Histochemical characteristics on minimodeling-based bone formation induced by anabolic drugs for osteoporotic treatment

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ABSTRACT

Modeling, the changes of bone size and shape, often takes place at the developmental stages, whereas bone remodeling—replacing old bone with new bone—predominantly occurs in adults. Unlike bone remodeling, bone formation induced by modeling i.e., minimodeling (microscopic modeling in cancellous bone) is independent of osteoclastic bone resorption. Although recently-developed drugs for osteoporotic treatment could induce minimodeling-based bone formation in addition to remodeling-based bone formation, few reports have demonstrated the histological aspects of minimodeling-based bone formation. After administration of eldecalcitol or romosozumab, unlike teriparatide treatment, mature osteoblasts formed new bone by minimodeling, without developing thick preosteoblastic layers. The histological characteristics of minimodeling-based bone formation is quite different from remodeling, as it is not related to osteoclastic bone resorption, resulting in convex-shaped new bone and smooth cement lines called arrest lines. In this review, we will show histological properties of minimodeling-based bone formation by osteoporotic drugs.

INTRODUCTION

Bone is a mineralized tissue that constitutes part of the vertebrate skeleton, protects bone marrow cells, stores minerals such as calcium and phosphates, and provides structure and support for the body, enabling the mobility of the body in cooperation with muscles. As an individual grows, each bone expands and is metabolized during bone modeling and bone remodeling.

Bone modeling is defined as a histomorphometric phenomenon involving changes in bone shapes and sizes. Modeling can hold back growth in some places while increasing it in others, consequently creating or maintaining the shapes and sizes of the bones, usually in response to physiological influences or mechanical forces. During bone development, the bone modeling of a whole bone (configuration of articular surface and cortical bone) is defined as
Throughout life, bone remodeling is the replacement of old bone with new bone in adults, which is based on cellular coupling between osteoclastic bone resorption and osteoblastic bone formation (Jee et al., 2007) (Table 1, Fig. 1). In bone remodeling, osteoclastic bone resorption always precedes osteoblastic bone formation. The boundaries between the old bone and new bone caused by bone remodeling, which are scalloped in shape, are called “cement lines”. In a normal state, bone remodeling—the replacement of old bone with new bone—keeps the size or shape of the replaced bone, due to balanced coupling between osteoclastic bone resorption and subsequent osteoblastic bone formation. In contrast, unlike bone remodeling, osteoclastic bone resorption does not precede bone formation at sites of modeling-based bone formation. During the histomorphometrical process of bone minimodeling, the quiescent osteoblasts (bone-lining cells) located on the pre-existing bone change into the active form of osteoblasts. The active form of osteoblasts shows a macromodeling because it can be seen at the macroscopic level. In contrast, it is called minimodeling when microscopic change of size and shape is observed in trabeculae of cancellous bone. Thus, modeling can be divided into macromodeling (macroscopic modeling) mainly in cortical bone and minimodeling (microscopic modeling) in trabeculae of cancellous bone. Unlike in bone remodeling, in the cellular process of minimodeling, bone-lining cells—the resting, flattened osteoblasts that cover bone surfaces—convert into active osteoblasts, which deposit new bone tissue onto the old bone without requiring preceding osteoclastic bone resorption (Table 1, Fig. 1). Historically, Frost assumed that, even on trabeculae, microscopic modeling might provide adaptation to excessive mechanical stress, a process that Frost termed “minimodeling” (Frost 1966, 1990; Jee et al., 2007). Modeling can usually be observed in the growing bones in fetal and neonatal stages, while most cancellous bones are formed by minimodeling. However, Frost (1990) postulated the possibility that minimodeling can continue in trabeculae throughout life.

In contrast to modeling, bone remodeling is the replacement of old bone with new bone in adults, which is based on cellular coupling between osteoclastic bone resorption and osteoblastic bone formation (Jee et al., 2007) (Table 1, Fig. 1). In bone remodeling, osteoclastic bone resorption always precedes osteoblastic bone formation. The boundaries between the old bone and new bone caused by bone remodeling, which are scalloped in shape, are called “cement lines”. In a normal state, bone remodeling—the replacement of old bone with new bone—keeps the size or shape of the replaced bone, due to balanced coupling between osteoclastic bone resorption and subsequent osteoblastic bone formation. In contrast, unlike bone remodeling, osteoclastic bone resorption does not precede bone formation at sites of modeling-based bone formation. During the histomorphometrical process of bone minimodeling, the quiescent osteoblasts (bone-lining cells) located on the pre-existing bone change into the active form of osteoblasts. The active form of osteoblasts shows a

### Table 1

|                  | Remodeling                                      | Minimodeling (modeling)                        |
|------------------|-------------------------------------------------|------------------------------------------------|
| **Coupling**     | Activation→resorption→formation                 | Activation→formation                            |
| **Appositional rate** | Slow                                               | Fast                                              |
| **Cement line**  | Scalloped                                       | Smooth                                           |
| **Occurrence**   | Throughout life span                             | Prominent during growth; less frequent in adults (rare event) |
| **Function**     | Maintenance and repair                           | Skeletal adaptation of microdamage to mechanical usage (shape and size) |

Modified from Jee et al., 2007.
cuboidal cell body including abundant rough endoplasmic reticulum and Golgi apparatus, and deposits new bone tissue onto the underlying bone surface. Thus, the activation and subsequent bone formation of osteoblasts are not coupled with osteoclasts. Therefore, the resultant new bone induced by minimodeling is convex in shape and is often referred to as focal boutons or bone buds in rat cancellous bone (Erben 2001; Li et al., 2004; Chen et al., 2008). The boundary between the pre-existing bone and newly deposited bone induced by minimodeling-based bone formation is typically smooth and without interruption by seams of collagen fibers known as “arrest lines” because they are found during the temporary arrest of osteoblastic activity (Fig. 1). Thus, as another histological evidence between remodeling and minimodeling, a site of remodeling-based bone formation is defined by the scalloped shape of cement line on the new bone, indicating previous osteoclastic bone resorption, while a site of minimodeling-based bone formation is characterized by a smooth cement line without interrupting collagen fiber seams (Erben 1996; Kobayashi et al., 2003; Lindsay et al., 2006; Ma et al., 2006). From a histochemical perspective, cement lines can be distinguished from arrest lines by detecting the activity of tartrate-resistant acid phosphatase (TRAP, a hallmark of an osteoclast). Since osteoclasts secrete abundant TRAP onto the resorption lacunae, the cement lines can be detected as TRAP-positive scalloped lines between the new bone and pre-existing bone (Yamamoto et al., 2016).

Among the drugs developed for osteoporotic treatment, eldecalcitol/alfacalcidol (vitamin D3 analogs), teriparatide (human recombinant parathyroid hormone, PTH [1-34]), and romosozumab (human sclerostin antibody) have recently been shown to induce minimodeling-based bone formation. In this review, histological aspects of minimodeling, mainly observed in animal models, will be introduced.

Minimodeling in growing rodents and human adults
Erben (1996) stated that the prevailing activity in the vertebral and tibial cancellous bone of aged rats was remodeling, whereas, in the rapidly growing tibia of 3-month-old rats, most of the cancellous bone-forming regions were minimodeling sites. By using rib biopsy specimens from 57 human adults, Takahashi et al. (1964) verified that the smooth cement lines on the trabeculae could have originated from the overfilling of resorption cavities, indicating that new bone in adult stages would be derived from bone remodeling rather than bone modeling. As individual grows, the reduced modeling and increased remodeling appear to be related to the reduction in longitudinal growth with aging, and therefore, minimodeling-based bone formation may come to be a rare phenomenon in normal adults.

However, Kobayashi et al. (2003) examined histological evidence of minimodeling in human bone using bone histomorphometry of iliac bones obtained from 34 patients subjected to total hip arthroplasty, and minimodeling was detected in 21 of the entire 34 specimens (62%), as well as 17 of the 27 specimens obtained from postmenopausal patients (63%). They noticed that the volume of osteoid (incompletely-mineralized areas beneath mature osteoblasts) of minimodeling sites comprised approximately 10% of the total osteoid volume, and the calcine-labeled surfaces on average constituted 25% to 50% of the entire labeled surface. Therefore, Kobayashi et al. (2003) emphasized that minimodeling should be taken into an account when dealing with parameters related to osteoid volume and mineralization, and therefore, supported Frost’s hypothesis that minimodeling can continue throughout human life. Sano et al. (2018) followed Kobayashi’s study to examine bone histomorphometry in 21 bone specimens collected during hip arthroplasty. They proposed that active modeling-based bone formation can be referred to as “forming minimodeling structure”. As a consequence, forming minimodeling structure was detected in 9 of 20 specimens (45%) and, interestingly, the bone volume was significantly higher in specimens with forming minimodeling structures compared with specimens without these structures. Consistent with Frost (1990) and Kobayashi et al. (2003), they postulated that the minimodeling-based bone formation on trabecular bone surfaces takes place in adults, even though the occurrence of minimodeling may be reduced.

Thus, minimodeling in cancellous bone appears predominant in growing bones during developing and young adult stages but is still observable in the adult stages of not only rodents but also humans. It is still possible that the overfilling of new bone originating from remodeling-based bone formation would show a short scalloped cement line followed by a longer smooth cement line, a mixed remodeling-minimodeling packet (Ma et al., 2006; Jee et al., 2007). Dempster et al. (2018) recently proposed three manners of bone formation—modeling-based bone formation, remodeling-based bone formation and overflow of modeling-based bone formation—by estimating the early effects of an established anabolic (teriparatide) versus antiresorptive (denosumab:
antibody to human receptor activator of NF-κB ligand) agent in human transiliac bone biopsies.

**Minimodeling induced by vitamin D3 analogs, alfacalcidol and eldecalcitol**

Shiraishi et al. (2000) who noticed the anabolic effect of alfacalcidol—a vitamin D3 analog that is not coupled with bone resorption, described the effect as “supercoupling”, in that it suppresses bone resorption while maintaining or stimulating bone formation. Li et al. (2004) previously stated that rats administered with alfacalcidol exhibited increased trabecular connectivity of cancellous bone in the proximal tibial metaphysis and the lumbar vertebral body. They found a strong correlation between minimodeling-based bone formation and the increased connectivity. Li et al. (2004) called the new bone induced by minimodeling “bone boutons” or “bone buds”, which are characterized by small, focal packets of newly formed bone emanating from the trabecular surface. Additionally, the lamellae of the bone buds induced by minimodeling did not run parallel to those of the trabecular plate to which they were attached. Thus, there were many reports on the anabolic effects of vitamin D analogs including alfacalcidol; however, many researchers at that time believed that vitamin D analogs reduced osteoporosis mainly by means of an anti-resorptive effect (Uchiyama et al., 2002).

We have clearly demonstrated minimodeling induced by eldecalcitol—a vitamin D analog (a next-generation of alfacalcidol) (de Freitas et al., 2011; Saito et al., 2013) (Fig. 2). When rats were administered with eldecalcitol, a range of mature osteoblasts were localized on the new minimodeling-induced bone showing calcein labeling. Despite the presence of mature osteoblasts, only a few alkaline phosphatase-reactive preosteoblasts were observed over the osteoblasts (Fig. 3). The index of preosteoblastic proliferation was significantly decreased in the eldecalcitol-treated rats compared with the control rats (de Freitas et al., 2011). We conjectured that eldecalcitol induces minimodeling more strongly than alfacalcidol, even though eldecalcitol also reveals anti-resorbing effects (Kikuta et al., 2013). When PTH [1-34] was administered in rodents, however, we observed a huge amount of bone volume, many calcein labeling indicative of bone mineral deposition, and a large network of BrdU-incorporating preosteoblasts as a result of their accelerated proliferation (Luiz de Freitas et al., 2009; Yamamoto et al., 2016) (Fig. 4). Unlike teriparatide, eldecalcitol-driven minimodeling did not seem to accompany Fig. 2 Histochemical images of minimodeling-based bone formation by eldecalcitol. A: Calcein-labeling (yellow-green) of trabeculae administered with eldecalcitol (a vitamin D analog). In the dark field of a fluorescence microscope, calcein-labeling, indicative of new deposition of calcium phosphate, can be seen as yellow-green colored lines associated with trabeculae (black). Minimodeling-induced new bone is indicated by white arrows. B: Light microscopic image of minimodeling-induced new bone in the toluidine blue-stained section. The convex shape of the bone (white arrows) can be seen on the trabeculae administered eldecalcitol. Note no mature cuboidal osteoblasts in the resting phase between the region of minimodeling-based bone formation. C: Higher magnification of minimodeling-induced new bone. The boundary (white arrows) between the new bone (asterisk) and pre-existing bone is smooth, and active osteoblasts (mature osteoblasts) of a cuboidal shape localize on the newly formed bone. Note, no developed preosteoblastic layer overlies the mature osteoblasts; instead, spherically shaped bone marrow cells are in the vicinity of the mature osteoblasts. Bars, A, B: 50 μm, C: 20 μm.
Minimodeling in bone

(Klein-Nulend et al., 2013; Stern and Nicolella 2013; Wang et al., 2014; Plotkin et al., 2015; Hinton et al., 2018), our conjecture on the relation between minimodeling and osteocytic network appears to be consistent with the postulation that minimodeling of cancellous bone may be an adaptation to acute changes in mechanical loading (Frost 1996; Jee et al., 2007). If this is the case, minimodeling may strengthen the geometric structures of cancellous bones enough to resist changes in mechanical stress.

the accelerated proliferation of preosteoblasts, despite mature osteoblasts would be activated to form new bone (Figs. 2 and 3). We assume that the stimulatory signal for inducing minimodeling would come from the interplay between osteoblasts and osteocytic network embedded in bone matrix, rather than from the cellular coupling between osteoclasts and osteoblasts on the bone surface. Since many researchers have reported that osteocytic network may serve for mechanosensing and mechanotransduction in bone (Klein-Nulend et al., 2013; Stern and Nicolella 2013; Wang et al., 2014; Plotkin et al., 2015; Hinton et al., 2018), our conjecture on the relation between minimodeling and osteocytic network appears to be consistent with the postulation that minimodeling of cancellous bone may be an adaptation to acute changes in mechanical loading (Frost 1996; Jee et al., 2007). If this is the case, minimodeling may strengthen the geometric structures of cancellous bones enough to resist changes in mechanical stress.

Fig. 3  Images of alkaline phosphatase, von Kossa staining and ultrastructure of minimodeling-based bone formation by eldecalcitol. A: Toluidine blue staining demonstrated the convex shape of new bone on the pre-existing bone surface induced by minimodeling. B: Immunohistochemistry of alkaline phosphatase (ALPase, brown color) conducted on the similar region as panel A. Brown-colored ALPase immunoreactivity (arrows) can be seen on osteoblasts located on the newly formed convex bone. C: von Kossa staining (dark brown) indicative of bone mineralization. The new bone induced by minimodeling showed well-mineralized bone matrix. D: Transmission electron microscopic (TEM) image of the minimodeling-induced bone. Despite the presence of mature osteoblasts (active form of osteoblasts that synthesize bone matrix), no developed preosteoblastic layer overlies the osteoblasts; instead, bone marrow cells can be seen close to the osteoblasts. E: Higher magnification of the superficial region of new bone induced by minimodeling in panel C. In the blue-colored osteoid, many round mineralized structures, i.e., mineralized nodules (arrows), are observed. F: Highly magnified TEM image of the osteoid in panel D. Notice the round electron-dense structures (arrows) identical to the demineralized trace of mineralized nodules that are also seen in the von Kossa staining (panel E). Bars, A, B: 50 μm, C, D: 20 μm, E: 15 μm, F: 10 μm.
Fig. 4  Histochemical images of remodeling-based bone formation by teriparatide. A, B: Calcein labeling indicative of calcium deposition in control mice (A) and mice administered PTH (B). Note the large amounts of calcein labeling in the PTH-administered bone. C, D: Immunohistochemistry of ALPase (brown) in control mice (C) and mice administered PTH (D). A thick layer of ALPase-reactive osteoblastic cells (ob) including preosteoblasts is seen in the PTH administered bone (D). E, F: Double-labeling of BrdU (brown) and ALPase (red-violet) in control mice (E) and mice administered PTH (F). Many brown colored BrdU-positive cells (white arrowheads) can be seen in the PTH-administered bone (F), indicating PTH administration increased proliferation and activity of osteoblastic cells. G, H: Double-labeling of Runx2 (brown) and ALPase (red-violet) in control mice (G) and mice administered PTH (H). In control bone, some ALPase-positive osteoblasts (red-violet, black arrows in G) show brown nuclei (white arrowheads in G) indicative of Runx2 reactivity. However, in PTH-administered bone, the thick layer of ALPase-positive osteoblastic cells (red-violet, black arrows in H) revealed more Runx2-immunoreactive nuclei (white arrowheads in H). I: Toluidine blue-stained semithin section of PTH-administered bone, showing the developed thick layers of preosteoblasts (a vertical white arrow) including many preosteoblasts (black arrows) and bone marrow cells (bmc). J: Transmission electron microscopic (TEM) image of PTH-administered bone. Consistent with the semithin section (panel I), many layers of preosteoblasts formed, including bone marrow cells (bmc). K: Statistical analysis of the percentage of bone/tissue area. The index of the bone area is considerably elevated in the PTH-administered bone compared with the control specimen. L: Statistical analysis of osteoblastic cell numbers per tissue area. The cell number index is significantly increased in the PTH-administered bone compared with the control specimen. Bars, A, B: 40 μm, C–H: 20 μm, I: 15 μm, J: 10 μm Panels A–H and J: Reproduced from Luiz de Fraitas et al., 2009.
due to the osteoporotic state.

As mentioned above, minimodeling takes place independently of osteoclastic bone resorption. Therefore, it seems likely that minimodeling is inducible not only in rodents with their high bone turnover rates, but also in humans that show lower bone turnover. Indeed, eldecalcitol-driven minimodeling-based bone formation has been reported in primates (Saito et al., 2015) and human patients (Hikita et al., 2016). Hikata et al. (2016) divided osteoporotic patients into three groups: non-treated, eldecalcitol-treated, and bisphosphonate-treated, and they found a trend toward enhanced minimodeling in eldecalcitol-treated patients and its suppression in bisphosphonate-treated patients compared with untreated patients. They therefore suggested that eldecalcitol and bisphosphonate have opposing effects on minimodeling. Taken together, eldecalcitol appears to induce minimodeling-based bone formation with regardless of turnover of bone remodeling, as well as to decrease osteoclastogenesis and subsequent bone resorption.

Minimodeling and remodeling induced by teriparatide

Teriparatide, human recombinant PTH [1-34], is a strong anabolic drug used for osteoporotic treatment. The basic mechanisms of the PTH-driven anabolic effect are believed to include accelerated bone turnover, with bone formation prevailing over bone resorption (Luiz de Freitas et al., 2009; Yamamoto et al., 2016). Using PTH-administered animal models, we have reported that PTH stimulates the proliferation of preosteoblastic cells (Luiz de Freitas et al., 2009) (Fig. 4), as well as differentiation of perivascular cells surrounding endomucin-positive blood vessels into osteoblastic progenitors (Zhao et al., 2021). Luiz de Freitas et al. (2009) have previously reported that intermittent PTH administration did not facilitate osteoblastic bone formation in the absence of osteoclasts, indicating that the PTH-driven anabolic effect depends on cell coupling from osteoclasts.

In osteoporotic women with teriparatide treatment, both Ma et al. (2006) and Lindsay et al. (2006) have reported increased cancellous bone by modeling-based bone formation as well as the overfilling of new bone originating from remodeling sites (bone formation over a smooth cement line contiguous with remodeling-based bone formation sites), which is latterly referred to as overflow of modeling-based bone formation (Dempster et al., 2018). Therefore, Ma et al. (2006) and Lindsay et al. (2006) suggested that analyzing the minimodeling packets with a longitudinal cutting orientation is critical to avoid classifying the formation phase of a remodeling packet as a minimodeling packet. Based on the classifying criteria, Ma et al. (2006) found minimodeling-induced bone, mixed remodeling/minimodeling, and remodeling-based formation of 3.8%, 3.9%, and 92.3%, respectively, compared to the placebo-treated group. Thus, teriparatide has the potential to induce minimodeling; however, the dominant anabolic activity of teriparatide is remodeling-based bone formation, rather than minimodeling-based bone formation.

When compared with teriparatide, mice treated with abaloparatide (a novel 34-amino acid peptide analog of parathyroid hormone-related peptide) showed a higher bone increase, while the effect on bone resorption was almost comparable between abaloparatide and teriparatide (Makino et al., 2020). However, it is not well-known if abaloparatide could show more minimodeling-based bone formation compared with teriparatide; it is currently assumed that the cellular mechanism by which abaloparatide showed a better bone turnover balance was partly due to the enhanced remodeling-based bone formation, although both teriparatide and abaloparatide could bind to the common receptor to PTH and PTHrP (PTH/PTHrP receptor). Bhattacharyya et al. (2019) have recently reported the possibility that teriparatide and abaloparatide have a similar affinity for the GTPγS-sensitive R state of PTH/PTHrP receptor. Teriparatide has a four-fold higher affinity for GTPγS-insensitive R state compared with abaloparatide, resulting in a prolonged cAMP signaling. They postulated that teriparatide causes an excess formation over resorption early on producing an anabolic “window” which will be lost as time goes, due to increased osteoclastic bone resorption to catch up with the formation. On the contrary, abaloparatide has an osteogenic effect accompanied by lesser resorptive and weak hypercalcemia rather than teriparatide, due to faster dissociation from PTH/PTHrP receptor than teriparatide. Taken together, abaloparatide may provide a better bone turnover balance by not excessive stimulation for osteoclastic bone resorption. This may imply that abaloparatide-driven anabolic effect also depends on remodeling-based bone formation, rather than minimodeling-based bone formation.

Yamamoto et al. (2016) of our team evaluated the histology of new bone in mice intermittently administered equivalent daily PTH doses but with variations in the frequency of injections: either one injection every two days, one injection once a day, two injections in one day, or four injections in one
day (Fig. 5). As a consequence, we found that highly frequent administration of PTH (four injections in a day) tended to induce a huge amount of fine trabeculae in a manner indicative of high bone turnover, implying remodeling-based bone formation; whereas low frequent PTH administration (one injection in a day) caused stout trabeculae featuring focally-convex shaped new bone with smooth arrest lines indicative of minimodeling-based bone formation, as well as trabeculae showing remodeling-based bone formation.

Taking over Yamamoto’s study, we have examined the biological effects of the combined administration of eldecalcitol and hPTH [1-34] on 9-week-old Wistar rats (Hasegawa et al., 2019). The rats were divided into a Sham-operated group (Sham group), ovariectomy (OVX) with vehicle (OVX group), OVX with 10 μg/kg/day of hPTH [1-34] (PTH group), OVX with 20 ng/kg/day of eldecalcitol (eldecalcitol group), and OVX with 10 μg/kg/day of hPTH [1-34] and 20 ng/kg/day of eldecalcitol (combined group). On analysis, the combined group showed a marked increase in bone volume/tissue volume, trabecular thickness, and trabecular number compared with the OVX group, and the highest bone mineral density compared with the other groups. The combined group displayed histological profiles of minimodeling-based bone formation and remodeling-based bone formation. Thus, the combined administration of eldecalcitol and hPTH [1-34] augmented the anabolic effects of the drugs by means of minimodeling and remodeling (Hasegawa et al., 2019).

Taken together, these findings indicate that PTH

![Figure 5](image_url)

**Fig. 5** Remodeling-based bone formation by highly frequent PTH administration and minimodeling-based bone formation by less frequent PTH administration. A: Bone with highly frequent administration of PTH (20 μg/kg, 4 times in a day in mice) showed a scalloped cement line (black arrows), active osteoblasts (mature osteoblasts), several preosteoblasts (preOB), osteoclasts, and newly formed bone with several embedded osteocytes (ocy). The inset shows scalloped-shaped calcein labeling (white arrowheads). Note, dotted line is the calcein-labeled new bone surface. BM: bone marrow. B: Bone with less frequent administration of PTH (20 μg/kg, once in a day in mice) showed a smooth cement line (arrest line, black arrows) and active osteoblasts (mature osteoblasts). However, there were few preosteoblasts and osteoclasts; instead, bone marrow cells can be seen adjacent to the mature osteoblasts. The inset demonstrates smooth calcein labeling (white arrowheads). Note, the dotted line is the calcein-labeled new bone surface. BM: bone marrow. Bars, A, B: 10 μm Reproduced from Yamamoto et al., 2016.
may predominantly affect osteoprogenitor cells including preosteoblasts that have committed to differentiation into mature osteoblasts, and consequently induce remodeling-based bone formation. However, PTH may still have potential to cause minimodeling-based bone formation, and does not seem to interrupt the eldecalcitol-driven minimodeling which is independent of the turnover of bone remodeling.

**Modeling-based bone formation induced by romosozumab**

Romosozumab, a humanized monoclonal sclerostin antibody used for osteoporotic treatment, increases modeling-based bone formation (McClung *et al.*, 2014). Ominsky *et al.* (2014) demonstrated that the sclerostin antibody increased bone volume by inducing modeling-based bone formation and prolonged the formation period at both modeling and remodeling sites while reducing bone resorption. Because romosozumab predominantly stimulates new bone formation at both cancellous bone and cortical surfaces (Ominsky *et al.*, 2014; Langdahl *et al.*, 2016), in this review, we have applied the term of modeling-based bone formation rather than minimodeling-based bone formation regarding romosozumab-induced new bone formation. The romosozumab-derived bone formation is rapidly and transiently induced as a result of the inhibition of sclerostin expression by osteocytes. Because sclerostin inhibits Wnt signaling, romosozumab activates the Wnt–β-catenin signaling pathway of osteoblasts to synthesize new bone (Semenov *et al.*, 2005; Ke *et al.*, 2012). In a normal state, the primary trabeculae showing abundant bone formation demonstrate few sclerostin-positive osteocytes, while diaphyseal cortical bone, which shows relatively slow bone formation compared with primary trabeculae, contains many osteocytes with intense sclerostin immunoreactivity (Hasegawa *et al.*, 2013). Therefore, it is feasible that sclerostin, an inhibitory factor for osteoblastic bone formation, would be abundantly expressed in the regions of less active bone formation.

In a phase 3 clinical trial of romosozumab (FRAME: ClinicalTrials.gov number, NCT01575834), 7180 women with postmenopausal osteoporosis were enrolled, and romosozumab significantly increased the bone mineral density of the spine and hip after one year’s treatment (Cosman *et al.*, 2017). It is also notable that new vertebral fractures were significantly decreased in romosozumab-administered patients than in placebo-administered patients. In the first human-dose study of sclerostin monoclonal antibody (AMG 785) in healthy men and post-menopausal women, Padhi *et al.* (2011) reported dose-related increases in the bone-formation markers containing procollagen type 1 N-propeptide, bone-specific alkaline phosphatase, and osteocalcin, along with a dose-related decrease in the bone-resorption marker serum C-telopeptide, resulting in a large anabolic window. Therefore, romosozumab appears to be a clinically potent drug for osteoporotic treatment, in which bone mass would increase in the manner of modeling-based bone formation.

In a comparative animal study of teriparatide, a lower osteoblast number index was associated with decreased osteoprogenitor numbers in rats administered the sclerostin antibody, strongly indicating that romosozumab stimulates the maturation of osteoblasts and subsequent osteoblastic bone formation but not the proliferation of osteoprogenitors such as preosteoblasts (Ominsky *et al.*, 2015). However, in a report by Boyce *et al.* (2018) the sclerostin antibody transiently increased the proliferation of osteoprogenitor cells in the early stage of treatment, coincident with the activation of modeling-based bone formation in rats; however, this proliferation would decrease during long-term treatment. Such romosozumab-driven modeling seems to be consistent with our previous report wherein eldecalcitol-driven minimodeling demonstrated the reduced proliferation of preosteoblasts and showed only a few preosteoblasts over the mature osteoblasts (de Freitas *et al.*, 2011). Although many clinical reports have suggested that romosozumab is a strong anabolic drug for osteoporotic treatment (McClung *et al.*, 2014; Cosman *et al.*, 2017; Padhi *et al.*, 2011), a drawback of romosozumab seems to be that it does not have long-lasting modeling-based bone formation.

**CONCLUDING REMARKS**

Minimodeling has been recently highlighted as new bone formation that could be induced by some osteoporotic drugs such as eldecalcitol and romosozumab. Unlike bone remodeling, without preceding osteoclastic bone resorption, minimodeling promotes bone-lining cells (i.e., resting, flattened osteoblasts) to change into mature osteoblasts for depositing new bone onto the old bone. It is necessary to further explore the biological and molecular mechanism for minimodeling-based bone formation induced by eldecalcitol or romosozumab.
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CONFLICTS OF INTEREST

Norio Amizuka had a financial interest in Chugai Pharmaceutical Co., Ltd, Asahi Kasei Pharma Co. Ltd, Teijin Pharma Ltd.

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