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Review

Animal models for osteoporosis

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ABSTRACT

The major types of osteoporosis in humans are postmenopausal osteoporosis, disuse osteoporosis, and glucocorticoid-induced osteoporosis. Animal models for postmenopausal osteoporosis are generated by ovariectomy. Bone loss occurs in estrogen deficiency due to enhanced bone resorption and impaired osteoblast function. Estrogen receptor α induces osteoclast apoptosis, but the mechanism for impaired osteoblast function remains to be clarified. Animal models for unloading are generated by tail suspension or hind limb immobilization by sciatic neurectomy, tenotomy, or using plaster cast. Unloading inhibits bone formation and enhances bone resorption, and the involvement of the sympathetic nervous system in it needs to be further investigated. The osteocyte network regulates bone mass by responding to mechanical stress. Osteoblast-specific *BCL2* transgenic mice, in which the osteocyte network is completely disrupted, can be a mouse model for the evaluation of osteocyte functions. Glucocorticoid treatment inhibits bone formation and enhances bone resorption, and markedly reduces cancellous bone in humans and large animals, but not consistently in rodents.

1. Introduction

Bone mass is determined by the balance between the activities of osteoblasts, which form bone, and those of osteoclasts, which resorb bone. In osteoblast differentiation, Runx2, Sp7, and canonical Wnt signaling play essential roles in the commitment of pluripotent mesenchymal cells to the osteoblastic lineage (Komori, 2011). After their differentiation, osteoblasts produce Col1a1 and non-collagenous bone matrix proteins, including osteopontin (Spp1), bone sialoprotein (Ibsp), and osteocalcin (Bglap). Mature osteoblasts are embedded into the bone matrix and finally become osteocytes (Komori, 2010). Monocytic cells differentiate into osteoclasts under the control of macrophage colony-stimulating factor and receptor activator of NF-κB ligand (Rankl, Tnfsf11), which are expressed in osteoblast lineage cells (Yasuda et al., 1998).

Osteoporosis, which is now one of the major age-related diseases, is caused by the imbalance of these two activities, which are influenced by diet, physical activities, hormonal status, cytokines, and clinical status, such as diabetes mellitus and glucocorticoid treatment. The major types of osteoporosis in humans are postmenopausal osteoporosis, disuse osteoporosis, and glucocorticoid-induced osteoporosis. Therefore, I focus on animal models for these three types of osteoporosis in this review.

2. Animal models for postmenopausal osteoporosis

2.1. Postmenopausal osteoporosis in humans

Recent research in humans using quantitative computed tomography has shown that a decrease of volumetric bone mineral density of cancellous bone begins in young adult women and men and continues throughout life, with acceleration during perimenopause in women. In contrast, the decrease in volumetric bone mineral density of cortical bone begins in middle age in women and after 70–75 years of age in men (Riggs et al., 2004, 2008). Although the reasons for the beginning of cancellous bone loss in young adult women and men remain to be clarified, the acceleration of cancellous bone loss and the decrease of cortical bone in women are closely correlated to estrogen deficiency. Indeed, estrogen replacement therapy in ovariectomized or menopausal women reduces bone loss and fractures (Christiansen and Lindsay, 1990). In postmenopausal osteoporosis, bone loss is most prominent in cancellous bone and the endocortical surface, and it is caused by increased bone turnover and a negative calcium balance (Christiansen and Lindsay, 1990). Estrogen deficiency enhances bone resorption. Although bone formation is also induced by

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enhanced bone resorption, osteoblasts, whose function is impaired by estrogen deficiency, cannot restore the resorbed bone, and bone loss occurs. These characteristics are required in animal models of estrogen deficiency.

2.2. Animal models by ovariectomy

The most popular animal model of postmenopausal osteoporosis is generated in mouse, rat, sheep, and nonhuman primates by ovariectomy (Fig. 1). In mice, the loss of cancellous bone but not cortical bone occurs soon after ovariectomy, and estrogen replacement with 17-β-estradiol in ovariectomized mice prevents bone loss (Jilka et al., 1992). In rat, the effects of ovariectomy were examined in detail. Significant bone loss after ovariectomy occurs in the proximal tibial metaphysis after 14 days, in the lumbar vertebral body after 60 days, and in the femoral neck after 30 days (Li et al., 1997; Wronski et al., 1988, 1989). In cortical bone, bone resorption is enhanced in the endosteum and bone formation is enhanced in the periosteum after ovariectomy, leading to an enlarged bone marrow cavity. The reduction of cortical thickness and enlargement of the bone marrow cavity begin between 90 and 120 days after ovariectomy (Danielsen et al., 1993). In sheep, the markers for bone resorption and bone formation are induced by ovariectomy at the highest levels after 3 months and 4 months, respectively. Ovariectomy increases the porosity and the eroded and osteoid surfaces in cortical bone, but has no effect on cancellous bone after 6 months (Chavassieux et al., 2001). There is technical difficulty in positioning sheep for repeated examination to see the longitudinal changes (Turner, 2002). Ovariectomy induces high bone turnover and rapid bone loss in monkeys, which can be seen at 3 months, and monkey ovariectomy models have been used to support the submissions of many new drugs for osteoporosis (Binkley et al., 1998; Smith et al., 2009). Since the differences in major bone parameters between ovariectomy and sham-operated groups are small, however, many animals per group are required to obtain statistically significant results (Smith et al., 2009).

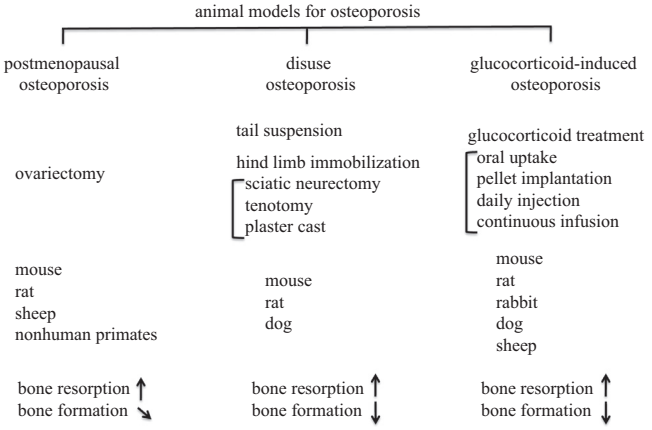

Fig. 1. The major methods and animals for the generation of postmenopausal osteoporosis, disuse osteoporosis, and glucocorticoid-induced osteoporosis.

2.3. Functions of estrogen receptor signaling in bone

The indirect and direct effects of estrogen are considered to cause postmenopausal osteoporosis. The production of inflammatory cytokines, including interleukin (IL)-1, IL-6, IL-7, tumor necrosis factor (TNF)-α, and granulocyte macrophage colony-stimulating factor (GM-CSF), by immune cells is enhanced in an estrogen-deficient state (Pacifici, 2008). These cytokines induce osteoclastogenesis and bone resorption. Secretion of the pituitary-derived follicle-stimulating hormone increases in estrogen deficiency. Neither follicle-stimulating hormone β (*Fshb*) nor follicle-stimulating hormone receptor (*Fshr*) null mice show bone loss in estrogen deficiency. Osteoclasts and their precursors possess follicle-stimulating hormone receptors, and follicle-stimulating hormone enhances osteoclastogenesis through these receptors (Sun et al., 2006).

As mutations of estrogen receptor α (*Esr1*) cause osteoporosis in humans (Quaynor et al., 2013; Smith et al., 1994), the mechanism of direct estrogen effects on bone has been extensively examined using various conditional knockout mice of *Esr1* (Fig. 2). In mature osteoclast-specific *Esr1* conditional knockout mice using Cre knock-in mice at the cathepsin K (*Ctsk*) gene locus, bone loss by ovariectomy was prevented in conditional knockout mice. Estrogen induced Fas ligand (Fasl) at the mRNA and protein levels in the presence of wild-type Esr1, and enhanced the apoptosis of osteoclasts. Therefore, estrogen was thought to protect bone by inducing osteoclast apoptosis through the transcriptional regulation of *Fasl* (Nakamura et al., 2007). Another group generated *Esr1* conditional knockout mice with targeted deletion of *Esr1* in osteoclast precursor cells using lysozyme M (*Lyz2*) Cre transgenic mice (Martin-Millan et al., 2010). In these mice, bone loss by ovariectomy was prevented in cancellous bone, but not in cortical bone. Therefore, estrogen prevents cancellous bone loss by inducing osteoclast apoptosis, but it prevents cortical

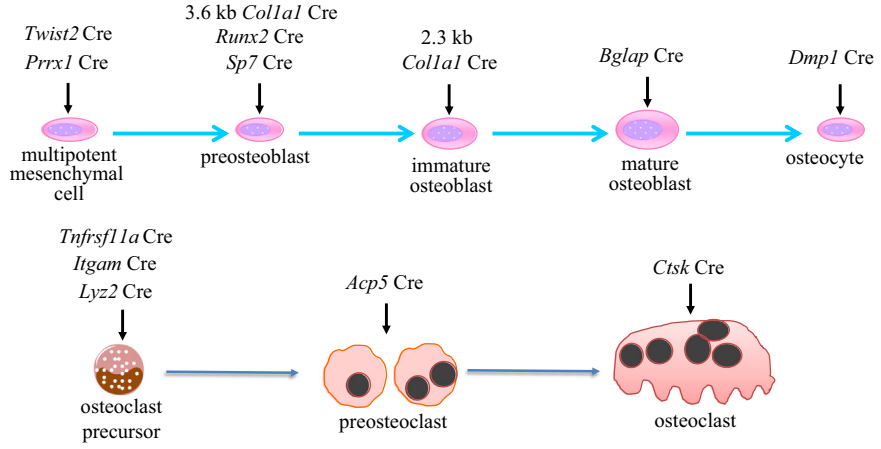

Fig. 2. Cre recombinase-mediated target gene deletion during osteoblast and osteoclast differentiation.
Approximate stages at which Cre recombinase starts to work in osteoblast and osteoclast differentiation are shown. *Twist2* (Dermo1) Cre knock-in mice (Yu et al., 2003), *Runx2* promoter Cre transgenic mice (Rauch et al., 2010), 3.6-kb *Col1a1* promoter Cre transgenic mice (Liu et al., 2004), *Tnfrsf11a* (RANK) Cre knock-in mice (Maeda et al., 2012), *Itgam* (CD11a) promoter Cre transgenic mice (Ferron and Vacher, 2005), and *Acp5* (TRAP) promoter Cre transgenic mice (Chiu et al., 2004) are not described in this manuscript.

bone loss by affecting other cells including osteoblasts and osteocytes. Furthermore, they failed to induce *Fasl* by estrogen, although *Fasl* was required for the induction of osteoclast apoptosis by estrogen, and estrogen effectively promoted osteoclast apoptosis in mice bearing an *Esr1* knock-in mutation that prevents binding to DNA. Therefore, estrogen seems to induce osteoclast apoptosis through the DNA binding-independent action of Esr1 (Martin-Millan et al., 2010).

The deletion of *Esr1* in *Prrx1* Cre or *Sp7* Cre transgenic mice, in which this gene is deleted in osteo-chondroprogenitors or preosteoblasts, respectively, resulted in the reduction in cortical bone, but not cancellous bone (Almeida et al., 2013) (Fig. 2). In these mice, bone formation was reduced in the periosteum. Bone resorption in the endosteum was enhanced in wild-type mice, but not in *Esr1* conditional knockout mice, by ovariectomy, and bone loss occurred in cancellous bone, but not in cortical bone, in *Esr1* conditional knockout mice. Therefore, Esr1 attenuates bone resorption in the endosteum through osteoblast lineage cells and enhances bone formation in the periosteum. Wnt3 enhanced the proliferation and differentiation of osteoblasts for wild-type osteoblasts, but not for *Esr1*-deleted ones. However, estrogen had no effect on the enhancement of proliferation and differentiation by Wnt3 in wild-type osteoblasts. Therefore, it was suggested that canonical Wnt signaling stimulates the proliferation and differentiation of osteoblasts through unliganded Esr1 (Almeida et al., 2013). In contrast, *Esr1* conditional knockout mice using 2.3-kb *Col1a1* promoter Cre transgenic mice, which directs Cre expression to osteoblasts, showed no changes in both cancellous bone and cortical bone, suggesting that Esr1 regulates bone formation in the periosteum and bone resorption in the endosteum through the regulation of osteoprogenitors or preosteoblasts (Almeida et al., 2013). Maatta et al. (2013) reported *Esr1* conditional knockout mice with targeted deletion of *Esr1* in mature osteoblasts using *Bglap* Cre transgenic mice. In these female mice, both cancellous and cortical bone was reduced, and the numbers of osteoblasts and osteoclasts were reduced. The further reduction of neither cancellous nor cortical bone by ovariectomy was observed (Maatta et al., 2013). Melville et al. (2014) also reported *Esr1* conditional knockout mice using the same Cre mice. In these mice, both cancellous and cortical bone was also reduced, and the number of osteoblasts but not osteoclasts was reduced. Therefore, the functions of Esr1 in osteoblasts are still controversial.

There are two reports of osteocyte-specific *Esr1* conditional knockout mice using the same *Dmp1* Cre transgenic mice (Fig. 2). Windahl et al. (2013) reported that male but not female *Esr1* conditional knockout mice show a reduction in cancellous bone due to decreased bone formation. In contrast, Kondoh et al. (2014) reported that female but not male *Esr1* conditional knockout mice show a reduction in cancellous bone, but not in cortical bone, due to decreased bone formation. In both cases, the number of osteoclasts was normal. Therefore, the functions of Esr1 in osteocytes are also controversial. Cre transgenic mice may cause these inconsistencies because the expression patterns and levels of Cre are not the same, even in mice using the same promoter. Furthermore, as the genetic background markedly affects bone mass and turnover, the comparison of knockout mice with a chimeric background to control mice frequently causes variable results.

3. Animal models for disuse osteoporosis

3.1. Animal models for unloading

The prevalence of disuse osteoporosis, which is caused by non-weight bearing, immobilization, or long-term bed rest, is rapidly increasing due to the increase in bedridden patients with age-associated diseases. Astronauts also lose bone during space flight with markedly increased bone resorption (LeBlanc et al., 2007). In long-term bed rest study for 90 days in humans, bone loss occurs due to the markedly increased bone resorption with mild increase in bone formation (Watanabe et al., 2004). As enhanced bone resorption should increase bone formation, however, the bone formation in long-term bed rest study in humans should have been mildly inhibited. The results from rats in spaceflight are quite different from those from astronauts in spaceflight, because the bone formation is reduced but bone resorption is not significantly affected in rats in spaceflight (Morey and Baylink, 1978; Vico et al., 1991). Unloaded conditions are generated by tail suspension or hind limb immobilization by sciatic neurectomy, tenotomy, or using plaster cast in rodents and dogs (Brighton et al., 1988; Globus et al., 1986; Hott et al., 2003; Shaker et al., 1989) (Fig. 1). In tail suspension experiments, mice are suspended in a head-down tilt at approximately 30°, and they have free access to food and water by the use of their forelimbs (Sakata et al., 1999). In tail suspension in rats and mice, bone formation is inhibited, while bone resorption is enhanced or unchanged (Apseloff et al., 1993; Bikle et al., 1994; Globus et al., 1986; Simske et al., 1994; Vico et al., 1991; Wronski and Morey, 1982). In our tail suspension experiments using C57BL/6 mice for 2 weeks, bone formation is mildly inhibited and bone resorption is markedly enhanced (Moriishi et al., 2012). Immobilization also reduces bone formation and enhances bone resorption (Sakai et al., 1996; Weinreb et al., 1989). It is noteworthy that the susceptibility to unloading is largely dependent on the genetic background, C57BL/6 mice are preferentially used for unloading experiments in mice, and unloading reduces bone formation and enhances bone resorption in C57BL/6 background (Amblard et al., 2003; Judex et al., 2004; Kodama et al., 1999) (Fig. 1). Indeed, peak bone mass is also affected by genetic background. Since unloading experiments using knockout mice with mixed genetic backgrounds cause variable results, the use of knockout mice with a C57BL/6 pure background is recommended. Age is also an important factor for the susceptibility to unloading, and the inhibition of bone formation by unloading seems to be prominent in growing rodents (Globus et al., 1986; Sakai and Nakamura, 2001). Plasma corticosterone levels are similar between normally loaded and unloaded rats, and adrenalectomy does not prevent bone loss in unloading, indicating that glucocorticoid is not responsible for the bone loss in unloading (Halloran et al., 1988).

3.2. Animal models for the analysis of bone regulation by sympathetic nervous system

The sympathetic nervous system has been suggested to be involved in bone loss by unloading because the treatment with non-selective β-adrenoceptor antagonist propranolol and/or guanethidine sulfate prevented cancellous bone loss by tail suspension (Kondo et al., 2005; Levasseur et al., 2003). However, the treatment with propranolol failed to prevent bone loss by sciatic neurectomy and bone gain by supra-physiological external mechanical loading (Marenzana et al., 2007). Propranolol is a non-specific β-adrenoceptor antagonist, which blocks three β-adrenoceptor subtypes. The phenotypes of these β-adrenoceptor gene knockout mice are controversial. Cancellous bone in vertebrae and femurs was increased due to increased bone formation and decreased bone resorption in β-adrenoceptor 2 $(Adrb2)^{-/-}$ mice (Elefteriou et al., 2005). Treatment with isoproterenol induced cAMP, *Rankl* expression, and osteoclastogenesis in co-culture with bone marrow macrophages in wild-type osteoblasts, but not in $Adrb2^{-/-}$ osteoblasts. Furthermore, neither bone loss nor increase in bone resorption markers by ovariectomy was observed in $Adrb2^{-/-}$ mice. These findings indicate that Adrb2 is responsible for the enhanced bone resorption by the sympathetic nervous system and Adrb2 is required for bone loss by estrogen depletion. Pierroz et al. examined $Adrb1^{-/-}$ mice, $Adrb2^{-/-}$ mice, and $Adrb1^{-/-}2^{-/-}$ mice

(Pierroz et al., 2012). Cancellous bone in vertebrae but not in femur was reduced and cortical thickness was unchanged in *Adrb1*$^{-/-}$ mice; cancellous bone was increased in vertebrae but reduced in femurs and cortical thickness was unchanged in *Adrb2*$^{-/-}$ mice; and cancellous bone was unchanged in both vertebrae and femurs and cortical thickness was reduced in *Adrb1*$^{-/-}$*2*$^{-/-}$ mice (Pierroz et al., 2012). The parameters for both bone formation and bone resorption were reduced in *Adrb1*$^{-/-}$*2*$^{-/-}$ mice. In *Adrb1*$^{-/-}$*2*$^{-/-}$*3*$^{-/-}$ mice, bone volume was increased in cancellous bone of vertebrae and femurs and in cortical bone of femurs at 6 weeks of age and only in cortical bone of femurs at 16 weeks of age due to the reduced bone resorption (Bouxsein et al., 2009). Tibiae from *Adrb2*$^{-/-}$ mice but not *Adrb1*$^{-/-}$ mice and *Adrb1*$^{-/-}$*2*$^{-/-}$ mice responded to axial compression loading similarly to those from wild-type mice (Pierroz et al., 2012). Furthermore, treatment with isoproterenol reduced cancellous and cortical bone in wild-type mice, but not in *Adrb1*$^{-/-}$*2*$^{-/-}$ mice (Pierroz et al., 2012). Moreover, bone loss by ovariectomy similarly occurred in wild-type and *Adrb1*$^{-/-}$*2*$^{-/-}$*3*$^{-/-}$ mice (Bouxsein et al., 2009). These findings indicate that the three β-adrenoceptor subtypes have different functions on bone, that Adrb1 but not Adrb2 is involved in the regulation of bone mass by the sympathetic nervous system, and that β-adrenoceptors are not required for bone loss by estrogen depletion.

Female mice but not male mice with chronic sympathetic hyperactivity owing to double knockout of adrenoceptors, *Adra2a* and *Adra2c*, which negatively regulate norepinephrine release, showed high bone mass due to decreased bone resorption and increased bone formation (Fonseca et al., 2011). An increase in bone mass was observed in cancellous bone in vertebrae and femurs and cortical bone in vertebrae. Following sympathetic activation, 80–90% of the norepinephrine released in synaptic clefts is cleared by norepinephrine uptake, which is performed by the norepinephrine transporter (Net), and the remaining extracellular norepinephrine diffuses into the circulation or is metabolized (Ma et al., 2013). *Net*$^{-/-}$ mice exhibited low sympathetic outflow due to central sympatho-inhibition by the upregulation of central norepinephrine extracellular levels and *Adra2a* expression. Male but not female *Net*$^{-/-}$ mice showed low bone mass due to reduced bone formation and increased bone resorption, indicating that low sympathetic outflow does not lead to high bone mass (Ma et al., 2013). Although the norepinephrine content in bone was low, serum norepinephrine levels were high in *Net*$^{-/-}$ mice. Chronic immobilization stress is a model of transient endogenous sympathetic activation and an anxiety/depression-like model in mice (Joo et al., 2009). Although endogenous serum norepinephrine levels were elevated in mice with chronic immobilization stress, no bone loss occurred (Ma et al., 2013). However, chronic immobilization stress reduced bone mass when Net was inhibited. Therefore, Net seems to play an important role in the protection against bone loss induced by sympathetic activation, and high serum norepinephrine levels may cause bone loss. However, the serum norepinephrine levels were high in *Adra2a*$^{-/-}$*2c*$^{-/-}$mice, which showed high bone mass. Thus, the elevated serum levels of norepinephrine cannot explain the bone loss in *Net*$^{-/-}$ mice (Fonseca et al., 2011; Ma et al., 2013). The regulation of bone mass by the sympathetic nervous system is complex and the involvement of the sympathetic nervous system in bone loss at unloading has to be proved by further investigation (Fig. 3).

3.3. Osteocyte network as a mechanosensory and mechanotransduction system

It is believed that mechanical stress is mainly sensed by osteocytes, mechanical energy is converted into electrical and/or biochemical signals, and they are transmitted to osteoblasts. Osteoblasts, which actively produce bone matrix proteins, have three kinds of destiny. They die by apoptosis, remain at the bone surface as lining cells, or are

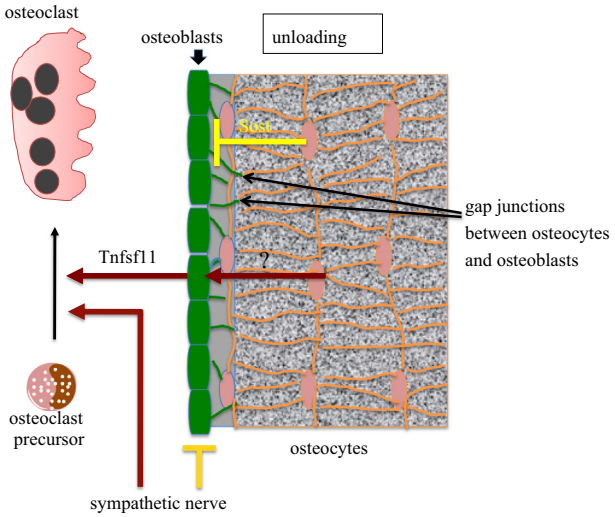

Fig. 3. Regulation of bone mass by osteocyte network in unloaded condition. Osteocytes form an intracellular network through processes and an extracellular network through canaliculi throughout bone, and the networks are extended to osteoblasts. In an unloaded condition, the osteocyte network inhibits bone formation by inducing Sost expression in osteocytes and enhances bone resorption by inducing *Tnfsf11* expression in osteoblasts. It still remains to be clarified how the osteocyte network stimulates *Tnfsf11* expression in osteoblasts. The sympathetic nervous system is generally considered to suppress bone formation and enhance bone resorption. However, the data are still controversial and its involvement in unloading-induced bone loss also needs to be investigated further.

embedded into bone matrix to be osteocytes. Osteocytes form extensive networks by their numerous processes and canaliculi, through which the processes pass. Therefore, osteocytes establish an extensive intracellular and extracellular communication system via gap junction-coupled cell processes and canaliculi and the communication system is extended to osteoblasts on the bone surface (Fig. 3). Small molecules pass through the network intracellularly using gap junctions; and soluble factors, which are secreted by osteocytes, pass through the network using canaliculi; and the soluble factors are finally released to the bone surface or vascular canals in bone (Komori, 2013).

Although osteocytes are believed to be responsible for mechanical stress-induced bone regulation, it has been difficult to prove this. The most convenient mouse model for such proof is osteocyte ablation (Tatsumi et al., 2007). As osteocytes embedded in bone are isolated from macrophages, however, dead osteocytes are not phagocytosed and all kinds of osteocyte death result in necrosis. In osteocyte necrosis, most of the intracellular content is released into the extracellular environment. The released intracellular content includes immunostimulatory molecules including the so-called damage-associated molecular pattern (DAMP) molecules, such as S100 family molecules, high-mobility group box 1 (HMGB1) protein, purine metabolites, heat-shock proteins, and uric acid (Lotze and Tracey, 2005; Zong and Thompson, 2006). These immunostimulatory molecules pass through canaliculi, reach the bone surface and vascular canals, and facilitate the recruitment and activation of macrophages, thereby promoting the production of proinflammatory cytokines including TNF-α, IL-6, and IL-1, which are the most important proinflammatory cytokines triggering inflammatory bone loss (Kong et al., 1999; O'Brien, 2010; Yasuda et al., 1998). Therefore, osteocyte ablation cannot be an appropriate mouse model for the evaluation of osteocyte functions (Komori, 2013).

3.4. Mouse models for the evaluation of osteocyte functions

A mouse model for the evaluation of osteocyte functions could be obtained by the osteocyte-specific disruption of *Gja1*. Gja1 (connexin 43) constitutes gap junction channels and hemichannels. Small

molecules are transmitted to neighboring cells through gap junction channels, and they also traffic between processes and canalicular spaces through hemichannels. Osteocyte-specific *Gja1* conditional knockout mice show an increase in apoptosis (Bivi et al., 2012). Osteocyte-specific transgenic mice expressing mutant *Gja1*, which blocks both gap junction channels and hemichannels, also show an increase in the apoptosis of osteocytes, whereas transgenic mice expressing mutant *Gja1*, which blocks gap junction channels but not hemichannels, do not show apoptosis. As previously reported, therefore, the hemichannels are required for preventing osteocyte apoptosis (Plotkin et al., 2002; Xu et al., 2014). Indeed, osteocyte death by the disruption of hemichannels induces osteoclastogenesis and bone resorption because the extracellular communication system is intact and the immunostimulatory molecules are released to the bone surface. In fact, osteoclast number is increased at the endocortical surface and the marrow cavity is enlarged in both *Gja1* conditional knockout mice and the mutated *Gja1* transgenic mice (Bivi et al., 2012; Xu et al., 2014). These findings indicate that both intracellular and extracellular communication systems have to be disrupted for the evaluation of osteocyte functions.

Another model for the evaluation of osteocyte functions is osteoblast-specific *BCL2* transgenic mice under the control of 2.3-kb *Col1a1* promoter, which highly induces the transgene expression in osteoblasts but very weakly in osteocytes. In *BCL2* transgenic mice, the number of osteocyte processes is severely reduced (Moriishi et al., 2011), probably due to the formation of a complex of BCL2, actin, and gelsolin, which reduces gelsolin-severing activity to increase actin polymerization (Ke et al., 2010). The reduction in the number of processes occurs in osteoblasts in *BCL2* transgenic mice because of the high transgene expression, and osteoblasts with reduced processes are embedded into bone matrix, becoming osteocytes. Indeed, the number of canaliculi is also severely reduced due to the severe reduction in osteocyte processes. Therefore, both intracellular and extracellular communication systems are impaired, osteocytes gradually die by apoptosis, and the frequency of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive lacunae reaches 80% at 4 months of age (Moriishi et al., 2012, 2011). However, the number of osteoclasts in the endosteum is reduced and bone resorption decreases in *BCL2* transgenic mice. The absence of the enhancement of bone resorption is due to the impaired extracellular communication system because immunostimulatory molecules released from dead osteocytes cannot reach the bone surface. The overexpression of *BCL2* in osteoblasts increases osteoblast number but inhibits osteoblast function in young mice, but it exerts no significant effect on osteoblast number and function at 4 months of age because the expression levels of the transgene are high at the growing age but low in adult mice. Furthermore, the overexpression of *BCL2* in osteoblasts has no effect on osteoclastogenesis (Moriishi et al., 2012, 2011). Although *BCL2* transgenic mouse is not an ideal model for the evaluation of osteocyte function, this is the only mouse model in which osteocyte networks are completely disrupted but no bone repair process begins. In an unloaded condition generated by tail suspension, bone loss occurs in wild-type mice due to enhanced bone resorption and reduced bone formation. In *BCL2* transgenic mice, however, no bone loss occurs, and both bone resorption and bone formation are unchanged in an unloaded condition. Therefore, the osteocyte network is really responsible for mechanosensing. Bone formation is reduced and bone resorption is enhanced in *BCL2* transgenic mice at 4 months of age, indicating that the osteocyte network inhibits bone formation and enhances bone resorption under physiological conditions. Therefore, the osteocyte network reduces bone mass in an unloaded condition by further inhibiting bone formation and enhancing bone resorption. These osteocyte functions are exerted at least partly through the enhanced sclerostin (Sost) expression in osteocytes and enhanced Rankl (*Tnfsf11*) expression in osteoblasts in an unloaded condition (Komori, 2013; Moriishi et al., 2012) (Fig. 3).

4. Animal models for glucocorticoid-induced osteoporosis

4.1. Rodent models for glucocorticoid-induced osteoporosis

Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis. In humans with glucocorticoid treatment, the early rapid decline in bone mineral density, probably caused by enhanced bone resorption, is followed by a slower progressive decline in bone mineral density, which is caused by the reduction in bone formation. Cancellous bone is preferentially lost in glucocorticoid-induced osteoporosis, and fractures frequently occur at sites enriched in cancellous bone, such as the vertebrae and femoral neck (Canalis et al., 2007). Glucocorticoid-induced osteoporosis was examined in animals, including mouse, rat, rabbit, dog, and sheep (Fig. 1).

Among the rodents, rat is frequently used to examine the effects of glucocorticoid in bone. However, the phenotypes vary depending on the age and dosage and the duration of glucocorticoid treatment. The oral uptake of a low dosage of prednisolone (0.5 mg/kg/day) in female Sprague Dawley rats at 2 or 3 months of age for 6 months had no effect on bone mineral density in both cancellous and cortical bone (Aerssens et al., 1994; Geusens et al., 1990). The daily injection of cortisol (32 mg/kg/day) in male rats for 16 days increased cortical bone volume (Ferretti et al., 1992). When 3-week-sustained pellets with 5 mg of prednisone were implanted into female rats at 2 months of age, cancellous bone was increased and cortical bone was reduced due to the decreased bone formation in both the periosteum and the endosteum (Turner et al., 1995). The continuous infusion of dexamethasone (16.25 mg/day) via a mini-osmotic pump for 19 days in adult male Sprague Dawley rats increased cancellous bone (King et al., 1996). The implantation of corticosterone pellets (25, 50, 100, or 300 mg) designed to release their content over a 60-day period in male Sprague Dawley rats at 7 weeks of age after adrenalectomy increased cancellous bone at a dosage of more than 50 mg, and the parameters of osteoblast surface, osteoclast surface, and bone formation rate were reduced (Li et al., 1996). The injection of methylprednisolone (1, 3, 6, 9 mg/kg/day) in female Wistar rats at 2 months of age for 90 days reduced cortical bone at low dose and cancellous bone at a high dose, and the bone formation was reduced in both cortical and cancellous bone (Ortoft et al., 1999). The injection of methylprednisolone (7 mg/kg/week) once a week in male Wistar rats at 32 weeks of age reduced both cancellous and cortical bone (Wimalawansa et al., 1997). In the oral administration of prednisone at a dose of 1.5 mg/kg/day, 3.0 mg/kg/day, or 6.0 mg/kg/day for 90 days in Sprague Dawley male rats at 3 months of age, cancellous bone mass and cortical bone mass at the diaphysis were unchanged, but cortical bone at the metaphysis was reduced, and parameters of bone formation and bone resorption were reduced in cancellous bone (Lin et al., 2014).

When dexamethasone (1 mg/kg/day) was injected five times a week for 4 weeks into ICR female mice at 3 weeks of age, cancellous bone volume but not cortical bone thickness was reduced (Altman et al., 1992). The implantation of slow-releasing pellets of prednisolone (2.1 mg/kg/day) into male Swiss Webster mice at 7 months of age for 27 days reduced cancellous bone mass and markers for bone formation without change in markers for bone resorption (Weinstein et al., 1998). In our experiments using male C57BL/6 mice at 7 weeks of age, the implantation of slow-releasing pellets of prednisolone (1.25 mg/kg/day) for 4 weeks increased cancellous bone and reduced cortical bone. The daily injection of dexamethasone (2 mg/kg/day) for 4 weeks in male

and female C57BL/6 mice at 8 weeks of age also increased cancellous bone and reduced cortical bone (unpublished results). Therefore, a major problem in rodent models of glucocorticoid-induced osteoporosis is that cancellous bone is mainly affected in humans, but the loss of cancellous bone is not consistently observed in rodents. Glucocorticoid suppresses the secretion of growth hormone, the production of insulin-like growth factor (IGF)-1, and the expression of IGFBP5, which is an anabolic growth factor; some of the bone phenotypes by glucocorticoid are reversed by growth hormone; and glucocorticoid treatment causes the delay in bone growth and the process of endochondral ossification (Altman et al., 1992; Kaufmann et al., 1988; McCarthy et al., 1990; Okazaki et al., 1994; Ortoft et al., 1999). Therefore, glucocorticoid treatment affects the modeling of cancellous and cortical bone during the growing age, and the controversial results in rats and mice during the growing age can be explained, at least in part, by the suppressive effects of glucocorticoid on the production of growth hormone, IGF, and IGFBP5. As the cancellous bone mass in elderly mice, especially C57BL/6 mice that are preferred as a knockout mouse strain, is low, however, the use of elderly mice makes it difficult to evaluate the precise effects of glucocorticoid using bone histomorphometry.

4.2. Large animal models for glucocorticoid-induced osteoporosis

In larger animals, oral administration of prednisolone (0.7 mg/kg/day) in female white rabbits at 6–7 months of age for 5 months reduced both cancellous and cortical bone (Grardel et al., 1994). The injection of methylprednisolone (1 mg/kg/day) in female New Zealand white rabbits at 8 months of age also reduced bone mineral density and bone volume in lumbar vertebrae (Baofeng et al., 2010). Prednisone administration (1.3 mg/kg) as a single oral dose each day for 29 weeks in male beagles at 1.6 years of age reduced the bone mineral density of vertebrae and diminished parameters of bone formation, without change in a parameter of bone resorption, in cancellous bone (Quarles, 1992). The injection of methylprednisolone (0.25 mg/kg/day) in sheep at 9 years of age for 3 months reduced parameters of bone formation and bone resorption, but not bone volume, in cancellous bone (Chavassieux et al., 1997). The injection of prednisolone (0.6 mg/kg/day, 5 times a week) in sheep for 7 months reduced cancellous bone volume in vertebrae and a marker of bone formation (Ding et al., 2010). Therefore, treatment for 7 months is needed for significant reduction in cancellous bone volume in sheep. The glucocorticoid treatment in these large animals shows a similar phenotype to that in humans.

5. Conclusions

Animal models for postmenopausal osteoporosis are generated in mouse, rat, sheep, and nonhuman primates by ovariectomy, and their phenotypes mimic postmenopausal osteoporosis in humans. Bone loss occurs in estrogen deficiency due to enhanced bone resorption and impaired osteoblast function (Fig. 1). The signal of estrogen is mainly exerted through Esr1. *Esr1*conditional knockout mice revealed that Esr1 inhibits bone resorption by inducing osteoclast apoptosis and enhances bone formation. However, the phenotypes of *Esr1*conditional knockout mice in the osteoblast lineage are controversial and further investigation is needed to clarify the function of Esr1 on osteoblasts and osteocytes. Animal models for unloading are generated by tail suspension or hind limb immobilization by sciatic neurectomy, tenotomy, or using plaster cast in rodents and dogs, and their phenotypes mimic those in disuse osteoporosis in humans. Unloading inhibits bone formation and enhances bone resorption (Fig. 1). Although the sympathetic nervous system may be involved in the bone loss in unloading, studies in knockout mice of β-adrenoceptors, α2-adrenoceptors, and norepinephrine transporter are not consistent and the functions of the sympathetic nervous system on bone need to be further investigated (Fig. 3). The osteocyte network senses unloading, enhances bone resorption, and inhibits bone formation (Fig. 3). The osteocyte network has intracellular and extracellular communication systems, and both have to be disrupted for the evaluation of osteocyte functions. Although there is no ideal mouse model for the evaluation of osteocyte function, both communication systems are disrupted in osteoblast-specific *BCL2* transgenic mice. Glucocorticoid-induced osteoporosis was examined in animals, including mouse, rat, rabbit, dog, and sheep (Fig. 1). Glucocorticoid treatment mainly reduces cancellous bone by inhibiting bone formation and enhancing bone resorption in humans. However, the loss of cancellous bone is not consistently observed in rodents. In large animals, glucocorticoid treatment consistently reduces cancellous bone.

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