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REVIEW ARTICLE

Modulation of SIRT6 activity acts as an emerging therapeutic implication for pathological disorders in the skeletal system

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SIRT6 activator

Abstract The skeletal system is a dynamically balanced system, which undergoes continuous bone resorption and formation to maintain bone matrix homeostasis. As an important ADP-ribosylase and NAD^+-dependent deacylase, SIRT6 (SIR2-like protein 6) is widely expressed on various kinds of bone cells, such as chondrocytes, osteoblasts, osteoclasts. The aberration of SIRT6 impairs gene expression (e.g., NF-κB and Wnt target genes) and cellular functions (e.g., DNA repair, glucose and lipid metabolism, telomeric maintenance), which disturbs the dynamic balance and ultimately leads to several bone-related diseases. In this review, we summarize the critical roles of SIRT6 in the onset and progression of bone-related diseases including osteoporosis, osteoarthritis, rheumatoid arthritis, and intervertebral disc degeneration, as well as the relevant signaling pathways. In addition, we discuss the advances in the development of SIRT6 activators and elucidate their pharmacological profiles, which may provide novel treatment strategies for these skeletal diseases.

Introduction

The skeletal system undergoes a dynamic balance of bone matrix metabolism with the osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Several kinds of cells, cytokines, hormones, and mechanical stimulation regulate bone remodeling and maintain the physiological functions in a normal statement. However, these cells

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may perform dysfunctional cytological events including cellular proliferation, cellular senescence, cellular metabolism, and inflammation once the dynamic balance is destroyed. These pathological changes might result in many bone-related diseases such as osteoporosis (OP), osteoarthritis (OA), rheumatoid arthritis (RA), intervertebral disc degeneration (IDD) and so on (Table 1, 2 and Fig. 1—3).

The deacetylation and acetylation of histones function an important role in gene expression, which are mainly catalyzed by histone deacetylases (HDACs) and histone acetylases (HATs), respectively.[1,2] The first discovered class III HDAC was silence information regulator 2 (sir2), which regulates lifespan and cellular aging in *Saccharomyces cerevisiae*.[3,4] Indeed, sir2 also appears in other species and is collectively known as sirtuins, which are composed of seven members SIRT1—7 in mammals.[5—7] SIRT6 (sir2-like protein 6), a NAD^+-dependent deacetylase, targets the acetylated lysines K9, K18, and K56 on histone H3.[8—10] SIRT6 enhances chromatin accessibility and thereby promoting transcription of target genes by pivotal deacetylase activity.[11,12]

Recent studies have shown that SIRT6 played multitasking roles, including cellular differentiation and senescence, anti-inflammation, cellular metabolism, and so on.[13—15] These regulatory functions also exist in the skeletal system. For instance, overexpression of SIRT6 suppresses the inflammatory response and bone destruction in a mouse model of arthritis and delays senescence and apoptosis of nucleus pulposus (NP) cells in the IDD mouse model.[16,17] Consistently, Zhang DM et al. demonstrated SIRT6 deficiency induces OP in mice.[18] Indeed, more and more evidence shows that SIRT6 performs crucial functions in skeletal diseases such as OP, RA, OA, and IDD. Therefore, in this review, we summarize the critical regulatory role of SIRT6 in multiple bone cells including chondrocytes, osteoblasts, osteoclasts, and NP cells, and discuss the mechanisms by which SIRT6 disorders mediate bone-related diseases. We believe that SIRT6 may serve as a novel therapeutic target to relieve the poor progression of these pathological disorders in the skeletal system.

Structural features and biological functions of SIRT6

The catalytic core region of SIRT6 is approximately 275 amino acids, and its length or sequence vary with the inconstant N-terminal and C-terminal extensions.[19] The N-terminus is crucial for intrinsic catalytic activity and

Table 1 Summary of the enzymatic and cellular features of SIRT6.

Targets	Brief mechanism of enzymatic functions	Down-regulated genes	Cellular functions	References
H3K56	Deacetylates H3K56 to inhibit Wnt/β-catenin pathway	*PPAR-γ*	Chondrogenesis ↑	45, 46
H3K9	Deacetylates H3K9 to inhibit NF-κB pathway	*NFATc1* *Runx2* and *Osx*	Osteoclastogenesis ↓ Osteoblastogenesis ↑	48, 51, 52 56
IκBα	Activates IκBα to inhibit NF-κB pathway	*NFATc1*	Osteoclastogenesis ↓	54
H3K9	Deacetylates H3K9	*TERT* and *TRF-1*	Cellular senescence ↓ Telomere integrity ↑	59, 60 8, 62
HIF1α	Negatively regulates the glycolysis related genes expression	*HIF1α*	Glucose homeostasis	96, 97, 99
H3K9	Deacetylates H3K9 to inhibit NF-κB pathway	*SOX9*, *BMP2*, and *HIF-2α*	Chondrogenesis ↑ Chondrocyte hypertrophy ↓ Cellular inflammation ↓	122, 123

Table 2 Several kinds of pathological bone diseases could be modulated by targeting SIRT6.

Brief mechanism of SIRT6	Cellular functions	Diseases attenuated	References
Inhibit Wnt/β-catenin pathway	Chondrogenesis ↑	Osteoarthritis	45, 46
Inhibit NF-κB pathway	Osteoclastogenesis ↓	Osteoporosis	108, 109
	Chondrocyte hypertrophy ↓	Osteoarthritis	126, 129
	Chondrogenesis ↑	Rheumatoid arthritis	16, 133
	Cellular inflammation ↓	Intervertebral disc degeneration	146, 147
	Cellular senescence ↓	Other bone-related diseases	
	Telomere integrity ↑		

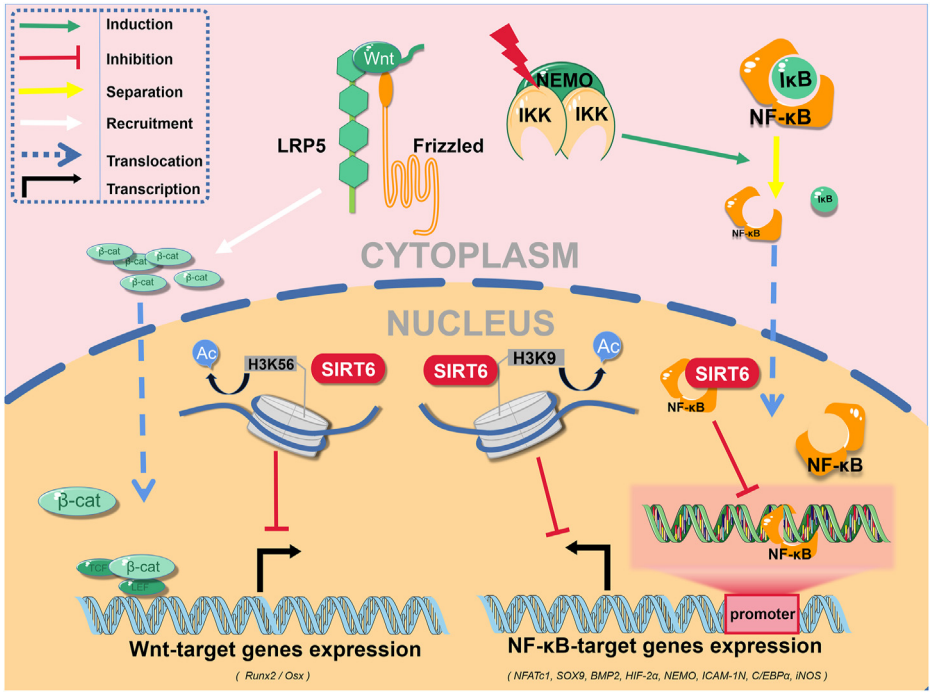

Figure 1 Schematic diagram of the mechanism of SIRT6 inhibition effect to Wnt and NF-κB signaling. Wnt binds to Frizzled receptor and LRP5 leads to the accumulation of β-catenin in cytoplasm. β-catenin which enters nucleus combines with transcription factors LEF/TCF, leads to the transcription of Wnt-target genes. Wnt signaling is inhibited by SIRT6 through deacetylating histone 3 at lysine 56 (H3K56). The core element of the NF-κB cascade, NEMO/IKKγ, is activated by stresses, then IκB separates from the NF-κB/IκB complex. However, SIRT6 blocks the combination between free NF-κB and the promoter of NF-κB-target genes, and deacetylates histone 3 at lysine 9 (H3K9). Then inhibits the Wnt-target and NF-κB-target genes expression.

chromatin association, and the C-terminus is responsible for proper nuclear localization.[20] In addition, SIRT6 includes three kinds of conservative components: two globular domains composed of eight α-helices, nine β-strands, a large Rossmann-fold for NAD^+ binding; and a smaller domain containing a zinc-binding motif.[21] Hydrogen bonds between the Rossman-fold and the zinc-binding motif stabilize the structural conformation of SIRT6.[22]

SIRT6 makes effects on DNA repair, gene expression, telomere integrity maintenance, nucleosome and chromatin remodeling, mitosis and meiosis, and cell cycle.[23–25] For instance, Van Meter M et al. showed that SIRT6 recruits poly (ADP-ribose) polymerase 1 (PARP1) to DNA break sites for repair of DNA double-strand breaks in response to oxidative stress.[26] SIRT6-mediated histone 3 at lysine 56 (H3K56) deacetylation promotes the localization of chromatin remodeler SNF2H to double-strand breaks damage, thereby maintaining efficient DNA repair.[27] In addition, Raul Mostoslavsky et al. demonstrated that SIRT6 promotes resistance to DNA damage and maintains genomic stability, functioning in base excision repair (BER).[28] Furthermore, SIRT6 plays an essential role in telomeric maintenance by binding to telomere chromatin and deacetylating histone 3 at lysine 9 (H3K9) specifically.[8] SIRT6-mediated deacetylation of H3K56 residues during S-phase associates the RECQ-like helicase WRN with telomere chromatin, participating in telomere metabolism and DNA replication.[29,30]

Physiologic functions of SIRT6 enzymatic activities in bone cells

Bone is composed of three major cell types including bone-forming osteoblasts, bone-resorbing osteoclasts and terminally differentiated osteocytes.[31] The constant intercellular communications among these bone cells maintain bone development, bone remodeling, and homeostasis in response to various external stimulation such as mechanical loading, inflammatory response, and hormone fluctuations.[32] Osteoblasts and osteoclasts actively participate in bone remodeling and homeostasis. Osteoblasts are mononuclear and terminally differentiated cells, which lay down mineralized extracellular matrix formed from calcium hydroxyapatite.[33,34] Osteocytes, found dispersed throughout the bone matrix, are derived from osteoblasts that are surrounded alongside bone surface.[35] In contrast, osteoclasts are capable of resorbing both the organic and inorganic components of bone, thereby playing critical roles in bone remodeling.[36] In adults, the balance between chondrocyte anabolism and catabolism maintains the homeostasis of articular cartilage.[37] The crosstalk among bone cells takes place throughout the process of bone development such as the formation of long bones from the cartilaginous template. During the formation of the long bone, blood vessels first invade the cartilage template, then osteoclasts enter and replace chondrocytes, and osteoblast

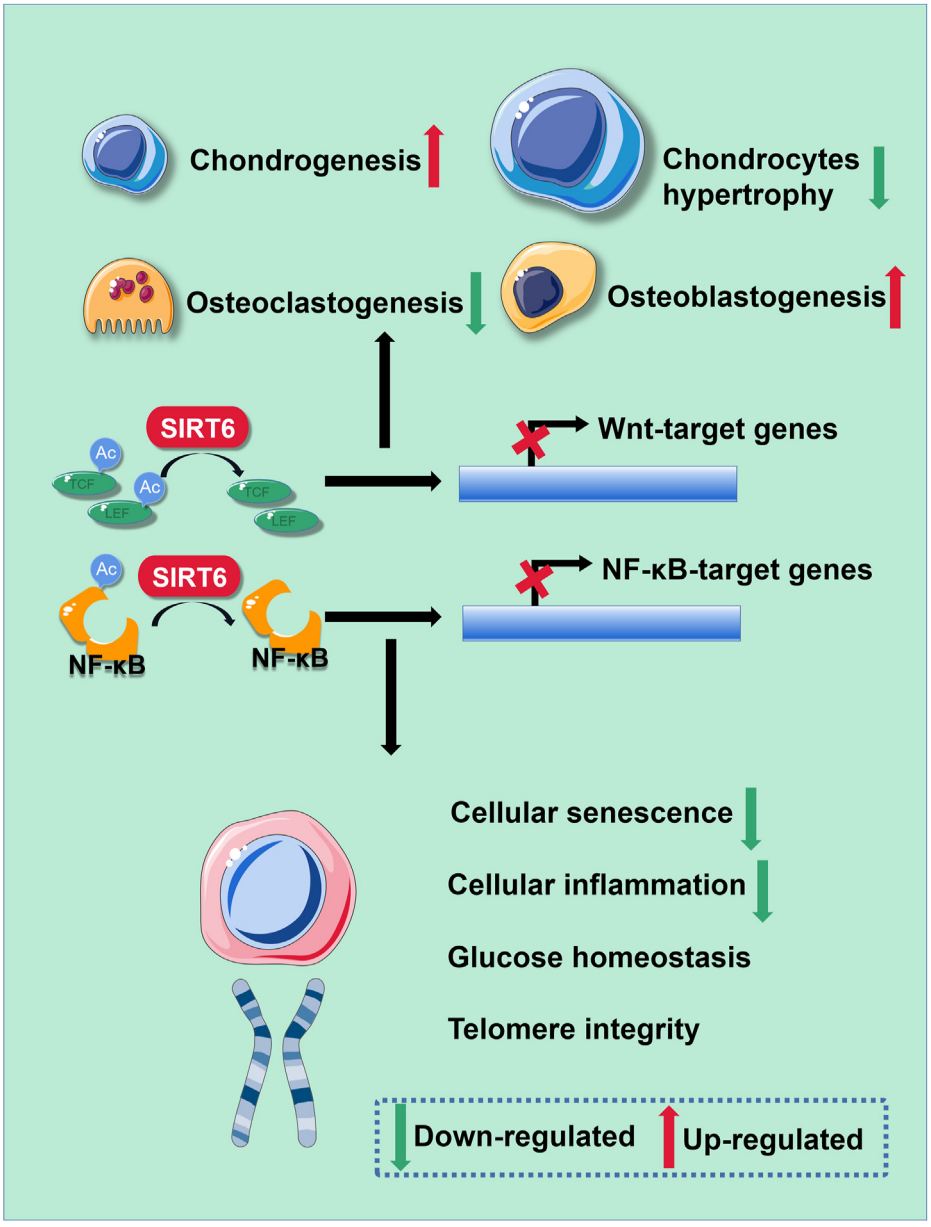

Figure 2 Schematic diagram of SIRT6 biological effects.

precursor cells enter into the hypertrophic area of cartilage to create a bone marrow cavity.[38] SIRT6 displays on multi-tasking abilities in bone cells of the skeletal system, such as cellular differentiation, cellular senescence, cellular metabolism, and cellular inflammation.

Cellular differentiation

The high plasticity and multipotential of MSCs permit the differentiation into osteoblasts, adipocytes, and chondrocytes. Several signaling pathways participate in regulating the various differentiation. The peroxisome-proliferator-activated receptor-γ (PPAR-γ) and β-catenin-dependent Wnt signaling have a reciprocal relationship in the regulation of adipogenesis and osteogenesis.[39] PPAR-γ promotes adipogenic differentiation and inhibits osteoblast differentiation in MSCs, whereas Wnt/β-catenin signaling usually plays an opposite role.[40–43] In the cytoplasm, β-catenin is accumulated by Wnt binding to Frizzled receptor and lipoprotein receptor-related protein 5 (LRP5) co-receptor. Stabilized β-catenin then enters the cell nucleus and associates with transcription factors LEF/TCF, promoting the transcription of Wnt-target genes.[44] The absence of β-catenin blocks the osteogenic differentiation and stimulates the MSCs differentiation into chondrocytes.[45] Hu Wang et al. demonstrated that SIRT6 interacted with transcription factor LEF1 and deacetylated H3K56, which ultimately inhibited the transcription of Wnt target genes.[46]

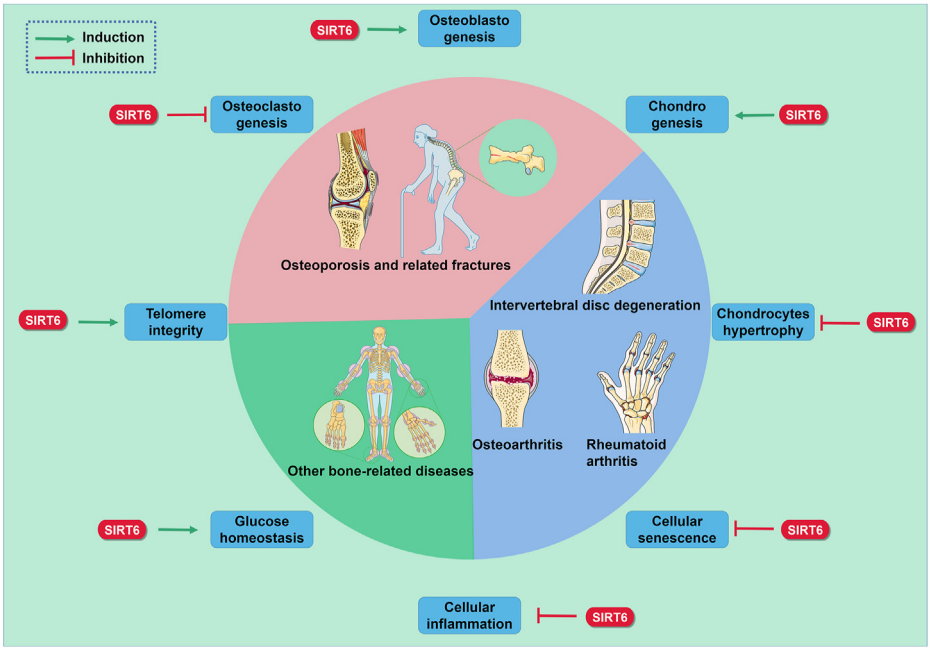

Figure 3 Schematic diagram of cellular functions and bone-related diseases could be modulated by SIRT6.

In addition, NF-κB signaling inhibits osteogenic differentiation *in vitro* and bone formation *in vivo* by accelerating the degradation of β-catenin.[47] Meanwhile, nuclear NF-κB binds to the promoter region of nuclear factor of activated T cell cytoplasmic 1 (NFATc1), which serves as a downstream transcriptional regulatory factor of NF-κB pathway to promote osteoclasts formation.[48] Lim R et al. revealed that the cells transfected with a vector expressing SIRT6 show restrained NF-κB transcriptional activity.[49] Consistently, Wu X et al. found that SIRT6 deletion upregulates Toll-like receptor 4 (TLR4) and reinforces activation of NF-κB signaling.[50] Mechanically, SIRT6 inhibits NF-κB signaling through different manners. On the one hand, SIRT6 directly mediates deacetylation of H3K9 near the promoters of NF-κB target genes, which significantly restrains transcription.[51,52] On the other hand, IκBα, a NF-κB repressor, combines with NF-κB tightly as a complex in the cytoplasm. Stress activates IκB kinase (IKK), which separates IκBα from the complex and promotes the translocation of solitary NF-κB into the nucleus, ultimately modulation expression of target genes.[53] SIRT6 induces cysteine monoubiquitination of the methyltransferase SUV39H1, which causes the isolation of SUV39H1 from IκBα gene. The expression of IκBα is increased and leads to NF-κB pathway inactivation.[54] Osterix (Osx) and Run-related transcription factor 2 (Runx2) are osteoblast-specific transcription factors, which promote osteogenic differentiation.[55] SIRT6 depletion results in H3K9 hyperacetylation on the promoters of Runx2 and Osx, and then promotes their expression in osteoblasts. Overexpression of Osx and Runx2 impairs osteoblastogenesis. Additionally, SIRT6 deficiency-mediated hyperacetylation also increases expression of osteoprotegerin and Dkk1, which respectively serve as a potent inhibitor of osteoclastogenesis and osteoblastogenesis, resulting in poor bone remodeling.[56]

Cellular senescence

Cellular senescence is an irreversible cell cycle arrest, characterized by increased expression of p16, p21, and p53, accumulated reactive oxygen species and increased senescence-related β-galactosidase activity.[57,58] Cellular senescence is caused by many mechanisms such as telomere shortening and DNA damage.[59] Telomere shortening is caused by a lack of telomerase, which leads to cellular senescence and mitochondria dysfunction.[60] Saeed et al. demonstrated that telomerase deficiency impairs osteoblast differentiation and leads to the loss of bone mass.[61] SIRT6 maintains telomere integrity by deacetylating H3K9 thereby upregulating telomerase reverse transcriptase (TERT) as well as telomere repeat binding factor (TRF)-1 in the heart.[8,62]

In addition, SIRT6 is implicated in the regulation of cell life span and aging through the NF-κB pathway. SIRT6 binds to the RelA/p65 subunit of NF-κB and mediates deacetylation of H3K9 at promoters of NF-κB target genes, which inhibits downstream gene expression of NF-κB.[63] Piao J et al. reported that SIRT6 deficiency impairs proliferation and differentiation of chondrocytes, reduces expression of Indian hedgehog (Ihh), and ultimately leads to a senescent phenotype in the tibial growth plate.[64] Ihh upregulates Smoothened (Smo) gene expression and then promotes the terminal differentiation of chondrocytes. Conversely, the decreased Ihh leads to the overexpression of parathyroid hormone-related protein (PTHrP), which facilitates

chondrogenesis.[65] Ihh regulates chondrocyte differentiation through a negative feedback loop formed with PTHrP.[66]

Anti-inflammation

The bone marrow microenvironment is composed of a variety of hematopoietic stem cells, lineage-specific downstream progenitors, non-hematopoietic cells, extracellular matrix. It serves as a barrier to isolate hematopoietic stem cells from many stimulating signals such as differentiation and apoptosis, which prevents the excessive proliferation of stem cells and tumorigenesis.[67,68]

Inflammation with excessive pro-inflammatory cytokines disrupts the balance of bone homeostasis, and the NF-κB signaling pathway regulates both inflammatory response and bone remodeling.[38] Mechanically, NF-κB and downstream target genes are activated by inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the inflammation microenvironment. These inflammatory cytokines induce receptor activator of NF-κB ligand (RANKL) expression in macrophages, which facilitates osteoclastogenesis and bone resorption.[69,70] NEMO/IKKγ, the core element of the NF-κB cascade, is activated by RANKL and TNF in both physiological and pathological (inflammatory) conditions to regulate osteoclastogenesis.[71,72]

Pro-inflammatory cytokines, including IL-1, IL-6, and TNF-α, are the main inflammatory mediator of bone marrow inflammatory microenvironment.[73] TNF-α, mainly secreted by activated macrophages, inhibits the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs), thereby promoting bone resorption.[74] TNF-α regulates osteogenic differentiation of BMSCs by the following mechanisms: 1) Wnt/β-catenin-dependent pathways promote the osteogenic differentiation by enhancing the expression of Runx2 and Osx.[75] TNF-α can inhibit the activation of the canonical Wnt pathway to a certain extent. 2) TNF-α inhibits the expression of bone morphogenetic protein 2 (BMP2) through activation of NF-κB, p38/MAPK, and c-Jun N-terminal kinase (JNK) signaling pathways, which ultimately restrains osteogenic differentiation of BMSCs.[76] 3) TNF-α inhibits the extracellular signal-regulated kinase (ERK) pathway and thereby affecting the osteogenic differentiation of BMSCs.[77]

Macrophages are predominant regulators in the initiation, progression, and elimination of inflammation.[78] Indeed, Macrophages are polarized into different phenotypes including pro-inflammatory M1 or anti-inflammatory M2 macrophages.[79] M1 macrophages, polarized from macrophages stimulated by IFN-γ and/or LPS, secrete pro-inflammatory cytokines such as TNF-α, IL-1β, and chemokines such as MCP-1, MIP-1α.[80] In addition, macrophages exposed to IL-4 or IL-13 are polarized into M2 macrophages, which secret anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist IL-1Ra.[81] The pro-inflammatory cytokines activate NF-κB pathway by the upstream kinases, and then NF-κB induces activation of kinase (NIK) and transforming growth factor-β activated kinase (TAK1).[82,83] In contrast, anti-inflammatory cytokines inhibit the NF-κB pathway.[84,85] In conclusion, M1 macrophages secret pro-inflammatory cytokines and then activate NF-κB pathway by the upstream kinases. NF-κB induces NIK and TAK1, which leads to osteoclastic bone destruction and inflammatory bone loss.

As mentioned above, the NF-κB signaling pathway is the core of inflammation, which leads to inflammatory bone loss and bone destruction via many different mechanisms. SIRT6 has been proved to restrain NF-κB-dependent gene expression through H3K9 deacetylation.[86] Therefore, SIRT6 may be an effective therapeutic target for a variety of inflammatory bone diseases. The recruitment of SIRT6 to chromatin prevents hyper-induction of NF-κB-dependent genes.

Cellular metabolism

Glycolysis is an essential metabolic pathway for meeting ATP demand during osteoblasts differentiation.[87] Indeed, osteoblasts metabolize glucose in the presence of sufficient oxygen, and aerobic glycolysis provides the necessary glycolytic intermediates to support cellular proliferation and differentiation.[88,89] In addition, osteoclast differentiation requires both aerobic glycolysis and mitochondrial respiration.[90] Chondrocytes also follow glycolytic metabolism at physiologic oxygen tensions and anaerobic glycolysis in low oxygen conditions and aerobic.[91]

SIRT6 appears to play a prominent role in regulating cellular metabolism.[92,93] For instance, SIRT6 regulates glucose homeostasis by sensing nutrient levels in the microenvironment.[94,95] HIF-1α is an important glycolytic regulator which inhibits mitochondrial oxygen consumption in response to nutrient and oxygen availability.[96,97] Zhong L et al. proposed that SIRT6 is a co-repressor of HIF-1α transcriptional activity which links SIRT6 to glycolysis. Mechanically, in normal nutrient conditions, SIRT6 inhibits the glycolysis gene expression and HIF1α activation to induce mitochondrial respiration.[98] Conversely, under nutrient stress conditions, inactivated SIRT6 induces HIF1α activation and increases expression of glycolytic genes, which enhances glycolysis and restrains mitochondrial oxygen consumption. As we mentioned before, SIRT6 works as a gatekeeper in glucose metabolism, regulating glycolysis and aerobic mitochondrial respiration in hypoxia and normal condition, respectively.[99]

Involvement of SIRT6 in various bone-related diseases

Osteoporosis

OP is a skeletal disorder characterized by bone mass loss, high bone fragility, and microarchitecture deterioration, resulting in bone fragility and fractures.[100–102] OP is widespread in the world and affects people of all ethnic backgrounds, most commonly in older women and men.[103] The imbalance caused by aberrantly increased osteoclasts formation and reduced osteoblasts differentiation or mineralization may be responsible for progressive bone loss and lytic lesions.[104] In addition, epidemiological evidence has indicated that estrogen deficiency leads to a decline in bone mass and strength during the late postmenopausal years.[105,106]

Osteoclasts, the major contributors of OP, are differentiated from monocyte/macrophage haematopoietic lineage under the stimulation of M-CSF and RANKL.[107] Zhang D et al. indicated SIRT6 deficiency increases the number of osteoclasts and decreased bone mass by upregulating NF-κB signaling-related gene NEMO, ICAM-1N, C/EBPα, and iNOS.[108] Furthermore, Moon YJ et al. reported that SIRT6 deacetylates lysine 171 and lysine 299 of estrogen receptor α (ERα) in preosteoclasts, thereby inhibiting related proteasomal degradation. ERα promotes transcription of Fas ligand in preosteoclasts to trigger the apoptosis of osteoclasts, ultimately inhibiting osteoclastic bone resorption.[109,110] Collectively, the activation of SIRT6 in osteoclasts may be an emerging therapeutic strategy of OP in elder and postmenopausal patients.

Bone homeostasis depends on osteoblasts-mediated bone formation and osteoclasts-mediated bone resorption. OP could be caused by the imbalance of this tightly coupled process.[111] Osteogenesis is the differentiation process of MSCs to osteoblasts that are involved in bone remodeling. The osteogenic activity of MSCs is negatively correlated to the degree of OP.[112] Zhao J et al. found that miR-128 expression in bone samples of OP patients is markedly higher than that of non-OP patients. Further studies confirmed that miR-128 targets SIRT6 to inhibit the osteoblastic differentiation of C2C12 cells, thereby accelerating the development of OP.[113] TRPV1 is a non-selective cation channel that induces the production of calcitonin gene-related peptide (CGRP). CGRP shows significant anti-osteoclastogenic effects.[114,115] Xiao F et al. revealed SIRT6 overexpression reduces expression of TRPV1 channel via ubiquitination. Therefore, SIRT6 is decreased in hMSCs to enhance TRPV1 expression, which promotes osteogenic differentiation and restrains osteoclastogenic effects via CGRP.[116]

Osteoarthritis

OA is a dominating cause of articular disability in the elderly, and its incidence increases significantly with aging and obesity.[117,118] The underlying molecular mechanisms of OA are not completely clarified yet. In this review, we aimed to conclude the role of SIRT6 changes that happened in OA, rather than illustrate all of the pathophysiological lesions completely. Hypertrophic differentiation of chondrocytes, known as increased cell size and volume, is the main feature of OA and manifested by high expression of collagen type X alpha 1 (Col10a1), Runx2 and MMP13.[119–122] NF-κB signaling induces hypertrophy of chondrocyte mainly by upregulating the expression of hypoxia-inducible factor-2α (HIF-2α), SRY-box transcription factor 9 (SOX9), and BMP2, ultimately promoting the progression of OA.[123]

Chondrocyte senescence shares various markers and processes with hypertrophy during OA.[124] Chondrocytes with secreted phenotype produce catabolic enzymes, pro-inflammatory mediators, and chemokines, which collectively serve as senescence-associated secretory phenotype (SASP) in senescent cells to destroy cartilage matrix integrity and form an inflammatory microenvironment in the OA joint.[125] Overexpression of SIRT6 suppresses cellular senescence in OA-related poor progress. Wu Y et al. revealed that SIRT6 reduces the MMP-13 level and attenuates the decreased expression of collagen-II in chondrocytes induced by IL-1β.[126] In addition, negatively modulating SIRT6 could promote the expression of MMP-1 and MMP-13 at the mRNA level, which induces the senescence of chondrocytes and increases the p16 level.[127] P16 protein also promotes senescence of human articular chondrocytes in OA.[128]

Furthermore, Sun H et al. demonstrated that SIRT6 knockdown activates the NF-κB pathway and significantly reduces the expression of several key osteogenic markers including ALP, Runx2, and osteocalcin, which decreases osteogenic differentiation ability and thereby accelerates the progression of OA.[129]

Rheumatoid arthritis

RA belongs to a chronic inflammatory, systemic and autoimmune disorder.[130,131] Apart from articular manifestations, RA patients suffer from cumulative comorbid risks such as myocardial infarction, stroke, and other cardiovascular events associated with autoimmune mechanisms.[131] Lee et al. demonstrated that overexpression of SIRT6 could reduce both the inflammatory response and tissue destruction in rheumatoid joints of mice.[16]

Proinflammatory cytokines such as TNF-α, GM-CSF, IL-1, IL-6, and chemokines such as IL-8 are abundant in patients with RA. Feldmann M et al. found that TNF-α neutralization restrains other proinflammatory cytokines, which suggests that the proinflammatory cytokines are linked in a network with TNF-α at its apex in RA.[132,133] In addition, TNF-α inhibits SIRT6 expression and thereby significantly enhances the expression of monocyte chemotactic protein 1 and IL-6 through ROS pathway and AKT pathway respectively in vascular adventitial fibroblasts.[134] Therefore, TNF-α inhibitors may be an effective drug that transforms the outlook for patients with RA. The Lee HS group demonstrated that SIRT6 overexpression can suppress excessive osteoclast activity, decreases the production of IL-1β and TNF-α in the model of collagen-induced arthritis.[16] However, Jiang H et al. demonstrated that SIRT6 removes the fatty acyl modification on K19 and K20 of TNF-α to promote its secretion, which reveals the complexity and contradictoriness of SIRT6 regulation.[135]

Intervertebral disc degeneration (IDD)

The intervertebral disc is important for movement, weight-bearing, and spinal flexibility.[136] It is like a sandwich in which two cartilaginous endplates clip annulus fibrosus with enfolded inner gelatinous nucleus pulposus cells.[137] IDD, as the main reason for low back pain in about 80% of people in their lifetime, is manifested by degeneration of nucleus pulposus, annulus fibrosus, and cartilaginous endplates.[138,139] Accumulating evidence illustrated that cellular senescence, apoptosis, inflammatory response, oxidative stress, and mitochondrial dysfunction in nucleus pulposus (NP) cells are closely involved in the pathological mechanisms of IDD.[140–143] Senescent NP cells generate ROS

and secrets pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which facilitate senescence of neighboring cells, accelerating apoptosis of cells of intervertebral disc and amplifying inflammation.[144,145] SIRT6 significantly suppresses the senescence of NP cells, functioning the critical role in mitigating IDD.[17] SIRT6 stabilizes telomeres and attenuates pre-mature senescence of NP cells by triggering autophagy of NP cells.[17,146,147]

SIRT6 acts as a potential therapeutic target in skeletal diseases

The above studies have proved SIRT6 plays central role in many cellular processes such as cell metabolism, DNA repair, aging, and inflammation. Therefore, SIRT6 may represent potential weapons for preventing the progression of multiple skeletal diseases. For instance, SIRT6 inhibits inflammatory response and chondrocyte senescence by suppressing NF-κB signaling, which significantly prevents OA progression.[126] Mechanically, SIRT6 destabilizes the binding of NF-κB-promoter complex to DNA through the deacetylation of NF-κB-promoter complex, which reduces phosphorylation of NF-κB and NF-κB target gene expression.[148] Since SIRT6 holds such therapeutic potential, researchers are identifying SIRT6 modulators as therapeutic agents for a wide range of skeletal diseases as well as several other diseases.

Gil R et al. revealed SIRT6 deacetylase activity is nucleosome-dependent and nucleosome association converts SIRT6 into an active structure.[149] Rahnasto-Rilla M et al. discovered the region near the binding site of acetylated peptide substrate may be the potential binding site of SIRT6 activators.[150] Moreover, the Yariv Kanfi group proved that SIRT6 increases with nutrient deprivation in cultured cells and animals fed a calorically restricted diet. Meanwhile, the transcription of SIRT6 is not enhanced, but the SIRT6 stabilization is increased.[95]

You W et al. yielded the first synthetic SIRT6 activators pyrrolo [1,2-a]quinoxaline derivatives, which directly bind to the SIRT6 catalytic core and activate SIRT6-dependent deacetylation of peptide substrates and complete nucleosomes.[151] Feldman JL et al. clarified that long-chain fatty acids potently enhance SIRT6 enzymatic activity.[152] Quercetin also increases SIRT6 activity at a high concentration by binding to the SIRT6-selective acyl binding channel.[153,154] Fucoidan belongs to a polysaccharide that is naturally present in brown algae and seaweeds, which exhibits dose-dependent SIRT6 stimulating activity and functions in healing inflammation.[154,155] Kaempferol, a flavonoid that is naturally found in green leafy vegetables, binds to the SIRT6-specific acyl binding channel to activate SIRT6.[156,157] Kaempferol also produces anti-inflammatory action by inhibiting NF-κB and activator protein-1 (AP-1) pathways and decreasing expression of TNF-α, IL-1, and IL-8.[158] Therefore, kaempferol may serve as an effective SIRT6 activator to delay the progression of inflammatory bone disease. Resveratrol (RES) is a polyphenolic phytoalexin found in many plants such as grapes, berries, and peanuts, which functions as a SIRT6 activator to delay the progression of atherosclerosis.[159,160]

In addition, the Mu W group elucidated that metformin promotes SIRT6 expression and suppresses phosphorylation of NF-κB remarkably in the proliferation and differentiation of murine pre-osteoblasts.[161] Cyanidin, a valid SIRT6 activator, inhibits the NF-κB signaling pathway in IL-1β-induced chondrocytes, which effectively attenuates the progression of OA induced by medial meniscus instability in mice.[162] Furthermore, RES also suppresses senescence of intervertebral disc cells in an inflammatory environment and regulates the extracellular matrix expression of NP cells via Wnt/β-catenin signaling pathway, which significantly prevents IDD progression.[163,164] In addition, caffeic acid increases SIRT6 expression in human lung fibroblast cells, and low-dose caffeic acid phenethyl ester abrogates bone resorption of a mouse cranial model.[165,166] Unfortunately, the efficacy, selectivity, and specificity of the mentioned SIRT6 activators remain to be fully established for the treatment of skeletal diseases. We need more investigation to develop potent drugs for the treatment of various bone-related diseases.

Conclusion and perspective

In conclusion, SIRT6 is an important epigenetic regulator that widely participates in DNA repair, telomere integrity maintenance, nucleosome and chromatin remodeling, and so on, through histone deacetylase activity. These SIRT6-mediated cellular processes are linked to regulation of differentiation, senescence, metabolism and inflammatory response in bone cells, functioning in bone health and bone-related disease prevention. Indeed, SIRT6 also mediates deacylation of the long-chain fatty, which may broaden the research depth of protein post-translational modification.[135] Additionally, the Michael Van Meter group found SIRT6 ribosylates KAP1, a nuclear corepressor protein interacting with HP1α and thereby repressing LINE1 retrotransposons.[167] LINE1 retrotransposons are a kind of transposable element that affects genome stability and functions in age-related pathologies such as cancer. These findings revealed two novel enzymatic activities of SIRT6: ADP-ribosylation and acyl hydrolysis of long-chain fatty acids. Researchers need further investigation to identify physiological targets for SIRT6 de-fatty-acylation and mono-ADP ribosylation and explore the regulation of SIRT6 in different fatty acylome and mono-ADP-ribosylome.

As mentioned above, SIRT6 works as a gatekeeper in glucose metabolism to regulate glycolysis and aerobic mitochondrial respiration in hypoxia and normal conditions, respectively. Indeed, SIRT6 modulates multiple metabolic pathways in addition to glycolysis, including gluconeogenesis, lipogenesis and fatty acid uptake, β-oxidation, etc.[168] Multiple studies demonstrated that aging and overnutrition result in decreased SIRT6 level and function, leading to abnormal glucolipid metabolism.[168] However, the specific mechanisms by which SIRT6 regulates glucolipid metabolism in bone cells remain unclear. Linking SIRT6-mediated

glycolipid metabolism and energy supply may provide novel therapeutic strategies for bone-related diseases.

SIRT6 has emerged as an important regulator of longevity in mammals. Many natural and synthetic products have been identified as activators of SIRT6 and are beneficial for aging, cancer, inflammation, and bone-related diseases.[169] However, it remains unclear that how SIRT6 retains or removes markers in specific substrates. Genomic or cellular contexts may influence SIRT6 to select different histone substrates, but this is a rough selection. Ideal therapeutics could activate or inhibit SIRT6 in specific cell types and cellular contexts to prevent unwanted side effects.

Conflict of interests

The authors declare no conflicts of interest.

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