

The Role of Cellular Senescence in the Pathogenesis of Osteoporosis and Research on Therapeutic Targets

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Abstract. Osteoporosis is an age-related bone metabolic disease, and cellular senescence is considered a common driver of its occurrence and development. This paper reviews the mechanism of cellular senescence in osteoporosis and advances in targeted therapy. Senescent bone marrow mesenchymal stem cells exhibit decreased osteogenic differentiation capacity and shifted adipogenic differentiation, degeneration of the lacunar-canalicular system of osteocytes and impaired mechanosensation, as well as imbalanced functions of osteoblasts and osteoclasts. The senescence-associated secretory phenotype disrupts the homeostasis of the bone microenvironment through pro-inflammatory factors and matrix metalloproteinases, activates the RANKL/OPG signaling axis, and inhibits the Wnt/ β -catenin pathway. Upstream triggers include DNA damage, oxidative stress, and epigenetic alterations. Targeted therapeutic strategies include the elimination of senescent cells by senolytics (dasatinib + quercetin, fisetin), inhibition of SASP secretion by senomorphics (metformin, rapamycin), and novel delivery systems based on exosomes and nanocarriers. Targeting cellular senescence provides a new direction for the precise prevention and treatment of osteoporosis.

Keywords: cellular senescence, osteoporosis, senescence-associated secretory phenotype, bone microenvironment, therapeutic targets

1. Introduction

1.1. Heterogeneity of osteoporosis and challenges of population aging

Osteoporosis (OP) is a systemic bone metabolic disease characterized by reduced bone mass, destroyed microstructure of bone tissue, and increased bone fragility, which can significantly raise the risk of fragility fractures. According to etiology, osteoporosis can be divided into two major categories: primary and secondary. Primary osteoporosis accounts for approximately 85%–90% of all cases, mainly including postmenopausal osteoporosis (PMOP, Type I) and senile osteoporosis (SOP, Type II).

With the accelerating process of global population aging, osteoporosis has become a major public health concern. Currently, there are approximately 60 to 80 million osteoporosis patients in China. Epidemiological data show that the prevalence of osteoporosis in women increases significantly with age: 41.2% in those aged 60–69 years, rising to 62.1% in those aged 70–79 years, and reaching

73.6% in those aged 80 years and above. However, the treatment initiation rate is only 21.0%, indicating obvious deficiencies in the diagnosis and treatment of this disease [1].

Osteoporosis exhibits significant biological heterogeneity. In recent years, genome-wide association studies (GWAS) have identified multiple genetic loci associated with bone mineral density and fracture risk in European populations, but fewer than 40% of these loci can be replicated in Asian and African populations [2]. In addition, the relationship between metabolic dysfunction-associated steatotic liver disease (MASLD) and bone mineral density has shown negative, no, or even positive correlations in different studies, suggesting that bone metabolism is co-regulated by multiple factors including age, gender, ethnicity, visceral fat distribution, and metabolic status [3]. Therefore, re-understanding the pathogenesis of osteoporosis from the perspective of aging biology is of great significance for achieving precise prevention and treatment.

1.1.1. Differences between postmenopausal osteoporosis (Type I) and senile osteoporosis (Type II)

Primary osteoporosis is not a disease that affects only one group of people. Its internal disease mechanisms show clear differences, which mainly lie in the distinctions between postmenopausal osteoporosis (Type I) and senile osteoporosis (Type II). Type I osteoporosis mostly occurs in women after menopause, and its core driving factor is the sudden lack of estrogen. Estrogen normally holds back the growth and activity of bone-resorbing cells. When this inhibitory effect disappears, bone breakdown becomes far more active than bone formation, and this mainly causes rapid loss of trabecular bone. But Type II osteoporosis is slow bone loss linked to aging, and it affects both men and women. Its mechanism is more complex, including a general drop in bone formation ability, changed vitamin D metabolism, and secondary hyperparathyroidism [4].

Studies using computer models offer a number-based view to understand these two sets of mechanisms. Researchers built a model of bone cell groups to copy the different effects of aging and estrogen shortage on natural bone renewal. In the model, aging effects are mainly shown through two measures. First, the level of transforming growth factor- β (TGF- β) in the bone matrix decreases. Second, cells that are not bone cells produce more sclerostin. Estrogen shortage is imitated by higher RANKL expression, lower osteoprotegerin (OPG) production, and greater sensitivity of bone-resorbing cells to RANKL. Simulation results show that during aging, the effect of increased sclerostin is stronger than that of decreased TGF- β . The three mechanisms copying estrogen shortage cause almost the same bone loss reaction. This shows that though the two types often appear together in clinical cases, their molecular driving mechanisms have different focuses. This provides a theoretical base for making targeted treatment plans.

1.1.2. Proposal of cellular senescence as a common driver of osteoporosis

Though Type I and Type II osteoporosis have different starting causes, both finally lead to the breakdown of the bone's inner microenvironment. In recent years, cellular senescence has been seen as a shared path that connects these different causes to final bone loss. Cellular senescence means a steady state where cells stop dividing, along with changes in their functions and a complex set of substances they secrete. As people age, senescent cells build up in bone tissue. They break the balance between bone formation and bone breakdown by releasing substances such as pro-inflammatory factors, and this pushes osteoporosis to start and develop. So, treating cellular senescence as the core driver of bone aging provides a unified theoretical system for understanding the differences among various osteoporosis cases.

1.2. Characteristic definition of cellular senescence

Cellular senescence refers to a stable and generally irreversible state of cell cycle arrest that cells enter under various endogenous and exogenous stimuli, including DNA damage, oxidative stress, telomere shortening, and activation of oncogenic signals, thereby losing the ability to continue dividing, proliferating, and growing [5]. As one of the important hallmarks of organismal aging, cellular senescence was initially regarded as a protective mechanism against tumorigenesis. However, as senescent cells continuously accumulate in tissues, their negative effects on tissue homeostasis and organ function have gradually emerged.

Senescent cells can be identified by multiple biomarkers, the most commonly used of which include elevated activity of senescence-associated β -galactosidase (SA- β -gal) and high expression of the cell cycle inhibitors p16^{INK4a}, p21^{CIP1/WAF1}, and p53 [6]. Nevertheless, cellular senescence is highly heterogeneous in nature, with significant differences in senescent phenotypes among different inducing factors, cell types, and tissue environments. Therefore, there is still a lack of universal biomarkers applicable to all senescent states, which poses a major challenge for the precise identification and targeting of senescent cells [7].

In addition to cell cycle arrest, senescent cells also develop a senescence-associated secretory phenotype (SASP). SASP consists of various pro-inflammatory cytokines, chemokines, growth factors, proteases, and other bioactive molecules, which can maintain the senescent state through autocrine signaling and influence the fate and function of neighboring cells via paracrine signaling [8]. In bone tissue, SASP is one of the core mechanisms by which senescent cells mediate bone microenvironment remodeling and imbalanced bone metabolism.

1.2.1. Cell cycle arrest (markers p16, p21, p53)

Irreversible cell cycle arrest is the most fundamental characteristic of cellular senescence, and its molecular basis mainly relies on two classic tumor suppressor pathways: p53/p21^{Cip1} and p16^{INK4a}/pRB. Cellular senescence is generally defined as a stable growth arrest state dominated by cell cycle regulatory factors such as p53, p21, and p16 [9].

Under stimuli such as DNA damage, oxidative stress, or telomere exhaustion, cells first activate the DNA damage response (DDR) and then initiate the p53-dependent checkpoint pathway. Activated p53 induces the expression of p21^{Cip1/WAF1}, which inhibits cyclin-dependent kinases (CDKs), prevents the phosphorylation of RB protein, maintains its active state, and thus suppresses the activity of E2F transcription factors, leading to cell arrest at the G1/S phase [10].

Another critical pathway is the p16^{INK4a}/pRB pathway. By inhibiting the activity of CDK4/6, p16^{INK4a} also maintains RB in a hypophosphorylated state, thereby blocking cell cycle progression and stabilizing the senescent phenotype [11]. In contrast to p21, which is more involved in the early establishment of senescence, p16^{INK4a} is usually closely associated with long-term and irreversible maintenance of senescence.

p16^{INK4a} is currently regarded as one of the most commonly used and reliable biomarkers for studying cellular senescence. Research by the team of Zhou Bin at the Chinese Academy of Sciences has indicated that p16^{INK4a} is barely expressed in young and healthy tissues but accumulates significantly under aging or tissue injury conditions. The genetic or pharmacological elimination of p16^{INK4a} cells can significantly ameliorate multiple age-related disease phenotypes [12]. In 24-month-old naturally aging mice, various bone-related cells, including T cells, myeloid cells, osteoprogenitor cells, and osteocytes, all show elevated expression of p16^{INK4a}, further confirming the age-dependent accumulation of senescent cells in bone tissue.

1.2.2. Accumulated activity of senescence-associated β -galactosidase (SA- β -gal)

In addition to cell cycle regulators, increased activity of senescence-associated β -galactosidase (SA- β -gal) is another widely used biomarker for senescent cells. SA- β -gal catalyzes the color development of its substrate at pH 6.0, and its activity originates from the upregulation of lysosomal β -galactosidase, reflecting the increased content and activity of lysosomes in senescent cells. Although the exact physiological function of SA- β -gal remains incompletely understood, its specific accumulation in most senescent cells makes it one of the gold standards for identifying senescent cells in cultured cells and tissue sections. In the serial subculture model of bone marrow mesenchymal stem cells (BMSCs), the proportion of SA- β -gal staining-positive cells gradually increases with the progression of replicative senescence, accompanied by the loss of cell proliferative capacity and functional decline.

2. Senescence phenotypes of various cells in the bone microenvironment

2.1. Senescence and lineage drift of bone marrow mesenchymal stem cells (BMSCs)

2.1.1. Loss of osteogenic differentiation potential: downregulated expression of transcription factor Runx2

Bone marrow mesenchymal stem cells (BMSCs) are the main source of bone-forming cells, and they play a key role in keeping bone balance and repairing bone damage. As age increases, BMSCs gradually go through replicative cell aging, with clear drops in their ability to divide, self-renew and turn into bone cells.

Runx2 is one of the most important regulatory proteins for bone cell differentiation. In aging BMSCs, many stress factors control the production and stability of Runx2. Oxidative stress and the loss of glutathione (GSH) can cause Runx2 protein to break down, and this holds back bone cell differentiation and new bone formation. But lowering the level of reactive oxygen species (ROS) can make Runx2 more stable and support bone growth [13]. Besides, reduced integrin-linked kinase (ILK) can also suppress Runx2 production, further lowering the activity and bone-building ability of bone-forming cells [14].

Aging BMSCs also have mitochondrial function problems, including lower membrane potential, excessive ROS buildup, and weaker antioxidant ability. These abnormal metabolic changes affect key regulatory proteins like Runx2, and finally cause a lasting drop in the cells' bone-forming potential.

2.1.2. Imbalanced adipogenic-osteogenic switch: bone marrow adiposity and its impact on bone quality

Another important feature of senescent bone marrow mesenchymal stem cells (BMSCs) is the shift in differentiation lineage, namely reduced osteogenic differentiation and enhanced adipogenic tendency, manifested as an imbalanced adipogenic-osteogenic switch. The differentiation fate of BMSCs directly determines the dynamic balance between bone formation and bone resorption; thus, this lineage drift is considered an important pathological basis of age-related osteoporosis.

With the progression of aging, BMSCs undergo systematic changes in endogenous transcriptional regulation, energy metabolism, and epigenetic status, and are continuously influenced by the senescence-associated secretory phenotype (SASP), which collectively drives their transition from

the osteogenic lineage to the adipogenic lineage. The result is abnormal accumulation of bone marrow adipose tissue (BMAT), namely bone marrow adiposity. Increased adipocytes not only occupy the space of trabecular bone and the hematopoietic microenvironment, but also inhibit osteogenesis by secreting adiponectin, free fatty acids, and inflammatory mediators, and promote local chronic inflammation and bone resorption, thereby further deteriorating the bone microenvironment.

A growing number of clinical studies have confirmed that the degree of bone marrow adiposity is closely related to bone loss and fracture risk. Most studies indicate that increased BMAT is significantly associated with reduced bone mineral density (BMD) and a higher prevalence of vertebral fractures. Proton density fat fraction (PDFF) measured by magnetic resonance imaging and changes in lipid composition can predict fracture risk independently of BMD [15]. Therefore, bone marrow adiposity is not only an important phenotype of bone aging, but also a potential imaging indicator for evaluating bone quality and fracture risk.

2.2. Senescence of osteocytes: dysfunction of the "commander" in bone tissue

2.2.1. Degeneration of the lacunar-canalicular system (LCN) and impaired mechanosensory function

Osteocytes rely on the LCN network to sense mechanical stress and exchange information with cells on the bone surface. During aging, the LCN undergoes obvious degenerative changes, including reduced osteocyte dendrite number, distorted canalicular structure, and decreased network connectivity.

Studies have shown that in aged mice and osteocyte-specific TGF- β receptor II knockout (T β RII^{ocy-/-}) mice, the number of osteocyte canaliculi decreases by 33%–45%, accompanied by reduced complexity of the network structure. Connectomic analysis reveals that this structural degeneration significantly reduces molecular diffusion efficiency in the bone matrix; computational fluid dynamics simulations further predict that the reduced canalicular surface area leads to a marked decrease in fluid shear stress on osteocytes, even when osteocyte number is not significantly reduced [16].

Therefore, LCN degeneration serves as an important structural basis for reduced mechanical sensitivity in aged bone, and also explains the phenomenon that declined bone quality does not entirely depend on bone loss.

2.2.2. Osteocyte apoptosis and senescence in regulating osteoclast recruitment

Senescent osteocytes not only passively lose sensory function but also actively participate in regulating local bone resorption. Senescent or apoptotic osteocytes can recruit and activate osteoclasts by secreting signaling molecules. Studies have shown that senescent osteocytes are also an important source of SASP. The pro-osteoclastogenic factors such as RANKL secreted by these cells can act directly on osteoclast precursors to promote their differentiation and maturation. Senescent osteocytes are involved in the regulation of TGF- β signaling. TGF- β signaling not only maintains osteocyte survival and LCN integrity, but also its downstream signaling disorder is closely related to LCN degeneration and reduced bone quality, forming a dual driving mechanism.

2.3. Senescence coupling of osteoblasts and osteoclasts

2.3.1. Impaired osteoblast maturation and reduced matrix mineralization capacity

Osteoblasts differentiated from senescent BMSCs exhibit significant impairment in both quantity and function. Senescent osteocytes show reduced responsiveness to osteogenic signals such as the Wnt/ β -catenin pathway, and downregulated expression of key transcription factors including Runx2, Osterix, and ATF4, leading to decreased synthesis of type I collagen (COL1A1), reduced alkaline phosphatase (ALP) activity, and insufficient mineralized nodule formation.

Animal studies further confirm that impaired osteoblast maturation can directly affect bone quality. Sh3bp2 mutant mice display decreased bone mineral content, reduced mineral crystallinity, abnormal collagen maturity, and a reduced number of mature osteoblasts, with significantly impaired differentiation and mineralization capacity of cultured osteoblasts *in vitro*, indicating that osteoblast dysfunction is an important basis for abnormal bone remodeling [17].

2.3.2. Induction of excessive osteoclast activation by the senescent microenvironment

Osteoclast activity is often excessively activated in the senescent microenvironment. The SASP microenvironment formed by accumulated senescent osteocytes, senescent immune cells, and other cell types is rich in pro-inflammatory factors such as IL-1 β , IL-6, and TNF- α . These factors can directly or indirectly (e.g., by inducing RANKL expression) promote osteoclastogenesis and activation. Bone remodeling coupling is disrupted during aging, resulting in a negative balance state.

3. Paracrine effects of SASP

The senescence-associated secretory phenotype (SASP) is one of the most typical functional features of cell aging, and a core process for aging cells to affect inner tissue environments and organ function. Though aging cells lose their ability to divide and grow, their internal metabolic activities do not stop. Instead, they continuously release a large number of bioactive substances, including pro-inflammatory cytokines (such as IL-1 β , IL-6, TNF- α), chemokines (such as CCL2, CCL3), growth factors (such as TGF- β), and various matrix metalloproteinases (MMPs) [8]. These secreted substances can maintain cells' aging state through autocrine signals, and act on nearby cells through paracrine signals to cause secondary aging, long-term inflammation and structural changes in tissues.

In bone tissue, SASP acts as an important link between cell aging and osteoporosis. Mature bone cells, bone marrow mesenchymal stem cells (BMSCs), bone-forming cells and immune cells can all develop SASP as aging progresses. Continuously built-up SASP factors not only hold back BMSCs' division and bone-forming differentiation and reduce the production of bone-related regulatory proteins like Runx2 and Osterix, but also boost bone-resorbing cell formation and bone breakdown by raising the RANKL/OPG ratio and turning on signaling pathways such as NF- κ B and MAPK, finally causing unbalanced bone renewal.

SASP can also cause a "cell aging spreading" effect. Inflammatory factors and reactive oxygen species from aging cells can damage DNA and disrupt normal function of nearby healthy cells, which then turn into new aging cells and keep increasing aging pressure in the local inner environment. This circular feedback process is especially important in bone tissue, as mature bone cells live long and renew slowly; once they enter an aging state, they can continuously break bone metabolic balance.

There is direct evidence that SASP from mature bone cells promotes bone-resorbing cell differentiation. Studies show that ionizing radiation (IR) in lab conditions can cause lasting DNA repair defects, cell dysfunction and typical aging features in mature bone cells. At the same time, the chemokine CCL3 (CC chemokine ligand 3) rises clearly in aging bone cells. When the culture liquid of these cells is used in co-culture tests, the differentiation ability of early bone-resorbing cells improves greatly, showing that aging bone cells directly speed up bone breakdown through SASP factors [18].

3.1. Components of SASP (senescence-associated secretory phenotype)

3.1.1. Cascade reaction of pro-inflammatory cytokines

Pro-inflammatory cytokines are the main parts of SASP. Among them, IL-1 β , IL-6 and TNF- α play key roles in controlling bone metabolic activities. IL-1 β can trigger the production of IL-6. The joint effect of IL-6 and TNF- α further raises RANKL expression levels. This greatly promotes the formation of bone-resorbing cells, while holding back the differentiation and mineral-forming function of bone-forming cells.

As BMSCs enter the aging state, IL-6 levels keep rising continuously. Externally added IL-6 not only causes DNA damage and stops the cell division cycle in young BMSCs, but also reduces their bone-forming ability. This shows that IL-6 plays an important role in the spread of cellular aging. Lab studies have found that using multiple inflammatory cytokines together increases the RANKL/OPG ratio by about two times, and clearly enhances the bone-breaking activity of bone-resorbing cells [19]. So, pro-inflammatory cytokines form a self-strengthening inflammatory network, which keeps driving bone renewal toward more bone breakdown.

3.1.2. Degradation of bone matrix by matrix metalloproteinases (mmps)

Besides cytokines, SASP also contains different kinds of matrix metalloproteinases (MMPs), such as MMP1, MMP3 and MMP9. MMPs can directly break down bone matrix components including type I collagen. They also damage the tiny inner structure of trabecular bone, and reduce the mechanical strength of bone tissue.

What is more, MMPs can cut apart growth factors stored in the extracellular matrix, and release hidden signal molecules. This further controls the behavior of bone-forming cells and bone-resorbing cells. Studies have shown that even after bone-resorbing cells are removed, cells from MSCs can still break down bone matrix in a way that depends on MMPs. This process can be effectively blocked by MMP inhibitors [20]. This suggests that proteases in SASP are not just tissue-damaging factors. They are also important carriers for spreading aging signals.

3.2. Bystander effect mediated by SASP

3.2.1. Senescence spreading in the local bone microenvironment

The "bystander effect" caused by SASP describes a common biological phenomenon. Aging cells release SASP factors and make nearby originally healthy cells enter an aging state. In this way, aging signals can spread and become stronger in a local area. In the inner environment of bone, aging BMSCs, bone cells and bone-forming cells secrete factors such as IL-6 and IL-8. These factors act on early bone marrow mesenchymal stem cells around them. They lead to the buildup of reactive oxygen species, trigger DNA damage responses, and stop the cell division cycle. As a result,

the number of aging cells grows larger. This circular feedback process speeds up the drop in function of the whole bone inner environment.

3.2.2. Relationship between systemic inflammation (inflammaging) and local bone loss

Inflammaging means a long-term, mild inflammatory state across the whole body as people grow older. It is marked by higher levels of pro-inflammatory substances in the blood, such as interleukins and tumor necrosis factor, in old people who have no clear infection. This state can cause tissue damage and physical weakness. Pro-inflammatory factors from the senescence-associated secretory phenotype (SASP) do not only work on local tissues. They also enter the blood circulation system and make this whole-body inflammation worse. The rise of whole-body inflammation with aging causes two kinds of harmful effects. On one hand, it changes the function of immune cells — for example, it makes T cells and myeloid cells show more inflammatory features — and indirectly worsens immune disorders in the skeleton. On the other hand, cytokines in the blood such as TNF- α and IL-6 can act directly on bone tissue. They stimulate the formation of bone-resorbing cells and hold back the function of bone-forming cells. This shifts the balance of bone renewal toward more bone breakdown and less bone formation.

Clinical studies have also proved the close link between inflammation and bone health. Patients with bone fractures have much higher levels of C-reactive protein (CRP) and systemic immune-inflammation index (SII) than people in the control group. The specific data are as follows: CRP level is 66.2 ± 70.3 mg/L in fracture patients, compared with 3.8 ± 4.0 mg/L in the control group ($p = 0.0008$); SII value is 1399.7 ± 1143.4 in fracture patients, compared with 751.4 ± 400.8 in the control group ($p = 0.025$). Besides, higher CRP levels have a clear negative connection with lower bone mineral density (BMD) in all tested parts. For the hip, the r value is about -0.63 ($p = 0.002$); for the spine, r is about -0.60 ($p = 0.005$); for the wrist, r is about -0.60 ($p = 0.005$) [21].

Besides, inflammaging and immune aging work closely with each other in both function and inner mechanisms. They together affect the natural renewal of bone tissue [21]. Inflammaging mainly shows two features: abnormal polarization of M1 macrophages, and more inflammatory cytokines in the blood. Immune aging means the body reacts worse to infections and vaccines, has weaker ability to fight bacteria, and has more aging B cells and memory T cells gathering in tissues. Inflammaging caused by Toll-like receptor (TLR) and changes in the adaptive immune system greatly affect the renewal of alveolar bone. They make age-related alveolar bone loss worse [21]. The aging immune system plays a key part in age-related alveolar bone loss. Local bone aging and inflammation form a harmful circular cycle with whole-body aging and inflammation. They together push forward the development of senile osteoporosis [21, 22].

3.3. Abnormal activation of signaling pathways

3.3.1. Imbalanced RANKL/OPG ratio

The RANKL/RANK/OPG signal system is the final common path that controls the formation and activation of bone-resorbing cells. In the aging inner environment of bone, many factors cause an imbalance between RANKL and its decoy receptor OPG. A lack of estrogen directly leads to higher RANKL expression and lower OPG production. Besides, SASP factors released by aging cells (especially mature bone cells), such as IL-6, TNF- α and prostaglandin E2, can strongly stimulate bone-forming cells and bone marrow stromal cells to produce RANKL while holding back OPG

production. The rise in the RANKL/OPG ratio is one of the core reasons why the aging microenvironment causes excessive activity of bone-resorbing cells.

A combined analysis of multiple studies shows that the OPG/RANKL ratio in the osteoporosis group is clearly lower than that in the control group (SMD = -0.29; 95% CI -0.57 to -0.02; $P < 0.05$). There is extremely low statistical difference among the included studies ($\text{Chi}^2 = 0.20$, $P = 0.66$, $I^2 = 0\%$) [23].

The discovery of the RANKL/RANK/OPG signaling pathway is an important breakthrough in understanding the control of bone-resorbing cell formation during the bone renewal cycle. RANKL binds to its receptor RANK on precursor cells of bone-resorbing cells. It drives the differentiation of these cells, and promotes their fusion, activation and survival. As a decoy receptor for RANKL, OPG is secreted by bone-forming cells and mature bone cells. It traps RANKL and blocks its binding to RANK, thus inhibiting bone breakdown caused by bone-resorbing cells. So the OPG/RANKL ratio is critical for regulating bone resorption, bone mass and the structural integrity of the skeleton [23].

3.3.2. Inhibition of the Wnt/ β -catenin signaling pathway

The Wnt/ β -catenin signaling pathway acts as a positive regulator for the differentiation of bone-forming cells and new bone formation. Aging suppresses the activity of this pathway at multiple levels. The most direct mechanism is the increase of sclerostin. Mature bone cells produce more sclerostin during aging, and this substance is a strong antagonist of the Wnt signaling pathway. Besides, certain inflammatory factors in SASP may also interfere with the transmission of Wnt signals. Suppressed Wnt signaling leads to blocked differentiation of bone-forming cells and weakened function, which forms a key molecular basis for the age-related drop in bone-forming capacity. The Wnt/ β -catenin pathway plays a crucial role in bone formation, making it a promising target for preventing bone loss related to osteoporosis.

PB greatly promotes the proliferation of bone-forming cells, speeds up cell cycle progression, and reduces cell death caused by DEX in a dose-dependent manner. Besides, PB enhances the differentiation and mineralization of bone-forming cells, and counteracts the inhibitory effects of DEX on ALP activity and calcium deposition. Western blot analysis confirms that PB restores β -catenin levels and activates the Wnt/ β -catenin pathway. Notably, the bone-forming effect of PB disappears when the Wnt inhibitor XAV939 is used, which further supports its Wnt-dependent working mechanism [24].

Compared with healthy controls, BMSCs from osteoporosis patients have higher expression levels of DANCR and miR-320a, while CTNNB1 expression is lower. Importantly, studies have proved that miR-320a and DANCR work independently of each other. Both of them inhibit CTNNB1 expression, and their inhibitory effects add up when miR-320a and DANCR are overexpressed at the same time. These findings show that DANCR and miR-320a regulate the Wnt/ β -catenin signaling pathway during osteogenic differentiation in osteoporosis by inhibiting CTNNB1 [25].

Overexpression of C4ST-1 raises the 4S/6S ratio and significantly inhibits the differentiation of bone-forming cells. To make this mechanism clear, researchers studied possible feedback loops. Increased 4-sulfated CS caused by C4ST-1 overexpression enhances Wnt3a expression, which in turn upregulates p53 expression. Using drugs to inhibit β -catenin and p53 partly restores Akp2 expression, which confirms their roles as key mediators. It is proposed that a high 4S/6S ratio activates the Wnt/ β -catenin-p53 axis, in which p53 acts as a "brake" on cell differentiation [26].

4. Upstream triggers inducing osteocyte senescence

4.1. DNA damage response and telomere attrition

The DNA damage response (DDR) is one of the classic mechanisms inducing cellular senescence. With an increasing number of cell divisions, telomeres at the ends of chromosomes gradually shorten. When telomeres shorten to a critical length, cells recognize this as DNA double-strand breaks, thereby activating the ATM/ATR-mediated DDR pathway, which subsequently triggers p53/p21 and p16^{INK4a}/Rb signaling and induces irreversible cell cycle arrest, known as replicative senescence.

In addition to telomere exhaustion, factors such as oxidative stress, ionizing radiation, and chemical toxicity can also directly cause DNA damage and induce stress-induced premature senescence. In bone tissue, the continuous accumulation of DNA damage is closely associated with bone loss. In Bmi1-deficient mice, the ATM–Chk2–p53 pathway is abnormally activated, accompanied by enhanced oxidative stress and osteocyte senescence, ultimately resulting in reduced osteogenesis, increased bone resorption, and significant bone loss [27]. Furthermore, during fracture repair, transient senescent cells in the callus can be detected with telomere-associated foci (TAF) and elevated expression of p16 and p21, suggesting that DDR plays an important regulatory role in bone tissue remodeling [28].

4.2. Oxidative stress and mitochondrial dysfunction

Accumulation of reactive oxygen species (ROS) is a key driver of cellular senescence, and mitochondrial dysfunction is both a major source and a primary target of ROS. With advancing age, mitochondrial membrane potential decreases, respiratory chain efficiency declines, ATP production reduces, and ROS generation increases in BMSCs and osteoblasts. This leads to continuous damage to mitochondrial DNA, lipids, and proteins, forming a vicious cycle of "mitochondrial damage–ROS accumulation–further damage".

Excessive ROS can activate signaling pathways such as p53/p21, p38 MAPK, and NF-κB, inducing cell cycle arrest and SASP secretion. Studies have shown that in Osx1-Cre-mediated Sod2-deficient mice, the mitochondrial antioxidant capacity of osteoblasts decreases, leading to impaired mitochondrial respiration, reduced NAD⁺ levels, and an obvious osteopenic phenotype [29]. In addition, oxidative stress is also closely related to a reduced number of osteoprogenitor cells and increased bone marrow adipose tissue (BMAT) [30]. Therefore, the mitochondrial-ROS axis serves as an important molecular hub linking energy metabolic disorders to bone aging.

4.3. Nutrient sensing and epigenetic alterations

4.3.1. Roles of SIRT1 and mtor pathways in regulating bone lifespan

Cells can sense their own energy levels and nutritional conditions. This ability serves as a key mechanism that controls the process of cell aging. Sirtuin 1 (SIRT1) is a deacetylase that relies on NAD⁺ to work normally. It can improve antioxidant ability, support the growth of mitochondria, promote autophagy and slow down cell aging. It works by modifying key proteins such as p53, FOXO, and PGC-1α.

In bone tissue, the activity of SIRT1 gradually drops as age increases. This change leads to weaker bone-forming ability of BMSCs and gradual loss of bone mass. SIRT1 activators such as

resveratrol have been proved to improve abnormal bone metabolism and slow down the progression of osteoporosis [31]. The mammalian target of rapamycin (mTOR) is another key system that controls protein production and cell growth, and its long-term activation is closely linked with the process of cell aging. Too much mTOR activation can hold back autophagy and promote the production of SASP. But rapamycin and its similar substances can reduce SASP release and improve the function of aging BMSCs. Studies have found that resveratrol can enhance mitophagy and protect bone-forming cells in osteoporosis models. It achieves this effect by activating SIRT1 and adjusting the PI3K/AKT/mTOR signaling pathway [31].

4.3.2. Regulation of senescence genes by histone modifications and non-coding rnas

Epigenetic alterations represent an important molecular basis of cellular senescence, including abnormal DNA methylation, remodeled histone modifications, and imbalanced non-coding RNA expression. Reduced heterochromatin markers (such as H3K9me3) commonly observed in senescent cells can lead to loose chromatin structure, transposon activation, and genomic instability.

Non-coding RNAs, particularly miRNAs and long non-coding RNAs (lncRNAs), play important regulatory roles in bone aging. For example, miR-29c-3p can promote BMSC senescence by targeting CNOT6 to activate the p53/p21 and p16/Rb pathways. The lncRNA Hottip participates in maintaining bone homeostasis by regulating histone methylation. Hottip-knockout mice exhibit decreased bone mass, impaired bone quality, and delayed fracture healing; in vitro experiments show that Hottip deficiency inhibits osteogenic differentiation and promotes osteoclastogenesis [32].

5. Novel therapeutic strategies for osteoporosis targeting cellular senescence

As the critical role of cellular senescence in the occurrence and development of osteoporosis has been gradually clarified, intervention strategies targeting senescent cells and their secretory phenotypes are becoming an important direction in anti-osteoporosis research. Different from traditional anti-resorptive or osteoanabolic drugs, anti-aging therapies aim to reduce senescent cell burden, inhibit chronic inflammation, and restore bone microenvironment homeostasis from the root cause of the disease.

5.1. Application of senolytics

5.1.1. Performance of dasatinib + quercetin (D+Q) in osteoporosis animal models

Senolytics selectively induce apoptosis of senescent cells by targeting senescent cell anti-apoptotic pathways (SCAPs), thereby reducing senescent cell burden and SASP levels. Dasatinib combined with quercetin (D+Q) is one of the most extensively studied senolytic combinations to date.

In naturally aged mice, intermittent administration of D+Q significantly reduces the number of p16⁺ cells in bone tissue, decreases inflammatory factor expression, and improves cortical and trabecular bone structure. In a type 2 diabetic osteoporosis (T2DOP) model, D+Q not only increases bone mass but also reshapes the gut microbiota, elevates short-chain fatty acid-producing bacteria (such as Lachnospiraceae and Bacteroides), and improves blood lipid and amino acid metabolism, suggesting its therapeutic effect may involve systemic metabolic regulation [33].

In a phase II randomized controlled trial of postmenopausal women, no significant improvement in bone turnover was observed in the overall population. However, exploratory analysis found that subjects with high baseline senescent cell burden showed elevated bone formation marker P1NP,

decreased bone resorption marker CTX, and significantly increased radial bone mineral density after receiving D+Q [34]. This result indicates that the clinical efficacy of senolytics may be closely related to the patient's internal senescent cell burden.

5.1.2. Potential anti-aging effects of natural products (e.g., fisetin)

Fisetin is a natural flavonoid widely found in fruits and vegetables, with favorable senolytic activity and safety. Compared with D+Q, fisetin exhibits superior anti-aging effects in certain models.

In *Zmpste24^{-/-}* progeroid mice, D+Q failed to significantly alleviate severe trabecular bone loss, whereas fisetin markedly attenuated the reduction in bone mineral density and damage to bone microarchitecture [35]. Theoretical modeling studies also suggest that early administration of fisetin may delay bone loss in postmenopausal women [36]. Due to its natural origin and low toxicity, fisetin is regarded as a highly translatable drug candidate.

5.2. Senomorphics

5.2.1. Targeted drugs inhibiting SASP secretion

Senomorphics aim to modulate the phenotype of senescent cells, particularly suppressing SASP secretion without killing the cells. JAK kinase inhibitors represent one class of SASP modulators. Studies have shown that intervention with JAK inhibitors reduces SASP factor levels in aged mice and alleviates bone mineral density loss. In addition, drugs targeting the NF- κ B pathway, the main regulator of SASP transcription, such as metformin and glucocorticoids, have also been proven to inhibit SASP production [37].

5.2.2. Protective potential of metformin and rapamycin on bone mineral density

Several existing drugs have been found to possess senomorphic properties. Metformin is a classic hypoglycemic agent that activates the AMPK signaling pathway, inhibits mTOR phosphorylation, reduces ROS generation, and downregulates miR-34a-3p, thereby alleviating DNA damage, delaying BMSC senescence, and suppressing SASP factor secretion [38]. Rapamycin directly inhibits mTORC1, reducing SASP production while inducing autophagy, enhancing the function of senescent BMSCs, increasing bone mineral density, and promoting osteogenesis in osteoporosis models. Resveratrol delays BMSC senescence and improves bone microarchitecture by activating SIRT1, regulating oxidative stress and inflammation, and preserving telomere integrity. These studies suggest that "drug repurposing" of approved pharmaceuticals may provide a rapid pathway for developing anti-osteoporosis therapies.

5.3. Novel delivery systems and gene therapy

5.3.1. Development of bone-targeting nanocarriers

Senolytics and senomorphics may take effect across the whole body, and they may cause potential toxic side effects. For this reason, improving the gathering efficiency of drugs in bone tissue has become a key research problem. In recent years, bone-targeted delivery systems based on liposomes, polymer nanoparticles and metal-organic frameworks have developed rapidly.

For example, biomimetic membrane-derived nanovesicles (mNVs) are coated with BMSC membranes. They can cross the bone marrow-blood barrier and help drugs reach and gather in the

bone marrow efficiently. In osteoporosis models, mNVs loaded with teriparatide greatly slow down bone loss and maintain normal bone mass [39]. Besides, ASP8-modified ZIF-8 nanoparticles carrying curcumin can both promote bone formation and hold back the activity of bone-resorbing cells. They effectively improve the inner micro-structure of bone tissue in ovariectomized mice [40].

5.3.2. Prospects of exosomes as cell-free therapeutic agents

Exosomes are nanoscale vesicles secreted by cells, naturally carrying miRNAs, mRNAs, and proteins, and play important roles in intercellular communication. Exosomes derived from healthy BMSCs or adipose mesenchymal stem cells can promote osteogenesis, inhibit osteoclastogenesis, and alleviate oxidative stress.

Numerous studies have shown that exosome therapy improves osteoblast viability and mineralization capacity, and mitigates bone loss induced by estrogen deficiency or glucocorticoids [41]. For example, adipose-derived stem cell exosomes promote osteogenesis by blocking the nuclear export of hsa_circ_0028877. Further engineering strategies, such as loading osteogenesis-related RNAs or proteins, surface modification with bone-targeting peptides, and cell pretreatment, can significantly enhance the targeting and therapeutic efficacy of exosomes [42, 43].

Compared with traditional cell therapy, exosomes possess advantages such as low immunogenicity, low toxicity, high stability, and ease of storage, and are considered one of the important cell-free strategies for the future treatment of primary osteoporosis [44].

6. Limitations, challenges and future perspectives

At present, the translation of basic research targeting cellular senescence into clinical practice still faces numerous bottlenecks. First, senescent cells in bone tissue are highly heterogeneous. Senescent phenotypes and SASP profiles differ among distinct cell types (BMSCs, osteocytes, immune cells, etc.) and change dynamically with aging stages. It remains challenging to identify universal biomarkers that can precisely target pathological senescent cells without affecting normally functioning cells. Second, the gap between experimental models and clinical reality warrants caution. In vitro senescence induction methods (e.g., radiation, serial passaging) do not fully replicate the natural aging process in humans; findings from different species and animal strains are also difficult to extrapolate directly to humans. For instance, IL-6 significantly inhibits osteogenesis in mouse BMSCs, whereas similar effects in human BMSCs may require much higher concentrations. Furthermore, the long-term safety and optimal dosing regimens of senolytics still need validation in large-scale clinical trials.

In the future, osteoporosis prevention and treatment from the perspective of precision medicine will heavily rely on the detection of senescence biomarkers and risk early warning. By measuring circulating levels of specific SASP factors (e.g., IL-6, MMP9), assessing p16^{INK4a} expression in peripheral blood cells, or using novel imaging techniques to evaluate bone marrow adiposity and osteocyte LCN integrity, it is promising to establish an individualized "bone aging" risk assessment model. Combined with these biomarkers, clinicians can identify high-risk populations before severe bone loss occurs and select the most appropriate intervention strategies—whether to eliminate senescent cells, inhibit SASP, or enhance autophagy and mitochondrial function—thus achieving a fundamental shift from "treating disease" to "delaying aging and preventing disease".

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