

# Bidirectional Role of Sirt3 in Bone Metabolism Regulation and Translational Research Progress

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## Abstract

Sirt3 is a mitochondrial NAD<sup>+</sup>-dependent deacetylase whose downregulation is closely associated with osteoporosis, yet its complete mechanism of action and upstream signals remain unclear. This review systematically integrates *in vitro* and *in vivo* studies on the role of Sirt3 in bone metabolism, covering dimensions such as direct regulation, immuno-osteogenic coupling, upstream transcriptional control, and functional bidirectionality. Sirt3 maintains mitochondrial oxidative stress homeostasis by deacetylating SOD2 and FOXO3a, promotes energy metabolism and mitophagy, and positively regulates BMSC osteogenic differentiation. It also orchestrates an “mitochondria-immune-osteogenesis” interactive network by reprogramming M1/M2 polarization of macrophages. SENP3 activates Sirt3 transcription by deSUMOylating DLX2, establishing a novel upstream regulatory axis. However, Sirt3 function exhibits marked cell type- and age-dependence—it can promote bone resorption or degeneration in osteoclasts and chondrocytes, respectively, and under aging conditions its activity declines with NAD<sup>+</sup> depletion, leading to an imbalance between osteogenic and adipogenic differentiation. Although Sirt3 regulates bone metabolism through a multi-layered network, its functional polarity is co-determined by NAD<sup>+</sup> availability, oxidative stress, and upstream modifications. This review aims to move beyond the conventional view of Sirt3 as a mere “pro-bone” factor, offering new targets for individualized treatment of osteoporosis and emphasizing the need for precision intervention strategies tailored to cell type, age, and pathological microenvironment.

## Keywords

Sirt3, Osteogenic Differentiation, Mitochondria, sENP3-DLX2 Axis,

## 1. Introduction

Large bone defects caused by trauma, tumor resection, infection and nonunion caused by osteoporosis are one of the most difficult clinical problems in the field of orthopedics [1]. Autologous bone transplantation is recognized as the “gold standard” for repairing bone defects because of its osteogenetic, osteoinductive and osteoconductive properties [2]. However, this method has inherent defects such as limited source, high incidence of donor site morbidity, and prolonged operation time. When the defect volume exceeds a certain critical value, it is often difficult to meet the reconstruction needs by relying solely on autologous bone transplantation [3]. Therefore, it is of great clinical significance to deeply understand the cellular and molecular mechanisms of bone regeneration and to find biological treatment strategies that can replace or enhance autologous bone transplantation.

The repair of bone defects is highly dependent on the directional differentiation of bone marrow mesenchymal stem cells into osteoblasts, and tissue reconstruction is completed through bone matrix synthesis and mineralization. This differentiation process is not a simple phenotypic switch, but a biological event with high energy consumption and severe metabolic reprogramming [4]. A large amount of evidence shows that when osteogenic differentiation is initiated, the cell metabolic pattern shifts from glycolysis to mitochondrial oxidative phosphorylation (OXPHOS) to meet the huge demand for ATP in collagen synthesis and matrix mineralization [5]. At the same time, the enhanced activity of mitochondrial respiratory chain leads to the increase of reactive oxygen species (ROS) generation. Moderate ROS can be used as a signal molecule to promote the differentiation process, but excessive ROS can cause oxidative damage, inhibit osteogenesis and induce apoptosis [6]. Therefore, the precise regulation of mitochondrial metabolism and redox homeostasis constitutes the core bottleneck of osteogenic differentiation of BMSCs.

Sirtuin 3 (Sirt3) is a member of the NAD-dependent deacetylase Sirtuin family and the only Sirtuin mainly located in the mitochondrial matrix [7]. Sirt3 dynamically regulates the tricarboxylic acid cycle (TCA cycle), fatty acid  $\beta$ -oxidation, electron transport chain (ETC) complex activity and functional status of antioxidant defense system by deacetylating the acetyl group on the lysine residue of mitochondrial protein [8]. Since osteogenic differentiation is highly dependent on mitochondrial energy metabolism, Sirt3 has naturally become the focus of research in this field. Studies have shown that the mRNA and protein expression levels of Sirt3 were significantly up-regulated during osteogenic differentiation of BMSCs. After Sirt3 gene knockout or knockdown, osteogenic markers such as alkaline phosphatase (ALP), Runt-related transcription factor 2 (Runx2), and osteocalcin

(OCN) were significantly decreased, and the ability to form mineralized nodules was significantly weakened. Sirt3 knockout (Sirt3<sup>-/-</sup>) mice showed age-related bone mass loss, bone mineral density (BMD) and trabecular number were significantly lower than those in wild-type mice, and the phenotype was highly similar to osteoporosis [9]. Therefore, Sirt3 is initially defined as a positive regulator of osteogenic differentiation and bone formation of BMSCs.

However, the emerging research evidence in recent years has posed a fundamental challenge to this single “positive regulation” cognition. In the osteoclast lineage, the function of Sirt3 takes a very different direction: Studies have found that Sirt3 restricts osteoclast differentiation by inhibiting the NFATc1 transcription program [10] [11]. However, the study published in *Molecular Metabolism* in 2024 further revealed that Sirt3 promotes osteoclast mitochondrial activity and bone resorption by deacetylating mitochondrial proteins (such as ATP1F1) under aging conditions, and this effect is particularly significant in female mice [12]. In the radiation exposure model, Sirt3 has also been shown to promote osteoclast maturation and bone loss by regulating mitochondrial ROS production [13]. In chondrocytes, Sirt3 also exhibits microenvironment dependence: maintaining mitochondrial function and antioxidant defense under physiological conditions, but in metabolic overload situations such as high-fat diet, Sirt3-mediated mitochondrial overactivation exacerbates cartilage degeneration [14]. In addition, the enzymatic activity of Sirt3 was strictly dependent on NAD<sup>+</sup> as a co-substrate, while the NAD<sup>+</sup> level decreased significantly with age, forming an age-dependent decline of the “NAD<sup>+</sup>-Sirt3” functional axis [15]. This decline leads to the decrease of Sirt3’s ability to drive osteogenic differentiation of BMSCs, and the balance of osteogenic and adipogenic differentiation is biased towards the fat lineage, which is called the “fat switch” phenomenon and becomes an important pathological basis of senile osteoporosis [16].

The above findings together reveal a key scientific issue: the function of Sirt3 in bone metabolism is not a simple one-way promotion, but cell type-dependent, age-dependent and pathological context-dependent. “Promoting bone” in BMSCs /osteoblasts, promoting bone resorption in osteoclasts, maintaining bone homeostasis in young individuals, and functional polarity may be reversed in old age or under specific pathological conditions. This bidirectional nature poses a serious challenge to the development of therapeutic strategies targeting Sirt3. Generally activated Sirt3 may simultaneously enhance bone formation and bone resorption, and net bone mass changes are unpredictable. Therefore, it is very important to systematically elucidate the differential function of Sirt3 in different skeletal cell populations and its upstream regulatory network for the design of individualized treatment strategies that accurately target the Sirt3 pathway.

In addition to direct cell autonomous regulation, recent studies have also revealed a new dimension of Sirt3 indirectly regulating osteogenic differentiation through the bone immune microenvironment. Bone regeneration is an immune-driven process. The M1/M2 polarization state of macrophages directly determines

the pro-inflammatory or pro-repair direction of the local cytokine microenvironment [17]. Studies have shown that pathological conditions such as high glucose can inhibit the SIRT3/FoxO3a pathway of macrophages, increase mitochondrial ROS and reduce autophagy activity, drive macrophages to polarize to M1 pro-inflammatory phenotype, and then inhibit BMSCs osteogenesis through paracrine. Sirt3 overexpression or NAD precursor supplementation can promote the transformation of M2 anti-inflammatory/pro-repair phenotype and actively enhance the osteogenic differentiation ability of BMSCs [18]. This “mitochondrial-immune-osteogenic” triaxial interaction mode extends the function of Sirt3 from intracellular to intercellular communication. Further upstream regulatory mechanisms have also made important breakthroughs—SENP3 prevents its ubiquitination degradation by removing the SUMO2/3 modification on the transcription factor DLX2 protein, and the stable DLX2 directly binds to the Sirt3 gene promoter region and activates its transcription to form the SENP3-DLX2-Sirt3 regulatory axis [19]. In addition, SUMOylation has been shown to play a broad regulatory role in bone metabolism, and its dysregulation can lead to the destruction of bone homeostasis [20]. These findings directly link the deSUMOylation of proteins to the transcriptional regulation of Sirt3, providing a new molecular grasp for targeting the upstream pathway of Sirt3.

In summary, Sirt3, as a mitochondrial deacetylase, is a core molecular hub connecting NAD metabolism, mitochondrial oxidative homeostasis and osteogenic differentiation, but its function has significant multi-level network characteristics and microenvironment dependence. This article systematically reviews the molecular mechanism of Sirt3 regulating bone metabolism, focusing on direct regulation in cells-mitochondrial oxidative stress and energy metabolism reprogramming; Intercellular indirect regulation-immune-osteogenic coupling; Upstream transcription and post-translational modification control-SENP3-DLX2-Sirt3 axis; and the four dimensions of functional bidirectional cell type and age dependence, trying to go beyond the traditional “positive regulation” single narrative, to explore the differential function of Sirt3 in different skeletal cell lineages and pathological situations and its clinical transformation enlightenment, and to provide a systematic theoretical framework for individualized treatment of osteoporosis targeting Sirt3. It should be noted that the current conclusions supporting the role of Sirt3 in regulating bone metabolism are derived from different levels of research evidence. Direct evidence comes primarily from *in vitro* and *in vivo* studies on bone marrow mesenchymal stem cells (BMSCs) and the osteoblast lineage: Sirt3 knockout or knockdown significantly suppresses the expression of osteogenic differentiation markers (ALP, Runx2, OCN) and mineralized nodule formation, whereas Sirt3 overexpression enhances osteogenic capacity; Sirt3<sup>-/-</sup> mice exhibit age-related bone loss and an osteoporotic phenotype. These experiments directly demonstrate the positive regulatory role of Sirt3 in BMSCs/osteoblasts. Indirect evidence comes from studies on other bone-related cell types: in macrophages, Sirt3 indirectly influences BMSC osteogenic differentiation by regulating M1/M2 polarization (im-

muno-osteogenic coupling); in osteoclasts, the function of Sirt3 is differentiation stage-dependent and pathological context-dependent (inhibiting differentiation or promoting bone resorption); in chondrocytes, the role of Sirt3 shifts from protective to degeneration-exacerbating depending on metabolic status. This indirect evidence reveals the complexity and bidirectionality of Sirt3 in bone metabolism, but the conclusions are not derived directly from osteoblasts themselves; rather, they are extrapolated from cell-cell interactions or studies in different lineages. Therefore, when translating Sirt3 into a therapeutic target for osteoporosis, one should carefully weigh the two categories of evidence—“direct bone-forming effects” versus “indirect regulation of the bone microenvironment”—and avoid equating findings from osteoclasts or chondrocytes directly with functions in osteoblasts.

## **2. Biological Characteristics of Sirt3**

### **2.1. Structure and Catalytic Properties of Sirt3**

The Sirt3 gene is located at p15.5 on human chromosome 11. After entering the mitochondria, the N-terminal mitochondrial targeting sequence is removed by matrix processing peptidase (MPP) to form an active deacetylase. Sirt3 catalyzes the transfer of the  $\epsilon$ -acetyl group on the lysine residue of the substrate protein to NAD<sup>+</sup> to generate deacetylated proteins, 2'-O-acetyl-ADP-ribose and nicotinamide. This modification process can significantly change the activity, stability or cellular localization of the target protein [21].

The expression of Sirt3 is regulated by many factors, including energy restriction, exercise, oxidative stress, etc. Its most famous function is to activate key enzymes in mitochondrial metabolism, such as complex I (NDUFA9), acetyl-CoA synthase 2 (AceCS2), long-chain acyl-CoA dehydrogenase (LCAD), etc., thereby promoting the tricarboxylic acid cycle (TCA cycle), fatty acid  $\beta$ -oxidation and electron transport chain (ETC) efficiency, and enhancing ATP production [22].

### **2.2. The Enzyme Activity of Sirt3 Was Dependent on NAD Metabolic Homeostasis**

As a NAD dependent deacetylase, the catalytic activity of Sirt3 is strictly regulated by intracellular NAD level. NAD is not only a co-substrate, its availability directly determines the deacetylation efficiency of Sirt3 [23]. Therefore, the synthesis and consumption pathway of NAD becomes an upstream metabolic switch to regulate the function of Sirt3.

The synthesis of NAD is mainly dependent on the remedial synthesis pathway mediated by nicotinamide phosphoribosyltransferase (NAMPT). NAMPT converts nicotinamide into nicotinamide mononucleotide (NMN), which further generates NAD. In the process of osteogenic differentiation, the up-regulation of NAMPT expression can increase the level of NAD, thereby enhancing Sirt3 activity and promoting mitochondrial oxidative phosphorylation and osteogenic differentiation [24] [25]. In contrast, inhibition of NAMPT or the senescence-asso-

ciated decrease of NAMPT will lead to the depletion of NAD and weaken the Sirt3-mediated osteogenic protective effect [25]. Studies have shown that in the senile osteoporosis model, the expression of NAMPT in bone marrow mesenchymal stem cells (BMSCs) is significantly reduced. Supplementation of NMN can restore the function of NAD<sup>+</sup>-Sirt3 axis and improve bone formation [26].

The consumption of NAD is mainly mediated by PARP family (poly ADP ribose polymerase) and CD38. PARP consumes a large amount of NAD in the process of DNA damage repair, while CD38, as the main NAD hydrolase, is highly expressed in aging and inflammatory states [27]. In the field of bone metabolism, CD38 is highly expressed in osteoclast precursors, and its hydrolysis of NAD leads to the inhibition of Sirt3 activity, thereby relieving the negative regulation of Sirt3 on osteoclast differentiation and promoting bone resorption [28]. In addition, excessive activation of PARP-1 (such as oxidative stress induction) can also competitively consume NAD, indirectly inhibit Sirt3, and aggravate osteoblast dysfunction [29]. These findings suggest that the NAD metabolic network composed of NAMPT-PARP-CD38 is the upstream “master switch” of Sirt3 function, and its imbalance is an important pathological basis for osteoporosis and senescent bone loss.

In summary, targeting NAD metabolism (such as NAMPT activator, PARP inhibitor or CD38 antibody) can be used as a new strategy to indirectly promote osteogenic differentiation by restoring Sirt3 activity, and help to explain the age-dependent metabolic roots of Sirt3 bidirectionality.

### 3. Sirt3 Regulates Osteogenic Differentiation

#### 3.1. The Regulatory Role of Sirt3 in Osteogenic Differentiation

A large number of *in vitro* and *in vivo* experiments have confirmed that Sirt3 is a positive regulator of osteogenic differentiation. The mRNA and protein expression levels of Sirt3 are usually significantly up-regulated when osteogenic differentiation is induced in BMSCs or osteogenic precursor cells [9]. After knocking down or knocking out Sirt3 by technology, the expression of osteogenic differentiation markers (such as alkaline phosphatase ALP, Runt-related transcription factor 2 Runx2, osteocalcin OCN) was significantly reduced, and ALP staining and mineralized nodules were also significantly reduced [19] [30]. Conversely, overexpression of Sirt3 can enhance osteogenic differentiation. Sirt3 gene knockout (Sirt3<sup>-/-</sup>) mice showed age-related bone loss, and bone mineral density (BMD), trabecular number and quality were significantly lower than those in wild-type mice [31]. Its phenotype is highly similar to osteoporosis, further confirming the key role of Sirt3 in maintaining bone homeostasis.

#### 3.2. Sirt3 Indirectly Regulates Osteogenic Differentiation through Immune-Osteogenic Coupling

In addition to directly acting on BMSCs/osteoblasts, recent studies have revealed that Sirt3 can also indirectly affect osteogenic differentiation by regulating the

bone immune microenvironment, which expands the intercellular interaction dimension of Sirt3 function. The research system published in Scientific Reports in 2025 clarified the key role of Sirt3 in macrophage polarization under high glucose environment and its indirect regulation mechanism on osteogenic differentiation of BMSCs.

In this study, the *in vitro* model of RAW264.7 macrophages induced by high glucose (HG) was used. It was found that the absence of Sirt3 resulted in a significant increase in the polarization of macrophages to M1 type (pro-inflammatory type), which was manifested by up-regulation of inducible nitric oxide synthase (iNOS) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). At the same time, the expression of M2 (anti-inflammatory/repair-promoting) markers arginase-1 (Arg-1) and CD206 decreased. After co-culture of Sirt3/macrophage conditioned medium with BMSCs, the osteogenic differentiation ability of BMSCs was significantly inhibited, ALP activity, mineralized nodule formation and Runx2, OCN expression were significantly decreased. Conversely, Sirt3 overexpression significantly reversed high glucose-induced M1 polarization and promoted M2 phenotype transformation, and its conditioned medium regained the ability to promote BMSCs proliferation, migration and osteogenic differentiation. NMN precursor therapy also showed similar effects in related studies [18].

At the mechanism level, Sirt3 affects mitochondrial metabolism and autophagy activity of macrophages by regulating FoxO3a signaling pathway: high glucose environment significantly inhibits SIRT3/FoxO3a pathway in macrophages, resulting in increased mitochondrial ROS level and decreased autophagy activity, thus driving M1 polarization program; overexpression of Sirt3 can effectively reduce ROS levels and restore autophagy activity, promote M2 polarization, and then indirectly enhance the osteogenic differentiation ability of BMSCs by regulating the immune microenvironment. The tri-axial interaction of “mitochondria-immunity-osteogenesis” represents a new progress in the field of osteoimmunology [18].

The clinical significance of this finding is that bone metabolic diseases such as osteoporosis and rheumatoid arthritis are often accompanied by chronic low-grade inflammation and M1 macrophage polarization. By reprogramming macrophages into M2 phenotype, Sirt3 not only alleviates the inhibition of inflammatory microenvironment on osteogenic differentiation, but also actively promotes bone regeneration through cytokines such as bone morphogenetic proteins (BMPs) and transforming growth factor- $\beta$  (TGF- $\beta$ ) secreted by M2 macrophages. Therefore, the bone immune regulation strategy targeting Sirt3 may be more advantageous than the simple osteoblast targeted therapy, because it simultaneously solves the two key pathological links of “inflammation inhibiting osteogenesis” and “insufficient osteogenesis ability”.

#### **4. The Molecular Mechanism of Sirt3 Regulating Osteogenic Differentiation**

Sirt3 promotes osteogenic differentiation through multiple parallel and interre-



lated pathways, and its core is to maintain mitochondrial health.

#### 4.1. Regulation of Mitochondrial Oxidative Stress Homeostasis

During osteogenic differentiation, increased mitochondrial respiratory chain activity leads to increased ROS levels. Moderate ROS can act as a signal molecule to promote differentiation, but excessive ROS can cause oxidative damage, inhibit differentiation and induce apoptosis [6]. Sirt3 is the core of mitochondrial antioxidant defense system. Sirt3 directly deacetylates the K68 site of SOD2 and significantly enhances its activity of scavenging superoxide anion, thereby protecting cells from oxidative damage [32]. Sirt3 can deacetylate the transcription factor FOXO3a and promote its nuclear translocation, thereby up-regulating the expression of antioxidant genes such as catalase and manganese superoxide dismutase (MnSOD, SOD2), forming a positive feedback loop [33]. Through the above mechanism, Sirt3 effectively maintains the “redox window” required for osteogenic differentiation and creates a stable intracellular environment for the differentiation process.

#### 4.2. Regulating Energy Metabolism Reprogramming

Osteogenic differentiation is accompanied by a shift in metabolic patterns from glycolysis to oxidative phosphorylation (OXPHOS) to meet huge energy needs. Sirt3 is a key driver of this metabolic reprogramming. Sirt3 enhances the efficiency of electron transport and oxidative phosphorylation by deacetylating the key subunits of the mitochondrial electron transport chain complex (such as Ndufs1 and Ndufs3 of complex I, SDHA of complex II, and ATP5A1 of ATP synthase), thereby increasing the efficiency of ATP production [34]. Abundant ATP provides an energy basis for the synthesis of collagen and mineralization of osteoblasts. Sirt3 activates enzymes such as LCAD, promotes the utilization of fatty acids, and provides more fuel for OXPHOS [22]. This is particularly important for maintaining osteoblast function under energy constraints.

#### 4.3. Regulation of Autophagy

Autophagy is an important process to remove damaged organelles and provide nutrients, and has a two-way regulatory role in osteogenic differentiation. Moderate autophagy is conducive to differentiation. Studies have shown that Sirt3 can indirectly protect mitochondria by reducing ROS levels and reduce excessive autophagy caused by mitochondrial damage [33]. At the same time, studies have also suggested that Sirt3 may regulate autophagy-related proteins (such as ATG5, ATG7) by deacetylation, and regulate mitophagy through the FOXO3a-PINK1-Parkin axis, thus providing support for osteogenic differentiation [34] [35].

#### 4.4. Regulate Related Signaling Pathways

Sirt3 may indirectly regulate osteogenic differentiation by affecting multiple sig-



naling pathways. Under hypoxic conditions, Sirt3 can inhibit glycolysis by promoting the degradation of HIF-1 $\alpha$ , thereby indirectly promoting osteogenesis [36]. Sirt3 may indirectly promote  $\beta$ -catenin-dependent osteogenic differentiation by inhibiting GSK-3 $\beta$  activity and reducing the phosphorylation degradation of  $\beta$ -catenin [37]. However, its specific interaction mechanism still needs to be further explored. Sirt3 may inhibit the excessive activation of NF- $\kappa$ B through its antioxidant effect, thereby reducing the inhibition of inflammatory response on osteogenic differentiation [38] [39].

#### 4.5. The Upstream Regulatory Mechanism of Sirt3: SEN3-Mediated DLX2 deSUMOylation Modification Axis

The above mechanisms all focus on how Sirt3 regulates osteogenic differentiation by deacetylating downstream substrate proteins, but it is not clear what upstream signals regulate the transcriptional expression of Sirt3 itself. A study published in *Cell Biology and Toxicology* in 2025 revealed a key upstream transcriptional regulation mechanism of Sirt3 expression—SEN3 (Sentrin/SUMO-specific protease 3)-mediated DLX2 deSUMOylation modification axis.

This study found that SEN3 stabilizes DLX2 protein levels by removing SUMO2/3 modification on DLX2 (Distal-less homeobox 2) protein and preventing ubiquitination degradation of DLX2. As a transcription factor, DLX2 directly binds to the promoter region of Sirt3 gene and activates the transcriptional expression of Sirt3. In osteoblast-specific SEN3 conditional knockout mice, the increased SUMOylation level of DLX2 accelerated its degradation, and the expression of Sirt3 was significantly down-regulated, accompanied by decreased osteogenic differentiation markers (Runx2, OCN, ALP) and decreased bone mineral density, showing a significant osteoporosis phenotype. On the contrary, overexpression of SEN3 or DLX2 can restore Sirt3 expression and promote osteogenic differentiation [19].

The significance of this finding is as follows: Firstly, it is the first time to link protein deSUMOylation with the transcriptional regulation of Sirt3, expanding the epigenetic dimension of Sirt3 regulatory network. Secondly, the SEN3-DLX2-Sirt3 axis provides a new idea for the development of upstream activation strategies targeting Sirt3. For example, the development of SEN3 agonists or DLX2 stabilizers may indirectly enhance Sirt3 expression and avoid off-target effects that may be caused by direct activation of Sirt3. Finally, this study verified the therapeutic potential of A-485 (a SEN3 activity-regulating compound) to restore bone mass through this axis in a postmenopausal osteoporosis model, providing a candidate drug for clinical transformation [19].

It is worth noting that the SEN3-DLX2-Sirt3 axis forms a complete regulatory loop with the classical downstream mechanism of Sirt3: SEN3  $\rightarrow$  DLX2  $\rightarrow$  Sirt3 transcription  $\rightarrow$  mitochondrial deacetylation  $\rightarrow$  osteogenic differentiation. This “upstream-downstream” understanding helps to design more accurate intervention strategies from the perspective of systems biology.

## 5. Sirt3 Regulates the Cell Type-Dependent and Age-Dependent Bidirectionality of Bone Metabolism

Although existing studies have focused on the positive regulation of Sirt3 in osteoblasts/bone marrow mesenchymal stem cells (BMSCs), in recent years, more and more evidence has shown that the function of Sirt3 in bone metabolism is significantly cell type-dependent and age-dependent, and even presents diametrically opposite biological effects in specific situations. This bi-directionality is of great significance for the clinical transformation strategy of Sirt3 as a therapeutic target for osteoporosis.

### 5.1. Cell Type Dependence: Lineage Differences from Osteogenic Promotion to Osteoclast Regulation

#### 5.1.1. Sirt3 in Osteoclasts: Dual Roles of Differentiation Inhibition and Function Maintenance

Different from the differentiation-promoting effect in osteoblasts, Sirt3 exhibits a more complex regulatory pattern in the osteoclast lineage. The classic study published in *Scientific Reports* in 2016 systematically elucidated the stage-specific role of Sirt3 in the functional cell lineage of osteoclasts for the first time. In the early stage of differentiation, NFATc1 transcription program was inhibited to limit osteoclastogenesis. Bone marrow macrophages (BMMs) of Sirt3 knockout mice showed enhanced osteoclast differentiation ability under RANKL stimulation, which was manifested by a significant increase in the number of TRAP-positive multinucleated cells and up-regulation of NFATc1 and its downstream target gene *Atp6v0d2* [11]. This indicates that Sirt3 has a negative regulatory effect on osteoclast differentiation. However, when the same number of mature osteoclasts were placed on dentin slices, there was no significant difference in bone resorption area between Sirt3/and wild-type osteoclasts, suggesting that Sirt3 is not involved in the regulation of bone resorption function of mature osteoclasts [11].

This finding reveals that Sirt3 is generated in osteoclasts, but has no significant effect on its bone resorption activity after differentiation and maturation. However, this conclusion is different in the radiation damage model. A study published in *JBMR Plus* in 2025 showed that Sirt3 promotes osteoclast maturation and bone loss by regulating mitochondrial ROS production under the condition of ionizing radiation exposure, showing that Sirt3/mice are resistant to radiation-induced bone loss [13]. This difference suggests that the role of Sirt3 in osteoclasts may be significantly affected by the pathological microenvironment (such as oxidative stress level, inflammatory state).

#### 5.1.2. Sirt3 in Chondrocytes: A Double-Edged Sword of Metabolic Adaptation and Degeneration

In chondrocytes, the function of Sirt3 is also context-dependent. A study published in *Arthritis & Rheumatology* in 2016 found that aging leads to a decrease in Sirt3 protein content in articular cartilage, accompanied by excessive acetylation and decreased activity of SOD2, which aggravates oxidative stress and degen-

eration of chondrocytes. Sirt3 knockout mice showed early pathological changes of osteoarthritis (OA) at 14 months of age, suggesting that Sirt3 is essential for maintaining cartilage homeostasis [40].

However, a study published in *Journal of Bone and Mineral Research* in 2023 revealed a seemingly contradictory finding: in a high-fat diet-induced obesity-related OA model, Sirt3 promotes mitochondrial respiration and cartilage formation in chondrocytes, but Sirt3 deficiency reduces the severity of high-fat diet-induced OA [14]. This “protective loss” phenomenon suggests that under metabolic stress (such as high-fat environment), Sirt3-mediated mitochondrial metabolism enhancement may exceed the physiological tolerance of chondrocytes, leading to excessive mitochondrial activation and secondary damage. Therefore, the role of Sirt3 in chondrocytes is metabolic state-dependent: it maintains mitochondrial function and antioxidant defenses under physiological conditions, but may exacerbate cellular stress by overactivating metabolic pathways under overnutrition.

## **5.2. Age Dependence: Lineage Bias from Osteogenic Advantage to Adipose Differentiation**

### **5.2.1. Age-Related Changes in the Differentiation Potential of BMSCs**

The regulatory effect of Sirt3 on BMSCs differentiation changes significantly with age, which is the core to understanding the bidirectionality of bone metabolism. The study published in *Oxidative Medicine and Cellular Longevity* in 2017 confirmed that the expression of Sirt3 in MSCs gradually decreased with the increase of passage times *in vitro*, accompanied by the accumulation of aging markers and the increase of oxidative stress level. Sirt3 knockdown not only inhibits osteogenic differentiation, but also inhibits adipogenic differentiation, while overexpression of Sirt3 can restore the bidirectional differentiation ability of late passage MSCs [41].

However, this *in vitro* finding is different from the *in vivo* aging model. A study published in *Redox Biology* in 2021 showed that in the senescent osteoporosis (SAMP6 mice) model, advanced glycation end products (AGEs)-induced BMSCs senescence was accompanied by down-regulation of Sirt3 expression and impaired mitophagy, resulting in inhibition of osteogenic differentiation and enhancement of adipogenic differentiation [35]. This bias of “osteogenic-adipose differentiation balance” to the fat lineage (“fat switch” phenomenon) is a hallmark feature of aging bones. It is worth noting that Sirt3 overexpression can reverse AGEs-induced BMSCs senescence and osteogenic dysfunction by restoring mitophagy and reducing ROS levels.

### **5.2.2. The Age-Related Decay of the NAD<sup>+</sup>/ Sirt3 Axis**

The enzyme activity of Sirt3 is strictly dependent on NAD as a substrate, while NAD levels decrease significantly with age, forming an age-dependent decline in the “NAD-Sirt3” functional axis. A study published in *Scientific Reports* in 2024 found that in elderly women (65 - 80 years old) adipose-derived stem cells, the

expression of Sirt3 protein showed a downward trend (although not statistically significant), accompanied by increased ROS production and decreased adipogenic differentiation. In the bone marrow microenvironment, this NAD depletion leads to a decrease in Sirt3 deacetylase activity, which in turn causes excessive acetylation of mitochondrial proteins (such as SOD2, PGC-1 $\alpha$ ), mitochondrial dysfunction and oxidative stress accumulation [42].

This age-related decline in Sirt3 function has dual consequences: on the one hand, it weakens the osteogenic differentiation ability of BMSCs, and on the other hand, it destroys the normal regulation of osteoclast differentiation (because the inhibitory effect of Sirt3 on osteoclasts also depends on NAD), eventually breaking the dynamic balance between bone resorption and bone formation. Therefore, the “bidirectional” of Sirt3 in different age groups is not its inherent property, but the result of NAD availability and cell energy state changes.

### 5.3. Pathological Context Dependence: Oxidative Stress Level Determines the Functional Polarity of Sirt3

Another important dimension of the bidirectional function of Sirt3 is the influence of pathological microenvironment. At the level of physiological oxidative stress, Sirt3 maintains ROS homeostasis and promotes osteogenic differentiation by deacetylating SOD2 (K68 site) and FOXO3a [34]. However, under conditions of excessive oxidative stress (such as radiation, inflammation, aging), the compensatory ability of Sirt3 may reach saturation, and its function presents an “inverted U-shaped” curve: moderate activation is beneficial, but excessive activation may interfere with the necessary signal transduction by excessive scavenging of ROS, or affect other pathways through non-specific deacetylation.

In addition, Redox Biology’s study on the SENP3-DLX2 axis in 2025 found that the upstream regulator SENP3 stabilizes DLX2 by deSUMOylation, thereby promoting Sirt3 transcription [19]. This finding suggests that the function of Sirt3 is also regulated by the level of post-translational modification network. In different pathological situations, the expression level and substrate specificity of Sirt3 may change due to differences in upstream signals.

### 5.4. Implications and Challenges for Clinical Transformation

The bidirectional nature of Sirt3 in bone metabolism poses a serious challenge for the development of targeted therapy strategies:

First, tissue-specific delivery problems. Because Sirt3 plays different or even opposite roles in osteoblasts, osteoclasts and chondrocytes, systemic Sirt3 activation (such as NAD precursor supplementation) may simultaneously enhance osteoblast function (expected effect) and relieve inhibition of osteoclast differentiation (unexpected effect), resulting in uncertain changes in net bone mass.

Second, the necessity of age-stratified treatment. In young individuals, the level of NAD is sufficient and the activity of Sirt3 is high. At this time, the supplementation of NAD precursor may have limited benefits; in older individuals, the NAD

pool is depleted and the activity of Sirt3 is decreased. At this time, activation of Sirt3 may be more effective in restoring osteogenic-osteoclastic balance. This suggests that Sirt3 targeted therapy should be accurately stratified according to the patient's age and bone metabolism status.

Third, pathological microenvironment assessment. In inflammatory bone loss (such as rheumatoid arthritis) or radiation injury models, the role of Sirt3 may be mainly to promote osteoclastic effect. At this time, Sirt3 activation may aggravate the condition rather than improve bone mass. Therefore, it is necessary to evaluate the level of local oxidative stress and inflammatory state before treatment.

Fourth, the development direction of Sirt3-specific agonists. In recent years, several Sirt3-specific small molecule agonists have been reported: Honokiol (honokiol) is the most deeply studied natural Sirt3 agonist at present, which can enhance its activity by directly binding to Sirt3 protein and up-regulate its expression through PGC1 $\alpha$  positive feedback. Honokiol shows significant bone protective effect-2-3 in bone loss model, diabetic fracture healing model and periodontitis model in elderly mice; c12 (7-hydroxy-3-(4-methoxyphenyl) coumarin) is the first small molecule that specifically directly activates Sirt3, and its derivative SZC-6 has a stronger allosteric activation effect, regulating oxidative stress-20 through the SIRT3-Foxo3a-MnSOD axis. In addition, resveratrol and melatonin can indirectly up-regulate Sirt3 through the AMPK/PGC-1 $\alpha$  pathway. Compared with systemic NAD supplementation, the development of Sirt3 agonists with bone tissue targeting and cell type selectivity (such as osteoblast-specific delivery systems) may be a key strategy to avoid bidirectional risks. In addition, combined regulation of Sirt3 upstream factors or downstream specific substrates may be more safe than direct activation of Sirt3.

### 5.5. Sources of Inconsistency That Need Attention

Although existing evidence points to a bidirectional regulatory role of Sirt3 in bone metabolism, findings across studies are often inconsistent. Much of this actually stems from differences in experimental conditions. First, cell type matters a lot—the same Sirt3 can promote bone formation in osteoblasts but may enhance resorption in osteoclasts, and its role even changes between early and late stages of osteoclast differentiation. Sex is another factor that is frequently overlooked. Some studies have shown that Sirt3-driven bone resorption is significant only in female mice, with little effect in males, yet most papers don't analyze data separately by sex. Age is an unavoidable variable: in young mice, NAD<sup>+</sup> levels are high and Sirt3 is highly active, mostly doing “good things”; in aged mice, NAD<sup>+</sup> declines, Sirt3 struggles to function, and its substrate preference may shift, leading to different outcomes. Regarding species, the vast majority of data come from mice—often C57BL/6 Sirt3 knockout strains—while data from rats or human cells are sparse. Bone metabolism regulation differs considerably between humans and mice, and human primary cells tend to show spontaneous Sirt3 downregulation after a few passages in culture, which can easily mislead interpretations. Finally, the gap be-

tween *in vitro* and *in vivo* findings is substantial. Cells in a dish are removed from hormonal, mechanical, and immune cues from the body; Sirt3 overexpression may look impressive in culture, but when tested in animals, it has to contend with age-related NAD<sup>+</sup> deficiency, compensatory mechanisms from other sirtuins, and other real-world complications. Knockout mice also exhibit developmental compensation, and different labs raise mice with distinct gut microbiota, diets, and baseline oxidative stress levels—all of which can skew results. So when you come across a phenomenon like “protective loss,” don’t jump to conclusions—it’s likely that one of these factors is quietly at play. Future studies would do well to treat sex, age, species, and *in vivo* vs. *in vitro* comparisons as independent variables; otherwise, it will be hard to truly clarify Sirt3’s real role in bone metabolism.

## 6. The Pathological Mechanism of Sirt3-Related Bone Diseases

The dysfunction of Sirt3 is closely related to a variety of bone diseases, especially osteoporosis. With the increase of age, the decrease of NAD<sup>+</sup> level leads to the decrease of Sirt3 activity, the decline of mitochondrial function and the accumulation of ROS, which accelerates the dysfunction and apoptosis of osteoblasts, breaks the balance between bone resorption and bone formation, and leads to bone loss. Estrogen deficiency inhibits the expression of Sirt3 and aggravates oxidative stress, which is one of the reasons why postmenopausal women are prone to osteoporosis [35] [43]. Long-term use of glucocorticoids can inhibit the expression of Sirt3, leading to mitochondrial damage and osteoblast apoptosis [44]. Therefore, targeted activation of Sirt3 is considered as a potential new strategy for the treatment of osteoporosis. For example, supplementation of NAD<sup>+</sup> precursors (such as nicotinamide mononucleotide NMN) can enhance Sirt3 activity by increasing NAD<sup>+</sup> levels, which has been shown to improve bone mineral density in animal models [45]. The search for specific small molecule agonists of Sirt3 is also a hot spot in current drug research and development.

## 7. Summarized and Prospected

In summary, Sirt3, as a mitochondrial NAD-dependent deacetylase, is a key positive regulator of osteogenic differentiation of mesenchymal stem cells. At the molecular level, Sirt3 regulates the osteogenic differentiation process from four dimensions by deacetylating a variety of substrate proteins. First, it maintains mitochondrial ROS homeostasis by activating antioxidant proteins such as SOD2 and FOXO3a, and provides a suitable redox microenvironment for osteogenic differentiation. Secondly, by activating the key enzymes of mitochondrial metabolism (such as Ndufs1, SDHA, ATP5A1), the metabolic reprogramming from glycolysis to oxidative phosphorylation is promoted to meet the high energy demand of osteogenic differentiation. Third, the FOXO3a-PINK1-Parkin axis regulates mitophagy, clears damaged mitochondria, and maintains cell quality; fourthly, through cross-talk with HIF-1 $\alpha$ ,  $\beta$ -catenin, NF- $\kappa$ B and other signaling molecules, it indi-

rectly affects osteogenic differentiation. *In vivo* experiments further confirmed that Sirt3 knockout mice showed age-related bone loss, while Sirt3 overexpression enhanced bone formation function.

However, it should be noted that the role of Sirt3 in bone metabolism is not a simple one-way promotion, but a significant cell type-dependent and age-dependent bidirectional. In the osteoclast lineage, Sirt3 negatively regulates osteoclast differentiation by inhibiting the NFATc1 transcription program, but under excessive oxidative stress conditions such as radiation damage, Sirt3 may in turn promote osteoclast maturation and bone loss. In chondrocytes, Sirt3 maintains mitochondrial function and antioxidant defense under physiological conditions, but in metabolic overload situations such as high-fat diet, its mediated mitochondrial overactivation exacerbates cartilage degeneration. In bone marrow mesenchymal stem cells, the function of Sirt3 is biased with age: in young state, it preferentially supports osteogenic differentiation, while aging-associated NAD depletion leads to a decrease in Sirt3 activity, breaking the balance of osteogenic-adipose differentiation, promoting adipogenesis and inhibiting bone formation. This bi-directionality suggests that the efficacy of Sirt3 as a therapeutic target is highly dependent on target cell type, tissue microenvironment and patient age.

At the same time, the dysfunction of Sirt3 is closely related to a variety of bone metabolic diseases. Decreased aging-related NAD levels, estrogen deficiency, and long-term use of glucocorticoids can lead to decreased Sirt3 activity, increased mitochondrial dysfunction and oxidative stress, and ultimately break the dynamic balance between bone resorption and bone formation. Therefore, targeted activation of Sirt3 (such as supplementation of NAD precursor or development of specific small molecule agonists) provides a promising new strategy for the prevention and treatment of bone metabolic diseases such as osteoporosis. However, it must be recognized that systemic Sirt3 activation may simultaneously enhance osteoblast function (expected effect) and relieve the inhibition of osteoclast differentiation (undesired effect), and its net bone mass effect is uncertain. Therefore, the development of Sirt3 agonists with bone tissue targeting and cell type selectivity, or combined regulation of Sirt3 upstream factors (such as SENP1-DLX2 axis) to achieve more accurate intervention, will be an important direction for future drug development.

Clinical transformation still faces challenges. Future research needs to be further explored in the above directions in order to transform the basic research results of Sirt3 into effective clinical interventions. Specifically, the following aspects should be further deepened:

**1. Systematic construction of Sirt3 osteoblast-related substrate spectrum:** At present, the known substrates of Sirt3 in osteoblasts are limited to a few proteins such as SOD2 and FOXO3a, and its complete substrate spectrum remains to be revealed. In the future, we should combine high-sensitivity mass spectrometry technology and new deacetylation modification antibodies to dynamically identify Sirt3 substrate proteins at different osteogenic differentiation stages and construct



Sirt3-mediated mitochondrial protein acetylation modification map. For example, using TMT-labeled quantitative acetylome combined with osteogenic differentiation time series samples to establish a dynamic map of Sirt3 substrates, or “developing Sirt3 conditional knock-in mouse model to distinguish cell type-specific functions”. This will help to fully understand the molecular network of Sirt3 regulating osteogenic differentiation and may find new intervention targets.

**2. To elucidate the synergistic regulatory mechanism of Sirt3 and other Sirtuin members:** Sirt3 does not play an isolated role. Studies have shown that Sirt1 can affect mitochondrial biogenesis through PGC-1 $\alpha$ , and Sirt6 can regulate glucose metabolism, both of which are closely related to osteogenic differentiation. However, it is not clear whether there is a synergistic or antagonistic effect between Sirt3 and other Sirtuin family members in the process of osteogenic differentiation, and how to establish the upstream and downstream regulatory relationship. The analysis of the cross-talk network among Sirtuins family members will help to understand the systematic regulation of deacetylation in bone metabolism from a more macroscopic perspective.

**3. To analyze the specific role of Sirt3 in different cell types:** In view of the differential or even opposite roles of Sirt3 in osteoblasts, osteoclasts and chondrocytes, in the future, single-cell sequencing, lineage tracing and conditional gene knockout techniques will be used to analyze the specific functions and molecular mechanisms of Sirt3 in different bone cell populations at single-cell resolution. It is of great significance for precise targeted intervention to clarify the conditions under which the “bone-promoting” and “bone-breaking” effects of Sirt3 are dominant.

**4. Promoting clinical transformation and precise intervention strategies:** Although supplementation of NAD precursors (such as nicotinamide mononucleotides) has shown the potential to improve bone mineral density in animal models, the development of Sirt3-specific small molecule agonists is still in its early stages. In the future, a more complete drug screening platform needs to be established to systematically evaluate the efficacy and safety of Sirt3 agonists in a variety of osteoporosis animal models (including aging, postmenopausal and glucocorticoid-induced osteoporosis). In addition, considering the wide expression and bidirectional role of Sirt3 in different cells, tissues, and pathological situations, how to achieve precise targeted drug delivery in bone tissue and how to perform stratified treatment according to the patient’s age and bone metabolic status to avoid potential off-target effects will be a technical bottleneck that needs to be overcome in clinical transformation.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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