Nuclear Receptor Regulation of Osteoclast and Bone Remodeling

Zixue Jin1, Xiaoxiao Li1, and Yihong Wan1

1 Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

Mini Review

Osteoclasts are bone resorbing cells essential for skeletal remodeling and regeneration. However, excessive osteoclasts often contribute to prevalent bone degenerative diseases such as osteoporosis, arthritis and cancer bone metastasis. Osteoclast dysregulation is also associated with rare disorders such as osteopetrosis, pycnodysostosis, Paget’s disease and Gorham-Stout syndrome. The nuclear receptor (NR) family of transcription factors functions as metabolic sensors that control a variety of physiological processes including skeletal homeostasis and serves as attractive therapeutic targets for many diseases. In this review, we highlight recent findings on the new players and the new mechanisms for how NRs regulate osteoclast differentiation and bone resorption. An enhanced understanding of NR functions in osteoclastogenesis will facilitate the development of not only novel osteoprotective medicine, but also prudent strategies to minimize the adverse skeletal effects of certain NR-targeting drugs for a better treatment of cancer and metabolic diseases.

Bone remodeling and osteoclast

Bone is a rigid organ. Yet it is regulated by a highly dynamic remodeling process that is carried out by bone degrading osteoclasts, bone forming osteoblasts and mechanical sensing osteocytes. Osteoclast is the only cell type that can remove bone, which is an important activity during both skeletal development and skeletal maintenance in adulthood (1–3). The coordinated action of osteoclasts and osteoblasts is critical for normal bone remodeling. When the balance between bone formation and degradation is lost, diseases occur.

Osteoporosis and other osteoclast-related diseases are highly prevalent in our aging society, and represent enormous personal and economic cost. In 2002, an estimated 44 million people aged 50 or older in the United States were at risk for fracture due to osteoporosis or low bone mass. If this trend continues and no effective treatments identified and widely implemented, it is estimated that by 2020 more than 61 million people will be at risk. Furthermore, according to American Academy of Orthopaedic surgeons, the projected costs for osteoporosis care over the next two decades are $474 billion.

Osteoporosis is the most common skeletal disease in which bone resorption exceeds bone formation, leading to bone loss and fractures (4, 5). Inflammatory arthritis is associated with periarticular and joint bone erosion, resulting from precocious recruitment of osteoclasts in the inflamed bone tissue (6). In cancer patients, excessive osteoclast activity promotes cancer metastasis to the bone, a frequent, debilitating and essentially incurable cancer complication, leading to osteolysis, bone pain, fractures and life-threatening hypercalcemia. Osteoclast overabundance is also associated with several less common diseases including Paget’s disease, named after Sir James Paget, an English surgeon and pathologist (7); as well as Gorham-Stout syndrome (GSS), also known as vanishing bone disease or phantom bone disease (8). In contrast, osteoclast deficiency results in rare disorders such as osteopetrosis and pycnodysostosis, the latter is also known as Toulouse-Lautrec syndrome, named after the French artist Henri de Toulouse-Lautrec (7, 9). In these disorders, bones are abnormally dense but brittle, due to defective
osteoclast function, reduced bone resorption, as well as diminished bone remodeling and repair (7, 9).

Osteoclasts originate from hematopoietic stem cells. The fusion of myeloid precursors gives rise to these multi-nucleated specialized macrophages in bone (10, 11). Macrophage colony-stimulating factor (CSF) (M-CSF) activation of its receptor MCSFR/c-Fms/CSF1R and receptor activator of NFκB ligand (RANKL) activation of its receptor RANK are two major events that trigger precursor proliferation and osteoclast differentiation, respectively. MCSFR signaling directs myeloid progenitors to monocytic-macrophage lineage, and the consequent expression of RANK in these cells signify them as osteoclast precursors (12–14). Once these receptors are activated, pro-osteoclastogenic transcription factors, such as c-fos, nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), the activator protein 1 (AP-1), micro-ophthalmia-associated factor of activated T-cells, cytoplasmic 1 (NFATc1), osteoclastogenic transcription factors, such as c-fos, nu-sors (12–14). Once these receptors are activated, prog-}

H2O2 plays an important role in osteoclast progenitor proliferation and mature osteoclast lifespan (23). Upon RANKL stimulation, FoxOs and catalase are down-regulated to trigger H2O2 accumulation; and osteoclast-spe-

Nuclear receptors (NRs) are a superfamily of structur-
ally conserved transcription factors that can be activated by lipophilic ligands. They typically contain a DNA-bind-
ing domain (DBD), a ligand-binding domain (LBD) and an N-terminal domain (31, 32). There are ~49 members of NRs in humans and mice. NRs are involved in broad physiological functions including development, growth, metabolism, immunity, reproduction, circadian rhythm, neuronal control, learning and behavior, as well as dis-

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eases such as obesity, diabetes, osteoporosis and cancer (32, 33). The most common NR ligands are steroid hormones and metabolites such as lipids (34). Orphan nuclear receptors refer to the NRs that still lack known endogenous ligands (35). Once their endogenous ligands have been identified, these receptors are “adopted”. This subfamily of NRs mainly includes receptors for retinoic acid, fatty acids and oxysterols (36, 37). NRs exert their transcription repression and activation through recruiting negative and positive regulatory proteins called co-activators and corepressors, respectively (38). The switch between transcription repression state and transcription activation state is often triggered by the binding of NRs to ligands that function as either agonists or antagonists, leading to up- or down-regulation of target genes, respectively (39). In general, unliganded or antagonist-bound NRs associate with corepressors whereas agonist-bound NRs recruit coactivators. In addition to the classic transcriptional regulation, NRs can also exert nongenomic functions. Because of the important roles of NRs in a variety of diseases including osteoporosis, cancer and metabolic disorders, they have become major targets for drug discovery. At the same time, just like most medicine, NR-targeting drugs also have limitations such as adverse effects in different organs including bone. Therefore, an improved understanding of how NRs regulate bone remodeling will be essential to design better treatment strategies. In this review, we highlight the recent findings in NR regulation of osteoclast and bone resorption.

1. PPARγ

Peroxisome proliferator-activated receptors (PPARs) are a family of adopted orphan NRs (13). There are three isoforms PPARα, PPARγ and PPARδ/β (34, 40). In the classical function of transcription regulation, PPARs form heterodimers with RXRs and bind to peroxisome proliferator response elements (PPRE) in the promoter of their target genes. In the absence of ligand, PPARs are associated with corepressors to inhibit transcription; upon ligand binding, corepressors are dissociated and co-activators are recruited to activate transcription (41, 42). PPARs are pivotal metabolic sensors because their natural ligands are fatty acids and fatty acid derivatives, and one of their key functions is to control glucose and lipid metabolism (34). Several synthetic ligands have also been developed for PPARs as drugs for metabolic diseases (43). Synthetic PPARγ ligands in the thiazolidinediones (TZDs) family such as rosiglitazone and pioglitazone are insulin-sensitizing drugs commonly used for the treatment of type 2 diabetes (44–50), revealing PPARγ as a previously unrecognized regulator of skeletal biology. In 2014, an updated meta-analysis of 24,544 participants with 896 fracture cases from 22 randomized controlled trials, based on searches in MEDLINE, Embase and the Cochrane Database, shows that TZD treatment is associated with an increased risk of fractures in women at lumbar spine, femoral neck and hip, with similar effects for rosiglitazone and pioglitazone that appears to be independent of age (51). Another study reported that pioglitazone significantly decreased BMD in the pelvis in both men and women, decreased BMD in the thoracic spine and ribs of women and the lumbar spine and legs of men (52).

The negative effects of PPARγ on bone are mediated by a simultaneous inhibition of bone formation and stimulation of bone resorption, as described in recent reviews (53–55). It was first discovered that PPARγ activation suppresses osteoblast differentiation from mesenchymal stem cells by favoring adipogenesis (56–60). Homozygous PPARγ-deficient ES cells fail to differentiate into adipocytes, but spontaneously differentiate into osteoblasts; furthermore, PPARγ heterozygous mice display a high bone mass due to increased osteoblast number and bone formation (56). A recent study also indicates that TZDs may induce osteocyte apoptosis by a G protein-coupled receptor 40-dependent mechanism (61).

In terms of PPARγ regulation of osteoclast, multiple mechanisms have been identified. In 2007, we have uncovered a cell-autonomous pro-osteoclastogenic function of PPARγ, by combining both genetic loss-of-function and pharmacological gain-of-function strategies (62). PPARγ deletion in the hematopoietic lineage impedes osteoclast differentiation, leading to decreased bone resorption and increased bone mass, revealing PPARγ as a physiologically significant regulator of osteoclastogenesis. Ligand activation of PPARγ by rosiglitazone significantly accelerates osteoclast differentiation in a receptor dependent manner both in vitro and in vivo (62). Mechanistically, PPARγ potentiates osteoclast differentiation by directly enhancing the expression of c-fos, an essential osteoclast transcription factor (62). Consistent with the findings using rosiglitazone, treatment with alogliptin, another PPARγ agonist, for 8 weeks also induces bone resorption in mice (63).

In two subsequent studies, our group has shown that PPARγ also promotes osteoclast lineage allocation, and osteoclast progenitors reside in the PPARγ-expressing hematopoietic bone marrow population (64); moreover, ligand activation of PPARγ by rosiglitazone also attenuates the antosteoclastogenic effects of the canonical Wnt/β-catenin signaling (65).
Furthermore, we have identified PGC1β (peroxisome proliferator-activated receptor-gamma coactivator 1b, Ppargc1b) as an essential coactivator for PPARγ stimulation of osteoclastogenesis (66). PGC1β is induced by RANKL and rosiglitazone during osteoclast differentiation, and PGC1β deletion in osteoclasts impairs osteoclastogenesis and bone resorption (66, 67). Importantly, osteoclastic PGC1β knockout mice are resistant to rosiglitazone-induced bone resorption and bone loss (66). Recently, we have also found that PPARγ activation by rosiglitazone reduces the level of a novel antiosteoclastogenic miRNA – miR-34a, which may contribute to its osteoclast-enhancing effects (68).

In addition to the osteoclast-autonomous regulation, PPARγ in osteoblasts or stromal cells has also been shown to indirectly promote osteoclast differentiation by increasing RANKL/OPG ratio (69). Bone marrow stromal cell lines that express PPARγ have high potential to support osteoclastogenesis (70). Together, these findings support a pro-osteoclastogenic and resorption-enhancing role of PPARγ andTZDs. Of note, several studies also reported an antosteoclastogenic effect of TZDs in vitro (71–74) or under inflammatory conditions (75–77), suggesting that TZD regulation of osteoclast differentiation and function is context-dependent.

Recent efforts have revealed potential strategies to separate the metabolic benefits of PPARγ from its deleterious side effects such as bone loss by identifying novel mechanisms and selective PPARγ modulators (SPPARMs). Choi et al reported that the insulin sensitizing effects of rosiglitazone are mediated by the inhibition of obesity-linked PPARγ phosphorylation at serine 273 by Cdk5 rather than the classical PPARγ transcriptional agonism (78). Subsequently, synthetic compounds that function as nonagonist or partial-agonist SSPPARMs were developed to specifically block Cdk5-mediated PPARγ phosphorylation but not transcriptional activation, including SR1664 and the optimized UHC1, which exhibit potent antidiabetic activity without causing the fluid retention and weight gain side effects of TZDs (79, 80). In vitro studies reveal that SR1664 is also spared from the antisteoblastic effects (79) or pro-osteoclastogenic effects (our unpublished observation) of rosiglitazone. Along the same line, telmisartan (TEL), an antihypertensive drug from the class of angiotensin receptor blockers, was found to decrease PPARγ Ser273 phosphorylation but not PPARγ Ser112 phosphorylation in a model of marrow MSC differentiation, thus exhibiting insulin-sensitizing activity but not antisteoblastic effects (81). Although the in vivo effects of these compounds on bone remain to be fully characterized, these exciting new findings uncover an opportunity for the development of a new generation of better antidiabetic drugs through PPARγ with limited bone loss side effects (82).

2. PPARδ/β

The role of PPARδ/β in osteoclastogenesis is also multifaceted based on the limited studies reported. Mano et al have shown that PPARδ/β mRNA is highly expressed in mature rabbit osteoclasts, and PPARδ/β activation by carboxyprostacyclin enhances osteoclastic gene expression and bone resorption in a receptor-dependent manner, suggesting that PPARδ/β plays a pro-osteoclastogenic role (83). In 2013, Scholtysek et al report that osteoblastic PPARδ/β knockout mice have decreased Wnt signaling activity, lower serum OPG, higher numbers of osteoclasts and osteopenia (84). Conversely, PPARδ/β activation in osteoblasts by GW501516 amplifies Wnt/β-catenin signaling and increases OPG expression, leading to an attenuation of osteoblast-mediated osteoclastogenesis. GW501516 treatment in ovarioctomized female mice rescues the altered RANKL/OPG ratio, leading to a rebalancing of bone turnover and the restoration of normal bone density (84). Although PPARδ/β in osteoclasts and osteoblasts may exert opposite effects on osteoclast differentiation, this in vivo pharmacological study suggests that the antosteoclastogenic effects may be dominant. PPARδ/β agonists are currently in clinical trials as lipid-lowering drugs (85), thus raising the hope for their potential as a new treatment for bone diseases such as osteoporosis.

3. ERRα

Estrogen-related receptor (ERR) α, β and γ are orphan NRs that currently have no known natural ligands (86). ERRα cannot bind to estrogen - sequence analysis shows that ERRα and ERα have 68% similarity in the DNA binding domain but only 36% similarity in the ligand binding domain (87). ERRα controls a diverse biological processes, including skeletal homeostasis (54, 66, 88), energy metabolism (89–91) and cancer (92, 93). Together with coactivators such as PGC1α and PGC1β, ERRα has been shown to promote mitochondrial biogenesis and oxidative phosphorylation (66, 94, 95). Recently, our group and Ishii et al have uncovered that PGC1β-mediated mitochondrial biogenesis is essential for osteoclast differentiation and activity (66, 67).

ERRα is expressed in both osteoblasts and osteoclasts. It has been shown that ERRα inhibits osteoblast differentiation and ERRα KO mice have increased osteoblast number and bone density (96). Moreover, female ERRα KO mice are resistant to age-induced bone loss, suggesting that ERRα inhibition may be a new strategy to attenuate osteoporosis (97). In addition, ERRα can modulate
RANKL/OPG ratio, implying that ERRα may also play a role in osteoclastogenesis (98). A recent study from the same group shows that ERRα knockdown in RAW264.7 macrophages disrupts osteoclast adhesion and transmigration (99).

In our recent study, we have found that rosiglitazone-activated PPARγ induces ERRα expression during osteoclast differentiation (66). Furthermore, bone marrow precursors from ERRα KO mice exhibit severely impaired osteoclastogenesis. As a result, ERRα KO mice display an increased bone mass due to a significantly decreased bone resorption. Mechanistically, PGC1β potentiates ERRα function to enhance osteoclast differentiation and activity by promoting mitochondrial biogenesis, fatty acid β-oxidation (FAO) and oxidative phosphorylation (OXPHOS) (66). These findings reveal ERRα as a novel yet critical regulator of osteoclastogenesis and bone resorption, thus opening a new chapter in future studies to further investigate the pathological role of ERRα in bone diseases and potential pharmacological strategies to inhibit ERRα for skeletal regeneration.

4. LXR

The liver X receptors, LXRα and LXRβ, are NR members that modulate cholesterol metabolism and suppress inflammation. They form heterodimers with RXR and can be activated by naturally occurring cholesterol derivatives such as oxysterols (37). They can also be activated by synthetic agonists such as GW3965 and T0901317. Preclinical studies in rodent models indicate that these synthetic LXRα agonists may be effective treatment for atherosclerosis, diabetes, inflammation, cancer and Alzheimer’s disease (100–107).

Recently, LXRs have also emerged as a possible regulator of osteoclast differentiation. Remen et al show that LXR activation by GW3965 significantly inhibits osteoclast differentiation and bone resorption in an LXRβ-dependent manner, potentially via suppressing the NFATc1/p38/MITF axis (108). Kleyer et al show that LXR agonists can protect female mice from OVX-induced bone loss by decreasing RANKL/OPG ratio (109). Similarly, two reports in 2013 show that LXR agonists may attenuate LPS-induced osteoclast differentiation by suppressing the activity of NFκB and c-fos to reduce the expression of osteoclast markers Acp5, Ctsk, Mmp-9, Dc-stamp and Itgβ3 (110, 111). In addition to inhibiting osteoclast differentiation, LXR agonists also accelerate apoptosis in mature osteoclasts through the induction of caspase-3 and –9 activity and Bim expression (110). These findings suggest that LXRs are important players in osteoclastogenesis and LXR agonists may represent potential novel treatment for bone degenerative diseases such as osteoporosis.

5. Retinoid Acid Receptors

Retinoic acids (RAs) function through two NRs: retinoic acid receptor (RAR) and retinoid X receptor (RXR). Each receptor has three isoforms (α, β and γ). Vitamin A and retinol can be converted into biologically active forms that include retinal and RA, which have diverse functions in vision, organ development and immune system (112–115). It has been long debated whether vitamin A or retinol intake can increase skeletal fragility (116–118). In some human studies, high intake of retinol is associated with osteoporosis (119). One study suggests that long-term high-retinol-diet may promote the development of osteoporotic hip fractures among postmenopausal women (116). In contrast, other studies found no changes in bone density or even a protective effect of RA on bone (120, 121).

Ingested Vitamin A is converted into active compounds including 11-cis-retinal (important for vision) and all-transretinoic acid (ATRA). ATRA accounts for most of the biological functions of vitamin A. It binds to RAR/RXR heterodimer and regulates specific RA response elements (32, 122). Vitamin A or ATRA have been shown to stimulate osteoclast differentiation and bone resorption in calvarial bones, at least in part by increasing RANKL/OPG ratio, predominantly through RARα (123–128). However, ATRA has also been reported to have the opposite effects on osteoclasts, where ATRA inhibits RANKL- and MCSF- induced osteoclast differentiation from bone marrow macrophages, splenocytes and RAW264.7 cells (129–131). This inhibition is mediated by the suppression of osteoclast progenitors and RANKL induction of osteoclast transcription factors such as NFATc1 (129–131). Moreover, an in vivo study in rodents indicates that retinoid administration increases bone resorption at subperiosteal sites in cortical bones while decreasing bone resorption in trabecular bones (132). The appeared discrepancy in these studies may be explained by the differences in the direct effects of ATRA on osteoclasts vs. the indirect effects of ATRA through osteoblasts, as well as the differences in the local milieu in cortical vs. trabecular bones. Future studies using cell type-specific RAR and/or RXR knockout mice will further delineate how vitamin A and the retinoid acid receptors regulate osteoclastogenesis and bone resorption.

6. Estrogen Receptors

Estrogen is a female sex steroid hormone that binds to two NRs: estrogen receptor α (ERα) and estrogen receptor β (ERβ) (133). Declined level of estrogen is a major...
cause for postmenopausal osteoporosis, the most common skeletal disorder in women (134). This disease can be experimentally modeled in female rodents by ovariectomy (OVX) (135). In older men, estrogen deficiency also contributes to bone loss (136–138). Several mechanisms have been proposed for how estrogens and ERs negatively regulate osteoclast differentiation and function, some of which are still controversial. Since this topic has been discussed in a recent review by Manolagas et al (139), here we will highlight the key findings and new observations.

A seminal study by Jilka et al first reveals that estrogen deficiency causes an increase in osteoclast precursors (140). This has been further supported in subsequent studies showing that both OVX-induced estrogen deficiency (64) and ERα deletion in the myeloid lineage (141) result in more abundant osteoclast precursors. These findings suggest that estrogen suppresses osteoclast precursor population.

Estrogen has also been proposed to decrease the levels of RANKL and TNFα. Untreated early postmenopausal women are reported to exhibit elevated RANKL in bone marrow cells compared with estrogen-replete women (142). In ovariectomized mice, TNFα produced by T cells can also potentiate RANKL-induced osteoclastogenesis; and T cell deficient mice are resistant to OVX-induced bone loss, indicating that T cell is a critical mediator of estrogen deficiency induced osteoporosis (143). In contrast to these reports, Lee et al found that OVX could still induce trabecular bone loss in three T lymphocyte-deficient (TLD) mouse models: nude mice, recombination activating gene 2-deficient (RAG2 KO) mice, and T cell receptor alpha chain-deficient (TCRα KO) mice (144). Moreover, Onal et al showed that RANKL derived from B- but not T-lymphocytes contribute to the increase in osteoclasts and the loss of cancellous bone upon estrogen deficiency (145). In addition, estrogen may directly stimulate OPG expression in human osteoblast cell lines, thus inhibiting osteoclast differentiation and reducing bone resorption (146). Recent evidence has also shown that estrogen suppresses RANKL-induced osteoclastogenesis through down-regulation of the AP1 transcription factor c-Jun (147). Estrogen has also been shown to protect bone and impede resorption by defending against reactive oxygen species (ROS) (148).

Estrogen not only inhibits osteoclast formation and function but also promotes osteoclast apoptosis. In 1996, Hughes et al found that 17β-Estradiol (E2) promoted apoptosis of murine osteoclasts both in vitro and in vivo (149). Estrogen-induced apoptosis was inhibited when TGFβ was blocked, indicating that TGFβ might mediate this apoptotic effect (149). Around the same time, Kameda et al showed that E2 was able to directly inhibit osteoclastic bone resorption by inducing osteoclast apoptosis in a dose- and time-dependent manner (150). In 2007, Nakamura et al showed that estrogen diminishes osteoclast survival by inducing Fas ligand (FasL) in osteoclasts (151). In contrast to this autocrine mechanism of estrogen regulation of FasL production, Krum et al reported in 2008 a paracrine mechanism in which estrogen promotes preosteoclast apoptosis through the upregulation of FasL in osteoblasts (and not osteoclasts) (152). In a recent study in 2013, Garcia et al found that E2 treatment in human and murine osteoblasts induced the expression of Matrix Metalloproteinase 3 (MMP3), which promoted FasL cleavage and osteoclast apoptosis (153).

The two estrogen receptors ERα and ERβ can form homo- and hetero-dimers. Many studies have demonstrated that ERα is the predominant estrogen receptor regulating bone (154), although ERβ may also modulate bone resorption in female mice but not male mice (155). To study estrogen’s effects on mature osteoclast, Nakamura et al generated osteoclast-specific ERα knockout mice using CTSK-Cre, and observed that female mutant mice exhibited trabecular bone loss due to increased osteoclast survival and down-regulation of FasL (151). Similar result was found by targeted deletion of ERα with lysozyme M (LysM)-Cre (141). Both studies showed that ERα deletion in the macrophage/osteoclast lineage only caused a loss of trabecular bone but not cortical bone, indicating that ERα in other cell types may indirectly suppress osteoclast activity and bone resorption. Indeed, a more recent study has shown that the effects of estrogens on trabecular vs. cortical bone mass are mediated by direct effects on osteoclasts and osteoblasts, respectively (139, 141).

Recent studies from the McDonnell group report that 27-hydroxycholesterol functions as an endogenous SERM and LXR ligand that negatively impact bone mineral density (BMD) in mice by simultaneously decreasing bone formation and increasing bone resorption, revealing that cholesterol metabolism may influence ER and estrogen regulation of osteoclastogenesis and bone resorption (156–158).

The critical antiresorptive roles of estrogen rationalize the strategies to prevent postmenopausal osteoporosis in women by E2 administration. Timely restoration of E2 levels via E2 supplement can prevent estrogen-dependent bone loss and significantly reduce the risk of fracture (159). However, ERs are expressed in many tissues, including the central nervous system (CNS), blood vessels and the reproductive system (160–162). Many side effects of E2 administration, including breast and uterine cancers, make it impractical to use E2 to treat estrogen-
dependent bone loss (163–165). Studies by Kousteni et al reveal that sex steroids protect the adult murine skeleton through a mechanism that is distinct from that used to preserve the mass and function of reproductive organs: the classical genotropic actions of sex steroid receptors are dispensable for their bone protective effects, but essential for their effects on reproductive tissues; a synthetic ligand (4-estren-3alpha,17beta-diol) that reproduces the nongenotropic effects of sex steroids, without affecting classical transcription, increases bone mass and strength in ovariectomized female mice without affecting reproductive organs (166, 167). Similarly, Bartell et al recently showed that a 17β-estradiol (E2) dendrimer conjugate (EDC), which is incapable of stimulating nuclear-initiated classical ERα actions, is as potent as an equimolar dose of E2 in preventing OVX-induced bone loss in the cortical compartment that represents 80% of the entire skeleton, without affecting OVX-induced cancellous bone loss or uterine weight reduction (168). Clinically, SERMs that behave as ER agonists in bone but ER antagonists in breast and uterus have been developed for the treatment of osteoporosis with limited breast or uterine cancer risk, including raloxifene and lasofoxifene.

7. Androgen Receptor

The male sex steroid hormone androgens bind to androgen receptor (AR) to positively regulate skeletal homeostasis. Androgens are important for male skeletal growth during puberty, and for bone maintenance in postpuberty men (169). Androgen deficiency due to either idiopathic hypogonadotropic hypogonadism or complete androgen insensitivity causes low bone mass in men (169, 170). Conversely, androgen treatment by dihydrotestosterone (DHT) suppresses osteoclast survival and protects against orchidectomy-induced bone loss (141, 166). In peripheral tissue, androgens can be converted into estrogens by aromatase. Since both ERs and ARs are expressed in bone tissues (171), androgens have the potential to activate both ERs and ARs. However, recent studies on mice with AR deletions shed new light on AR’s specific role in bone. Kawano et al (172) showed that global AR deletion caused severe osteopenia in male mice, with reduction in both trabecular and cortical bones. They observed an increased bone turnover with both high bone formation and high bone resorption. However, bone marrow cells derived from AR KO mice showed normal ex vivo osteoclast differentiation and function, so the in vivo difference in bone resorption may result from indirect effects on osteoclasts. Indeed, Kawano and colleagues found that AR deletion in osteoblast increased RANKL expression (172). Later, Chiang et al showed that specific AR deletion in mature osteoblasts led to an increased osteoclast surface in the vertebra; however, they did not observe any changes in osteoclast surface in the femur or serum bone resorption markers (169). Two other studies also showed that AR deletion in mature osteoblasts and osteocytes reduces bone mass without affecting osteoclast numbers (173, 174), suggesting that other tissues and/or cell types may contribute to the antiresorptive effects of androgen and AR.

Similar to ERα, the bone protective function of AR has been proposed to be achieved through nongenotropic effects that are outside of the nucleus and independent of transcription (166, 167). In contrast, it has also been shown that osteoblast-specific deletion of AR exon 3, which encodes the second zinc finger of the DNA-binding domain, results in trabecular bone loss in adult male mice, suggesting that androgen maintains trabecular bone directly through DNA binding-dependent AR actions in mature osteoblasts (173). Therefore, future studies are required to further dissect the cellular and molecular mechanisms underlying AR regulation of osteoclast and bone resorption.

8. Glucocorticoid Receptors

Glucocorticoid receptors (GRs) are members of the NR superfamily that mediate the effects of glucocorticoids (GCs). GCs are commonly used as immunosuppressive and anti-inflammatory drugs to treat diseases such as allergies, autoimmune disorders and rheumatoid arthritis (RA). However, they have severe adverse effects on the skeleton manifested as bone loss (175, 176). Glucocorticoid-induced osteoporosis (GIO) is the second most common osteoporosis, affecting 30%–50% of patients receiving GC therapy (176). In clinical studies, these patients show increased bone resorption and decreased bone formation (177).

The main mechanism by which GCs induce bone loss is to inhibit osteoblast proliferation and differentiation, as well as promote apoptosis of osteoblasts and osteocytes (178) (179). A recent study by Rauch et al show that these effects are mediated by monomeric GR because GC treatment still reduces bone formation in mice carrying a mutation that only disrupts GR dimerization (180, 181). Nonetheless, an increase in osteoclast markers has also been observed in GC therapy, indicating that accelerated bone resorption also contributes to GC-induced bone loss (182, 183). It has been reported that GCs increase the levels of M-CSF and RANKL but decrease the level of OPG in stromal and osteoblast cells, thus promoting osteoclast differentiation (183, 184). However, some studies show that GC treatment with prednisolone has no effect on RANKL or OPG expression (185). Selective GR agonists (SEGRAs) have been developed as novel thera-
pies that impose less risk on bone. Recently, a novel GR modulator Compound A (CpdA) is reported to maintain anti-inflammatory function without altering RANKL/OPG ratio (186, 187). Because bone formation and bone resorption are coupled, in late stage of GCs treatment, osteoblasts can no longer generate sufficient signals for osteoclast differentiation, and osteoclastogenesis eventually decreases.

Besides the indirect effects of GCs on osteoclasts via osteoblasts, it has been shown that GCs can also directly prolong osteoclast life span (188). Osteoclast-specific overexpression of 11beta-hydroxysteroid dehydrogenase type 2, an enzyme that inactivates GCs, largely prevents the loss of bone density associated with GC treatment (185). However, Kim et al report that while GCs extend the longevity of osteoclasts, they actually suppress osteoclast bone resorbing capacity due to failure to organize cytoskeleton in response to MCSF in vitro (189). Moreover, their results show that osteoclast-specific GR knockout mice (GRoc−/−) are protected from not only the impact of GCs on osteoclasts and their precursors but also GC suppression of osteoblasts and bone formation, suggesting that osteoclast is the major cell type that mediates GIO (189). Together, these studies reveal that multiple cell types and mechanisms may contribute to GC regulation of osteoclast and bone resorption; nonetheless, clinical evidence and mouse genetic analyses (180, 185) suggest that the proresorptive function of GCs may be dominant over its antiresorptive effects in vivo, leading to a net increase in osteoclast activity and bone resorption.

9. Vitamin D Receptor

Vitamin D is a group of fat-soluble secosteroids that are important for calcium absorption and bone homeostasis (190, 191). The active form 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) binds and activates the NR member Vitamin D receptor (VDR), which forms a heterodimer with another NR member retinoid X receptor (RXR) to regulate the transcription of target genes. Vitamin D is used as antiresorptive agent that prevents bone fracture (192). Mutations in the genes regulating Vitamin D function or metabolism are associated with several human bone abnormalities (193–195). Vitamin D-dependent hereditary rickets type II (VDDR-II) is a rare autosomal recessive disorder caused by mutations in the VDR gene (196, 197). VDDR-II patients have low bone mass, growth retardation and elevated level of 1,25(OH)2D3. Vitamin D-dependent rickets type I (VDDR-I) is caused by mutation in the gene for 25-hydroxyvitamin D (136) 1alpha-hydroxylase (CYP27B1), which is an enzyme important for converting 25-hydroxyvitamin D (136) to 1,25(OH)2D3 (198). VDDR-I patients have similar symptoms as VDDR-II patients but without elevation of 1,25(OH)2D3 (199, 200).

Various laboratories have generated genetic mouse models to mimic human VDDR type I and type II. Type I 1α(OH)ase−/− and type II VDR−/− mice develop hyperparathyroidism, growth retardation and skeletal abnormalities in a manner similar to human VDDR-I and VDDR-II (201–204). Interestingly, the impaired bone mineralization in VDR−/− mice can be rescued by dietary means that normalize blood calcium and phosphate levels (205, 206). Furthermore, transgenic expression of VDR in the intestine of VDR global knockout mice can restore calcium absorption and bone density to normal levels (207). Together, these findings indicate that the major biological role of VDR in bone is to promote intestinal calcium and phosphate absorption.

In a study published in 2012, Lieben et al generated and characterized intestine- and osteoblast-specific VDR knockout mice (208). Decreased intestinal calcium absorption in intestine-specific VDR knockout mice resulted in severely reduced skeletal calcium levels to ensure normal serum calcium levels by increasing 1,25(OH)2D3 production to both stimulate bone turnover and suppress bone matrix mineralization (208). Ablation of osteoblast VDR precluded this calcium transfer from bone to serum, leading to better preservation of bone mass and mineralization (208). Therefore, it was concluded that maintaining normocalcemia has priority over skeletal integrity, and that to minimize skeletal calcium storage, 1,25(OH)2D3 not only increases calcium release from bone, but also inhibits calcium incorporation in bone (208).

It is well known that 1,25(OH)2D3 enhances osteoclastogenesis in vitro by increasing RANKL expression in osteoblasts, and administration of high doses of 1,25(OH)2D3 can increase bone resorption in mice (209, 210). When spleen-derived osteoclast precursors from WT mice were cocultured with osteoblasts from VDR−/− mice, no osteoclast could form, demonstrating that VDR in osteoblasts is critical for RANKL expression (211). In 2006, Masuyama et al report that VDR in chondrocytes also promotes osteoclastogenesis by inducing RANKL expression, and chondrocyte VDR knockout mice exhibit reduced osteoclast number and higher trabecular bone mass in juvenile mice (212). Interestingly, these mice also display defects in mineral homeostasis due to lower FGF23 expression in osteoblasts, leading to higher serum phosphate and 1,25(OH)2D3 levels (212). Together, these findings support a bone catabolic function of Vitamin D.

However, many active vitamin D compounds such as calcitriol, alfalcacidol and eldecalcitol are used as drugs to inhibit osteoclastogenesis and improve BMD (213, 214).
This has been demonstrated in vivo that daily administration of eldecalcitol increases BMD by suppressing RANKL expression in trabecular bone (215). Eldecalcitol can prevent the risk of osteoporotic fractures without side effects such as hypercalcemia, and is in phase III clinical trials (214). Together, these findings indicate that the antiresorptive functions of Vitamin D outside of bone, such as elevating intestinal calcium absorption and reducing PTH production, may be more dominant over the pro-osteoclastogenic functions of Vitamin D within bone, and exert indirect effects on skeletal homeostasis to give rise to a net outcome of suppressing osteosteatogenesis and bone resorption.

Conclusions

Developing better drugs to inhibit osteoclast differentiation and activity requires a deeper understanding of the genetic and pharmacological regulation of osteoclast biology. Nuclear receptors represent promising drug targets for osteoporosis due to their multifaceted roles in skeletal remodeling, their possibility to be modulated by small molecule agonists and antagonists, as well as the availability of already existing NR-targeting drugs that could potentially be used for bone diseases. Moreover, a better understanding of the relationship between osteoclast and nuclear receptors helps identifying potential adverse skeletal effects of NR-targeting drugs. This review offers an up-to-date summary of our current understanding of how nuclear receptors regulate osteoclast differentiation and function. In addition to the classical steroid receptors that are well-known regulators of bone remodeling such as ER, AR, GR and VDR, recent studies have identified new NR members as novel regulator of osteoclastogenesis, bone resorption and skeletal homeostasis including PPARγ, PPARδ/β, ERRα and LXRα. Future investigations promise to discover more NR players in bone physiology and diseases.

Our understanding of the cellular and molecular mechanisms for how NRs regulate osteosteatogenesis is far from complete. Future studies are needed to further address some of the key questions and challenges. 1) What are the cell-autonomous vs. systemic (both neuroendocrine and metabolic) regulation of osteosteatogenesis, and which mechanism plays a dominant role. This may explain some of the conflicting observations between in vitro and in vivo studies. 2) Both genetic and pharmacological studies are required for a complete and accurate assignment of the functional roles of each NR: genetic approaches permit a gene- and cell type-specific dissection but may risk developmental defects and compensation; pharmacological approaches permit a global assessment of the net effect of NR activation or inhibition but may risk receptor-independent artifacts at high doses. Current understanding of osteosteatosis regulation by certain NRs, such as RARs, is mainly based on ligand treatment, and thus mouse genetic models will be an important future direction. 3) What are the relative contributions of transcriptional regulation vs. nongenomic mechanisms such as post-translational modification in NR functions? The important findings that ER regulation of bone is independent of transcriptional regulation and PPARγ regulation of bone is independent of Cdk5-mediated Ser273 phosphorylation begs future investigation of how other NRs act in bone. 4) What are the physiological ligands for NR regulation of bone and osteoclast, including agonist, antagonist and selective modulators? The endogenous ligands for many orphan NRs are still unknown or controversial, yet will be crucial to delineate how metabolism control bone physiology and diseases. 5) How to design synthetic NR ligands that protect bone with limited adverse effects in other tissues such as reproduction, cancer and metabolism; similarly, how to design synthetic NR ligands that maintain their therapeutic benefits in other diseases such as diabetes without bone loss. Recent discoveries of tissue-specific or function-specific ER and PPARγ modulators provide the inroad to reach this goal. 6) Finally, only a small subset of NRs has been carefully examined in the context of osteosteatosis biology, while the vast majority of the rest of 49 nuclear receptors have not been fully characterized. For example, thyroid hormone receptors (TRs) have a known effect on the skeletal system (216, 217), but it is unclear whether and how TR modulates osteosteatosis differentiation and activity. The recent identification of a membrane-bound TRα isoform (p30 TRα1) that mediates rapid, transcription-independent nongenomic effects to promote osteoblast proliferation and survival provides a new trail to understand how thyroid hormone may directly or indirectly modulate osteosteatosis (218). Therefore, the intersection between nuclear receptors and osteosteatosis biology offers ample opportunities for future research and discovery.

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Received October 7, 2014. Accepted December 26, 2014. Address all correspondence and requests for reprints to: Yihong Wan (yihong.wan@utsouthwestern.edu), Yihong Wan, Ph.D., Assistant Professor, Email: yihong.wan@utsouthwestern.edu, Univ of Texas Southwestern Medical Center - Department of Pharmacology, 6001 Forest Park Road, Rm ND8.502B, Dallas, TX, UNITED STATES, 75390–9041, Phone: 214–645-6062.

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