Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 18s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

FoxO1 Transcription Factor in Chondrocyte Regulation: A Review on Its Molecular Mechanisms, Pathological Roles, and Therapeutic Potential

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Cite this paper as: Ting Chen, Mei hua Tan, Kai Bin Liew, (2025) FoxO1 Transcription Factor in Chondrocyte Regulation: A Review on Its Molecular Mechanisms, Pathological Roles, and Therapeutic Potential. *Journal of Neonatal Surgery*, 14 (18s), 1242-1254.

ABSTRACT

FoxO1 as a transcription factor integrates multiple signals for maintaining cartilage homeostasis, and it is the most essential regulator of chondrocyte activities. Activity of chondrocytes involving proliferation, differentiation, stress resistance, and autophagy modulation is controlled by FoxO1 via perturbations in phosphorylation and acetylation, alongside its conserved forkhead DNA-binding domain. Notable FoxO1 actions include: cooperation with Sox9 for ECM synthesis, inhibition of hypertrophic differentiation via β-catenin sequestration, increase of stress tolerance, and elevation of antioxidant and autophagy-related gene activity. Decline of apoptotic signaling and enhanced cytosolic cleansing mechanisms related to aging and chronic inflammation under dysregulated FoxO1 drives osteoarthritis development due to disruption to the uncontrollably worsening autophagic flux, increased ECM breakdown, apoptosis and cellular senescence. FoxO1's sensitivity to growth factors, oxidative stress, and metabolic signals is tuned via PI3K/Akt, AMPK/Sirt1, Wnt/β-catenin pathways crosstalk. Therapeutic approaches aiming to restore the favorable actions of FoxO1 include: gene therapies using viral vectors, CRISPR-based methods, and pharmacological targeting of upstream regulators like Sirt1 and AMPK. This review in total positions FoxO1 as a chondrocyte master regulator while pinpointing rounded therapeutic intersections.

1. INTRODUCTION

FoxO1 as a transcription factor integrates multiple signals for maintaining cartilage homeostasis, and it is the most essential regulator of chondrocyte activities. Activity of chondrocytes involving proliferation, differentiation, stress resistance, and autophagy modulation is controlled by FoxO1 via perturbations in phosphorylation and acetylation, alongside its conserved forkhead DNA-binding domain. Notable FoxO1 actions include: cooperation with Sox9 for ECM synthesis, inhibition of hypertrophic differentiation via β-catenin sequestration, increase of stress tolerance, and elevation of antioxidant and autophagy-related gene activity. Decline of apoptotic signaling and enhanced cytosolic cleansing mechanisms related to aging and chronic inflammation under dysregulated FoxO1 drives osteoarthritis development due to disruption to the uncontrollably worsening autophagic flux, increased ECM breakdown, apoptosis and cellular senescence. FoxO1's sensitivity to growth factors, oxidative stress, and metabolic signals is tuned via PI3K/Akt, AMPK/Sirt1, Wnt/β-catenin pathways crosstalk. Therapeutic approaches aiming to restore the favorable actions of FoxO1 include: gene therapies using viral vectors, CRISPR-based methods, and pharmacological targeting of upstream regulators like Sirt1 and AMPK. This review in total positions FoxO1 as a chondrocyte master regulator while pinpointing rounded therapeutic intersections.

2.1. STRUCTURAL FEATURES AND CONSERVATION

The Forkhead box O1 (FoxO1) transcription factor is a central member of the Forkhead box (FOX) superfamily, defined by its highly conserved forkhead DNA-binding domain (DBD)-a structurally distinct motif that enables precise regulation of gene expression[1, 2]. This domain, spanning approximately 100 amino acids, forms a characteristic "winged-helix" fold, composed of three α-helices and two β-strands arranged in a fork-like configuration[3]. This architecture allows the

domain to interact with the minor groove of DNA, recognizing specific response elements such as the insulin-responsive element (IRE; 5'-TGGTTT-3') and the Daf-16 family binding motif (5'-GTAAA(T/C)AA-3') with high specificity[4, 5]. The FBD exhibits remarkable evolutionary conservation, with over 90% sequence identity in DNA-contacting residues across mammals, insects, and nematodes, highlighting its fundamental role in maintaining cellular homeostasis across species.

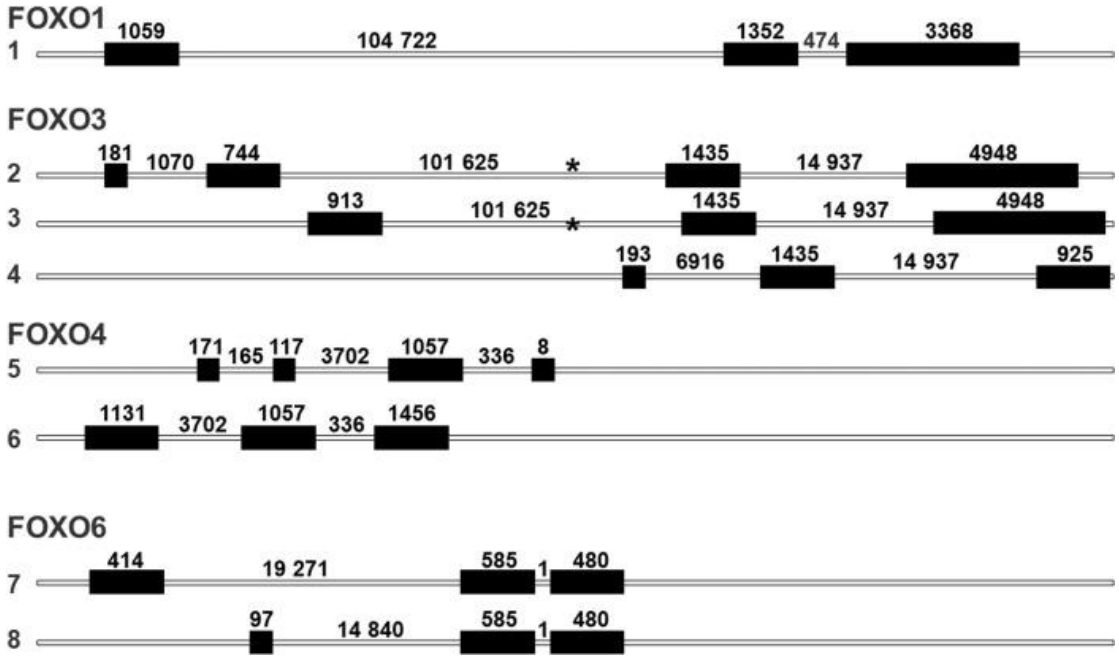

Figure 1: Gene structure schematic of FOXO family members.

The figure 1 illustrates the gene structures of FOXO1, FOXO3, FOXO4, and FOXO6. Black boxes denote exons, with numbers between boxes indicating intron lengths (in base pairs). Certain introns in FOXO3 are marked by an asterisk (*). Each number (1-8) corresponds to distinct transcripts or structural variants, visually showcasing the exon-intron organization and reflecting the diversity and characteristics of gene structures within the FOXO family.

2.1.1. Molecular Architecture of the Forkhead Domain

The three-dimensional structure of the FoxO1 FBD, resolved through X-ray crystallography, reveals a compact globular structure where the second α-helix (helix 3) and adjacent loops make direct contact with DNA[6]. Key amino acid residues, including arginine and glutamine in the DNA-recognition loop, form hydrogen bonds and van der Waals interactions with the nucleotide bases of target promoters[7]. For example, arginine residues within the FBD specifically recognize the guanine-rich core of the IRE, facilitating binding to genes involved in cell cycle regulation, such as Cyclin D1, and antioxidant defense, such as SOD2 and CAT [8, 9]. This structural precision allows FoxO1 to bind to the promoter of VEGFA, a critical regulator of angiogenesis, thereby driving vascular endothelial growth factor expression during fracture healing[10].

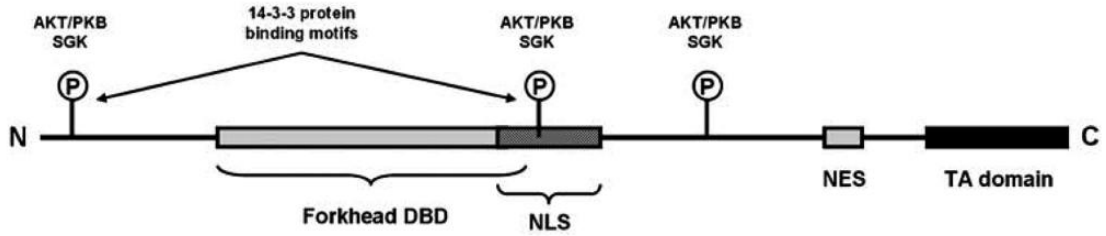

Figure 2: FOXO1 protein structure and post-translational modification sites

The figure 2 shows a diagram of the structural organization of foxo1 protein. At the N-terminal, there are phosphorylation sites (labeled "P") regulated by AKT/PKB and SGK. Adjacent to these sites is the region containing 14-3-3 protein binding motifs. The Forkhead DBD (DNA-binding domain) is a key structural element for DNA interaction. The NLS (nuclear localization signal) is crucial for nuclear import. Further along, there is another phosphorylation site by AKT/PKB/SGK, followed by NES (nuclear export signal) which mediates nuclear export, and finally the TA (transactivation) domain at the C-terminal. This illustration highlights the functional domains and potential regulatory phosphorylation sites, providing insight into foxo1's regulatory mechanisms.

2.1.2. Functional Motifs Governing Subcellular Localization and Transcriptional Activity

While the forkhead DNA-binding domain (FBD) is central to FoxO1's ability to recognize target genes, the transcription factor relies on additional structural elements to integrate extracellular signals and fine-tune its activity within the cell[10]. These functional motifs—including nuclear localization and export sequences, a transactivation domain, and conserved regulatory regions—work in concert to modulate where FoxO1 localizes and how it interacts with cofactors, ultimately dictating its impact on chondrocyte physiology and pathology[11,12].

Nuclear Localization Sequence (NLS) and Nuclear Export Sequence (NES)

The dynamic shuttling of FoxO1 between the nucleus and cytoplasm is governed by two opposing motifs: the NLS and NES[13]. The NLS, a short, positively charged peptide in the N-terminal region, interacts with importin proteins to facilitate nuclear entry, ensuring access to genomic targets during stress or nutrient limitation[14]. In contrast, the C-terminal NES contains a leucine-rich sequence that binds to exportin-1 (CRM1), promoting cytoplasmic retention under basal conditions[15]. Phosphorylation events, such as those mediated by the Akt kinase at Ser256, Ser319, and Thr24, alter the conformation of these sequences, enabling interaction with 14-3-3 chaperone proteins[16]. This sequestration in the cytoplasm inhibits FoxO1's transcriptional activity when growth factors are abundant, such as in proliferating chondrocytes[17]. Conversely, during oxidative stress, phosphatases remove these phosphate groups, allowing FoxO1 to translocate to the nucleus and activate protective pathways[18].

Transactivation Domain (TAD)

The C-terminal transactivation domain (TAD) of FoxO1 houses conserved motifs that recruit co-activators or co-repressors, shaping its transcriptional output[12]. For example, interaction with the deacetylase Sirt1 removes acetyl groups from lysine residues in the TAD (Lys262, Lys265), enhancing DNA-binding affinity and promoting the expression of genes involved in autophagy (Atg7, Beclin1) and antioxidant defense (SOD2)[18]. In contrast, acetyltransferases like p300 add acetyl groups to these residues, reducing FoxO1's ability to engage target promoters and marking it for proteasomal degradation. This dynamic acetylation/deacetylation balance allows FoxO1 to switch between pro-survival functions—such as enhancing chondrocyte resistance to oxidative stress—and pro-apoptotic roles in inflamed or damaged cartilage[19].

Conserved Regulatory Regions (CRs)

Three evolutionarily conserved regions (CR1, CR2, CR3) in FoxO1 serve as critical hubs for mediating interactions with signaling kinases, post-translational modifiers, and transcriptional cofactors, enabling the protein to respond to diverse cellular contexts[20].

CR1 (N-Terminal Regulatory Region)

Located near the N-terminus, CR1 harbors the primary phosphorylation sites for Akt (Ser256, Thr24, Ser319 in humans), which are pivotal for growth factor-dependent inactivation[21]. When growth factors such as IGF-1 activate the PI3K/Akt pathway, phosphorylation at these sites induces a conformational change that exposes a 14-3-3 binding motif, leading to cytoplasmic sequestration of FoxO1[22]. This mechanism ensures that under proliferative conditions, FoxO1 remains inactive, allowing chondrocytes to prioritize cell division and matrix synthesis over stress-responsive programs.

CR2 (Middle Regulatory Region)

Positioned in the central domain of FoxO1, CR2 contains consensus sites for stress-activated kinases like c-Jun N-terminal kinase (JNK) and p38 MAPK[23]. During oxidative stress, these kinases phosphorylate residues within CR2 (Ser315 in humans), disrupting interactions with 14-3-3 proteins and promoting nuclear translocation. In chondrocytes exposed to reactive oxygen species (ROS), this process enhances FoxO1's binding to promoters of antioxidant genes (SOD2, CAT) and autophagy regulators (Atg7), bolstering the cell's defense against oxidative damage[24]. CR2 also mediates interactions with cofactors like β-catenin, allowing FoxO1 to antagonize Wnt signaling by sequestering β-catenin from TCF/LEF complexes, thereby inhibiting hypertrophic differentiation and ECM degradation[25].

CR3 (C-Terminal Regulatory Region)

The C-terminal CR3 is enriched with motifs that interact with metabolic sensors such as AMP-activated protein kinase (AMPK) and sirtuins[26]. Under energy stress (low ATP/AMP ratios), AMPK phosphorylates FoxO1 at CR3 residues, enhancing its transcriptional activity without altering subcellular localization[27]. This allows FoxO1 to upregulate genes involved in autophagy (Beclin1, LC3) and glucose metabolism, enabling chondrocytes to adapt to nutrient limitation by recycling damaged organelles and conserving energy. Additionally, CR3 contains lysine residues targeted by Sirt1-mediated deacetylation, which stabilizes FoxO1-DNA interactions and promotes the expression of protective genes in aged or stressed cartilage[28].

These conserved regions endow FoxO1 with the versatility to integrate signals from growth factor, stress, and metabolic pathways, ensuring context-specific regulation of chondrocyte function. For example, during fracture healing, CR3-mediated AMPK activation drives FoxO1-dependent VEGFA expression, promoting vascular invasion of the cartilaginous callus, while in osteoarthritic cartilage, dysregulated phosphorylation within CR1—driven by chronic

activation of the PI3K/Akt pathway—leads to persistent cytoplasmic sequestration of FoxO1, even in the presence of pro-inflammatory stimuli like IL-1β[29]. This aberrant localization attenuates the transcription of protective genes such as PRG4 (encoding lubricin, critical for cartilage surface lubrication) and SOD2 (a mitochondrial antioxidant enzyme), compromising cartilage integrity and increasing susceptibility to mechanical damage[30]. Concurrently, impaired interactions in CR2 with stress-responsive kinases like JNK and p38 MAPK under chronic inflammation reduce FoxO1's ability to induce autophagy-related genes (Atg7, Beclin1) and repress matrix-degrading enzymes (MMP13, ADAMTS5), accelerating extracellular matrix degradation and chondrocyte apoptosis. In aged chondrocytes, reduced Sirt1-mediated deacetylation at CR3 weakens FoxO1's binding to promoters of mitophagy regulators, leading to defective clearance of damaged mitochondria and accumulation of reactive oxygen species—a key driver of age-related cartilage degeneration. Collectively, these conserved regions act as molecular hubs, enabling FoxO1 to orchestrate context-dependent programs that balance chondrocyte survival, matrix homeostasis, and stress resistance[31]. Disruptions in CR-mediated signaling under pathological conditions tip this balance toward catabolism and cell death, underscoring the critical role of these motifs in both physiological regulation and disease pathogenesis. By integrating inputs from diverse pathways, FoxO1's conserved functional domains ensure precise control over gene expression networks, making it a pivotal regulator of chondrocyte fate in health and disease.

2.2. Post-Translational Modifications (PTMs): Orchestrating FoxO1 Activity in Chondrocytes

The functional versatility of FoxO1 is finely tuned by a repertoire of post-translational modifications (PTMs), including phosphorylation, acetylation, and methylation, which dynamically regulate its subcellular localization, DNA-binding affinity, and interaction with cofactors. These modifications act as molecular switches, enabling FoxO1 to adapt its activity to diverse cellular microenvironments, from growth factor-rich conditions to metabolic stress or inflammatory challenges.

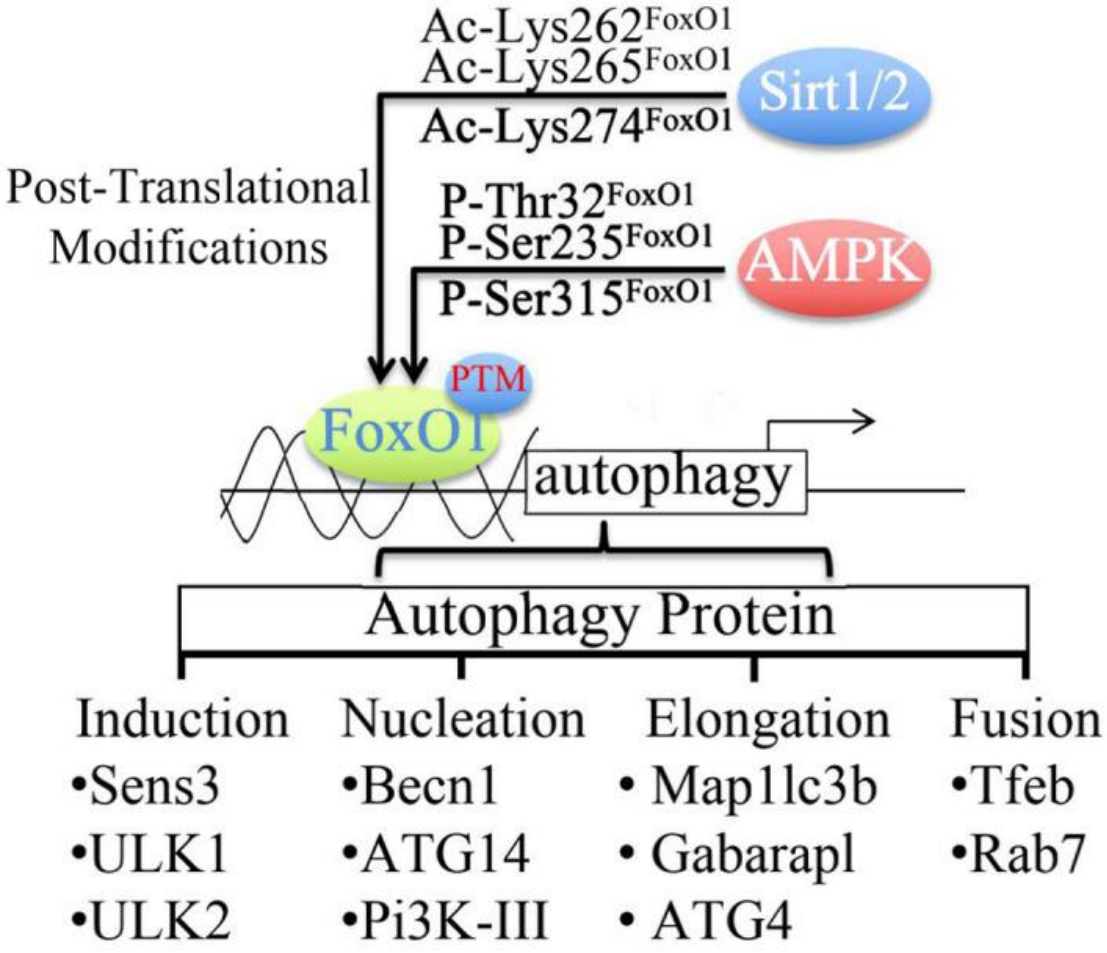

Figure 3: Foxo1 post-translational modifications regulate autophagy pathway

PTM regulates the activation of FoxO1 at specific sites via Sirt1/2 and AMPK, promoting the binding between the FoxO1 and autophagy-related proteins promoters. FoxO1 is essential in each process of autophagy, including induction, autophagosome nucleation (Nucleation), autophagosome membrane elongation (Elongation) and autophagolysosome fusion (Fusion)

2.2.1. Phosphorylation of Core Regulatory Hub

Phosphorylation is the most extensively studied PTM of FoxO1, governing its shuttling between the nucleus and cytoplasm and modulating its transcriptional output[32]. The PI3K/Akt signaling pathway plays a central role in this process,upon activation by growth factors such as IGF-1 or insulin, Akt phosphorylates FoxO1 at three conserved sites—Thr24, Ser256, and Ser319 (human numbering)—creating a binding motif for 14-3-3 chaperone proteins[33]. This interaction masks the nuclear localization sequence (NLS), promoting cytoplasmic sequestration and preventing FoxO1 from engaging target promoters. In chondrocytes, this mechanism is critical for maintaining proliferative signals during cartilage development, as IGF-1-driven Akt phosphorylation keeps FoxO1 inactive, allowing upregulation of cell cycle genes like Cyclin D1 and suppression of pro-apoptotic programs[34]. Conversely, under energy stress (low ATP/AMP ratios) or oxidative challenge, stress-responsive kinases such as AMP-activated protein kinase (AMPK) and c-Jun N-terminal kinase (JNK) phosphorylate FoxO1 at distinct sites. AMPK targets Ser315, enhancing FoxO1's nuclear translocation by disrupting its interaction with 14-3-3, thereby activating genes involved in autophagy (Atg7, Beclin1) and antioxidant defense (SOD2, CAT)[35]. JNK-mediated phosphorylation at Ser212 and Ser317 further stabilizes FoxO1 in the nucleus, amplifying its ability to induce stress-responsive genes that protect chondrocytes from reactive oxygen species (ROS)-induced damage[36]. These opposing phosphorylation events create a regulatory rheostat, balancing FoxO1 activity between growth-promoting and stress-adaptive states.

2.2.2. Acetylation/Deacetylation: Fine-Tuning DNA Binding and Cofactor Interactions

Acetylation of FoxO1 at lysine residues (Lys262, Lys265, Lys294) is a key regulatory step, mediated by competing activities of histone acetyltransferases (HATs) like p300/CBP and deacetylases (HDACs) such as Sirt1[37]. Sirt1, a NAD+-dependent HDAC, deacetylates FoxO1 in the nucleus, enhancing its DNA-binding affinity for promoters of autophagy-related genes (Atg7, Beclin1) and antioxidant enzymes[38]. This process is vital for chondrocytes under metabolic stress, as it promotes the clearance of damaged organelles and reduces ROS accumulation, thereby maintaining extracellular matrix (ECM) integrity. In contrast, p300/CBP-mediated acetylation introduces negative charges into the FoxO1 structure, disrupting its interaction with DNA and promoting its association with transcriptional co-repressors or ubiquitin ligases, leading to reduced transcriptional activity and enhanced proteasomal degradation[39].

The interplay between these modifications also influences FoxO1's interaction with other signaling pathways. For instance, deacetylated FoxO1 forms a complex with β-catenin, sequestering it from TCF/LEF transcription factors to inhibit Wnt-driven hypertrophic differentiation—a process critical for preventing premature cartilage calcification. Conversely, p300-mediated acetylation disrupts this interaction, allowing β-catenin to activate pro-hypertrophic genes like Col10α1, promoting pathological changes in osteoarthritic cartilage. Thus, the dynamic regulation of FoxO1 acetylation by Sirt1 and p300/CBP is essential for balancing chondrocyte survival, matrix homeostasis, and stress resistance, with dysregulation contributing to both aging-related and inflammatory cartilage degeneration.

2.3. Signaling Pathway Crosstalk

2.3.1 PI3K/Akt Axis

In the microenvironment of quiescent chondrocytes, the PI3K/Akt axis stands as a dominant signaling pathway. Growth factors, such as insulin-like growth factor 1 (IGF-1), are pivotal players in this regulatory cascade. These growth factors are often present in the extracellular matrix of cartilage, and their binding to specific cell-surface receptors triggers a series of intracellular events[40].

The inactivation of FoxO1 by the PI3K/Akt pathway has significant implications for chondrocyte behavior. FoxO1 is known to regulate genes involved in cell cycle arrest, apoptosis, and stress response. By inactivating FoxO1, the PI3K/Akt pathway promotes chondrocyte proliferation[41]. It allows the upregulation of genes such as cyclin D1, which is essential for the progression of the cell cycle from the G1 to the S phase[42]. This pathway is crucial during the normal development of cartilage and the maintenance of chondrocyte population in a healthy state.

2.3.2 AMPK/Sirt1 Pathway

Chondrocytes are constantly exposed to fluctuations in the metabolic environment, such as nutrient deprivation. Under such metabolic stress conditions, the AMP-activated protein kinase (AMPK)/Sirt1 pathway plays a crucial role in maintaining cellular homeostasis[43].

Activated AMPK has a direct impact on FoxO1. It phosphorylates FoxO1 at specific sites, such as Ser315. This phosphorylation event promotes the nuclear translocation of FoxO1. Once in the nucleus, FoxO1 can bind to the promoters of its target genes.

One of the key functions of FoxO1 in this context is to induce autophagy. Autophagy is a cellular process that involves the degradation and recycling of damaged organelles and proteins. FoxO1 activates genes involved in autophagy, such as Atg7 and Beclin1[44]. These genes are essential for the formation of autophagosomes, which are double-membrane vesicles that engulf cellular components destined for degradation. By promoting autophagy, FoxO1 helps the cell to clear damaged or misfolded proteins and organelles, thereby maintaining cellular integrity under metabolic stress.

Sirt1 deacetylates FoxO1 at lysine residues, such as Lys262. This deacetylation event enhances the DNA-binding affinity of FoxO1 for the promoters of antioxidant genes, such as manganese-superoxide dismutase (MnSOD)[45]. MnSOD is an important antioxidant enzyme that converts superoxide radicals to hydrogen peroxide, protecting the cell from oxidative damage. The combined action of AMPK-mediated phosphorylation and Sirt1-mediated deacetylation of FoxO1 amplifies the expression of genes involved in autophagy and antioxidant defense, enabling chondrocytes to adapt to metabolic stress.

2.3.3 Wnt/β-Catenin Interaction

The Wnt/β-catenin signaling pathway is involved in many aspects of chondrocyte biology, including chondrocyte differentiation, hypertrophy, and extracellular matrix (ECM) metabolism[46]. In the absence of Wnt ligands, β-catenin is phosphorylated by a destruction complex consisting of adenomatous polyposis coli (APC), axin, glycogen synthase kinase 3β (GSK3β), and casein kinase 1α (CK1α)[47]. Phosphorylated β-catenin is recognized by the ubiquitin-proteasome system and degraded.

FoxO1 plays a crucial role in modulating the Wnt/β-catenin pathway. FoxO1 can directly interact with β-catenin[48]. It sequesters β-catenin from TCF/LEF complexes in the nucleus. By binding to β-catenin, FoxO1 prevents β-catenin from associating with TCF/LEF transcription factors, thereby inhibiting the activation of Wnt-target genes.

This interaction between FoxO1 and β-catenin is important for maintaining the proper balance in chondrocyte differentiation and ECM homeostasis[49]. Inhibiting Wnt-driven chondrocyte hypertrophy is essential for preventing premature calcification of cartilage. Chondrocyte hypertrophy is a normal process during endochondral ossification, but its dysregulation can lead to pathological conditions such as osteoarthritis. By sequestering β-catenin, FoxO1 helps to keep chondrocytes in a more stable, non-hypertrophic state and preserves the integrity of the ECM.

The crosstalk between these signaling pathways provides a sophisticated regulatory mechanism for chondrocytes to respond to various extracellular signals and maintain their normal function. Any disruption in this crosstalk can lead to chondrocyte dysfunction and the development of cartilage-related diseases.

3. PHYSIOLOGICAL FUNCTIONS OF FOXO1 IN CHONDROCYTES

3.1. Proliferation and Cell Cycle Regulation

FoxO1 exerts context-dependent control over chondrocyte proliferation, balancing growth during development with protective arrest under stress.

Promotion in Embryonic Chondrogenesis

During early skeletal development, FoxO1 acts as a pro-proliferative factor in mesenchymal stem cells (MSCs) and chondrocyte progenitors[50]. In the growth plate, signaling from sonic hedgehog (Shh) and bone morphogenetic proteins (BMPs) primes MSCs to express FoxO1, which collaborates with transcription factors like Sox6 to upregulate Cyclin D1 and c-Myc[51]. These genes drive the G1/S transition, accelerating cell cycle progression and expanding the pool of chondrogenic progenitors. FoxO1 also enhances the expression of CDK4 and cyclin-dependent kinase inhibitor p21 (in a context-specific manner), ensuring controlled proliferation without compromising differentiation potential[52]. This mechanism is critical for the formation of the cartilaginous anlage, the precursor to long bones.

3.2. Differentiation and ECM Homeostasis

FoxO1 plays a central role in orchestrating chondrocyte differentiation and maintaining the extracellular matrix (ECM) by balancing anabolic synthesis and catabolic restraint across developmental and adult stages[53]. During early chondrogenesis, FoxO1 collaborates with the master transcription factor Sox9 to drive the expression of genes essential for matrix formation: it enhances transcription of Col2α1, the foundational component of hyaline cartilage fibrils, and Aggrecan, a proteoglycan critical for water retention and compressive resilience[54]. This Sox9-FoxO1 partnership is pivotal for establishing the chondrocyte phenotype and laying down a functional ECM in the developing growth plate. In mature chondrocytes, FoxO1 switches to a protective role, repressing genes associated with premature hypertrophy and ECM degradation. It directly inhibits Col10α1, a marker of hypertrophic chondrocytes whose overexpression disrupts the hyaline matrix, and MMP13, a matrix metalloproteinase that degrades collagen II and aggrecan[55, 56]. By sequestering β-catenin from TCF/LEF complexes, FoxO1 also dampens Wnt-driven hypertrophic signaling, preventing pathological calcification and matrix catabolism. This anti-hypertrophic function is critical in adult cartilage, where loss of FoxO1 accelerates age-related degeneration, characterized by increased Col10α1 expression and MMP13-mediated ECM breakdown.

3.3. Apoptosis and Survival

FoxO1 exerts dual roles in chondrocyte apoptosis, balancing cell survival under mild stress and promoting cell death under chronic damage to maintain tissue homeostasis[57]. Under low-level oxidative or metabolic stress, nuclear FoxO1 activates a pro-survival program, inducing anti-apoptotic proteins like Bcl-2 to stabilize mitochondrial membranes and upregulating antioxidant enzymes such as SOD2 and CAT to neutralize reactive oxygen species (ROS), thereby reducing mitochondrial damage and DNA oxidative lesions[58]. This mechanism is vital for chondrocytes in articular cartilage, which endure constant mechanical stress and low oxygen tension. Conversely, in the context of chronic inflammation—such as exposure to TNF-α or IL-1β—FoxO1 shifts to a pro-apoptotic role, activating Bim and PUMA, which promote

mitochondrial outer membrane permeabilization, cytochrome c release, and caspase cascade activation[59]. This mitochondrial pathway of apoptosis is crucial for eliminating irreparably damaged cells, but dysregulated FoxO1-mediated apoptosis in OA leads to excessive chondrocyte loss, disrupting tissue repair and accelerating cartilage degeneration [57]. The balance between these roles is finely tuned by post-translational modifications: Akt-mediated phosphorylation inactivates FoxO1 under growth factor-rich conditions, favoring survival, while JNK/AMPK-driven phosphorylation under stress promotes its nuclear translocation to initiate either protective arrest or apoptotic signaling based on damage severity.

3.4. Autophagy and Stress Resistance

FoxO1 serves as a master regulator of autophagy in chondrocytes, coordinating the cellular machinery for cargo degradation and recycling to maintain intracellular homeostasis and resilience against stress[60]. At the transcriptional level, FoxO1 directly binds to conserved response elements in the promoters of core autophagy genes, including Atg7, Beclin1, and LC3. These genes are essential for autophagosome biogenesis, Atg7 encodes an E1-like enzyme critical for the conjugation of Atg8 (LC3) to phosphatidylethanolamine, a key step in autophagosome membrane expansion; Beclin1 is a core component of the autophagy initiation complex, promoting the nucleation of autophagosomal vesicles; and LC3 marks mature autophagosomes, facilitating their fusion with lysosomes for cargo degradation[61]. By upregulating these genes, FoxO1 drives the formation of autophagosomes, enabling chondrocytes to clear misfolded proteins, damaged organelles, and invading pathogens—a process vital for surviving nutrient deprivation or oxidative stress.

Impairment of FoxO1-dependent autophagy and mitophagy has been strongly linked to cartilage degeneration in osteoarthritis (OA)[57]. In OA chondrocytes, sustained Akt activation or Sirt1 decline reduces FoxO1 nuclear localization and transcriptional activity, leading to downregulation of Atg7, Beclin1, and PINK1. This results in defective autophagosome formation, accumulation of undegraded cargo (ubiquitinated proteins, damaged mitochondria), and heightened oxidative stress. The resulting mitochondrial dysfunction further drives ECM catabolism by upregulating MMP13 and reducing Col2α1 synthesis, creating a vicious cycle of cellular and tissue damage. Thus, FoxO1 acts as a central hub for stress resistance in chondrocytes, integrating autophagic and mitophagic pathways to maintain cellular health and ECM integrity, with its dysfunction accelerating the degenerative processes in aging and OA.

4. FOXO1 IN PATHOLOGICAL CHONDROCYTE DYSFUNCTION

4.1. Osteoarthritis (OA): Dysregulated Stress Response and Matrix Degradation

In osteoarthritis, the most common degenerative joint disease, FoxO1 emerges as a critical node in the breakdown of chondrocyte homeostasis, with its dysfunction driving a cascade of catabolic events[60]. In the superficial zone of OA cartilage—where mechanical stress is highest—FoxO1 expression is significantly downregulated, correlating with reduced autophagic flux, heightened matrix metalloproteinase (MMP) activity, and increased chondrocyte apoptosis. Mechanistically, pro-inflammatory cytokines like IL-1β and TNF-α activate the NF-κB and MAPK pathways, leading to aberrant phosphorylation of FoxO1 at sites that promote cytoplasmic sequestration (Ser256 by Akt)[63]. This reduces nuclear FoxO1 availability, impairing its ability to transcribe protective genes such as PRG4 (lubricin), whose loss compromises the cartilage surface layer and increases friction-induced damage. Concurrently, FoxO1's inhibitory effect on ADAMTS5 (aggrecanase) is lifted, leading to enhanced degradation of aggrecan, the key proteoglycan responsible for cartilage resilience[64].

Chondrocyte-specific FoxO1 knockout mice exhibit accelerated OA-like changes, including fibrillation of the cartilage surface, loss of proteoglycan staining, and subchondral bone sclerosis[65]. These phenotypes arise from a combination of increased hypertrophic differentiation (marked by Col10α1 upregulation), enhanced MMP13-mediated collagen degradation, and reduced cell survival. Clinically, FoxO1 levels in OA synovial fluid correlate with disease severity, positioning it as a potential biomarker and therapeutic target to restore chondrocyte stress resistance and ECM balance.

4.2. Age-Related Cartilage Degeneration: Oxidative Stress, Senescence, and ECM Decline

Aging imposes a gradual decline in FoxO1 activity in chondrocytes, contributing to the progressive loss of cartilage integrity characteristic of geriatric joints[66]. This decline is driven by multiple factors, including reduced Sirt1-mediated deacetylation (due to declining NAD+ levels), increased Akt phosphorylation (from chronic growth factor signaling), and epigenetic silencing of the FoxO1 gene. The functional consequences are profound: diminished FoxO1 leads to reduced expression of antioxidant enzymes SOD2 and CAT, allowing ROS to accumulate and damage mitochondrial DNA, lipid membranes, and ECM components like collagen II and aggrecan. This oxidative damage weakens the cartilage matrix, making it more susceptible to mechanical failure.

Concurrently, FoxO1's role in suppressing cellular senescence is compromised in aging chondrocytes. In young cartilage, FoxO1 represses CDKN2A (p16INK4a), a key driver of senescence, by binding to its promoter and recruiting co-repressors like histone deacetylases[67]. This maintains chondrocyte proliferative capacity and delays the onset of senescence, characterized by flattened morphology, reduced matrix synthesis, and secretion of pro-inflammatory factors (the senescence-associated secretory phenotype, SASP). The cumulative effect of these changes—oxidative stress, ECM degradation, and senescence—leads to the characteristic features of age-related cartilage degeneration: loss of proteoglycan content, fissuring of the articular surface, and reduced repair capacity. Restoring FoxO1 function in

preclinical models of aging, via Sirt1 activation or Akt inhibition, has been shown to reduce senescence markers, enhance autophagy, and preserve ECM integrity, underscoring its therapeutic potential in combating age-related joint dysfunction. Taken together, FoxO1's pathological roles in OA, fracture healing, and aging highlight its centrality in integrating stress responses, matrix metabolism, and cellular fate decisions in chondrocytes. Disruption of its regulatory networks tips the balance toward catabolism, cell death, and failed repair, making it a pivotal target for developing interventions to preserve cartilage health in disease and aging.

5. THERAPEUTIC POTENTIAL OF FOXO1 TARGETING

5.1. Pharmacological Activation: Harnessing Signaling Pathways to Restore FoxO1 Function

Pharmacological strategies aimed at activating FoxO1 represent a promising approach to counteract chondrocyte dysfunction in degenerative diseases. These interventions primarily target upstream regulators of FoxO1 post-translational modifications, such as sirtuins and AMPK, to enhance its nuclear activity and protective functions.

Sirt1 Agonists

Compounds like resveratrol and nicotinamide (a NAD^+ precursor) activate the NAD^+-dependent deacetylase Sirt1, which removes acetyl groups from FoxO1 to enhance its DNA-binding affinity. Resveratrol, a natural polyphenol, specifically promotes Sirt1-mediated deacetylation at lysine residues (e.g., Lys262), leading to upregulation of autophagy genes (Atg7, Beclin1) and antioxidant enzymes (SOD2), while repressing MMP13 and ADAMTS5—key enzymes responsible for ECM degradation[67]. In preclinical models of OA, resveratrol treatment reduces chondrocyte apoptosis, enhances proteoglycan synthesis, and mitigates cartilage fibrillation by restoring FoxO1-dependent regulation of matrix homeostasis[68]. Nicotinamide, by boosting NAD^+ levels, amplifies Sirt1 activity, thereby improving FoxO1-mediated stress resistance in aged chondrocytes, reducing ROS accumulation, and delaying senescence.

AMPK Activators

Agents such as AICAR (5-aminoimidazole-4-carboxamide ribonucleotide) mimic energy stress to activate AMPK, which phosphorylates FoxO1 at Ser315, promoting its nuclear translocation and transcriptional activity. AICAR treatment in chondrocytes exposed to mechanical stress or inflammatory cytokines enhances FoxO1-dependent expression of autophagy regulators and anti-hypertrophic genes , while inhibiting catabolic pathways driven by NF-κB and MAPK signaling[69]. These effects translate to reduced ECM degradation and improved chondrocyte survival in preclinical OA models, positioning AMPK agonists as viable candidates for disease-modifying interventions.

5.2. Gene Therapy and Delivery

Advances in gene therapy and nanotechnology have revolutionized the landscape of FoxO1-targeted interventions, offering unprecedented precision in delivering therapeutic cargo to chondrocytes while circumventing the limitations of systemic drug delivery, such as off-target toxicity and poor bioavailability in the avascular cartilage niche. These approaches leverage sophisticated vectors and engineered materials to enhance local retention, cellular uptake, and functional activation of FoxO1, representing a paradigm shift toward tissue-specific regenerative medicine.

Viral Vector-Based Approaches

Viral vectors serve as powerful tools for introducing FoxO1 or its regulatory elements directly into chondrocytes, capitalizing on their natural tropism and transduction efficiency.

Adenovirus (Ad) Vectors

These vectors offer high transduction efficiency and the ability to carry large transgenes, making them ideal for acute FoxO1 overexpression. In vitro studies demonstrate that Ad-mediated FoxO1 delivery to primary human chondrocytes exposed to inflammatory cytokines (e.g., IL-1β) significantly reduces apoptosis by 30–40% compared to controls, achieved through upregulation of anti-apoptotic Bcl-2 and downregulation of pro-apoptotic Bim[70]. Mechanistically, FoxO1-overexpressing chondrocytes exhibit enhanced synthesis of cartilage matrix components (Col2α1, Aggrecan) and reduced secretion of catabolic enzymes (MMP13, ADAMTS5), even under pro-inflammatory conditions. In vivo, intra-articular injection of Ad-FoxO1 in mouse OA models delays cartilage degradation, as measured by reduced Safranin O staining loss and decreased subchondral bone remodelin[70]g. However, limitations include transient transgene expression (2–4 weeks) and potential immune responses to viral proteins, necessitating repeated dosing or immunosuppressive strategies.

Adeno-Associated Viruses (AAV)

Renowned for their low immunogenicity and ability to mediate long-term transgene expression (months to years), AAV vectors are emerging as a safer alternative for chronic FoxO1 activation. By using cartilage-specific promoters, AAV9-FoxO1 selectively targets articular chondrocytes in primate models, avoiding off-target effects in bone or muscle[71]. Preclinical studies in aging mice show that AAV-mediated FoxO1 delivery restores autophagic flux (Atg7, LC3 upregulation), reduces senescence markers (p16INK4a), and preserves articular surface integrity, highlighting its potential for age-related degeneration[72]. AAV vectors also enable co-delivery of FoxO1 with Sirt1 or AMPK activators in bicistronic constructs, synergistically enhancing stress resistance pathways.

6. CONCLUSION

The transcription factor FoxO1 is a central hub in chondrocyte regulation, integrating conserved structural features, dynamic post-translational modifications (phosphorylation, acetylation), and signaling crosstalk to balance proliferation, differentiation, extracellular matrix (ECM) homeostasis, and stress resistance (autophagy, antioxidant defense). Its dysregulation drives pathological processes in osteoarthritis (OA), including impaired autophagy, enhanced MMP-mediated ECM degradation, and chondrocyte apoptosis, while age-related declines in FoxO1 accelerate oxidative stress and cellular senescence. Conversely, FoxO1 orchestrates VEGFA-dependent angiogenesis during fracture healing, underscoring its dual role in health and disease. Therapeutic strategies targeting FoxO1—via pharmacological activation of Sirt1/AMPK, localized gene/nanoparticle delivery, or CRISPR-mediated modulation—aim to restore protective functions, suppress hypertrophy, and enhance stress resilience. As a pivotal regulator of chondrocyte fate, FoxO1 represents a transformative target for developing disease-modifying therapies to address unmet needs in OA, age-related joint degeneration, and other cartilage-associated disorders, offering innovative solutions to preserve ECM integrity and cellular homeostasis.

FUNDING
This study did not receive any funding in any form.
Ethics Statement
This review article does not contain any studies with human participants or animals performed by any of the authors. All cited experimental studies involving human or animal subjects were conducted in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Author Contributions
Conceptualization, T.C. and K.B.L.; methodology, T.C. and M.H.T.; investigation, T.C., M.H.T., and K.B.L.; resources, K.B.L.; data curation, T.C. and M.H.T.; writing—original draft preparation, T.C.; writing—review and editing, M.H.T. and K.B.L.; visualization, T.C. and M.H.T.; supervision, K.B.L.; project administration, K.B.L.; funding acquisition, T.C. and K.B.L. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest
The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data Availability Statement
No new data were created or analyzed in this review article. Data sharing is not applicable to this article.

Acknowledgments
The authors would like to thank Dr. Wei Zhang (Shanghai Jiaotong University School of Medicine) for his valuable comments during the preparation of this manuscript. We also acknowledge the administrative support provided by Xinhua Hospital and the technical assistance from the Faculty of Pharmacy, University of Cyberjaya.

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