphorylation, SIRT1-mediated deacetylation, and MDM2-mediated monoubiquitination [47, 57, 60]. In contrast, under high levels of oxidative stress, NLK-inhibited FOXO4 monoubiquitination causes downregulation of FOXO4 activity and, consequently, inactivates the antioxidant defense program and raises the rate of cell death [50]. In addition, studies have suggested that

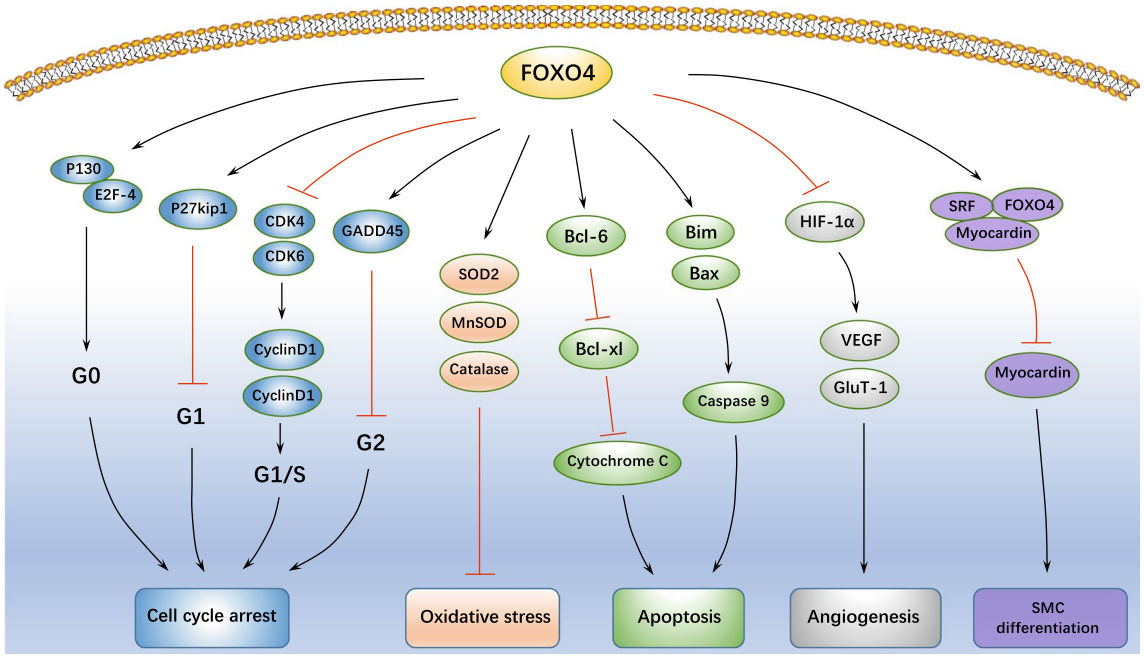

Fig. 3 The roles and underlying mechanisms of FOXO4 in various cellular processes. FOXO4 negatively regulates cell proliferation by increasing the transcription of genes involved in arrest at different phases of the cell cycle, and inhibits SMC differentiation by interacting with SRF and myocardin to form a ternary complex that represses the ability of myocardin. FOXO4 regulates the transcription of catalytic subunits of the antioxidative machinery and promotes apoptosis by increasing the expression of genes including Bcl-6, Bim, and Bax. Moreover, FOXO4 prevents HIF-1α and thereby decreases VEGF and GluT-1 expression, which in turn leads to the inhibition of angiogenesis

the transcriptional activity of FOXO4 can be affected by the formation of intermolecular disulfides with TNPO1 and p300. Cytosolic ROS-induced TNPO1–FOXO4 interaction enhanced FOXO4 nuclear translocation and upregulated antioxidants, whereas FOXO4–p300 interaction occurring in the nucleus inhibited the function of FOXO4 [56, 63, 73]. Taken together, these findings suggest that the different states and subcellular localizations of ROS mediate different FOXO4 activities.

Cell cycle arrest

An intact cell cycle starts from the G_0 phase (quiescence) and goes through four main phases: G_1 phase, S-phase, G_2 phase, and M phase. FOXO4 has been found to cause cell cycle arrest by activating a number of genes in each phase. Studies have shown that FOXO4 activation upregulated retinoblastoma-like p130 expression and increased p130/E2F-4 complex formation, which induce cells to enter a quiescent phase [74]. Meanwhile, the activation of FOXO4 increases expression levels of the cell cycle inhibitor p27kip1, leading to cell cycle suppression at the G_1 phase [67]. In addition, a p27kip1-independent mechanism underlying the cell cycle suppression of FOXO4 was identified to occur through the inhibitory effect on D-type cyclins D1 and D2, which are required for normal G_1/S-phase transition. FOXO4 downregulated their expression, leading to an impaired capacity of CDK4 to phosphorylate and inactivate the S-phase repressor pRb [75]. In mouse myoblastic cells, FOXO4 directly binds to the promoter of growth-arrest and DNA damage-response protein 45 (GADD45) in response to oxidative stress, resulting in the upregulation of GADD45 and thereby G_2 phase arrest [76]. As described previously, these effects of FOXO4 on cell cycle arrest can be reversed by activation of the PI3K/AKT pathway and suppression of the Ras/Ral pathway-mediated repression of FOXO4 activity.

Apoptosis

Apoptosis is highly regulated and controlled via two main pathways: the intrinsic pathway mediated by mitochondria, resulting in the activation of caspase-9, along with the extrinsic pathway, which is mediated by the activation of death receptors and involves the activation of caspase-8 [77]. B-cell lymphoma extra-large (Bcl-xl) is a transmembrane molecule in the mitochondria. It acts as an anti-apoptotic protein by preventing the release of mitochondrial contents such as cytochrome c, which leads to caspase activation and consequently programmed cell death [78]. Initial investigation showed that FOXO4 can bind to the

promoter of B-cell lymphoma 6 (Bcl-6) and activate its transcription. Subsequently, the upregulated Bcl-6 targets the promoter of Bcl-xl and inhibits its expression. In other words, FOXO4 triggers apoptosis by indirectly suppressing the levels of anti-apoptotic Bcl-xl through the transcriptional repressor Bcl-6 [79]. Recently, one study reported that the overexpression of FOXO4 significantly increased the apoptotic rate of clear-cell renal carcinoma cells, along with the activation of Bcl-2 interacting mediator (Bim) and Bcl-2 associated X protein (Bax), resulting in increased mitochondrial membrane permeability and caspase-9 activation [10]. Thus, FOXO4 can induce cell death through the intrinsic apoptotic pathway mediated by mitochondria. During myocardial ischemia–reperfusion injury, excessive oxidative stress induced an increase in FOXO4 expression, as a result of activating apoptosis [80].

FOXO4 regulates muscle homeostasis

Foxo4 is expressed most abundantly in muscle tissues, implying its role in muscle homeostasis. Endothelial precursors can differentiate into endothelial cells, smooth muscle cells (SMCs), and fibroblasts. Vascular SMCs exhibit phenotypic plasticity and are capable of a wide range of different phenotypes in response to extracellular environmental cues such as growth factors, as well as vascular injury. Many molecules involved in the mechanical control of the differentiation of SMCs have been identified, including the serum response factor (SRF) and its coactivator gene myocardin. Previous studies indicated that FOXO4 interacts with both SRF and myocardin to form a ternary complex that represses the ability of myocardin to activate the expression of endogenous SMC marker genes and, thus, FOXO4 has been proposed to inhibit SMC differentiation [81]. This is consistent with the finding that PI3K/AKT signaling and X-box binding protein 1 unspliced form stimulate the nuclear export of FOXO4, thereby attenuating the repression of myocardin, leading to SMC differentiation and preventing aortic aneurysm formation, respectively [82–84]. However, FOXO4 can promote the migration of vascular SMCs and neointimal hyperplasia by activating the transcription of matrix metalloproteinase 9 [85].

Furthermore, FOXK1 interacts with and represses FOXO4, resulting in decreased FOXO4 target gene expression and increased proliferation of muscle progenitor cells [86]. It has been shown that mice with FOXO4 deficiency have increased proliferation of gastrocnemius muscles and altered regeneration of smaller myofibers following cardiotoxin injury [86]. Taken together, these findings indicate that FOXO4 acts as a negative regulator of muscle cell proliferation and differentiation.

FOXO4 paradoxically acts as a tumor suppressor

There is an increasing body of evidence suggesting that FOXO4 functions as a tumor suppressor in the development and progression of cancer. Indeed, the pivotal anti-tumoral activities of FOXO4 have been elucidated and shown to be linked to the regulation of genes with anti-proliferative (e.g., p27kip1, cyclin D1, GADD45) and pro-apoptotic functions (e.g., Bim, Bcl-6, Bax), consequently inhibiting invasion and metastasis and promoting the apoptosis of diverse cancer cells [12]. However, multiple recent studies actually indicated that FOXO4 also acts as a tumor promoter. It was found that upregulated FOXO4 expression levels in response to chemotherapeutic treatment in B-cell lymphoma are associated with a poor prognosis [87]. In fact, the expression and activity of FOXO4 apparently varied among different cancer cells depending on the cellular conditions, especially the level or subcellular localization of ROS [88]. As mentioned previously, a low level of ROS induced the activation of FOXO4, which subsequently enhanced SOD2 and catalase-mediated stress resistance, and attenuated Bcl-6 and Bim-mediated FOXO4-dependent cell death. These features apparently promote the aberrant evasion of apoptosis in tumor progression (reviewed elsewhere [12, 89]).

In addition to the induction of cell cycle arrest and apoptosis, it is noteworthy that FOXO4 is also involved in the regulation of angiogenesis, erythropoiesis, and glucose metabolism, processes that are essential for tumor expansion. Under hypoxic conditions, nuclear activation of FOXO4 resulted in the downregulation of hypoxia-inducible factor 1α protein (HIF1α) and, thereby, suppressed vascular endothelial growth factor (VEGF), erythropoietin, and glucose transporter 1 expression, which finally inhibited angiogenesis [90]. In line with these effects, the intramuscular injection of endothelial pro-angiogenic cells deficient in FOXO4 into a rat showed increased neovascularization capacity in ischemic limbs [91]. However, another FOXO member, FOXO1, was shown to exert a pro-angiogenic function because FOXO1-knockout mice died at the embryonic stage as a result of severely impaired vascular development [92]. Therefore, FOXOs appear to exert both pro- and anti-angiogenic effects. Against this background, it is proposed that FOXO4 functions as a factor that regulates cellular homeostasis in both healthy and cancer cells, rather than just acting as a tumor suppressor (for a detailed review, see [93]).

Dysregulation of FOXO4 in disease development

The dysregulation of FOXO4 has been implicated in many pathological processes. Since FOXO4 plays important roles in cellular responses to oxidative stress, and cell damage

caused by accumulated ROS is depicted to be causative of aging-related dysfunction, it is proposed that FOXO4 may influence aging and age-related diseases, such as Alzheimer's disease and Parkinson's disease, by governing the antioxidant capacity of cells [94]. In endothelial progenitor cells, ROS/JNK-mediated nuclear translocation of FOXO4 is correlated with increased transcriptional activation of p21 and subsequent activation of cellular senescence [46, 95]. Furthermore, in senescent cells, FOXO4 can directly bind to p53 in response to DNA damage and also leads to the activation of the p53-dependent transcription of the senescence-associated p21 gene [19, 96]. These studies provide evidence that deleterious stimuli can enhance FOXO4 activation of cellular senescence, which serves as a defense mechanism to prevent cell hyper-proliferation. Therefore, apart from FOXO4 function in apoptosis, cellular senescence also forms the basis for the process of cell renewal. However, evidence for FOXOs playing the opposite role has also emerged, showing that FOXO1 and FOXO3 act in resisting senescence. In cardiac microvascular endothelial cells and vascular SMCs, activation of the PI3K/ATK pathway inhibited FOXO1 and FOXO3 transcriptional activity on antioxidant, leading to increased cellular ROS levels and senescence [97, 98]. As shown above, increased ROS associated with FOXO1 and FOXO3 inactivation might be involved in JNK-induced activation of FOXO4 and the subsequent senescence process [95]. Within the FOXO family, FOXO4 shows the opposite effects of FOXO1 and FOXO3 in cellular senescence, which depends on the individual FOXO activation mediated by the particular prevailing conditions of cellular stress. Furthermore, FOXOs themselves play roles underlying feedback mechanisms in response to different cell statuses [19]. Therefore, the balance in activation among distinct FOXOs serves to preserve the homeostasis of cellular senescence.

Sarcopenia is a common form of age-associated disorder recognized as involving the degenerative loss of skeletal muscle mass. In acute atrophy, caloric restriction increases the mRNA and protein levels of FOXO4 and prevents the aging process in the skeletal muscles [26]. However, beyond age-related loss of muscle mass, AKT activated by the IGF-1 receptor is responsible for the inactivation of FOXO4 and subsequent inhibition of the transcripts of muscle atrophy F-box (MAFbx/atrogin-1) and muscle ring finger 1 (MuRF1), which are muscle-specific ubiquitin E3 s for protein degradation during muscle atrophy [99]. However, it was found that exogenous TNF increased atrogin-1 mRNA via the activation of FOXO4 independent of AKT signaling [100]. Recently, an interesting study showed that FOXO4 is involved in regulating muscle atrophy through the Ras/Ral pathway during animal hibernation [101]. Moreover, with-no-lysine kinase (WNK) positively regulates skeletal muscle cell hypertrophy by mediating the activity of FOXO4. WNK1 knockdown was revealed to promote FOXO4 nuclear translocation and increase the levels of MAFbx and MuRF1 transcripts in C2C12 mouse skeletal muscle cells, implying that the WNK1–FOXO4 axis is a potential therapeutic target in human diseases causing sarcopenia [102].

Diabetes mellitus and its associated complications are a leading cause of mortality worldwide. FOXO4 acts as a vital regulator of diabetes, given its close association with cellular metabolism. The mRNA level of FOXO4 was found to be high in the hepatic tissue of diabetes model rats [103]. In addition, in patients with diabetes, activation of FOXO4 was shown to drive the expression of Bcl2L11 and enhance the apoptosis of kidney podocytes mediated by advanced glycation end-products (AGEs) [104, 105]. Interestingly, several studies revealed that the liver-specific deletion of hepatic FOXO 1/3/4 in mice significantly impacts glucose homeostasis through regulating glucose-6-phosphatase and glucokinase, which are associated with the processes of gluconeogenesis and glycolysis [106, 107]. Moreover, the deletion of FOXO 1/3/4 in pancreatic β cells reduced insulin secretion, resulting in early-onset diabetes [108]. However, the independent contributions of FOXO4 to insulin secretion and the regulation of hepatic glucose production remain to be clarified.

FOXO4 in perspective clinical applications

The FOXO4 transcription factor participates in the cellular response to chemotherapy and regulates tumor cell resistance or sensitivity to conventional antitumor drug treatment. Notably, PI3K/AKT and MAPK are oncogenic signaling pathways that are commonly activated in various cancers and target FOXO4 in similar manners to inhibit its tumor suppressor function. Previous studies identified that activation of the PI3K/AKT or MAPK signaling pathway induced the phosphorylation of FOXO4 and resulted in its export from the nucleus into the cytoplasm with a reduction of DNA-binding activity [109, 110]. Thus, many natural and pharmacological agents activating FOXO4 via inhibition of these signals have been evaluated in experimental as well as clinical settings. For instance, sulforaphane, an active anti-proliferative compound, was shown to inhibit PI3K/AKT and MAPK/ERK pathways, and in turn activated the FOXO4-induced expression of p21CIP and p27KIP1, leading to cell cycle arrest and apoptosis in pancreatic cancer [110]. Analogously, isoorientin and momordin Ic induced cell death through the upregulation of FOXO4, mediated by inhibition of the PI3K/AKT and MAPK pathways in human hepatoblastoma cancer cells [111, 112]. Again, imatinib inhibits BCR–ABL through PI3K/AKT/FOXO4 in chronic myeloid leukemia, and curcumin increases FOXO4 activity through the AKT/PTEN pathway in hepatocellular carcinoma, which both result in apoptosis and autophagy [113,

114]. Resveratrol has recently drawn extensive interest because of its chemopreventive properties against cancers. It has been shown that FOXO4 can be activated by resveratrol-induced inhibition of the PI3K/AKT and mTOR pathway in prostate cancer cells [115]. Moreover, resveratrol may also modulate FOXO4 activity through the activation of SIRT1, which increases the expression of apoptosis-related genes [116]. The chemotherapeutic drug doxorubicin has been widely used for treating a spectrum of cancers. Although doxorubicin administration led to the phosphorylation of AKT and inactivation of FOXO4 in colon carcinoma cells, increased expression and nuclear translocation of FOXO4 can enhance doxorubicin-mediated cytotoxicity and apoptosis, suggesting that FOXO4 activity may play a vital role in sensitizing cancer cells to cytostatic drugs [117]. Other compounds, such as trastuzumab, tamoxifen, and α-tocopheryl succinate, may also have anti-cancer activities involving FOXO4-mediated apoptosis [118–120]. In addition, some antitumor drugs exert their cytotoxic effects via the promotion of oxidative stress in cancer cells [121], whereas FOXO4 is well established as an effector of antioxidation. This, to a certain extent, antagonizes the role of antitumor drugs by protecting cancer cells from their cytotoxic effects. Therefore, further large-scale studies are required to confirm the utility of FOXO4 as a target for cancer treatment.

Conclusions

A range of studies have provided evidence that FOXO4 is involved in multiple physiological and pathological processes by directly regulating the expression of genes associated with antioxidative stress, cell cycle arrest, and apoptosis. Post-translational modification is a key regulator of FOXO4 transcriptional activity and its translocation between the cytoplasm and the nucleus. Moreover, FOXO4 activity is also influenced by direct interactions with other proteins or signaling pathways. The dysregulation of FOXO4 protein is associated with the development and progression of many disorders, including sarcopenia, age-related diseases, metabolic disorders, and tumorigenesis. Notably, studies have shown that FOXO4 is a potential therapeutic target for a wide range of cancers. The PI3K/AKT signaling pathway is relatively stable and not commonly mutated in cancers. Therefore, many natural and designed antitumor drugs manipulate FOXO4 activity by targeting a downstream node of the PI3K/AKT pathway. In colon carcinoma cell lines, FOXO4 activation is beneficial for attenuating the resistance of cells against chemotherapeutic agents. Although the well-established anti-proliferative and apoptotic functions support the notion that FOXO4 could be a promising target for cancer treatment, only rare alterations involving FOXO4 have been identified in tumors compared with the case for classical tumor suppressors. Therefore, it is necessary to address the following issues: First, the tumor suppressor role of FOXO4 has not been confirmed by cancer genetics and in most human tumor samples. Second, it is unclear whether FOXO4 alteration is an early event that causes tumorigenesis or is a consequence of the disease process. Third, since FOXO4 is involved in resistance to antitumor drugs, the priority between anti- and pro-tumoral activities of FOXO4 in specific settings needs to be demonstrated. Overall, more basic and clinical studies are required to elucidate the potential for using FOXO4 as a therapeutic target for preventing the progression of diseases.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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