Drug Design, Development and Therapy

Dovepress
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REVIEW

The Roles of Forkhead Box O3a (FOXO3a) in Bone and Cartilage Diseases – A Narrative Review

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Abstract: Bone and cartilage diseases are significantly associated with musculoskeletal disability. However, no effective drugs are available to cure them. FOXO3a, a member of the FOXO family, has been implicated in cell proliferation, ROS detoxification, autophagy, and apoptosis. The biological functions of FOXO3a can be modulated by post-translational modifications (PTMs), such as phosphorylation and acetylation. Several signaling pathways, such as MAPK, NF-κB, PI3K/AKT, and AMPK/Sirt1 pathways, have been implicated in the development of bone and cartilage diseases by mediating the expression of FOXO3a. In particular, FOXO3a acts as a transcriptional factor in mediating the expression of various genes, such as MnSOD, CAT, BIM, BBC3, and CDK6. FOXO3a plays a critical role in the metabolism of bone and cartilage. In this article, we mainly discussed the biological functions of FOXO3a in bone and cartilage diseases, such as osteoporosis (OP), osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis (AS), and intervertebral disc degeneration (IDD). FOXO3a can promote osteogenic differentiation, induce osteoblast proliferation, inhibit osteoclast activity, suppress chondrocyte apoptosis, and reduce inflammatory responses. Collectively, up-regulation of FOXO3a expression shows beneficial effects, and FOXO3a has become a potential target for bone and cartilage diseases.

Keywords: FOXO3a, osteoporosis, osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, intervertebral disc degeneration

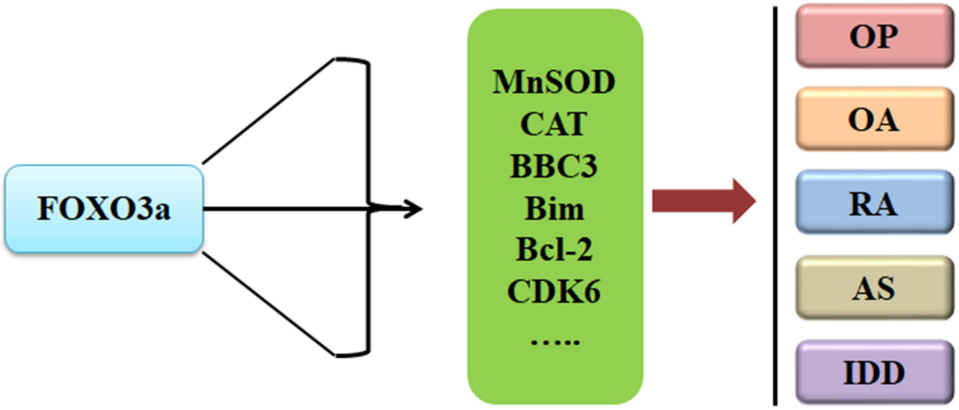
Introduction

Bone and cartilage diseases, the main causes of musculoskeletal disability, are characterized by bone and cartilage structural destruction, degradation, and remodeling. Bone and cartilage diseases, such as osteoporosis (OP), osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis (AS), and intervertebral disc degeneration (IDD), greatly affect patients' life quality and impose social and economic burden worldwide.[1] OP affects appropriately 10% of individuals aged ≥50, with a rise to about 13.6% by the year 2030.[2] An estimated 240 million patients in the world have symptomatic OA.[3] Extensive research has been explored, and several therapeutic strategies have been used to manage bone and cartilage diseases. For example, several disease-modifying drugs, such as tofacitinib (a Janus kinase inhibitor) and tocilizumab (a non-tumor necrosis factor inhibitor), have been explored for RA treatment. However, no absolute cure is available.[4] This might be explained by the lack of a full understanding of the molecular mechanism of bone and cartilage diseases.

The pathological mechanisms of bone and cartilage diseases are regulated by a network of signaling pathways, which disrupt the homeostasis of bone and cartilage by increasing catabolism and decreasing anabolism. A review study reports that MAPK, NF-κB, Wnt/β-catenin, and AMPK/Sirt1 signaling pathways have been implicated in the pathogenesis of bone and cartilage diseases.[5] It is reported that the MAPK/NF-κB signaling pathway is involved in osteoclastogenesis in RANKL-treated bone marrow-derived macrophages (BMMs), disrupting the balance between osteoblast-regulated bone formation and osteoclast-controlled bone resorption.[6] Similarly, the MAPK/NF-κB signaling pathway plays a crucial role

Received: 5 September 2024
Accepted: 20 February 2025
Published: 27 February 2025

Graphical Abstract



in inflammation and immune modulation, which regulate the pathogenesis of human OA, RA, and SA.[7,8] Wnt/β-catenin signaling pathway has been reported to regulate cellular differentiation, proliferation, and apoptosis. The roles of the Wnt/β-catenin signaling pathway in bone and cartilage diseases have been intensively studied in mice.[9,10] The AMPK/Sirt1 signaling pathway plays a key role in mediating mitochondrial energy metabolism and oxidative stress, which are involved in the pathological development of mouse bone and cartilage diseases.[11,12]

The FOX family, a transcription factor superfamily, has a DNA-binding forkhead (FH) domain and contains 19 subclasses of FOXA-FOXS with 50 or more members in mammals.[13] The FOXO family in mammals has four members, including FOXO1, FOXO3a, FOXO4, and FOXO6. They share more than 30% of identity in amino acid sequences and appropriately 90% of similarity in their DNA-binding FH domain (Figure 1). FOXO1, FOXO3a, and FOXO4 are commonly expressed, but with different preferences, in a multitude of mammalian tissues and organs. Interestingly, the expression of FOXO6 in human beings is originally and merely found in the brain. However, it is recently reported that FOXO6 can be found in human liver.[14] The activities of FOXOs can be mediated by a variety of factors, such as insulin,[15] insulin-like growth factor,[16] nutrients,[17] and oxidative stress.[18] For example, oxidative stress can stimulate the expression of FOXOs, which in turn mediate the transcription of genes encoding antioxidants, cell cycle mediators, and pro-apoptotic factors.[18] Activation of FOXOs may lead to the induction of cell survival or cell death.[19] FOXO3a, also

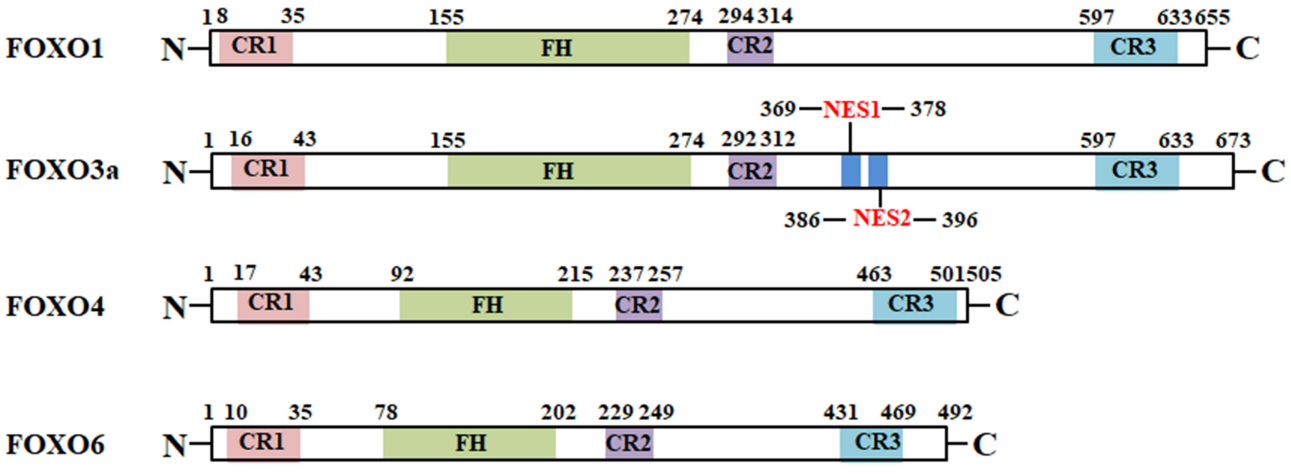


Figure 1 The sequence and functional domains of FOXO family members are shown. Four FOXO family members (FOXO1, FOXO3a, FOXO4, and FOXO6) have more than 30% of identity in amino acid sequences and appropriate 90% of similarity in the FH domain. There are three CRs, including CR1, CR2, and CR3, in four FOXO family members. FOXO3a contains NES1 and NES2 sequences.

known as FOXO3, has been extensively studied as a critical mediator in stress responses, cellular homeostasis, and lifespan. FOXO3a has been implicated in cell proliferation, ROS detoxification, autophagy, and apoptosis.[20,21] Various studies have shown that FOXO3a plays a critical role in bone and cartilage diseases by cross-talking with a network of signaling pathways.[22–25] In this article, we mainly discuss the biological activities of FOXO3a in bone and cartilage diseases. FOXO3a shows potential effects, and it becomes a therapeutic target for bone and cartilage diseases. As far as we know, no review articles have been reported to advance the knowledge in this field.

The Structure and Regulation of FOXO3a

The Structure of FOXO3a

The members of the FOXO family are characterized by the "wing-like spiral structure", which includes three intertwined α helices, three β chains, a ring structure, and C-terminus. The FOXO3a protein, with a molecular weight of 71 kDa, has five structural domains, including a highly conserved forkhead wing-turn-helix DNA domain (FH), two nuclear localization sequences (NLS), a nuclear export sequence (NES), and a C-terminal transactivation domain (TAD).[26] The FH domain is surrounded by some large and disordered C- and N-terminal regions, which are called intrinsically disordered regions (IDRs). There are some conserved regions (CRs) within IDRs, such as CR1, CR2, and CR3[27] (Figure 1). Particularly, there are AKT-phosphorylation sites and 14-3-3-binding regions within CR1 and CR2, which play an important role in mediating the stability of the complex composed of FOXO3a and DNA.[28] The FH domain is the region responsible for the interaction of FOXO3a with estrogen receptor α (ERα) and p53.[29] All FOXO family members can recognize the consensus DNA motifs 5′-GTAAA(T/C)AA-3′ and 5′-(C/A)(A/C)AAA(C/T)-3′. The TAD domain is critical for the transactivation of target genes of FOXO3a.[30] Therefore, these results show that various domains in the FOXO3a protein exhibit different biological functions, including interaction with mediators and DNA.

The Regulation of FOXO3a

The protein structure of FOXO3a indicates that FOXO3a has various biological functions and mediates various cellular processes, such as proliferation, autophagy, and apoptosis, by regulating the expression of downstream factors. There are binding regions for FOXO3a in the gene promoters of various factors, such as BIM,[31] BBC3 (also named as PUMA),[32] tumor necrosis factor-related apoptosis-inducing ligand (TRAIL),[33] PINK1,[34] intercellular cell adhesion molecule-1 (ICAM1),[35] CDK6,[36] manganese superoxide dismutase (MnSOD),[37] and catalase (CAT).[37] In peroxide-treated human primary tenocytes cultured in low serum media, FOXO3a can protect against cell apoptosis and inhibit cell proliferation. However, FOXO3a may promote cell apoptosis when cells are cultured in high serum. In particular, proliferating cells are more susceptible to the pro-apoptotic actions of FOXO3a.[38] Therefore, these results show that the distinctive functions of FOXO3a are associated with different cellular environments and cell lines.

In addition, the biological functions of FOXO3a may be mediated by post-translational modifications (PTMs), such as phosphorylation (Figure 2) and acetylation.[30] Phosphorylation modification is the most critical mechanism in mediating the transcriptional functions of FOXO3a. There are several serine (Ser)/threonine (Thr) residues in the FOXO3a protein that can be phosphorylated. For example, the residue Thr32, Ser253, and Ser315 of FOXO3a can be phosphorylated by AKT in the nucleus (Figure 2). Interestingly, Thr32 and Ser253 phosphorylation of FOXO3a are essential for its binding to 14-3-3 proteins, and this interaction may lead to its cytoplasmic translocation from the nucleus.[39,40] However, the deficiency of Ser315 phosphorylation does not seem to affect this interaction.[39] AKT can phosphorylate the 14-3-3 protein, induce the dissociation of 14-3-3, and trigger the nuclear translocation of FOXO3a.[41]

Serum and glucocorticoid-inducible kinase (SGK) can also phosphorylate FOXO3a at the residues of Thr32, Ser253, and Ser315 (Figure 2). In contrast to AKT, SGK prefers to phosphorylate the residues of Thr32 and Ser253, leading to the nuclear export of FOXO3a.[42] It has been reported that p53-mediated SGK activation can promote FOXO3a nuclear export after DNA damage. The crosstalk between p53 and FOXO3a results in FOXO3a phosphorylation and nuclear export in an AKT-independent manner.[43] Mammalian sterile 20-like kinase 1 (MST1) can be activated under oxidative stress. The residues Ser207 and Ser212 of FOXO3a can be phosphorylated by MST1, which inhibits the interaction of FOXO3a with 14-3-3 and promotes the nuclear accumulation of FOXO3a.[44] Another study reports that MK5 can

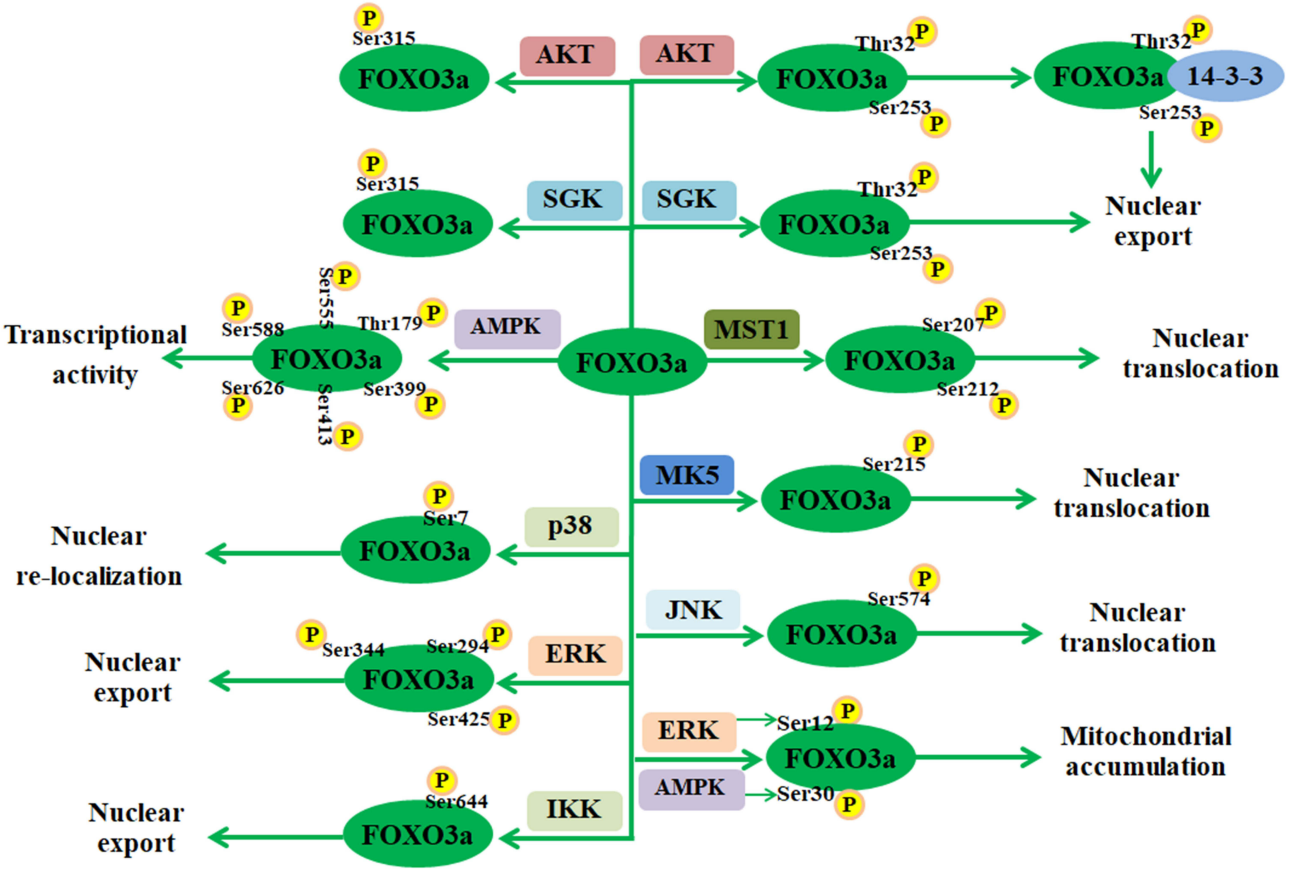


Figure 2 The phosphorylation modification of FOXO3a has been shown. FOXO3a can be phosphorylated by AKT, SGK, AMPK, MST1, MK5, p38, JNK, ERK, and IKK. Phosphorylation at Thr32, Ser253, Ser294, Ser344, Ser425, and Ser644 can induce nuclear export. In contrast, phosphorylation at Ser7, Ser207, Ser212, and Ser574 can induce nuclear translocation. ERK-induced Ser12 phosphorylation and AMPK-induced Ser30 phosphorylation promote the mitochondrial accumulation of FOXO3a.

phosphorylate FOXO3a at Ser215 and increase its nuclear localization and DNA binding activity in U2OS cells.[45] It has been reported that AMP-activated protein kinase (AMPK) can phosphorylate at least six residues (Thr179, Ser399, Ser413, Ser555, S588, and Ser626) of FOXO3a (Figure 2). AMPK-mediated phosphorylation does not affect the subcellular localization of FOXO3a but activates the transcriptional activity of FOXO3a.[46]

The phosphorylation of FOXO3a is also mediated by the MAPK/NF-κB signaling pathway (Figure 2). It has been reported that the residue Ser7 of FOXO3a can be phosphorylated by p38 MAPK, which induces the nuclear re-localization of FOXO3a and promotes its transcriptional activity.[47] However, SB202190, a specific inhibitor of p38 MAPK, can also promote FOXO3a nuclear accumulation and transcriptional activity.[48] It has been reported that JNK can induce FOXO3a nuclear localization and inhibit the expression of Bcl-2 by phosphorylating the residue Ser574 of FOXO3a.[49] The residues Ser294, Ser344, and Ser425 of FOXO3a can be phosphorylated by ERK, which can induce FOXO3a nuclear export and promote MDM2-mediated ubiquitin-proteasome degradation.[50] Another study shows that ERK-mediated Ser12 phosphorylation and AMPK-mediated Ser30 phosphorylation are essential for FOXO3a to accumulate in mitochondria in metabolically stressed cancer cells.[51] IκB kinase (IKK), a critical factor in the NF-κB signaling, can phosphorylate the residue Ser644 of FOXO3a, induce its nuclear export, and promote its protein degradation.[52] IKK-mediated Ser644 phosphorylation is essential for βTrCP-mediated ubiquitin-proteasome degradation of FOXO3a.[53] Therefore, these results show that phosphorylation modification at different sites in FOXO3a can lead to its activation, inactivation, or even degradation.

Acetylation and deacetylation are also the PTMs for mediating the biological functions of proteins. FOXO3a is also mediated by CBP/p300-mediated acetylation and Sirt-mediated deacetylation. CBP/p300 can interact with the N-terminus of FOXO3a and induce its acetylation.[54] In comparison to the phosphorylation modification, the role of

deacetylation modification in mediating the expression of FOXO3a is controversial. It has been reported that Sirt1 and Sirt2 can promote Skp2-mediated ubiquitination and proteasomal degradation of FOXO3a.[55] In non-small cell lung cancer (NSCLC) A549 and H1435 cells, Sirt1-mediated deacetylation consistently induces the ubiquitin-proteasome degradation of FOXO3a.[56] Sirtinol, a Sirt1-specific inhibitor, can enhance the levels of FOXO3a in NSCLC H1299 cells.[57] However, there are amounts of evidence to support that Sirt-mediated acetylation promotes the nuclear localization and activation of FOXO3a.[58–60] It has been reported that naringenin can promote the deacetylation and protein expression of FOXO3a. Sirt1 siRNA transfection can abolish the protein expression and acetylated levels of FOXO3a.[60] Sirt2 promotes the deacetylation and transactivation activity of FOXO3a in NIH3T3 cells.[61] Sirt3 and Sirt5 can also mediate the deacetylation of FOXO3a. It is reported that the nuclear localization of FOXO3a is significantly decreased in Sirt3-knockout mice.[62] Overexpression of Sirt3 can decrease the acetylation of FOXO3a and promote the accumulation of FOXO3a in LPS-treated BEAS-2B cells.[63] Consistently, Sirt2 and Sirt5 have been reported to promote the nuclear translocation of FOXO3a in oocytes.[64] Therefore, these results show that the roles of acetylation/deacetylation modification in FOXO3a still need more investigation.

The biological activity of FOXO3a can also be mediated by the interacting partners, such as estrogen receptors (ERα and ERβ), β-catenin, and Smads. It has been reported that ERα may interact with the N-terminal region (amino acids 1–300) and ERβ binds to the C-terminal region (amino acids 301–673). The interaction between FOXO3a and ER may lead to the inhibition of ER transcriptional activity. Mechanistically, FOXO3a competes with ERs to bind to the ER-binding sites at the promoter of target genes.[65] The physical interaction between FOXO3a and β-catenin may result in the suppression of the Wnt/β-catenin signaling pathway and the down-regulation of target gene expression.[66] The FOXO3a/p-Smad3/Smad4 complex can initiate the transcriptional expression of the CCNG2 gene.[67] FOXO3a and FOXM1 are the two antagonistic factors that exhibit opposite biological activities. FOXO3a competitively interacts with the FH transcriptional response element (FHRE) in the promoter of VEGF and inhibits its transcription.[68] Interestingly, FOXO3a can suppress the transcriptional expression of FOXM1 by recruiting HDAC1 and HDAC2.[69] Therefore, these results show that the biological functions of FOXO3a can be mediated by PTMs and interactions with regulators, and this mediation for FOXO3a can be involved in bone and cartilage diseases due to the dysregulation of signaling pathways.

The Roles of FOXO3a in OP Development

OP, a bone disorder, has been associated with disrupted bone microarchitecture and decreased bone mass and strength. There are many factors contributing to the pathogenesis of OP. Aging and estrogen deficiency play critical roles in pathological alterations. In particular, menopause-related estrogen deficiency promotes bone metabolic imbalance, which is shown by decreased bone formation and increased bone resorption.[70] FOXO3a has been reported to be a crucial factor in mediating osteogenic differentiation and osteoblast apoptosis under oxidative stress in patients with postmenopausal OP (PMOP).[71] 17β-estradiol (17β-E2), a sex hormone, has been proven to promote osteoblast differentiation and protect against OP. It is reported that 17β-E2 can up-regulate Sirt1 expression, promote autophagy by activating the AMPK/mTOR signaling, and inhibit osteoblast apoptosis by activating FOXO3a expression[22] (Table 1). However, estrogen/17β-E2 replacement for disease treatment may cause severe adverse complications. Therefore, these results show that FOXO3a plays a key role in protecting against OP development.

The Roles of FOXO3a in Osteogenic Differentiation

The physiological levels of reactive oxygen species (ROS) might be beneficial for the maintenance of bone homeostasis. However, the imbalance between ROS overproduction and the antioxidant defense system can lead to bone diseases. During cell differentiation, increased mitochondrial biogenesis may produce more ROS.[95] Oxidative stress decreases osteogenic differentiation in cell lines during OP development. It has been established that Sirt1 can inhibit oxidative stress-induced osteogenic differentiation inhibition and osteoporotic phenotype. Hydrogen peroxide (H_2O_2) induces mesenchymal stem cell (MSC) lineage commitment towards adipogenesis instead of osteogenesis by decreasing the expression of Sirt1, which up-regulates the expression of Runx2 by activating FOXO3a[72] (Table 1). In Sirt1-transgenic ($Sirt1^{TG}$) mice, the skeletal size, bone volume, osteoblast number, alkaline phosphatase (ALP) activity, and osteogenesis-

 https://doi.org/10.2147/DDDT.S494841

Table 1 FOXO3a Exhibits the Biological Functions of in Different Bone and Cartilage Models

Category	Models	Mechanisms	Biological Functions	Ref.
OP	hFOB1.19 cells	17β-E2-induced Sirt1 activates autophagy by activating FOXO3a.	FOXO3a inhibits osteoblast apoptosis	[22]
	H_2O_2-treated MSCs	Sirt1 suppresses oxidative stress and enhances Runx2 expression by activating FOXO3a.	FOXO3a promotes osteogenic differentiation	[72]
	$Sirt1^{TG}$ $Bmi\text{-}1^{+/-}$ MSCs	Sirt1 reduces FOXO3a acetylation and increases FOXO3a and SOD2 levels.	FOXO3a promotes osteogenic differentiation	[73]
	HG-treated ESCs	HG shifts β-catenin away from FOXO3a towards the LEF/TCF complex.	FOXO3a promotes osteogenic differentiation	[74]
	hMSCs	SeNPs increased antioxidant levels and activates the JNK/FOXO3a pathway.	FOXO3a promotes osteogenic differentiation	[75]
	Alcohol-treated primary rat MSCs	FOXOs bind and sequester β-catenin	FOXO3a promotes osteogenic differentiation	[76]
	H_2O_2-treated MC3T3-E1 cells	NAM improves mitochondrial functions and suppresses oxidative stress by activating the Sirt3/FOXO3a pathway	FOXO3a inhibits apoptosis and promote osteoblast differentiation	[77]
	H_2O_2-treated HOB and MC3T3-E1 cells	TRIM3 interacts with FOXO3a and suppresses its acetylation and degradation	FOXO3a inhibits oxidative stress-induced apoptosis	[78]
	H_2O_2-treated MC3T3-E1 cells	rmACLP(N) increases p38 phosphorylation and FOXO3a nuclear translocation	FOXO3a increases osteoblast survival and bone formation	[79]
	TNFα-treated MC3T3-E1 cells	FOXO3a reduces Cyr61 expression, CCL2 secretion, and macrophage recruitment.	FOXO3a attenuates bone resorption and periapical lesions	[80]
	HG-treated hFOB1.19 cells	NIPA2 attenuates PINK1/Parkin-mediated mitophagy by activating the PGC1α/FOXO3a pathway	FOXO3a increases osteoblast differentiation	[81]
	1,25-D3-treated MC3T3-E1 cells	1,25-D3 up-regulates FOXO3a expression and promotes its nuclear translocation.	FOXO3a decreases calcium uptake and prevents osteoblast differentiation	[82]
OA	IL-1β-treated human chondrocytes	Activated AMPK/FOXO3a pathway inhibits oxidative stress and inflammatory responses.	FOXO3a inhibits oxidative stress and catabolism	[24]
	OVX-OA osteoblasts	AICAR rescues SPARC-induced catabolism by activating the AMPK/FOXO3a pathway	FOXO3a inhibits chondrocyte degradation	[83]
	IL-1β-treated chondrocytes	TMF inhibits chondrocyte apoptosis and aggrecan degradation by activating the Sirt1/FOXO3a pathway.	FOXO3a inhibits chondrocyte apoptosis and ECM degradation	[84]
	IL-1β-treated chondrocytes	miR-30b-5p promotes apoptosis and NLRP3 inflammasome activation by inhibiting Sirt1/FOXO3a pathway	FOXO3a inhibits apoptosis and inflammasome activation	[85]
	IL-1β-treated chondrocytes	TMF-activated FOXO3a inhibits chondrocyte hypertrophy by suppressing the BMPER/BMP4 pathway	FOXO3a inhibits OA chondrocyte hypertrophy	[86]
RA	Collagen-induced rat arthritis	Overexpression of FOXO3a suppresses pro-inflammatory cytokine production.	FOXO3a inhibits inflammation	[87]
	Human RA FLSs	FOXO3a inhibits miR-155-up-regulated expression of IL-1β, IL-6, and TNFα	FOXO3a inhibits inflammation	[25]
	TNFα-treated RASFs	FOXO3a decreases Cyr61 expression by binding to the promoter of Cyr61.	FOXO3a inhibits inflammation	[88]
	$IL\text{-}1Ra^{-/-}$ mice	PLD inhibitors suppress p21Cip1 and p27Kip1 by activating FOXO3a	FOXO3a promotes cell cycle and proliferation	[89]
	HUVEC cells	Inhibition of the PI3K/AKT and MEK/ERK signaling pathways inhibit cell migration and capillary tube formation.	FOXO3a exhibits antiangiogenic effects	[90]

(*Continued*)

Table 1 (Continued).

Category	Models	Mechanisms	Biological Functions	Ref.
AS	Jurkat cells	FOXO3a decreases IL-8, IL-17A, and IL-23 and increases SOD, CAT, and T-AOC	FOXO3a inhibits inflammatory responses and oxidative stress	[91]
	Jurkat cells	METTL14 activates autophagy and alleviates inflammatory responses by up-regulating FOXO3a expression	FOXO3a inhibits inflammation and promotes autophagy	[92]
IDD	IL-1β-treated NP cells	ZIP4 promotes inflammation, oxidative stress, and ECM degradation by decreasing Sirt1 and FOXO3a expression	FOXO3a inhibits inflammation, oxidative stress, and ECM degradation	[93]
	LPS-treated NP cells	$CRL4^{DCAF6}$ E3 ligase promotes BBC3 expression by activating FOXO3	FOXO3a promotes BBC3-induced apoptosis	[94]

related gene expression (collagen I, Runx2, and osteocalcin) are significantly enhanced, compared to those in the wide-type mice. Mechanistically, Sirt1 reduces the acetylation of FOXO3a, increases the levels of FOXO3a and SOD2, and promotes osteogenesis in MSCs[73] (Figure 3). One study reports that Sirt1-activated FOXO3a can bind to the FOXO response elements (FRE) in the promoter region of Runx2 and promote its transcriptional expression in human MSCs (hMSCs).[96]

Wnt/β-catenin signaling has been implicated in skeleton formation, and dysregulation of the Wnt/β-catenin signaling is associated with the pathological alterations in bone disorders. The role of Wnt/β-catenin signaling in inducing osteogenesis can be regulated by FOXO3a. Interaction of FOXO3a with β-catenin sequesters β-catenin away from the LEF/TCF complex.[97] It is interesting to find that high glucose (HG) inhibits osteogenic differentiation of embryonic stem cells by diverting β-catenin from FOXO3a to the LEF/TCF complex and enhancing the activity of AKT in embryonic stem cells (ESCs).[74] MiR-155 plays a crucial role in vascular calcification and promotes the expression of osteogenic factors, such as Runx-2 and OPN, in human vascular smooth muscle cells (VSMCs). MiR-155 deficiency promotes cell

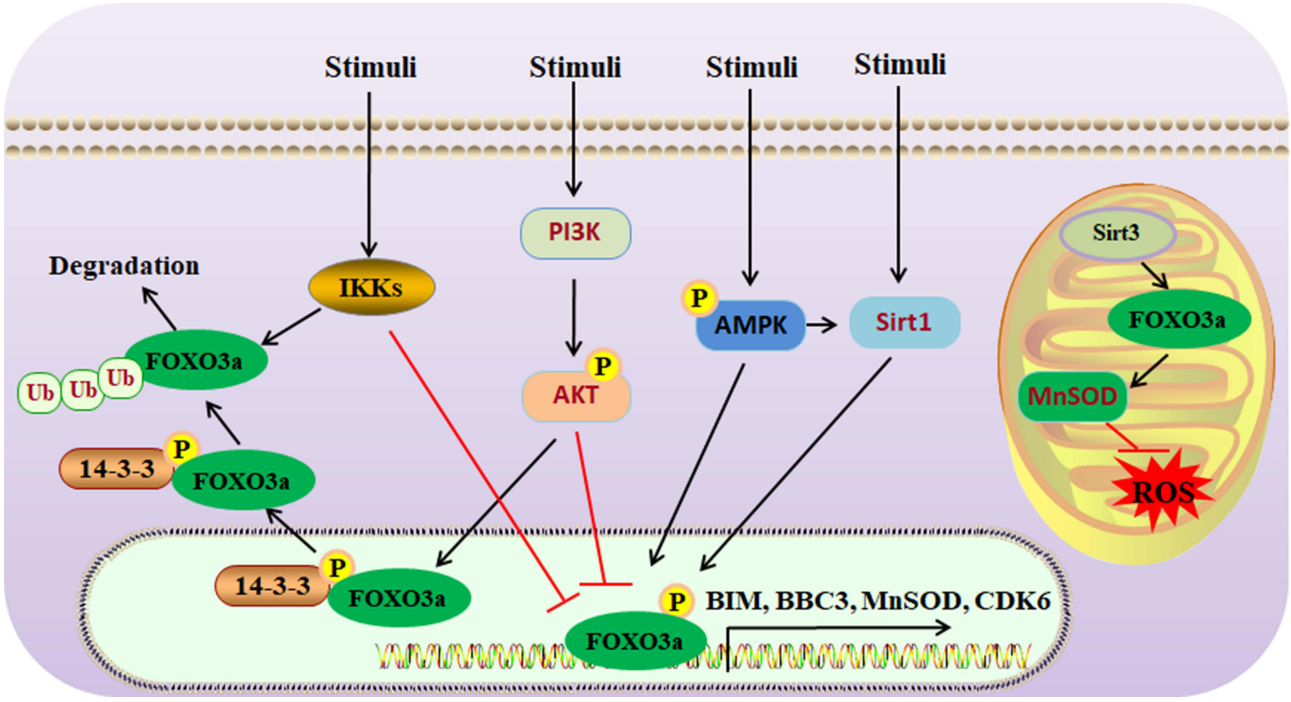


Figure 3 A schematic diagram shows the regulation of FOXO3a and its role in transcriptional activity. Activated PI3K/AKT can induce FOXO3a phosphorylation and the interaction of p-FOXO3a and 14-3-3, leading to nuclear export. Then, FOXO3a undergoes ubiquitination and proteasomal degradation. IKKs also promote the proteasomal degradation of FOXO3a. However, AMPK and Sirt1 promote FOXO3a nuclear translocation and transcriptional activity in mediating the downstream factors, such as BIM, BBC3, MnSOD, and CD6. Mitochondrial Sirt3 activates FOXO3a, which decreases ROS production by up-regulating MnSOD expression.

https://doi.org/10.2147/DDDT.S494841

survival and migration and inhibits cell apoptosis in VSMCs by activating AKT and promoting phosphorylation and degradation of FOXO3a, thus inhibiting vascular calcification.[98]

Oxidative stress-mediated expression of FOXO3a is also affected by various external factors, such as alcohol administration and cigarette smoke.[76,99] Alcohol administration has been associated with delayed fracture healing, due to oxidative stress. It has been reported that treatment of primary rat MSCs with alcohol (50 mm) can down-regulate the expression of osteogenic and chondrogenic lineage markers. Knockdown of FOXO1/3a can rescue the effects of alcohol (50 mm) on primary rat MSCs. However, inhibitors of FOXO1/3a can partially rescue the expression of pro-osteogenic lineage markers. Under oxidative stress, FOXOs bind and sequester β-catenin, which is crucial for the initiation of MSC osteochondral differentiation.[76] Cigarette smoke extract (CSE) can significantly reduce bone density, induce aging, and suppress osteogenic differentiation of BMSCs in vivo and in vitro. Mechanistically, CSE induces ROS generation, stimulates oxidative stress, and inhibits mitophagy by mediating the AKT/FOXO3a/Pink1/Parkin axis.[99] It has been reported that selenium nanoparticles (SeNPs) are antioxidants that induce hMSC differentiation toward osteogenesis over adipogenesis by activating the JNK/FOXO3a signaling pathway[75] (Table 1). However, one study reports that H_2O_2 may promote osteogenic differentiation of VSMCs by up-regulating the expression of Runx2. Consistently, dietary salt increases the NADPH oxidase 4 (NOX4)-mediated generation of H_2O_2 by activating the AKT/FOXO3a signaling pathway.[100] This discrepancy might be associated with the different cell lines and microenvironment. Therefore, these results show that oxidative stress inhibits osteogenic differentiation and promotes OP development by negatively regulating the expression of FOXO3a.

The Roles of FOXO3a in Osteoblasts

An increase in oxidative stress can be a crucial pathogenic factor for bone loss. It has been demonstrated that H_2O_2 treatment can cause the inhibition of osteoblast differentiation in MC3T3-E1 cells.[101] Nicotinamide (NAM) is the water-soluble form of vitamin B3 and one of the precursors of NAD^+ that is a coenzyme in mediating energy metabolism.[102] NAM can improve mitochondrial metabolism, reduce mitochondrial oxidative stress, inhibit apoptosis, and enhance osteoblast differentiation by activating the Sirt3/FOXO3a signaling pathway in H_2O_2-treated MC3T3-E1 cells[77] (Figure 3). The tripartite motif (TRIM) family is a class of E3 ubiquitin ligases. It has been reported that TRIM33 promotes osteoblast proliferation and differentiation.[103] Interestingly, TRIM3 can physically interact with FOXO3a and suppress its acetylation and degradation in H_2O_2-treated osteoblasts, leading to the inhibition of osteoblast apoptosis induced by oxidative stress[78] (Table 1).

JNK mediates the phosphorylation of FOXO3a, the translocation of the cytoplasm into the nucleus, and the transcriptional activity in up-regulating the expression of antioxidant enzymes, Runx2, and ALP.[75,104] It has been reported that aortic carboxypeptidase-like protein (ACLP) is high expression in skeletal muscles and promotes bone formation. The recombinant N-terminal mouse ACLP [rmACLP(N)] increases osteoblast survival, phosphorylated p38MAPK, and nuclear translocation of FOXO3a in H_2O_2-treated MC3T3-E1 cells.[79] Cysteine-rich-61 (Cyr61, also known as CCN1) mediates cell adhesion, chemotaxis, vascular restenosis, and angiogenesis. FOXO3a functions as a transcriptional repressor and interacts with the promoter of Cyr61.[105] It has been reported that TNFα up-regulates Cyr61 expression and enhances FOXO3a phosphorylation in MC3T3-E1 cells. Overexpression of FOXO3a compromises the effects of TNFα on Cyr61 expression, CCL2 secretion, and macrophage recruitment, leading to attenuation of bone resorption and alleviation of periapical lesions[80] (Table 1).

Non-imprinted in Prader-Willi/Angelman syndrome region protein 2 (NIPA2), a highly selective magnesium transporter, has been negatively associated with the development of type 2 diabetes mellitus (T2DM)-related OP. Magnesium is tightly correlated with mitochondrial physiology by mediating the AMPK signaling pathway.[106] Knockout of NIPA2 may result in a deficiency of magnesium. NIPA2 positively regulates the osteogenic capacity by inactivating PINK1/Parkin-mediated mitophagy via activation of the PGC1α/FOXO3a signaling pathway in HG-treated human fetal osteoblastic (hFOB1.19) cells.[81] Iron overload has been associated with OP pathogenesis. In ferric ammonium citrate (FAC)-treated MC3T3-E1 cells, the expression of dual-specificity phosphatase 14 (DUSP14) is down-regulated. FOXO3a interacts with the promoter of DUSP14 and enhances its expression and increased expression of DUSP14 protects osteoblasts from iron overload.[107]

In mice with genetic deletion of 25-hydroxyvitamin D-1α hydroxylase ($1\alpha OHase^{-/-}$ mice), down-regulated expression of Sirt1 and FOXO3a, increased oxidative stress, and up-regulated expression of osteocyte senescence-related markers are observed. Overexpression of Sirt1 in $1\alpha OHase^{-/-}$ mice may reverse these pathological alterations by up-

regulating FOXO3a expression.[108] 1,25-dihydroxyvitamin D3 (1,25-D3) promotes osteoblast activity and bone mineralization by increasing calcium absorption. It has been reported that 1,25-D3 up-regulates FOXO3a expression and promotes its nuclear translocation. However, FOXO3a overexpression decreases calcium uptake and prevents osteoblast differentiation in 1,25-D3-treated MC3T3-E1 cells[82] (Table 1).

Osteoblast apoptosis has been associated with OP pathogenesis. Unexpectedly, it is reported that osteoblast differentiation is suppressed in osteoblast-specific Bcl-2-transgenic mice.[109] Bcl-2 deficiency may lead to accelerated osteoblast differentiation by enhancing the expression and activities of FOXO3a via AKT inactivation[23] (Figure 3). One study reports that fluid shear stress (FSS) may inhibit TNFα-induced apoptosis in MC3T3-E1 cells. Mechanically, FSS activates ERK5, which phosphorylates the downstream factor AKT. Activated AKT induces the phosphorylation of FOXO3a and suppresses its transcriptional activities in regulating the expression of FasL and Bim.[110]

Endogenous glucocorticoids (GCs) at a low dose (10^{-8} M) have been shown to enhance osteoblast viability, decrease apoptosis, and promote autophagy by activating the expression of serum- and glucocorticoid-induced kinase-1 (SGK-1), which phosphorylates and inactivates FOXO3a in MC3T3-E1 cells.[111] Hepcidin, an endogenous hormone peptide, regulates iron homeostasis. Hepcidin deficiency suppresses osteoblast differentiation but does not affect osteoclast differentiation. In addition, the hepcidin deficiency diverts the binding of β-catenin from TCF4 to FOXO3a. Inhibition of FOXO3a can rescue the loss of bone mass in hepcidin-knockout mice, in which iron is accumulated.[112] Therefore, these results show that FOXO3a activation has been associated with osteoblast differentiation, proliferation, and apoptotic inhibition. Many factors, such as oxidative stress and dysregulated signaling pathways, may compromise FOXO3a activation and inhibit osteoblast activity.

The Roles of FOXO3a in Osteoclasts

During the differentiation of macrophages into osteoclasts, the expression of caspase-2 is decreased. Caspase-2 deficiency does not affect the early stage but promotes the late stage of differentiation. Caspase-2 deficiency-mediated osteoclastogenesis is mediated by the overproduction of ROS and the reduction of FOXO3a and its target antioxidants.[113] OP-D down-regulates the expression of serum osteoclastic markers, such as CTX-1 and TRAP, by activating the FOXO3a/β-catenin signaling pathway. In addition, OP-D promotes the production of GSH and decreases the generation of ROS and MDA in RAW264.7 cells.[114] Therefore, these results show that down-regulation of FOXO3a expression promotes osteoclastogenesis.

The Roles of FOXO3a in Osteocytes

Osteocytes are the orchestrators connecting with osteoblasts and osteoclasts for bone remodeling. An increase in osteocyte apoptosis in bone might be the potential mechanism underlying bone deterioration.[115] Persistent hyperglycemia-induced oxidative stress can induce osteocyte apoptosis in diabetes. Adiponectin (APN) and its receptors, such as AdipoR1 and AdipoR2, can be found in osteoblasts, osteoclasts, and chondrocytes. APN up-regulates the expression of Runx-2, osteocalcin, and alkaline phosphatase (ALP) and promotes osteogenic differentiation.[116] In addition, APN promotes cellular proliferation and suppresses apoptosis by activating the AMPK/FOXO3a signaling pathway and attenuating oxidative stress in high glucose-treated MLO-Y4 cells.[117] Therefore, these results show that FOXO3a activation can inhibit apoptosis and promote proliferation in osteocytes.

The Roles of FOXO3a in OA Development

OA, a progressive and degenerative joint disease, has been associated with chronic inflammation and reduced physical functions. There are more than 250 million patients with OA worldwide,[118] and the incidence rate of knee OA in people over 65 years is about 60% in China.[119] Particularly, women after menopause have a higher incidence of OA than age-matched men.[120] OA decreases the patients' life quality and has caused a great economic burden on OA patients. Due to no effective drugs to cure it, OA has become a health concern. Chondrocyte, the unique cell type in the cartilage, is responsible for the metabolism of extracellular matrix (ECM) and the homeostasis of cartilage. It has been reported that chondrocyte apoptosis and ECM degradation are the main pathological alterations in OA pathogenesis.[121] Matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondins (ADAMTSs) are responsible for degrading collagens and aggrecans, respectively, which are the main components in the cartilage.[122]

AMPK, a master regulator of energy homeostasis, is the heterotrimeric complex composed of many subunits, such as AMPKα, AMPKβ, and AMPKγ. Activated AMPK can limit oxidative stress, attenuate the pro-catabolic responses, and protect against OA development by up-regulating the expression of FOXO3a and PGC1α in OA chondrocytes[24] (Figure 3). Estrogen deficiency has been associated with accelerated bone loss and cartilage damage.[123] Interestingly, co-culture of ovariectomy (OVX)-OA subchondral osteoblasts with chondrocytes can significantly decrease the anabolism of chondrocytes and promote the degeneration of chondrocytes by down-regulating the AMPK/FOXO3a signaling pathway. Treatment with AMPK agonist AICAR can partially rescue the catabolic effects of OVX-OA osteoblasts on OA chondrocytes[83] (Table 1). Osteonectin (SPARC), a glycoprotein secreted by osteoblasts, can promote mineralization during bone formation. Recently, it has been reported that SPARC may increase the expression of MMPs and angiogenesis. SPARC released from OVX-OA osteoblasts promotes chondrocyte degradation by activating the NF-κB signaling. AICAR rescues SPARC-induced catabolism by activating the AMPK/FOXO3a signaling pathway.[83,124]

FOXO3a can interact with C/EBPβ and suppress its activity.[125] It has been reported that the naturally occurring flavonoid TMF can inhibit chondrocyte apoptosis and suppress ADAMTS5-mediated aggrecan degradation by attenuating C/EBPβ expression via activating the Sirt1/FOXO3a signaling pathway in IL-1β-treated chondrocytes.[84] TMF also mediates ABCA1-mediated cholesterol metabolism and protects against cholesterol accumulation-induced chondrocyte apoptosis by activating the Sirt1/FOXO3a signaling pathway in OA chondrocytes.[126] DL-3-n-butylphthalide (NBP) is a small molecule isolated from celery seeds. It is reported that NBP can enhance the expression of ECM-related components, such as collagen II, aggrecan, and proteoglycan 4 (PRG4), and alleviate the apoptosis of human OA chondrocytes by inhibiting the PI3K/AKT signaling and up-regulating FOXO3a expression.[127]

The PH-domain leucine-rich protein phosphatase 1 (PHLPP1), an atypical protein phosphatase, has been involved in negatively mediating the anabolism-related pathways, such as the AKT and PKC pathways. In OA chondrocytes, the expression of PHLPP1 is significantly increased. This might be due to the low levels of CpG methylation on the promoter of PHLPP1.[128] It is interesting to find that FOXO3a mediates the expression of PHLPP1. FOXO3a inhibition decreases the transcriptional activity of the Phlpp1 promoter and mRNA levels and increases GAG staining.[129] However, FOXO1/3/4 deficient mice demonstrate OA-like alterations, such as cartilage degradation, indicating the important role of FOXO in cartilage homeostasis. Particularly, the knockout of FOXO3a increases the severity of OA in mice.[130] MicroRNA has been implicated in OA development. The expression of miR-30b-5p in OA chondrocytes is up-regulated. Overexpression of miR-30b-5p promotes apoptosis and NLRP3 inflammasome activation in IL-1β-treated chondrocytes by degrading Sirt1. Overexpression of Sirt1 compromises the effects of miR-30b-5p on OA chondrocytes by promoting FOXO3a expression[85] (Table 1).

Endochondral ossification, a controlled developmental process, has been associated with the switch of chondrocytes at the growth plate from a resting state to an active state where chondrocytes continue to proliferate to hypertrophy. Hypertrophic chondrocytes secrete collagen X and mineralize the ECM. Next, osteoblasts, osteoclasts, and blood vessels invade the ECM to form the primary ossification center. Subsequently, hypertrophic chondrocyte initiates apoptosis.[131] FOXO3a is a transcription factor that is involved in this process. Mice with the complete loss of FOXO show an increase in hypertrophic zone length and hyperkyphosis phenotype at a higher age. This suggests a crucial role of FOXO in normal endochondral ossification.[132] BMP-binding endothelial regulator (BMPER), a secreted glycoprotein, directly interacts with BMP4 to modulate its functions. BMPER is a downstream factor of FOXO3a. It has been reported that TMF-activated FOXO3a can inhibit OA chondrocyte hypertrophy by suppressing the BMPER/BMP4 signaling pathway[86] (Table 1).

However, there are opposite comments on the roles of FOXO3a in OA pathogenesis. Transglutaminase 2 (TG2), a calcium-dependent enzyme expressed in the cartilage and growth plate, up-regulating the expression of MMP-3 and MMP-13 by promoting the phosphorylation of AMPK and FOXO3a and the nuclear translocation of FOXO3a. This suggests that FOXO3a is responsible for the production of ECM catabolism and the development of OA by up-regulating the expression of MMP-3 and MMP-13.[133] The explanation might be associated with the binding of FOXO3a to the promoter of MMP-13 and the activation of its transcriptional expression in VSMCs.[134] Therefore, these results show that FOXO3a activation promotes ECM anabolism, inhibits chondrocyte apoptosis, and protects against cartilage degradation.

The Roles of FOXO3a in RA Development

RA, a chronic inflammatory autoimmune disease, is characterized by swollen joints, synovial inflammation, and bone destruction. Genetic and environmental factors have been included in the pathogenesis of RA. However, the exact cause of RA remains unknown. It is well documented that fibroblast-like synoviocytes (FLSs) and synovial tissue-specific cells are the critical effectors in RA development. It has been reported that RA FLSs are responsible for the synthesis of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNFα.[135] FOXO3a in the immune system has multiple potential roles in mediating cell cycle and cell apoptosis. FOXO3a deficiency leads to mild systemic autoimmunity, as shown by T-cell hyper-activation and increased cytokine production.[136] However, another study reports that the transcript levels of FOXO3a in the peripheral blood mononuclear cells (PBMCs) from RA patients show no significant difference compared with normal controls.[137]

The expression of FOXO3a is attenuated in RA synovial tissues and lipopolysaccharide (LPS)-treated FLSs. Overexpression of FOXO3a suppresses LPS-induced production of pro-inflammatory cytokines and inhibits collagen-induced arthritis in rats.[87,138] Consistently, the decreased expression of FOXO3a is associated with the increased expression of miR-155 in RA synovial tissues. Overexpression of FOXO3a compromises the regulatory activity of miR-155 in up-regulating the expression of IL-1β, IL-6, and TNFα.[25] Tripartite motif-containing protein 3 (TRIM3) regulates the innate immune responses and carcinogenesis. FOXO3a can interact with the promoter of TRIM3 and stimulate its activity. In addition, FOXO3a compromises the effects of LPS on TRIM3 expression, protecting against RA development[87] (Table 1).

Cyr61 has been implicated in RA pathogenesis. It is reported that Cyr61 is up-regulated in RA synovial tissues and plays a crucial role in the IL-17-regulated proliferation of RA synovial fibroblasts (SFs) by mediating the αvβ5/AKT/NF-κB signaling pathway.[139] TNFα up-regulates Cyr61 expression and decreases FOXO3a nuclear translocation. However, FOXO3a overexpression can reverse TNFα-induced Cyr61 expression by directly binding to the promoter of Cyr61 in RASFs. Simvastatin protects collagen-induced inflammatory arthritis by activating the Sirt1/FOXO3a signaling and inhibiting Cyr61 expression.[88] FOXO3a suppresses the activation of NF-κB, which is responsible for T-cell hyperactivity. FOXO3a mediates helper T-cell activation and tolerance by inhibiting inflammatory responses.[140] In FOXO3a-knockout mice, intraperitoneal injection of serum derived from KRN transgenic mice induces severe inflammatory arthritis. FOXO3a maintains neutrophil vitality, and FOXO3a-deleted neutrophils increase Fas ligand expression and stimulate apoptosis.[141]

Angiogenesis is one of the critical underlying mechanisms for sustaining a chronic inflammatory state in early RA pathogenesis. IL-1β significantly promotes the expression of angiogenic factors, such as VEGF, and induces synovial angiogenesis. Phospholipase D (PLD) is significantly up-regulated in IL-1β-treated synoviocytes from RA patients. Mechanistically, IL-1β increases the binding of NF-κB and ATF2 to the promoter of PLD and leads to the enhancement of angiogenesis and inflammation. However, activation of FOXO3a induced by PLD inhibitors can abolish IL-1β-induced angiogenesis and inflammation in RA FLSs.[89] Consistently, another study supports that inhibition of the PI3K/AKT and MEK/ERK signaling pathways and subsequent activation of FOXO3a can enhance the antiangiogenic effects of resveratrol in human umbilical vein endothelial cells (HUVECs)[90] (Table 1). Resveratrol is a natural compound and has been widely used in protecting against cancer, diabetic retinopathy, psoriasis, and cardiovascular diseases. Interestingly, whether resveratrol-mediated FOXO3a activation is associated with angiogenesis inhibition in these diseases still needs to be verified.

Therefore, these results show that FOXO3a activity is attenuated in RA synovial tissues, and increased FOXO3a expression can ameliorate inflammatory responses, inhibit angiogenesis, and protect against RA development.

The Roles of FOXO3a in AS Development

Ankylosing spondylitis (AS), a chronic inflammatory autoimmune disease, may cause joint pain, limited mobility, and even disability. Particularly, low-back pain and morning stiffness are the symptoms at the early stage of AS, and spinal stiffness and deformity may occur at the late stage.[142] The global prevalence of AS is 0.2–1.4%, and most patients are young men.[143] In the clinic, there are no effective therapeutic strategies to cure AS. In addition, the potential molecular mechanisms in the pathogenesis of AS are complicated and remain unclear.

Earlier studies on AS development have identified human leukocyte antigen (HLA)-B27 as a genetic risk factor for AS. A recent study suggests that HLA-B27 can only account for 20% of overall genetic susceptibility to AS.[144] The relationship between FOXO3a gene polymorphisms and AS susceptibility has been explored in the Eastern Chinese Han population. FOXO3a polymorphisms rs12212067 and rs3800232 can be risk factors for AS development.[145] DNA methylation plays a critical role in AS pathogenesis. It has been reported that differential methylation has been observed in 19 CpG sites in the promoter of FOXO3a, and the CpG4 and CpG5 islands are significantly hypomethylated in patients with AS. The mRNA expression levels of FOXO3a are decreased and negatively correlated with the methylation of the CpG2 island in AS patients.[146]

It has been reported that FOXO3a inhibits inflammatory responses and oxidative stress in Jurkat cells, as shown by decreased levels of IL-8, IL-17A, and IL-23 and increased levels of SOD, CAT, and T-AOC. Bioinformatics analysis shows that FOXO3a can bind to the promoters of TGFβ and HO-1 and transcriptionally activate their expression[91] (Table 1). T-cell immune imbalance-induced inflammatory responses and tissue damage play a critical role in AS pathogenesis. It has been reported that methyltransferase-like 14 (METTL14)-regulated N^6-methyladenosine (m6A) modification in T cells from AS patients is significantly decreased. METTL14 plays a critical role in alleviating inflammatory responses and activating autophagy by up-regulating the downstream factor FOXO3a expression in an m6A-dependent manner[92] (Table 1). Therefore, these results show that FOXO3a polymorphism is associated with AS pathogenesis, and up-regulation of FOXO3a expression can inhibit AS development by suppressing inflammatory responses and oxidative stress.

The Roles of FOXO3a in IDD Development

Intervertebral disc (IVD) degeneration (IDD) is a spinal-related disorder with low-back pain that is associated with inflammation, oxidative stress, and catabolism.[147] Dysregulation of ECM catabolism in the IVD represents a critical pathophysiological feature of IDD. Zinc homeostasis is important for cellular metabolism. ZIP4, a member belonging to the SLC39A family, is a zinc transporter that plays a critical role in mediating nucleus pulposus (NP) physiological actions. It has been reported that ZIP4 expression is up-regulated in the NP cells of IDD patients. Enhanced expression of ZIP4 is associated with inflammation, oxidative stress, and ECM degradation by up-regulating HDAC4 expression and down-regulating Sirt1 and FOXO3a expression in the NP cells[93] (Table 1).

Apoptosis, a fundamental biological process, contributes to the pathogenesis of IDD. Increased apoptosis of NP and annulus fibrosus (AF) cells has been observed in IDD.[148] The expression of pro-apoptotic factor Bcl-2-binding component 3 (BBC3, also known as PUMA) is mediated by FOXO3a and up-regulated in IVD cells from IDD patients. Mechanically, CUL4A and CUL4B, the scaffold proteins, recruit DDB1, RBX1, and DCAF6 to assemble a $CRL4^{DCAF6}$ E3 ligase that ubiquitinates and degrades the transcriptional co-repressors CtBP1/2 and consequently releases FOXO3a. TSC01131, an inhibitor of $CRL4^{DCAF6}$ E3 ligase, can suppress IVD cell apoptosis mediated by the FOXO3a/BBC3 axis[94] (Table 1). The potential activities of leaf extracts from Violina pumpkin (*Cucurbita moschata* Duch.) on the recovery of degenerated IVD cells have been investigated. The leaf extracts show anabolic effects on bone, as shown by the stimulation of osteoblast differentiation and the inhibition of osteoclast activity.[149] Furthermore, the fraction of leaf extracts, consisting almost entirely of p-coumaric acid, enhances the expression of cellular homeostasis and stress response regulators, such as Sirt1, FOXO3a, NRF2, and SOD2.[150] Therefore, these results show that FOXO3a expression exhibits protective activity against IDD development.

FOXO3a Becomes a Potential Target for Bone and Cartilage Diseases

FOXO3a has been used as a potential diagnostic indicator for RA. Sixty RA patients and thirty healthy subjects have been included in a study that FOXO3a expression in the serum is correlated with the disease activity of RA.[151] Consistently, the genetic variation in FOXO3a may regulate the production of inflammatory cytokines, and it is suggested to predict the prognosis in RA. However, the single-nucleotide polymorphism (SNP) in FOXO3a is not associated with disease susceptibility but related to a milder course of RA.[152,153] Interestingly, a non-coding polymorphism in FOXO3a (rs12212067: T > G) at which the minor (G) allele has been identified. Rs12212067 variation decreases LPS-induced production of proinflammatory cytokines, such as TNFα, IL-1β, IL-6, and IL-8, and increases the anti-inflammatory factor IL-10 in peripheral blood mononuclear cells (PBMC).[152]

FOXO3a has become a potential biomarker to evaluate chondrocyte differentiation. Autologous chondrocyte implantation (ACI), a popular surgical approach for repairing osteochondral defects, is challenged by the de-differentiation of chondrocytes. Interestingly, chondrocytes embedded into the soft collagen and alginate (Col/Alg) hydrogels initiate to re-differentiate, as indicated by up-regulated expression of Col2a1, aggrecan, and FOXO3a.[154] Another study also reports that overexpression of FOXO3a promotes the expression of chondrocyte differentiation markers, such as collagen II, aggrecan, and SOX9, and enhances early chondrogenesis in ATDC5 cells or undifferentiated MSCs.[155]

Aging increases the susceptibility of the body to various chronic degenerative diseases, such as OP and OA. S-adenosyl-L-methionine (SAM) has been reported to promote osteogenic differentiation and ameliorate senescence by activating the PI3K/AKT/FOXO3a signaling pathway in H_2O_2-treated adipose-derived MSC (ADSC).[156] Melatonin suppresses oxidative stress and promotes osteogenic differentiation in hMSCs by activating the AMPK/FOXO3a signaling pathway.[157] Resveratrol, an activator of Sirt1, can significantly improve bone microarchitecture, promote osteogenesis, and suppress osteoclastogenesis by up-regulating the expression of FOXO3a and activating the Wnt/β-catenin signaling pathway.[158]

Recently, it has been reported that several anti-hypertensive drugs, particularly angiotensin-converting enzyme inhibitors, can alleviate bone fracture. Further study shows that angiotensin II causes mitochondrial DNA damage and triggers oxidative stress by inhibiting the Sirt1/FOXO3a signaling pathway in osteoblasts.[159] Ophiopogonin D (OP-D), obtained from *Radix Ophiopogon japonicus* (L.f). Ker-Gawl, acts as an antioxidant in protecting against OP development. Mechanically, OP-D promotes cellular proliferation and up-regulates the expression of osteogenic genes, such as BGLAP, Col1a1, and SPP1, by up-regulating the expression of FOXO3a and promoting the nuclear translocation of FOXO3a and β-catenin in H_2O_2-treated MC3T3-E1 cells.[114]

The natural compound 2,3,5,4-tetrahydroxystilbene-2-o-β-D-glucoside (TSG) inhibits oxidative stress and apoptosis by mediating the FOXO3a/β-catenin signaling in H_2O_2-treated MC3T3-E1 cells.[160] Consistently, tanshinol rescues oxidative stress-reduced osteoblast differentiation by inhibiting FOXO3a expression and activating the Wnt/β-catenin signaling in H_2O_2-treated MC3T3-E1 cells.[161] In addition, activation of the PI3K/AKT/FOXO3a and Wnt/β-catenin signaling pathways has been involved in the regulatory activity of different TiO_2 nanotubes in promoting osteoblast differentiation under oxidative stress.[162]

Conclusions

FOXO3a plays a critical role in skeletal biology, as it mediates bone and cartilage events, such as structural destruction, degradation, and remodeling. Particularly, FOXO3a has been implicated in osteogenesis, osteoclastogenesis, chondrogenesis, and apoptosis, which are important events during the pathological development of bone and cartilage diseases. FOXO3a has become a potential target for managing bone and cartilage diseases.

The biological effects of FOXO3a are mediated by PTMs (mainly including phosphorylation and acetylation) or the interacting partners. For example, phosphorylation at different sites of FOXO3a protein may determine its fates: activation and nuclear translocation for transcriptional activity or inactivation for ubiquitin-proteasome degradation. Deacetylation may promote the nuclear accumulation of FOXO3a, while acetylation induces its nuclear export. The PTMs of FOXO3a can be mediated by several signaling pathways, such as MAPK, NF-κB, PI3K/AKT, AMPK, and Sirt1 pathways, which have been implicated in the pathogenesis of OP, OA, RA, AS, and IDD.

Indirectly, FOXO3a can act as a co-activator or co-repressor to affect a variety of downstream factors, exhibiting different biological actions. Interestingly, FOXO3a can act as a transcriptional factor to regulate the expression of its target gene expression. For example, FOXO3a can bind and activate the promoter activity of MnSOD, CAT, BIM, BBC3, and CDK6. These target factors are correlated with oxidative stress, apoptosis, or cell cycle, respectively, and may play different effects on cellular differentiation and functions of osteoblasts, chondrocytes, and FLSs.

However, there are limitations of this review article. Most studies are focusing on the biological activities of FOXO3a under a specific condition, and the research data may be controversial. FOXO3a directly or indirectly regulates the pathological changes of bone and cartilage diseases. The direct roles of FOXO3a in bone and cartilage should be further investigated. Several endogenous or exogenous agents have been explored to treat bone and cartilage diseases by indirectly mediating the expression of FOXO3a. However, drugs directly interacting with FOXO3a and mediating its

activities are still absent. Data from conditional FOXO3a-knockout animals in bone and cartilage diseases are still needed. It is important to verify the specific biological functions of FOXO3a in different animal models. More comprehensive protocols are needed to elucidate the roles of FOXO3a in bone and cartilage diseases.

Abbreviations

FOXO3a, Forkhead box O3a; OP, osteoporosis; OA, osteoarthritis; RA, rheumatoid arthritis; AS, ankylosing spondylitis; IDD, intervertebral disc degeneration; PTMs, post-translational modifications; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κ-B; AMPK, adenosine 5′-monophosphate (AMP)-activated protein kinase; Sirt1, sirtuin 1; ERα, estrogen receptor α; BIM, Bcl-2-like protein 11; BBC3, Bcl2 binding component 3; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; ICAM1, intercellular cell adhesion molecule-1; MnSOD, manganese superoxide dismutase; CAT, catalase; SGK, Serum and glucocorticoid-inducible kinase; MST1, mammalian sterile 20-like kinase 1; PMOP, postmenopausal osteoporosis; MSC, mesenchymal stem cell; CSE, cigarette smoke extract; NOX4, NADPH oxidase 4; T2DM, type 2 diabetes mellitus; APN, adiponectin; OCN, osteocalcin; ALP, alkaline phosphatase; ECM, extracellular matrix; MMPs, Matrix metalloproteinases; ADAMTSs, a disintegrin and metalloproteinase with thrombospondins; FLSs, fibroblast-like synoviocytes; LPS, lipopolysaccharide.

Data Sharing Statement

The data used to support the findings of this study are included within the article.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was financially supported by the Science and Technology research project of the Education Department of Jiangxi Province (GJJ2201451), Ganzhou Municipal "Science and Technology + National Regional Medical Center" Joint Project (2023LNS17449, 2022-RC1328, and 2022-YB1395).

Disclosure

The authors declare no conflict of interests.

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