CHAPTER THREE

Nuclear Receptors in Skeletal Homeostasis

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Abstract

Nuclear receptors are a family of transcription factors that can be activated by lipophilic ligands. They are fundamental regulators of development, reproduction, and energy metabolism. In bone, nuclear receptors enable bone cells, including osteoblasts, osteoclasts, and osteocytes, to sense their dynamic microenvironment and maintain normal bone development and remodeling. Our views of the molecular mechanisms in this process have advanced greatly in the past decade. Drugs targeting nuclear receptors are widely used in the clinic for treating patients with bone disorders such as osteoporosis by modulating bone formation and resorption rates. Deficiency in the natural ligands of certain nuclear receptors can cause bone loss; for example, estrogen loss in postmenopausal women leads to osteoporosis and increases bone fracture risk. In contrast, excessive ligands of other nuclear receptors, such as glucocorticoids, can also be detrimental to bone health. Nonetheless, the ligand-induced osteoprotective effects of many other nuclear receptors, e.g., vitamin D receptor, are still in debate and require further characterizations. This review summarizes previous studies on the roles of...
nuclear receptors in bone homeostasis and incorporates the most recent findings. The advancement of our understanding in this field will help researchers improve the applications of agonists, antagonists, and selective modulators of nuclear receptors for therapeutic purposes; in particular, determining optimal pharmacological drug doses, preventing side effects, and designing new drugs that are more potent and specific.

1. INTRODUCTION

1.1 Bone and Bone Cells

Bone is a multifunctional organ that affects many aspects throughout the life. During embryonic and juvenile stages, bone development determines body size and provides support for skeletal muscle and organs. After bone growth is completed, bone stays in a homeostatic state, which is controlled by dynamic bone formation and bone resorption (Rodan, 1998). Bone also plays essential roles in endocrine functions, blood cell production, and mineral metabolism. Bone mainly consists of solid bone tissues and bone marrow. The solid bone tissues include cortical bone and cancellous bone (also called trabecular bone); both contain three major bone cell types, osteoblast, osteocyte, and osteoclast, which cooperate to maintain bone homeostasis.

Osteoblasts, the major bone-forming cells, produce type I collagen (Col1), osteocalcin, osteopontin (OPN), and alkaline phosphatase (ALP) that contribute to bone matrix formation and mineralization. Osteoblasts are derived from mesenchymal stem cells (MSCs). MSCs first go through a preosteoblast or immature osteoblast stage, and then a mature osteoblast stage, and at last become bone-lining cells and osteocytes (Fig. 1). Experimentally, differentiation stage-specific marker gene expression enables the detection of altered osteoblastogenesis and the development of conditional osteoblast stage-specific gene knockout tools. Paired-related homeobox gene 1 (Prx1) and transcription factor Sox9 are osteoblast progenitor markers (Akiyama et al., 2005; Nohno et al., 1993). Preosteoblasts express transcription factors Runt-related transcription factor 2 (Runx2) and osterix (Osx) (Nakashima et al., 2002; Otto et al., 1997), while Col1 and osteocalcin (Ocn) serve as protein makers for mature osteoblasts.

Osteocytes are derived from mature osteoblasts that are embedded into bone matrix. They sense mechanical loading and control bone adaptation to it. Osteocytes also have endocrine functions in regulating bone homeostasis by secreting proteins such as fibroblast growth factor 23 (FGF-23),
Dickkopf-related protein 1 (DKK1), and sclerostin (SOST). Dentin matrix protein 1 (DMP1) is commonly used as an osteocyte marker gene (Toyosawa et al., 2001).

Osteoclasts are the only bone-resorbing cells. These multinucleated cells are differentiated from monocyte/macrophage precursors in the myeloid lineage, which originate from hematopoietic stem cells (Fig. 1). The process of osteoclast differentiation—osteoclastogenesis—is under the control of several cytokines produced by osteoblasts, osteocytes, and stromal cells. Two major cytokines, macrophage colony-stimulating factor and receptor activator of nuclear factor-κB ligand (RANKL), activate their receptors M-CSFR and RANK, respectively, to stimulate osteoclast differentiation (Boyle, Simonet, & Lacey, 2003; Fig. 1). Osteoprotegerin (OPG) is a RANKL decoy receptor that impedes osteoclast differentiation (Khosla, 2001; Fig. 1). Activated RANKL/RANK signaling upregulates nuclear factor of activated T cells, cytoplasmic 1 (NFATc1), a master transcription factor for osteoclast differentiation (Takayanagi, 2007), as well as enzymes for

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**Fig. 1** Osteoblasts, osteocytes, and osteoclasts in bone. Bone-forming osteoblasts are differentiated from mesenchymal stem cells. Osteoblasts are then further matured into bone-lining cells and osteocytes that are embedded in bone matrix. Osteoblasts and osteocytes express M-CSF, RANKL, and OPG to control the differentiation of bone-resorbing osteoclasts from hematopoietic stem cells. Osteoclastogenesis from myeloid precursors is driven by M-CSF and RANKL, but counteracted by OPG, a RANKL decoy receptor. The orchestrated performance by osteoblasts, osteocytes, and osteoclasts maintains skeletal homeostasis. Cell type-specific protein markers are shown in orange.
bone matrix degradation including tartrate-resistant acid phosphatase (TRAP) and cathepsin K (CTSK).

During bone development and remodeling, the differentiation and activity of osteoblasts, osteoclasts, and osteocytes are tightly controlled to maintain normal bone morphology. Bone cells sense environmental factors by expressing their respective receptors, among which nuclear receptor (NR) superfamily members play critical roles. Dysfunction of one or more of these bone cell types, resulted from genetic alterations of NR genes or environmental changes of NR ligands, may cause bone disorders such as osteoporosis, osteopetrosis, bone fracture, and rickets.

1.2 Nuclear Receptors

NRs are a superfamily of transcription factors that share similar structures containing a DNA-binding domain, a ligand-binding domain (LBD), a hinge region, and an N-terminal domain (Mangelsdorf et al., 1995). NR superfamily consists of 48 members in human (Evans & Mangelsdorf, 2014; Fig. 2). They are divided into steroid receptors, nonsteroid receptors, and orphan NRs that have no known endogenous ligand. NRs form complexes with coregulators, including corepressors and coactivators, and exhibit efficient transcriptional regulation by coregulator exchange upon ligand binding or dissociation (Glass & Rosenfeld, 2000; McKenna, Lanz, & O’Malley, 1999).

The important roles of NRs in many biological processes make them ideal drug targets for numerous metabolic diseases such as diabetes and osteoporosis. Thus, a number of NR-specific agonists, antagonists, and modulators have been designed to control aberrant NR functions in patients (Moore, Collins, & Pearce, 2006). A better understanding of the fundamental mechanisms of NR function and regulation will eventually benefit patients by the discoveries of new drugs and the improvement of currently utilized drugs. This review summarizes the current understanding of the roles of several NRs in regulating bone health, which may provide new insights for disease therapy to scientists in this field.

2. NRs AND BONE HOMEOSTASIS

2.1 Estrogen Receptor

2.1.1 Estrogens and Bone Health

The steroid hormone estrogens play important roles in regulating various physiological processes, such as reproductive organ development, energy
Fig. 2 Nuclear receptor classification and their members mentioned in this chapter. Nuclear receptor superfamily consists of 48 members. They are divided into three subfamilies, steroid receptors, nonsteroid receptors, and orphan receptors. Upon ligand binding, steroid receptors form homodimers to exert their transcriptional regulations. The members in this subfamily discussed here are estrogen receptor (ER) \( \alpha \) and \( \beta \), androgen receptor (AR), glucocorticoid receptor (GR), and progesterone receptor (PR). Nonsteroid receptors form heterodimers with retinoid X receptors (RXRs). Vitamin D receptor (VDR), thyroid hormone receptor (TR) \( \alpha \) and \( \beta \), and retinoic acid receptor (RAR) \( \alpha \), \( \beta \), and \( \gamma \) are classified as nonpermissive receptors because their heterodimers are only activated by ligands of RXR partners. Other nonsteroid receptors, including peroxisome proliferator-activated receptor (PPAR) \( \alpha \), \( \delta/\beta \), and \( \gamma \), liver X receptor (LXR) \( \alpha \) and \( \beta \), and steroid and xenobiotic receptor (SXR) (murine ortholog pregnane X receptor (PXR)), are permissive receptors, and their heterodimers can be activated by ligands of either RXR or RXR partners. Orphan nuclear receptors have no known endogenous ligand when initially identified. They regulate gene transcription as monomers, homodimers, or heterodimers with RXR. The orphan receptors mentioned in this chapter include estrogen-related receptor (ERR) \( \alpha \), \( \beta \), and \( \gamma \), nuclear receptor 77 (Nur77) (alternative name, nerve growth factor-induced gene B (NGFI-B)), nuclear receptor-related factor 1 (Nurr1), neuron-derived orphan receptor 1 (NOR1), RAR-related orphan receptor (ROR) \( \alpha \), \( \beta \), and \( \gamma \), and short heterodimeric partner (SHP).
metabolism, and bone homeostasis. Impaired estrogen levels in postmenopausal females or aged males result in decreased bone formation and increased bone resorption, leading to osteopenia and osteoporosis (Falahati-Nini et al., 2000). Bone loss observed in estrogen-deficient women and rodent models, such as ovariectomized (OVX) rats and mice (Thompson, Simmons, Pirie, & Ke, 1995), can be significantly diminished by estrogen treatment (Bain, Bailey, Celino, Lantry, & Edwards, 1993; Lindsay et al., 1976).

NRs for estrogen, ERα and ERβ, are the major mediators of estrogen actions (Barros & Gustafsson, 2011). Upon estrogen binding, nuclear ERα and ERβ form homodimers, associate with estrogen response elements (EREs), and then activate or repress target gene transcriptions (Cowley, Hoare, Mosselman, & Parker, 1997). In addition to the classical transcriptional regulations, estrogen receptors (ERs) also have nonclassical functions. They can modulate the activities of other transcription factors such as the activator protein-1 (AP-1) and Sp1 (Marino, Galluzzo, & Ascenzi, 2006). ERs on plasma membrane can also rapidly induce multiple signaling pathways upon ligand activation, such as ERK (Acconcia et al., 2005). Interestingly, although NRs have been viewed as the major ERs, a G protein-coupled estrogen receptor, also known as GPR30, has been reported to contribute to estrogen actions (Martensson et al., 2009).

2.1.2 ER Knockout Models
ERα and ERβ are expressed in osteoblasts, osteoclasts, osteocytes, as well as chondrocytes (Bord, Horner, Beavan, & Compston, 2001), suggesting that ER activation may regulate the functions of multiple bone and cartilage cells. Several animal models have been established to study the roles of ERs in bone cells and skeletal homeostasis. Global knockout of ERα led to increased trabecular bone mineral density (BMD), decreased cortical BMD, and lower bone turnover rate in both male and female mice (Sims et al., 2002). Complete deletion of ERβ resulted in increased trabecular BMD, unchanged cortical BMD, and attenuated bone resorption in female mice, while bone morphology was unaltered in male mice (Sims et al., 2002). In contrast, decreased trabecular and cortical BMD as well as elevated bone turnover rate were observed in estrogen-deficient women after menopause or ovariectomy, in aged men, and in men with a mutated ERα (Riggs, Khosla, & Melton, 1998; Smith et al., 1994). The reason for the differences between bone phenotypes of ER knockout mice and estrogen/ER–deficient human is still unclear.
Osteoblasts in a specific differentiation stage express several protein markers, e.g., Prx1 in MSCs, osterix1 (Osx1) in early differentiating osteoblasts, Ocn and collagen 1a1 (Col1a1) in mature osteoblasts, and Dmp1 in osteocytes—mature osteoblasts that migrate into bone matrix after finishing mineralization (Fig. 1). To determine the functions of an NR in osteoblast differentiation, maturation, mineralization, and mechanical response, Cre–Lox recombination technology is widely utilized. Specific deletion of an NR in a certain bone cell type at a specific developmental stage can be achieved via the expression of Cre recombinase driven by the gene promoters of these specific protein markers in engineered NR flox mice. Specific deletion of ERα in osteoblast progenitors with Prx1-Cre reduced cortical bone thickness and unchanged cancellous bone mass (Almeida et al., 2013). ERα deletion in immature osteoblasts with Osx1-Cre reduced the thickness of both cortical and trabecular bone (Almeida et al., 2013). ERα deletion in mature osteoblasts and osteocytes with Col1a1-Cre did not alter bone mass in either male or female mice (Almeida et al., 2013). However, osteocalcin–Cre-mediated ERα deletion in mature osteoblast and osteocytes reduced both trabecular and cortical bone volume in female mice (Maatta et al., 2013; Melville et al., 2014) and reduced trabecular bone volume in male mice (Maatta et al., 2013). Dmp1-Cre-induced osteocyte-specific ERα knockout showed decreased trabecular bone volume in male mice (Windahl, Borjesson, et al., 2013). Summarizing these observations, ERα in the osteoblast lineage generally plays protective roles in bone mass accrual and maintenance.

In contrast, ERβ in osteoblasts may have an opposite role to ERα. Increased trabecular bone volume and unchanged cortical bone were observed in mice with Prx1-Cre-mediated ERβ deletion in osteoblast progenitors (Nicks et al., 2016). These opposite effects from loss of ERβ vs loss of ERα may be due to the differences in their transcriptional regulation and target genes, as well as their different affinities for transcription coregulators (An, Tzagarakis-Foster, Scharschmidt, Lomri, & Leitman, 2001; Katzenellenbogen & Katzenellenbogen, 2000). These findings suggest that selective ERα agonists may be better therapeutic choices than dual agonists.

ERα knockout in osteoclast precursors using LysM–Cre led to increased osteoclast number, decreased cancellous bone mass, and unchanged cortical bone mass (Martin–Millan et al., 2010). ERα deletion in mature osteoclasts by Ctsk–Cre resulted in decreased trabecular bone volume and unchanged cortical bone in female but not male mice (Nakamura et al., 2007). These
findings indicate that ERα in osteoclasts suppresses osteoclast function in trabecular bone but not cortical bone.

In summary, ERα and ERβ have little effect on bone development during embryonic stage and in young mice (Sims et al., 2002) but modulate bone homeostasis in adult and old mice through both direct and indirect pathways. ERα defect in the osteoblast/osteocyte lineage reduces bone mass of trabecular bone and/or cortical bone. This reduction is mostly due to attenuated osteoblast differentiation followed by decreased bone formation. ERα defect in the osteoclast lineage dramatically enhances osteoclastogenesis and thus accelerates bone resorption. More evidence is required for drawing a conclusion on the direct effect of ERβ on bone cells. Besides ER in bone cells, ER in other organs also mediates the indirect regulation on bone development. Global ERα or ERα/β deletion significantly elevated circulating levels of estradiol and testosterone (Sims et al., 2002). On the other hand, estrogen deficiency in female reduced intestinal calcium absorption, increased renal calcium excretion, and increased the secretion of parathyroid hormone (PTH) (Riggs et al., 1998), which enhances calcium release from bone. These effects of ER on sex hormone production, calcium homeostasis, and PTH secretion are mostly not mediated through bone cells but through ovary, liver, parathyroid gland, and other organs. All these findings may explain the differences of the bone phenotypes between global and conditional knockout mice, and between knockout mouse models and human.

2.1.3 Mechanisms

Several mechanisms have been proposed for osteoblast regulation by estrogen and ERs. It was reported that estrogen treatment enhanced osteoblast differentiation from mouse bone marrow MSCs through upregulating osteoblastic makers such as ALP and osteocalcin (Qu et al., 1998). It was also shown that ERα stimulated osteoblast differentiation by directly binding to and activating the transcription factor Runx2 (McCarthy, Chang, Liu, & Centrella, 2003).

Multiple mechanisms may also account for osteoclast regulation by estrogen and ERs. First, estrogen treatment decreased the RANKL/OPG ratio in human osteoblasts (Bord, Ireland, Beavan, & Compston, 2003; Hofbauer et al., 1999), which could lead to less osteoclast differentiation and bone resorption. Second, osteoclast-specific ERα deletion inhibited osteoclast apoptosis by abolishing estrogen induction of Fas ligand (FasL) in osteoclasts, leading to increased osteoclast number (Nakamura et al., 2007). Third, FasL was reported to be a direct ERα target gene in osteoblast. Estrogen-induced
FasL expression in osteoblasts was sufficient to increase osteoclast apoptosis (Krum et al., 2008). Fourth, bone loss in osteoclast-specific ERα knockout mice was shown to be rescued by osteoclast-specific deletion of hypoxia-inducible factor 1 alpha (HIF1α), a proosteclastogenic factor that was destabilized by estrogen but became stabilized upon estrogen deficiency (Miyauchi et al., 2013). In summary, evidence so far indicates that estrogen inhibits osteoclast differentiation by lowering RANKL/OPG ratio and destabilizing osteoclastic HIF1α and at the same time also inhibits osteoclast survival by inducing FasL and facilitating osteoclast apoptosis.

Ligand-activated nuclear ERs may regulate bone cell differentiation and activity by directly stimulating or repressing the transcription of target genes. A set of 3665 ERα-binding sites were found in human genome (Carroll et al., 2006), indicating a genome-wide regulation of gene transcription by estrogen, although different cell types may exhibit distinct ER targets. Future studies are required to systematically identify ERE and ER-binding sites, as well as functionally characterize ER target genes in each type of bone cells.

Besides direct promoter binding and target gene transcription regulation, ERs also physically interact with other transcription factors and modulate their transcriptional activities. For example, it was shown that the association of ERα with NF-κB and C/EBPβ repressed the transcription of interleukin-6 (IL-6) (Galien & Garcia, 1997; Stein & Yang, 1995), which promotes bone loss (Poli et al., 1994). Future studies are also needed to systematically identify ER-binding partners in bone cells, determine whether the binding is DNA dependent or DNA independent, and assess whether the binding is functionally significant.

It has been shown that ERs located on plasma membrane have similar ligand-binding affinities as nuclear ERs, and membrane ERs can rapidly transduce estrogen signaling via G proteins, ERK and c-Jun (Razandi, Pedram, Greene, & Levin, 1999). A series of studies have begun to determine the functional significance of membrane ERs in bone and delineate the mechanisms of gene transcription regulation via kinase-initiated regulation by membrane ERα vs classical genotropic regulation by nuclear ERα (Almeida, Han, O’Brien, Kousteni, & Manolagas, 2006; Almeida et al., 2010; Bartell et al., 2013; Kousteni et al., 2007, 2001, 2002, 2003).

ERs can also act in a ligand-independent manner during mechanical strain-induced bone response (Windahl, Saxon, et al., 2013). ERα mediates strain-induced osteoblast proliferation (Galea et al., 2013) and enhances loading-induced cortical bone increase in female mice (Saxon, Galea,
Meakin, Price, & Lanyon, 2012). ERβ mediates strain-induced Sost down-regulation (Galea et al., 2013), leading to enhanced Wnt/β-catenin signaling and osteoblast differentiation (Sharifi, Ereifej, & Lewiecki, 2015). However, ERβ was also shown to impair loading-induced cortical bone increase in both male and female mice (Saxon et al., 2012).

It should be noted that G protein-coupled estrogen receptor was reported to express in osteoblasts, osteocytes, and osteoclasts (Heino, Chagin, & Savendahl, 2008). Its deletion led to reduced bone growth (Martensson et al., 2009). Thus, G protein-coupled estrogen receptor should be considered during the design of estrogen-based drugs targeting nuclear ERs, as well as during the investigations of nuclear ER functions in bone cells upon estrogen treatment or depletion.

2.2 Androgen Receptor

Androgen is another steroid hormone that affects reproductive system, skeleton development, and many other aspects in both male and female (Chang, Lee, Wang, Yeh, & Chang, 2013). Upon androgen binding, androgen receptors (ARs) form homodimers and bind to a specific DNA sequence to regulate gene transcription. AR is expressed in osteoblasts (Colvard et al., 1989), osteoclasts (Mizuno et al., 1994), and osteocytes (Abu, Horner, Kusec, Triffitt, & Compston, 1997). Global knockout of AR in male mice reduced trabecular and cortical bone mass and upregulated RANKL expression in osteoblasts (Kawano et al., 2003). However, female mice with AR deficiency showed no bone abnormality (Kawano et al., 2003). Deletion of AR from osteoblast progenitors by Prx1-Cre in male mice led to decreased trabecular number, unchanged cortical bone, and increased osteoclast number (Ucer et al., 2015). Specific AR knockout in mature osteoblasts and osteocytes with osteocalcin-Cre triggered a reduction of trabecular bone volume and cortical bone thickness in young mice but not in adult mice (Chiang et al., 2009). Deletion of AR in mineralizing osteoblasts with Col2.3-Cre resulted in decreased trabecular bone volume and number, while no change was observed in cortical bone (Notini et al., 2007). Osteocyte-specific deletion of AR using Dmp1-Cre led to decreased trabecular bone mass and unchanged cortical bone in male mice (Sinnesael et al., 2012). Therefore, AR in all stages of the osteoblast/osteocyte differentiation prevents loss of trabecular bone and may have no effect on cortical bone mass. The reduced cortical bone thickness by osteocalcin-Cre-mediated AR deletion in young mice may need further
investigations. Specific AR deletion in osteoclast precursors by LysM-Cre or in mature osteoclasts by Ctsk-Cre had no effect on bone mass or osteoclast number (Sinnesael et al., 2015; Ucer et al., 2015). Taking together, these results indicate that AR mainly acts in the osteoblast lineage to protect bone. Future studies are needed to elucidate AR direct target genes and downstream signaling events in osteoblasts, as well as explore whether AR beyond bone cells may indirectly modulate skeletal homeostasis via neuroendocrine mechanisms.

2.3 Glucocorticoid Receptor

Glucocorticoid receptor (GR) mediates actions of glucocorticoids (GCs) on gene transcriptional regulation. GCs are widely used in treating diseases such as autoimmune disorders. However, GC-induced osteoporosis is a severe side effect for these patients (den Uyl, Bultink, & Lems, 2011). High level of GCs impairs osteoblast differentiation and induces osteoblast apoptosis (Ishida & Heersche, 1998; Weinstein, Jilka, Parfitt, & Manolagas, 1998). Interestingly, a recent study shows that monomeric GR, rather than dimeric GR, is responsible for GC suppression of osteoblast differentiation and bone formation (Rauch et al., 2010). GR deletion in osteoblasts by Runx2-Cre abolishes the ability of GCs to repress bone formation because GCs can no longer induce osteoblast apoptosis or inhibit osteoblast proliferation and differentiation (Rauch et al., 2010). In contrast, mice carrying a mutation that only disrupts GR dimerization (GRdim mice) are still sensitive to GC repression of bone formation. The antiosteoblastic effects of GCs may involve a suppression of cytokines, such as interleukin-11, via an interaction of the monomeric GR with AP-1, but not NF-κB (Rauch et al., 2010). GR deletion in osteoclast precursors by LysM-Cre diminishes the inhibitory effect of GCs on bone formation as well as osteoclastic bone resorption (Kim et al., 2006). These studies provide new mechanistic insights to how GC causes bone loss and how GR regulates bone.

2.4 Peroxisome Proliferator-Activated Receptor

Peroxisome proliferator-activated receptors (PPARs) are activated by various natural ligands such as unsaturated fatty acids and derivatives (Ahmadian et al., 2013; Evans & Mangelsdorf, 2014). They are key regulators of glucose and lipid metabolism; as such, their synthetic ligands such as rosiglitazone in the thiazolidinedione (TZD) class of drugs are widely used for the treatment of insulin resistance and type 2 diabetes (Ahmadian et al., 2013; Evans &
Mangelsdorf, 2014). Three PPAR isoforms have been discovered in human and mouse: PPARα, PPARδ (named PPARβ in mouse), and PPARγ. PPARα and PPARδ/β participate in energy combustion, while PPARγ facilitates energy storage. All three members form heterodimers with retinoid X receptor (RXR) and bind to peroxisome proliferator response element (PPRE) in target DNA. PPRE consists of a direct repeat AGGTCA that are separated by one base pair (Michalik et al., 2006). A number of coregulators participate in the transcriptional regulation by PPAR/RXR (Viswakarma et al., 2010), for example, PPARγ coactivator-1α (PGC-1α) (Vega, Huss, & Kelly, 2000). Ligand binding to PPAR/RXR heterodimer induces the exchange of corepressors for coactivators followed by transcriptional activation of target genes (Viswakarma et al., 2010).

2.4.1 Peroxisome Proliferator-Activated Receptor α

PPARα expression was found in osteoblasts, osteoclasts, and chondrocytes (Chan et al., 2007; Giaginis, Tsantili-Kakoulidou, & Theocharis, 2007). A global PPARα knockout mouse model was generated (Lee et al., 1995), but no significant change in bone was observed (Wu et al., 2000). However, treatment with PPARα agonists fenofibrate and Wyeth 14643 in rats increased whole body and femoral BMD and protected OVX rats from osteoporosis (Stunes et al., 2011). In vitro treatment with fenofibrate not only increased osteoblast differentiation (Still, Grabowski, Mackie, Perry, & Bishop, 2008) but also decreased osteoclast number (Chan et al., 2007). These findings suggest that activated PPARα protects bone through promoting bone formation while suppressing bone resorption.

2.4.2 Peroxisome Proliferator-Activated Receptor δ/β

PPARδ/β expression was detected in osteoblasts, osteoclasts, and chondrocytes (Giaginis et al., 2007). PPARδ/β knockout mice had smaller body size than wild-type control mice (Peters et al., 2000). In a mouse model with PPARδ/β deleted in all tissues except placenta by Sox2-Cre, reduced trabecular bone mass was observed (Scholtysek et al., 2013). At the same time, lower Wnt signaling activity, less serum OPG, and more osteoclasts were also detected in this mouse model (Scholtysek et al., 2013), suggesting that PPARδ/β deletion upregulates osteoblast-mediated osteoclastogenesis. Administration of PPARδ/β agonist GW501516 prevented bone loss in OVX mice (Scholtysek et al., 2013). Therefore, similar to PPARα, PPARδ/β also has a protective role in bone homeostasis.
2.4.3 Peroxisome Proliferator-Activated Receptor γ

There are two PPARγ isoforms, PPARγ1 and PPARγ2, due to alternative splicing and promoter usage. Because of the critical roles of PPARγ in regulating energy metabolism, several PPARγ agonists, such as TZD family members rosiglitazone and pioglitazone, have been developed to treat type 2 diabetes (Mayerson et al., 2002; Miyazaki et al., 2002). However, side effects of bone loss and increased risk of fracture arise from long-term TZD treatment (Bilezikian et al., 2013; Billington, Grey, & Bolland, 2015; Jin, Li, & Wan, 2015; Schwartz et al., 2015; Soccio, Chen, & Lazar, 2014; Wan, 2010), indicating a negative impact of activated PPARγ on bone.

PPARγ expression was found in osteoblasts, osteoclasts, and chondrocytes (Giaginis et al., 2007). Although homozygous PPARγ deficiency is lethal, higher bone mass, enhanced bone formation, and increased osteoblast differentiation were observed in heterozygous PPARγ-deficient mice (Akune et al., 2004). Deletion of PPARγ in mesenchymal progenitor cells with Col3.6–Cre increased BMD, bone volume, trabecular bone number, and osteoblast number (Cao et al., 2015). Deletion of PPARγ in osteoblast by Osx–Cre also led to increased trabecular bone number, unchanged cortical bone, and increased osteoblast differentiation (Sun et al., 2013). PPARγ deletion in osteoclast progenitor cells and endothelial cells with Tie2-Cre increased bone mass due to a reduction in osteoclast differentiation and bone resorption (Wan, Chong, & Evans, 2007).

Pharmacologically, treatment with TZDs such as rosiglitazone increases bone resorption and decreases bone formation by enhancing osteoclastogenesis and inhibiting osteoblastogenesis, in both humans and mice (Bilezikian et al., 2013; Billington et al., 2015; Jin et al., 2015; Schwartz et al., 2015; Wan, 2010). Moreover, it has been shown that rosiglitazone treatment also increases circulating osteoclast precursors but decreases circulating osteoblast precursors in postmenopausal women with type 2 diabetes mellitus (Rubin et al., 2014).

PPARγ cooperates with transcription coregulators to modulate bone cell differentiation. In osteoblasts, without ligand binding, PPARγ2 was shown to interact with small leucine zipper protein (sLZIP), which acted as a transcriptional corepressor by enhancing the association of PPARγ2 and HDAC3 to compete with PGC-1α recruitment (Kim & Ko, 2014). As a result of PPARγ2 suppression by sLZIP, osteogenesis was increased, while adipogenesis was decreased (Kim & Ko, 2014). In osteoclasts, PPARγ coactivator PGC–1β is indispensable during PPARγ stimulation of
osteoclastogenesis, as PGC-1β knockout mice showed complete resistance to bone loss after rosiglitazone treatment (Wei et al., 2010). These findings further deepen our understanding of how PPARγ suppresses osteoblast differentiation and enhances osteoclastogenesis to induce bone loss.

In addition, chondrocyte-specific PPARγ knockout in mice by Col2-Cre increased chondrocyte apoptosis and cartilage degradation, resulting in accelerated osteoarthritis (OA) (Vasheghani et al., 2015). PPARγ agonists showed antiinflammatory, antimatrix metalloprotease, and antiapoptotic effects in vitro (Fahmi, Martel-Pelletier, Pelletier, & Kapoor, 2011). In vivo treatment of PPARγ agonists decreased cartilage lesions in OA animals (Fahmi et al., 2011). These results suggest that activated PPARγ may be protective in the cartilage. Future studies are required to examine whether this is the case in human patients taking the TZD drugs.

2.5 Vitamin D Receptor

Vitamin D is a key regulator of serum calcium homeostasis and skeletal development. Vitamin D deficiency in children results in rickets. In human physiology, vitamin D₃ can be obtained through ultraviolet-driven biosynthesis or diet intake and can be converted into its active form of metabolite, 1α,25(OH)₂D₃. Upon 1α,25(OH)₂D₃ binding, vitamin D receptor (VDR) forms a heterodimer with RXR to activate target gene transcription through binding to vitamin D response elements (VDREs) that consist of two direct hexameric repeats separated by three base pairs (Heikkinen et al., 2011). In addition to the roles of vitamin D in maintaining serum calcium levels, VDR was detected in osteoblasts, osteocytes, and chondrocytes, but not in osteoclasts (Wang, Zhu, & DeLuca, 2014). Thus, vitamin D regulates bone homeostasis through both indirect effects on calcium and direct effects on bone cells.

The roles of VDR in bone have been investigated using several animal models. In global VDR knockout mice, femoral length was reduced; growth plate, osteoid width, and trabecular bone volume were increased, while osteoclast number was decreased (Masuyama et al., 2003; Yoshizawa et al., 1997). The abnormal bone phenotypes in these mice were mainly due to impaired intestinal calcium absorption (Van Cromphaut et al., 2001). A diet supplement of higher calcium over phosphorus ratio or intestine-specific reexpression of VDR rescued the bone defects in VDR knockout mice (Masuyama et al., 2003; Xue & Fleet, 2009). Under normal serum calcium levels, VDR regulation of bone homeostasis is mainly
mediated by controlling intestinal calcium absorption, whereas VDR signaling in osteoblasts and osteocytes is secondary (Lieben et al., 2012). However, low serum calcium triggers calcium release from bone to ensure a normal serum calcium level (Lieben et al., 2012). In this situation, vitamin D accelerates bone turnover, induces osteopenia, and suppresses bone mineralization (Lieben et al., 2012).

The negative role of VDR signaling in bone cells to decrease bone mass has been confirmed in conditional knockout mice. Col1a1-Cre-mediated VDR deletion in mature osteoblasts was reported to increase bone mass by reducing bone resorption and RANKL expression without affecting bone formation (Yamamoto et al., 2013). VDR deletion in osteocytes by Dmp1-Cre had no effect on bone (Lieben et al., 2012). VDR deletion in chondrocytes did not alter growth plate development, but increased trabecular bone mass through downregulating RANKL expression in chondrocytes and reducing osteoclastogenesis (Masuyama et al., 2006). In contrast to the observations in conditional VDR knockout mice, VDR transgenic overexpression driven by osteocalcin promoter in mature osteoblasts significantly increased trabecular bone volume by reducing bone resorption and osteoclastogenesis in mice (Baldock et al., 2006; Gardiner et al., 2000; Lam et al., 2014).

Besides the genetically modified animal models, conflicting data were also reported for the pharmacological effects of vitamin D on bone cells. High dose of vitamin D inhibits osteoblast mineralization in vitro (Yamaguchi & Weitzmann, 2012). Discontinuation of high vitamin D diet leads to gains of BMD in osteoporosis patients (Adams & Lee, 1997). It has been shown that activated VDR in osteoblasts directly upregulates the expression of OPN (Lieben et al., 2012), which is a negative regulator of osteoblast differentiation (Huang et al., 2004), by binding to the VDRE in opn promoter (Meyer, Goetsch, & Pike, 2010). It has also been reported that treatment with 1α,25(OH)2D3 downregulates Runx2 in osteoblasts to inhibit osteoblastogenesis and instead promote adipogenesis (Kim et al., 2016). Vitamin D also promotes osteoblast- and chondrocyte-driven osteoclast differentiation by increasing RANKL production (Lee, Kalinowski, Jastrzebski, & Lorenzo, 2002; Masuyama et al., 2006).

Nonetheless, opposite results were reported in other studies. Vitamin D-activated VDR in a human osteoblast cell line increased ALP activity and osteocalcin expression, as well as enhanced osteoblast differentiation and mineralization (van Driel et al., 2006). Vitamin D was also reported to inhibit osteoclast differentiation by suppressing expressions of NFATc1,
HIF1α, and c-Fos (Sakai et al., 2009; Sato et al., 2014; Takasu et al., 2006). Moreover, vitamin D was shown to block osteoclastogenesis by promoting migration of osteoclast precursors from bone to blood through suppressing S1PR2 expression in these cells (Kikuta et al., 2013).

The actions of vitamin D and VDR in maintaining serum calcium balance are very complicated involving their functions in the intestine, parathyroid gland, kidney, and bone. Thus, in order to elucidate tissue-specific and cell-specific VDR functions, multiple factors should be considered during in vivo and in vitro investigations, such as diet supplement, serum levels of calcium and vitamin D, tissue distribution of functional VDR, concentrations of calcium and vitamin D in cell culture medium, and cross talk among different organs and cell types.

2.6 Retinoid Acid Receptor and RXR

Retinoic acid (RA), including all-trans- and 9-cis-RA, belongs to vitamin A metabolites and fundamentally modulates vertebrate development especially organogenesis. RA functions as ligands for retinoid acid receptors (RARs) (RARα, RARβ, and RARγ) and RXRs (RXRα, RXRβ, and RXRγ). All-trans-RA only associates with RARs, while 9-cis-RA binds to both RARs and RXRs (Chambon, 1996). RARs and RXRs need to form RAR/RXR heterodimers to respond to RA and act as transcriptional factors. RAR/RXR heterodimers bind to specific RA response element and interact with corepressors in the absence of RA. RAR agonists are able to activate RAR/RXR complex by triggering exchange of corepressor with coactivator, while RXR agonists alone cannot (Germain, Iyer, Zechel, & Gronemeyer, 2002; Love et al., 2002).

In addition, 9-cis-RA induces the homodimer formation and transcriptional activation of RXRs (Zhang et al., 1992). Moreover, RXRs are also involved in the activation of other members of the NR superfamily by forming heterodimers with them. These heterodimers are divided into permissive and nonpermissive heterodimers. Activation of permissive heterodimers is induced by the ligand binding of either RXR or RXR partner (PPARs, LXRα, FXR, SXR/PXR, and CAR), while nonpermissive ones are only activated by the ligands of RXR partner (TRs, VDR, and RARs) (Evans & Mangelsdorf, 2014).

RARα and RARγ are expressed in osteoblasts and osteoclasts, whereas RARβ is detectable only after RA stimulation (Conaway et al., 2011; Inoue et al., 1996; Kindmark, Torma, Johansson, Ljunghall, & Melhus, 1993; van...
Beek, Lowik, Karperien, & Papapoulos, 2006). Global RARα or RARβ knockout did not cause any significant change in bone (Li, Sucov, Lee, Evans, & Jaenisch, 1993; Luksin et al., 1993; Mendelsohn et al., 1994). However, global RARγ knockout mice had reduced trabecular bone mass and increased osteoclastogenesis (Green et al., 2015), suggesting that RARγ is a negative regulator of bone resorption. In light of the potential functional redundancy within RARs and RXRs, several double global knockouts have been generated and characterized (Lohnes et al., 1994). In addition, chondrocyte-specific RAR double deletion by Col2a1-Cre demonstrated that RARα−/−;RARγ−/− mice and RARβ−/−;RARγ−/− mice showed growth plate defect (Williams et al., 2009), indicating a central role of RARγ in the cartilage.

High vitamin A diet and high serum retinol level were reported to be positively correlated with a higher risk of bone fracture in humans and animals (Feskanich, Singh, Willett, & Colditz, 2002; Lind et al., 2013; Melhus et al., 1998; Michaelsson, Lithell, Vessby, & Melhus, 2003; Whiting & Lemke, 1999). However, a number of reports showed no correlation between serum retinol level and fracture risk, or even a protective effect of retinol on bone (Ambrosini et al., 2014; Caire-Juvera, Ritenbaugh, Wactawski-Wende, Snetselaar, & Chen, 2009; Holvik et al., 2015; Ribaya-Mercado & Blumberg, 2007). Thus, the relationship between serum retinol level and fracture risk remains unclear.

Several in vitro studies demonstrated that RA negatively regulated osteoblast differentiation and mineralization (Iba, Chiba, Yamashita, Ishii, & Sawada, 2001; Lind et al., 2013; Ohishi et al., 1995). For example, RA treatment in vitro resulted in decreased mineralization and proliferation of osteoblasts accompanied by RAR-mediated reduced expressions of ALP, osteocalcin, Runx2, and Osx (Lind et al., 2013). However, RA treatment was also shown to enhance osteoblast differentiation (Gazit, Ebner, Kahn, & Derynck, 1993; Skillington, Choy, & Derynck, 2002; Song et al., 2005). Similar to the ambiguous roles of RA in osteoblast differentiation, RA treatment led to increased osteoclastogenesis in some reported studies (Conaway et al., 2011; Kneissel, Studer, Cortesi, & Susa, 2005; Saneshige et al., 1995) but was shown to decrease osteoclastogenesis in others (Balkan, Rodriguez-Gonzalez, Pang, Fernandez, & Troen, 2011; Hu, Lind, Sundqvist, Jacobson, & Melhus, 2010; Kneissel et al., 2005). RA-activated RARs were shown to negatively regulate longitudinal bone growth by suppressing growth plate chondrogenesis (Ballock et al., 1994; De Luca et al., 2000), but another group reported that RA increased
chondrocyte maturation and mineralization (Iwamoto et al., 1993). To understand these differences and elucidate the bona fide roles of RARs/RXRαs in bone will require more in depth in vitro and in vivo future studies.

2.7 Estrogen Receptor-Related Receptor

Estrogen receptor-related receptors (ERRs), including ERRα, ERRβ, and ERRγ, belong to the subfamily of orphan NR and share sequence homology with ERs. However, ERRs do not respond to ligands of ER such as estradiol (Horard & Vanacker, 2003). ERRα is the oldest orphan NR that has long been thought to not require an endogenous ligand, because it is active as long as the cells or tissues express its coactivators such as PGC-1α/β. Recently, our group has identified cholesterol as a potential endogenous ligand for ERRα that increased ERRα transcriptional activity (Wei et al., 2016). This discovery explains why ERRα is constitutively active because cholesterol is ubiquitous. Nonetheless, ERRα activities can be fine-tuned by altering cellular cholesterol levels. ERRα functions are diminished under cholesterol-depleted conditions such as following statin or bisphosphonate treatment, which are rescued by cholesterol add back (Wei et al., 2016). Moreover, ERRα is a key mediator of statin and bisphosphonate actions in bone, muscle, and macrophages (Wei et al., 2016). These findings deorphanize ERRα and provide new insights to the physiological regulation of ERRα activities, thereby revealing potential pharmacological strategies to control ERRα-related disorders.

In light of the multiple roles of ERRs in physiology and diseases, several synthetic small-molecule ligands have been developed. These ligands have been shown to bind to the LBD of ERRs (Wang et al., 2006) and function as agonists (GSK4716 and DY131) (Yu & Forman, 2005; Zuercher et al., 2005) or inverse agonists (4-hydroxytamoxifen and diethylstilbestrol) (Coward, Lee, Hull, & Lehmann, 2001; Tremblay et al., 2001). These findings not only provide useful chemical tools to probe ERR functions but also facilitate the development of ERR-targeting therapies.

Using mouse genetic models, ERRα was found to suppress osteoblastogenesis but promote osteoclastogenesis. Global ERRα knockout mice had increased femoral cancellous bone volume and density as well as enhanced osteoblast differentiation (Delhon et al., 2009). Female mice with specific deletion of ERRα in mature osteoblasts with Col1a1-Cre were completely resistant to bone loss induced by ovariectomy (Gallet et al., 2013). Global ERRα knockout mice also showed osteoclastogenesis defects
with reduced mitochondrial biogenesis during osteoclast differentiation, leading to lower bone resorption and higher bone mass (Wei et al., 2016, 2010). Therefore, ERRα is a proosteoclastogenic NR and transcription factor. The identification of cholesterol as a natural ERRα agonist reveals that cholesterol-stimulated osteoclastogenesis, high cholesterol diet-induced bone loss, and bisphosphonate-mediated osteoprotection are ERRα dependent (Wei et al., 2016).

Homozygous ERRγ deletion is lethal and heterozygous ERRγ deletion elevates bone mass in male mice by increasing trabecular number, trabecular thickness, and osteoblast number (Cardelli & Aubin, 2014). This suggests that like ERRα, ERRγ also exerts negative effects on bone by suppressing bone formation. Homozygous ERRβ deletion was also lethal due to its high expression in placenta (Luo et al., 1997). Sox2-Cre-mediated deletion of ERRβ in all tissues except placenta altered body weight and physical activity (Byerly, Swanson, Wong, & Blackshaw, 2013), but no bone phenotype has been reported in this animal model. Future studies using conditional ERRγ/β knockout mice will further delineate their specific functions in each bone cell type and potentially also in nonbone cell types during skeletal maintenance.

2.8 NR4A Orphan NRs

Nuclear receptor subfamily 4 group A (NR4A) is a group of orphan NRs consisting of three members: Nur77 (NR4A1, NGFI-B), Nurr1 (NR4A2), and NOR1 (NR4A3). They exert their transcriptional activities as monomers or homodimers (Philips et al., 1997; Wilson, Fahrner, Johnston, & Milbrandt, 1991). In addition, Nur77 and Nurr1, but not NOR1, are able to form heterodimers with RXR and promote 9-cis-RA-induced RXR activation (Perlmann & Jansson, 1995; Zetterstrom, Solomin, Mitsiadis, Olson, & Perlmann, 1996). In the protein structures of Nur77 and Nurr1, there is no classical ligand-binding pocket as in other NRs (Flaig, Greschik, Peluso-Iltis, & Moras, 2005; Wang et al., 2003). However, cytosporone B (Zhan et al., 2008) and ethyl[2,3,4-trimethoxy-6-(1-octanoyl)phenyl] acetate (Liu et al., 2010) have been reported as ligands for Nur77. Unsaturated fatty acids, such as docosahexaenoic acid, have also been reported as ligands for both Nur77 and Nurr1 (de Vera et al., 2016; Vinayavekhin & Saghatelian, 2011). Although careful studies, for example, using knockout mice, are required in the future to examine the specificity of these potential NR4A ligands, these interesting findings provide the opportunities to
deorphanize NR4A receptors and develop therapeutic drugs targeting NR4A in associated diseases.

Nur77, Nurr1, and NOR1 are expressed in osteoblasts (Lammi, Huppunen, & Aarnisalo, 2004; Pirih, Nervina, Pham, Aghaloo, & Tetradas, 2003; Tetradas, Bezuglaia, Tsingotjidou, & Vila, 2001). Their expression is stimulated by PTH and fibroblast growth factor 8b (FGF-8b) (Lammi & Aarnisalo, 2008; Pirih et al., 2003; Tetradas, Bezuglaia, & Tsingotjidou, 2001; Tetradas, Bezuglaia, Tsingotjidou, & Vila, 2001). In vitro Nurr1 knockdown in osteoblasts downregulated the expressions of osteocalcin and Col1a1, as well as ALP activity, while Nurr1 overexpression showed opposite effects (Lee, Choi, Gil, & Nikodem, 2006). These findings indicate that Nurr1 may play positive roles in promoting bone formation.

Opposite effects of NR4A on osteoblasts were also reported. Overexpression of Nur77, Nurr1, or NOR1 in osteoblasts was shown to activate OPN promoter (Lammi et al., 2004; Pirih, Tang, Ozkurt, Nervina, & Tetradas, 2004). OPN, one of major noncollagen proteins in bone matrix, is a negative regulator of osteoblast differentiation (Huang et al., 2004) and a mediator of bone resorption (Yoshitake, Rittling, Denhardt, & Noda, 1999). However, it remains unclear whether the NR4A-induced OPN expression is functionally significant for the overall impact on bone formation and bone resorption. Another study showed that overexpression of Nur77, Nurr1, or NOR1 in the U2-OS osteoblastic cell line suppressed β-catenin transcriptional activity and β-catenin in turn also inhibited the transcriptional activities of NR4As (Rajalin & Aarnisalo, 2011). Despite the important functions of β-catenin in osteoblasts, the impact of this potential cross talk between NR4As and β-catenin on osteoblast activity and bone formation has not been demonstrated. Future studies are required to determine the physiological relevance and functional significance of these interesting in vitro observations.

Nur77 expression is elevated during osteoclast differentiation, while the expression of Nurr1 and NOR1 is very low in osteoclasts (Li et al., 2015). Work from our lab shows that global Nur77 knockout reduced bone mass and increased osteoclastogenesis (Li et al., 2015), indicating a resorption-suppressive and osteoprotective effect of Nur77. Mechanistically, we uncover that Nur77 prevents excessive osteoclastogenesis by mediating an NFATc1 self-limiting regulatory loop. NFATc1 induces Nur77 expression at late stage of osteoclast differentiation; in turn, Nur77 transcriptionally upregulates E3 ubiquitin ligase Cbl-b, which triggers NFATc1 protein degradation (Li et al., 2015). These findings not only
identify Nur77 as a key player in bone turnover and a new therapeutic target for bone diseases but also elucidate a previously unrecognized NFATc1 → Nur77 → Cbl-b → NFATc1 feedback mechanism that confers NFATc1 signaling autoresolution.

3. CONCLUSIONS

Generally, activation of ERα, AR, PPARα, PPARδ/β, Nur77, and Nurr1 protects bone, while activation of ERβ, GR, PPARγ, and ERRα/γ causes bone loss. Conflicting roles of VDR and RAR in bone have been reported. Among other NRs that are not discussed here, SXR/PXR (Azuma et al., 2010), RORα (Benderdour, Fahmi, Beaudet, Fernandes, & Shi, 2011; Lyashenko et al., 2010; Meyer, Kneissel, Mariani, & Fournier, 2000), and SHP (Jeong et al., 2010) may play positive protective roles in bone, while PR (Rickard et al., 2008) negatively regulates bone homeostasis. In vivo analysis of global knockout mice indicates that LXRαs may exert negative detrimental effects on bone, in part by elevating osteoclast functions (Robertson et al., 2006); but in vitro data show that activation of LXRβs inhibits both osteoblastogenesis (Prawitt et al., 2011) and osteoclastogenesis (Kim et al., 2013; Kleyer et al., 2012; Remen, Henning, Lerner, Gustafsson, & Andersson, 2011; Robertson Remen, Gustafsson, & Andersson, 2013; Robertson Remen, Lerner, Gustafsson, & Andersson, 2013). The effect of thyroid hormone and thyroid hormone receptors, TRα and TRβ, on bone development and remodeling has been reviewed in detail recently (Bassett & Williams, 2016). Hypothyroid delays bone growth and maturation in children, while thyrotoxicosis accelerates these processes in children and causes osteoporosis in adult (Bassett & Williams, 2016). Global TRα deletion resulted in delayed bone development of young mice and increased bone mass with reduced osteoclast differentiation in adult mice (Bassett et al., 2007; Bassett & Williams, 2016). Global TRβ deletion led to elevated thyroid hormone levels, accelerated ossification and short stature in young mice, and reduced bone mass with increased osteoclast differentiation in adult mice, all of which may be due to hyperactivation of TRα by elevated thyroid hormone levels in these mice (Bassett et al., 2007; Bassett & Williams, 2016). Therefore, normal levels of thyroid hormone and functions of its receptors are necessary for both early bone development and adult bone maintenance. We have summarized the reported bone phenotypes in NR knockout animals (Table 1).
<table>
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<tr>
<th>NR</th>
<th>Cell Type</th>
<th>Cre</th>
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<th>C.b.</th>
<th>Ob</th>
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<td>Sims et al. (2002)</td>
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<td>Almeida et al. (2013)</td>
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<td>Mature Ob/Ocy</td>
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<td>Mature Oc</td>
<td>Ctsk</td>
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<td>Nicks et al. (2016)</td>
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<td>Col2.3</td>
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<td>LysM</td>
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</table>
NRs are essential in controlling gene expressions during bone development and remodeling in response to the rise and fall of ligand concentration. NRs can act as constitutive transcriptional activators when agonists are abundant and/or ubiquitous, or can be induced to activation by binding to ligands.

<table>
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<td>PR</td>
<td>Global</td>
<td></td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
<td>Rickard et al. (2008)</td>
</tr>
<tr>
<td>LXRα</td>
<td>Global</td>
<td></td>
<td>→</td>
<td>↑</td>
<td>→</td>
<td>↓</td>
<td>Robertson et al. (2006)</td>
</tr>
<tr>
<td>LXRβ</td>
<td>Global</td>
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<td>→</td>
<td>→</td>
<td>↑</td>
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<td>Robertson et al. (2006)</td>
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<tr>
<td>PXR</td>
<td>Global</td>
<td></td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>Azuma et al. (2010)</td>
</tr>
<tr>
<td>RORα</td>
<td>Global</td>
<td></td>
<td>↓</td>
<td>↓</td>
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<td>Lyashenko et al. (2010) and Meyer et al. (2000)</td>
</tr>
<tr>
<td>SHP</td>
<td>Global</td>
<td></td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>Jeong et al. (2010)</td>
</tr>
</tbody>
</table>

C.b., cortical bone; Ob, osteoblast; Oc, osteoclast; Ocy, osteocyte; Ocn, osteocalcin; T.b., trabecular bone; ↑, increase; ↓, decrease; →, no change.
to steroid/thyroid hormones, biological metabolites, vitamins, and other lipophilic natural or synthetic small molecules. Alterations of NR activities, resulted from abnormal levels of either receptors or ligands, can lead to bone disorders; for example, estrogen deficiency or GC treatment causes osteoporosis. NR modulation of skeletal homeostasis may involve mechanisms beyond direct transcriptional regulation, for example, membrane ERα-mediated kinase-initiated regulation. Moreover, NR control of bone remodeling may involve anatomic sites outside of bone that mediates neuronal, endocrine, and metabolic regulation on bone; for example, VDR acts in intestine to regulate calcium absorption. Therefore, the multifaceted roles of each NR in regulating bone homeostasis and the bone beneficial concentration ranges for their agonists, antagonists, or modulators need to be determined in future studies. NRs are excellent targets for drug design due to their broad functions in various biological processes and their functional tunability through small-molecule ligands. This review provides a timely update on our latest understanding of NR regulation of bone health. This new knowledge will facilitate the development of novel osteoprotective medicine and effective strategies to eliminate the bone loss side effects of current drugs.

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