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Osteoporosis, a Result of an "Aged" Bone Microenvironment

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Abstract

Osteoporosis is an age-related progressive bone disease. Recent advances in epigenetics, cell biology, osteoimmunology, and genetic epidemiology have unraveled new mechanisms and players underlying the pathology of osteoporosis, supporting a model of age-related dysregulation and crosstalk in the bone microenvironment.

Keywords

osteoporosis; aging; osteoimmunology; epigenetics; Wnt signaling

Osteoporosis is a "silent bone disorder" characterized by low bone mass and bone fragility, contributing to an increased public health and economic burden for our aging population [Box 1]. A significant number of osteoporotic cases go undiagnosed until the first bone fracture. Current treatment options, mostly antiresorptive agents (estrogen, bisphosphonates and denosumab) are limited in their ability to restore bone loss, once it is diagnosed. Newer and more effective treatment modalities for osteoporosis hinge on our evolving understanding of the players and mechanisms underlying this progressive bone loss.

Box 1

The Burden and Disease Etiology of Osteoporosis

Afflicting over 200 million worldwide, osteoporosis is by far the most common bone disease, leading to over 9 million fractures annually[1]. With 1 in 3 women and 1 in 5 men over 50 years old at risk, cause significant mortality (20–30% associated with first hip fracture) and morbidity to elderly individuals. Given the aging population, by 2025, the annual healthcare cost of osteoporotic fractures are predicted to reach $25.3 billion in the United States alone. The primary causes of osteoporosis are related to intrinsic age-

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related changes in bone metabolism, and have been historically associated with post-menopausal estrogen deficiency in women and with slowing production of testosterone in men. A growing number of underlying diseases (e.g. congenital connective tissue defects, metabolic and hematologic disorders, hypogonadal states, inflammatory diseases) nutritional deficiencies (e.g. Vitamin D and malabsorption) or drugs (e.g. corticosteroids and thyroid replacement) are recognized as secondary causes for osteoporosis, and may be key etiological factors in premenopausal women and men. A better understanding of the molecular mechanisms driving this multifactorial bone loss is evolving from an estrogen-centric paradigm to one focusing on age-related changes within the bone microenvironment.

The Forces at Stake: Osteoporosis Is Driven by Age-Related Mechanisms

The orchestrated balance between bone resorption by osteoclasts, and bone formation by osteoblasts, maintains a relatively stable bone mass in adulthood. In osteoporosis, accelerated osteoclastic resorption overwhelms compensatory bone formation, leading to net bone loss. Up until the last decade, the predominant cause of osteoporosis was attributed to estrogen deficiency. However, this estrogen-centric view has been challenged and revised in the recent decade, with our enhanced understanding of the skeletal aging process [2]. In both genders, trabecular bone loss occurs despite sex steroid sufficiency, suggesting intrinsic aging-related mechanisms at play.

Chronic, low-grade inflammation is a hallmark of aging. With advanced age, accumulating cytokines such as IL-6, TNF-α and IL-1 render the bone marrow (BM) increasingly pro-inflammatory[3]. The connection between inflammation and osteoporosis has long been established *in vitro* and in animal research. For instance, the transcription factor nuclear factor kappa B (NF-κB) is activated in most inflammatory responses. While activation of NF-κB signaling is a key step required in osteoclast differentiation, it can also potently inhibit osteoblastic bone formation [4]. Three recent large-cohort human epidemiological studies confirmed this immunological link, whereby a 1.5–3 fold increase in osteoporotic fracture risk was associated with a higher level of inflammatory “markers” [5]. Consistently, estrogen-withdrawal promotes T-cell activation and immune cytokine production in both rodents and humans. Furthermore, oxidative stress (OS) arises during aging with the accumulation of excess intracellular reactive oxygen species. Mounting *in vivo* evidence in rodents suggests that age-induced OS may contribute to osteoporotic bone loss[2]. Both estrogen-deficiency via ovariectomy (OVX) and aging-related bone loss result in increased OS markers. The buildup of OS also leads to the activation of NF-κB in various aging tissues. Hence, age-related chronic inflammation of the bone microenvironment could be a unitary driving force in the pathogenesis of osteoporosis. However, it remains unclear how intrinsic changes in aged BM niches lead to chronic inflammation, or if osteoporosis and altered bone metabolism in turn, exacerbate the inflammatory states of an aged BM.

The Players: Crosstalk between Skeletal, Immune Systems and Beyond

As alluded above, the onset of osteoporotic bone loss involves aberrant activation of the adaptive immune system. One of the most intense areas of research focuses on delineating the osteoimmunological interactions between various cell types residing in the bone microenvironment.

Osteoblasts and osteoclasts are long known to be coupled to the physiological maintenance of bone mass. Insulin-like growth factor I (IGF-1) and transforming growth factor β are classical matrix-derived coupling agents released by osteoclastic resorption to stimulate bone formation. A series of osteoclast-derived clastokines including PDGF-BB, have also been recently shown to promote bone formation [6]. In contrast to current osteoclast-targeting antiresorptive agents, odanacatib, a small-molecule inhibitor of cathepsin-K, suppresses resorption without affecting osteoclast survival. In fact, odanacatib exploits the coupling between osteoclasts and osteoclasts, by increasing the number of osteoclast precursors, and thereby promoting the secretion of osteogenic PDGF-BB[6].

Since the turn of this millennium, T- and B-lymphocytes have been recognized to play an indispensable role in the onset of osteoporosis by regulating bone cell functions. Estrogen depletion reportedly stimulates T/B-cell expansion and production of osteoclastogenic cytokines TNF-α and RANKL. T cells, normally associated with osteoclast activation, have recently been shown to reciprocally suppress osteoclasts both *in vitro* and *in vivo* via CTLA-4[7]. Tightly regulated interactions between the immune and skeletal systems have reaffirmed that aberrant immune responses have a strong potential of driving the disequilibrium of bone metabolism in osteoporosis. However, these findings also raise intriguing questions: 1) how does aging disrupt osteoimmune feedbacks, thereby leading to osteoporosis? 2) Could age-related weakening of the immune system prime the aging bone for osteoporotic bone loss?

Other important constituents of the bone microenvironment are adipocytes, derived from the same mesenchymal stem cell (MSC) progenitor pools as osteoblasts. The lineage commitment towards osteoblasts and adipocytes is considered mutually exclusive. In osteoporosis or skeletal aging, aberrant lineage allocation of MSCs leads to overwhelming marrow adipose tissue (MAT) accumulation at the expense of bone formation. The hormone leptin is secreted by adipocytes, and its peripheral/local effects on bone metabolism remain controversial[8]. Intriguing findings from a recent Prx1cre-Lepr$^{fl/fl}$ mouse model showed that local leptin signaling in limb bone marrow MSCs promoted adipogenesis while inhibiting osteogenesis[8], suggesting that aberrant increases in MAT could influence MSC lineage decisions. In addition, as the bone responds to various environmental cues during aging, epigenetic regulation of MSC lineage specification may also play a role in osteoporosis. Histone demethylases KDM4B and KDM6B favor osteogenesis over adipogenesis from human MSCs, by removing gene-silencing H3K9me3 and H3K27me3 chromatin marks on the promoters of osteogenic master regulator genes[9]. Indeed, KDM6B knockout mice exhibit impaired osteoblastogenesis[10]. Furthermore, H3K9me3 and H3K27me3 expression is elevated in BM MSCs of aged and OVX mice[9]. Concordantly, EZH2, an H3K27-specific methyltransferase, is upregulated in BM MSCs from OVX mice;

its suppression has been shown to effectively prevent bone loss and adipogenesis following OVX [11]. In the future, it will be important to determine how and if, high levels of H3K9me3 and H3K27me3 lead to a bone and fat functional switch that results in osteoporosis and skeletal aging.

The Cure: Novel Therapeutics on the Horizon Targeting WNT Signaling

To date, antiresorptive treatments can only reduce non-vertebral fracture risk by 30–40% [1]. Moreover, continued decrease of bone turnover carries long-term adverse effects such as osteonecrosis of the jaw. Efforts in osteoporosis treatment are shifting to new paradigms favoring bone restoration. Presently the only anabolic drugs approved for osteoporosis are recombinant forms of parathyroid hormone (PTH), which effectively increases bone mineral density (BMD) and reduces fracture risks. However, limitations of PTH include potential carcinogenicity and plateauing of BMD increase after two years of treatment[1]. Certain regulatory signaling pathways shared between various cells in the bone microenvironment may indeed emerge as novel therapeutic targets. It is known that WNT signaling is a central pathway favoring osteoblast differentiation while inhibiting adipogenesis. WNTs are secreted growth factors that can be classified into canonical and non-canonical ligands based on their dependence on transduction through β-catenin. As early as 2002, mutations involving several canonical WNT pathway components featuring LRP5 and SOST have been reported to result in high bone mass, generating tremendous interest in targeting WNT signaling to treat osteoporosis. Recent waves of genome-wide association studies (GWAS) of age-related osteoporosis have corroborated the validity of this approach, since *LRP5, DKK1, SOST* and other genes encoding components of the WNT pathway have emerged as contributing factors to bone mass variations[12]. Furthermore, several inhibitors of WNT antagonists including DKK1, WIF-1 and SOST have been suggested as putative candidates [1]. However, recent reports have demonstrated that SOST inhibition can exacerbate TNF-dependent inflammation in three rodent models of rheumatoid arthritis [13]. These findings suggest that caution should be taken when targeting WNT antagonists for the treatment of osteoporosis patients with inflammatory comorbidities. Another potential concern for indiscriminately activating the canonical WNT pathway is the tumorigenicity risk in non-skeletal tissues, given that stabilization of β-catenin has been associated with several malignancies.

An alternative strategy might involve targeting the non-canonical WNT pathway whose signaling transduction is independent of β-catenin. The *WNT4* locus has been identified as a genetic determinant of BMD and fracture risk (13). Indeed, transgenic mice expressing *Wnt4* in osteoblasts have been found to present increased bone formation and attenuated bone loss either from OVX treatment, aging or TNF-induction[14]. Mechanistically, WNT4 inhibited NF-κB activation in the bone microenvironment, thereby suppressing osteoclasts while promoting osteoblasts [14]. Consequently, targeted delivery of WNT4 may represent a promising approach for bone restoration, provided it bears the dual-action capability of restoring bone loss while attenuating local inflammation and lacking tumorigenic potential. *WNT16* has also been recognized as a BMD-associated gene locus in several GWAS studies in humans[12]. Global as well as osteoblast-specific deletion of *Wnt16* in mice was shown to result in reduced cortical, but not trabecular, bone mass [15]. Of note, osteoblast-derived

Wnt16 can inhibit osteoclasts in mice directly via non-canonical signaling, or indirectly, by raising OPG levels in osteoblasts via canonical and non-canonical WNT signaling [15]. Altogether, the therapeutic efficacy and safety of administering non-canonical WNTs, as well as the effects on surrounding immune system and non-skeletal tissues remain to be elucidated.

Concluding Remarks

Mounting evidence supports osteoporosis as a multifactorial age-related disease resulting from dysregulated interactions within the bone microenvironment such as aberrant osteoimmune responses and MSC lineage commitment (Figure 1). With the aid of large-scale GWAS studies, our renewed understanding of the pathogenesis of osteoporosis may facilitate the identification of novel putative targets and inform therapeutic decisions to overcome this long-standing challenge.

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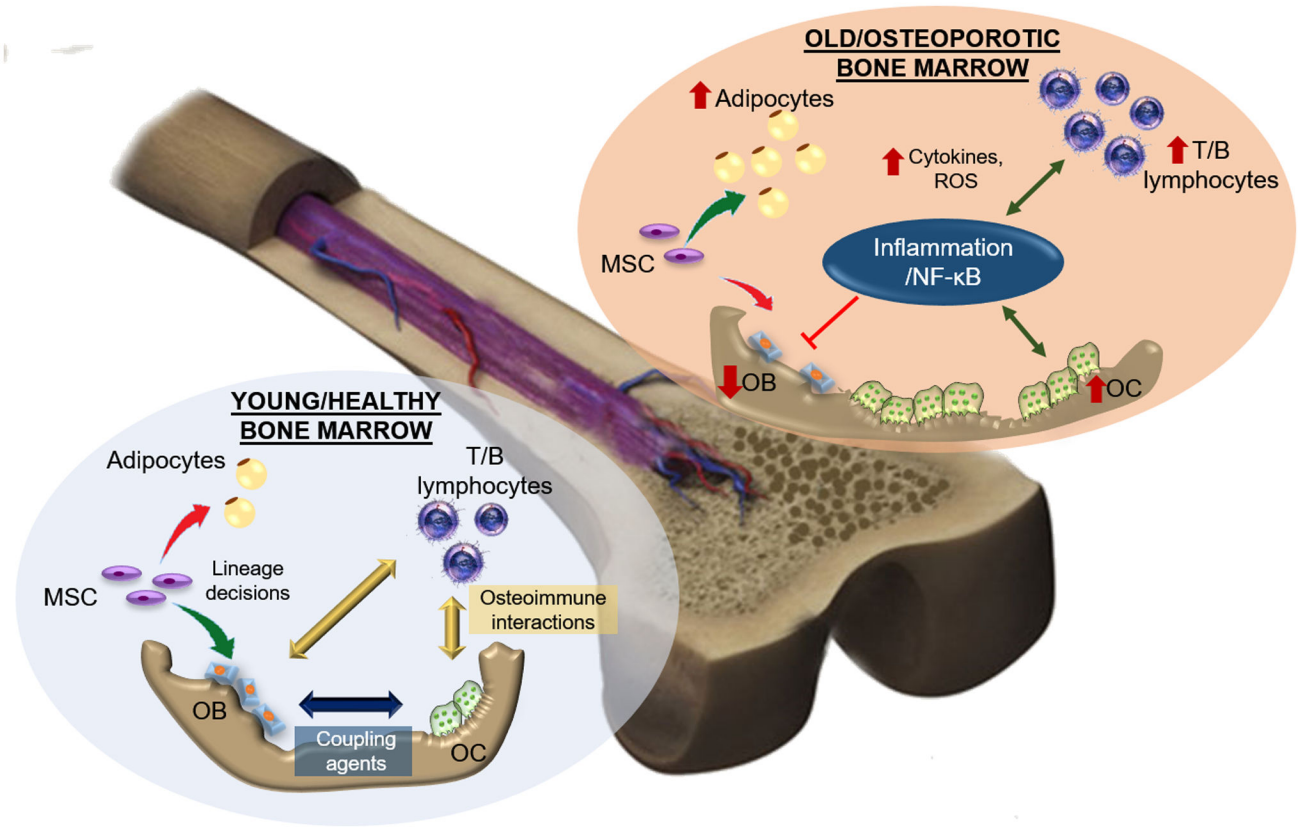

Figure 1. Overview of Age-Related Changes in the Bone Marrow Microenvironment

In young/healthy marrow, osteoblasts and osteoclasts are balanced via coupling agents such as RANKL, IGF-1 and PDGF-BB. Immune T/B cells reciprocally regulate bone cells through osteoimmune interactions, and MSCs are more prone to differentiate towards an osteolineage rather than into adipocytes. In old/osteoporotic marrow, accumulating ROS and pro-inflammatory cytokines can create a chronic inflammatory state. NF-κB activation can leads to T/B cell expansion, enhanced numbers of osteoclasts and suppressed numbers of osteoblasts. Epigenetic dysregulation can also result in an MSC shift from osteolineage to adipocytes. Thus, those changes, among many, can lead to a bone matrix that becomes thin and porous (osteoporotic) and susceptible to fracture. Abbreviations: OB, osteoblast; OC, osteoclast; MSC, mesenchymal stem cell; ROS, reactive oxidative species.