REVIEW

Osteoblasts in osteoporosis: past, emerging, and future anabolic targets

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Abstract

Objective: Age-related bone loss is associated with significant changes in bone remodeling characterized by decreased trabecular and periosteal bone formation relative to bone resorption, resulting in bone fragility and increased risk of fractures. Prevention or reversal of age-related decrease in bone mass and increase in bone fragility has been based on inhibition of bone resorption using anticatabolic drugs. The current challenge is to promote osteoblastogenesis and bone formation to prevent age-related bone deterioration.

Methods: A limited number of approved therapeutic molecules are available to activate bone formation. Therefore, there is a need for anabolic drugs that promote bone matrix apposition at the endosteal, endocortical, and periosteal envelopes by increasing the number of osteoblast precursor cells and/or the function of mature osteoblasts. In this study, we review current therapeutics promoting bone formation and anabolic molecules targeting signaling pathways involved in osteoblastogenesis, based on selected full-text articles searched on Medline search from 1990 to 2010.

Results and discussion: We present current therapeutic approaches focused on intermittent parathyroid hormone and Wnt signaling agonists/antagonists. We also discuss novel approaches for prevention and treatment of defective bone formation and bone loss associated with aging and osteoporosis. These strategies targeting osteoblastic cell functions may prove to be useful in promoting bone formation and improving bone strength in the aging population.

Introduction

Bone remodeling is a physiological process that maintains the integrity of the skeleton by removing old bone and replacing it with a young matrix. During aging, the rate of bone turnover (i.e. activation frequency) increases in both genders, and at the tissue level, there is an impaired osteoblastic bone formation compared with osteoclastic bone resorption caused by decreased number and activity of individual osteoblastic cells (1, 2). The impaired osteoblastic bone formation with age translates into decreased newly formed trabecular bone, as shown by reduced mean wall thickness. The age-related osteoblast dysfunctions may be caused by extrinsic mechanisms that are mediated by age-related changes in bone microenvironment, such as changes in levels of hormones and growth factors, and intrinsic mechanisms caused by osteoblast cellular senescence (2–4). As a consequence, both trabecular and periosteal bone formation decline with age in males and females (5). The age-related progressive bone loss is exaggerated in patients with osteoporosis, a disease characterized by decreased bone mass, increased bone fragility, and increased risk of fractures (1).

Based on the observation that bone loss during aging results from an imbalance between bone resorption and bone formation, potent anticatabolic drugs that include estrogen, SERMS, amino-bisphosphonates, and RANKL-inhibitor have been the main therapies for osteoporosis. These drugs reduce bone resorption and secondarily bone formation due to the coupling phenomenon during bone remodeling and thus maintain bone mass (6). Although anticatabolics are efficient in stabilizing bone mass, there is a need for anabolic drugs that target osteoblastic cells to increase bone formation and bone strength (7). In this study, we review the current limited number of anabolic drugs as well as novel therapies for targeting specific signaling pathways involved in osteoblast differentiation and function.

Osteoblastic cells and bone formation

Bone formation is dependent on the recruitment of sufficient number of osteoblasts as well as the activity of...
individual osteoblasts. Osteoblastic cells are recruited to bone forming surfaces mainly from a group of skeletal stem cells with osteogenic differentiation potential (referred to as skeletal, mesenchymal stem cells (MSC), or stromal stem cells). Although the exact location of MSC in vivo is still in debate, recent evidence suggests that some of these cells are pericytes located on the outer surface of blood vessels and sinusoids in the bone marrow (8). Also, recent studies suggest that MSC reach bone surfaces from the circulation through vascular channels in association with bone remodeling sites (9). Once they arrive at the bone surface, osteoblastic cells produce bone matrix that becomes mineralized. Finally, osteoblasts die by apoptosis or become embedded in bone matrix as osteocytes. The anabolic therapies can increase bone formation by increasing the number or activity of MSC and mature osteoblasts or by preventing their apoptosis.

**Past anabolic therapy**

Sodium fluoride (NaF) was the first agent to show a dramatic effect on bone formation and bone mass in osteoporosis. NaF increases osteoblast number and matrix deposition (10, 11) through inhibition of a fluoride-sensitive phosphotyrosine phosphatase resulting in a sustained activation of the MAPK mitogenic signaling pathway (12). Also, treatment with NaF was shown to increase osteoblast number and bone formation in ovariectomized (OVX) rats (13) and osteoporotic patients (14, 15). However, treatment with NaF did not reduce fracture risk in patients with osteoporosis despite marked increase in vertebral bone mineral density and thus was dismissed as a useful drug for osteoporosis (15, 16). Discrepancy in the biological effects of NaF on bone mass and bone strength is caused by the accumulation of abnormal and unmineralized matrix (17, 18). These first therapeutic attempts to promote bone formation demonstrated that an efficient anabolic drug should not only increase the amount of bone matrix but also enhance the bone quality (microarchitecture and matrix mineralization).

**Present anabolic therapies**

Parathyroid hormone (PTH) is the only approved anabolic therapy for osteoporosis. Its use is based on the seminal finding that intermittent (and not sustained) low-dose PTH increases bone formation more than bone resorption, leading to increased bone mass. At the tissue level, intermittent PTH administration increases the number and activity of osteoblasts, enhances the mean wall thickness and trabecular bone volume, and improves bone microarchitecture by establishing trabecular connectivity and increasing cortical thickness (19). The anabolic effects of PTH on bone formation are mediated through PTH receptor-dependent mechanisms. PTH enhances osteoblastic cell proliferation and function, extends mature osteoblast life span through antiapoptotic effects, enhances Wnt signaling through inhibition of Wnt antagonist sclerostin, and enhances the local production of bone anabolic growth factors such as insulin-like growth factor 1 (IGF1) (20). Also, PTH improves the functional abilities of aged osteoblastic cells in mice by antagonizing the age-associated increase in oxidative stress in osteoblastic cells (21). Clinical studies have demonstrated beneficial effects of intermittent PTH therapy on increasing bone mass and diminishing bone fragility associated with osteoporosis resulting from aging, sex hormone deficiency, and glucocorticoids therapy (22). One of the potential side effects of anabolic therapy with PTH is the over-stimulation of osteoblastic cells with a potential risk for developing osteosarcoma, as reported in long-term PTH therapy in rodents. However, human data do not support this hypothesis (23).

An alternative approach to promote bone formation is to stimulate endogenous PTH secretion using oral calcium-sensing receptor (CaSR) antagonists (calcilytics) that antagonize the parathyroid cell calcium CaSR, thereby stimulating the endogenous release of PTH (24). In aged OVX rats with established osteopenia, a calcilytic molecule was shown to increase PTH secretion and bone mass in the presence of an antiresorptive agent (25). Recently, an orally active CaSR antagonist was shown to increase bone formation and bone strength in OVX rats (26). Thus, calcilytic molecules may prove to be useful in promoting bone formation in osteopenic disorders. The clinical efficiency of CaSR antagonists in increasing bone mass and decreasing bone fragility compared with exogenous PTH therapy remains to be determined in humans.

Interestingly, strontium ranelate, an approved antosteoporotic drug, (27–29) was found to activate the CaSR in osteoblasts (30), resulting in activation of osteoblastic cell replication, differentiation, and survival (31, 32). In OVX rats, strontium ranelate-treated animals exhibited increased bone formation and decreased bone resorption, resulting in prevention of trabecular bone loss, improved bone microarchitecture, and strength (33, 34). In postmenopausal osteoporotic women, strontium ranelate treatment increased bone mineral apposition rate and improved trabecular microarchitecture (35), which was associated with reduced fracture risk (27, 29). Thus, specific activation of the CaSR in bone cells may be efficient to increase bone mass and strength independently of changes in PTH secretion.

**Therapies in pipeline**

**Targeting Wnt signaling**

Wnt/β-catenin signaling in bone is one of the main mechanisms controlling bone formation and bone formation. These signaling effects are mediated through Wnt antagonists, such as sclerostin, which negatively regulate bone formation by inhibiting Wnt signaling. Recent studies have shown that PTH may reduce the expression of sclerostin, thereby enhancing bone formation through Wnt signaling. Furthermore, calcilytics, which stimulate endogenous PTH secretion, may also increase Wnt signaling by reducing the expression of sclerostin, resulting in increased bone formation. Thus, targeting Wnt signaling in bone may provide a novel approach to increase bone formation and bone mass in osteoporosis.

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mass (36). Several genetic studies indicate that LRP5/Wnt signaling pathway is anabolic for bone (37, 38). In vitro and in vivo studies showed that activation of the canonical Wnt/β-catenin pathway promotes osteoblastic cell proliferation or differentiation and reduces adipogenic differentiation in MSC (39, 40). In addition, Wnt signaling promotes osteoblast survival (41) and interacts with bone morphogenetic protein 2 (BMP2) (42) and PTH signaling (43) to increase osteoblastogenesis. Also, changes in Wnt signaling contribute to age-related bone loss in mice (44). Mechanical loading upregulates Wnt signaling in MSC (45), suggesting that the combination of reduced β-catenin signaling and decreased mechanical stimulation with age may contribute to the age-related decline in bone formation. The important role of Wnt signaling in the control of bone formation suggests that this pathway may be a potential therapeutic target. Accordingly, activation of the canonical Wnt signaling using glycogen synthase kinase 3 inhibitors results in enhanced bone formation and increased trabecular and cortical bone density and bone strength in aged or OVX osteopenic mice (46, 47). Although targeting the Wnt pathway may be a promising approach to promote bone formation, the therapeutic use of Wnt signaling antagonists in clinical settings is limited due to the ubiquitous role of Wnt signaling. Given the potential implication of excessive Wnt signaling in cancer development, indirect targeting of Wnt signaling would be more specific and safe (48). An indirect approach is to inhibit Wnt antagonists. Sclerostin, the product of the SOST gene expressed by mature osteocytes, is a physiological negative modulator of bone formation (49, 50). Sclerostin binds only weakly to BMPs and acts by antagonizing Wnt binding to LRP4-6 co-receptors (51, 52). The pre-clinical observation that loss-of-function mutation of sclerostin results in increased bone formation and bone mass (53) led to the interesting concept that targeting sclerostin may increase bone formation in vivo (54). Systemic injections of an antiscerostatin MAB led to increased bone formation, bone mass, and strength in monkeys and OVX rats (55) and to increase bone formation markers in postmenopausal women (56). Therefore, it is envisaged that this strategy for targeting Wnt signaling may be efficient in increasing bone formation and bone mass in humans.

Besides sclerostin, other Wnt antagonists such as DKK1 could be targeted to promote bone formation. The observations that mice lacking DKK1 show increased bone formation and bone mass (57) whereas mice overexpressing DKK1 in osteoblasts are osteopenic (58) suggest that pharmacological DKK1 antagonists may increase bone formation and bone mass. sFRP1, another Wnt antagonist that acts by binding Wnt proteins, (59) could also be targeted because overexpression of sFRP1 inhibits bone formation whereas deletion of sFRP1 increases bone mass in mice (60). Oncostatin may also be targeted to promote bone formation because this cytokine produced by osteoblasts and osteocytes promotes bone formation via activation of leukemia inhibitory factor receptor and decreased sclerostin production (61). Recent data indicate that LRP5 may not play a major role in osteoblasts and that bone mass is rather regulated by Wnt-independent effects of LRP5 on gut-derived serotonin. Pharmacological inhibition of gut-derived serotonin promotes bone formation and prevents bone loss in OVX mice (62, 63). If confirmed, these findings may possibly provide a novel therapeutic approach in osteoporosis therapy, in addition to target LRP5/6 in osteoblasts for promoting bone formation in osteopenic disorders (64).

Future anabolic therapies

A large number of studies in the recent years have identified a number of potential pathways that can be targeted to increase bone formation and bone mass (Table 1 and Fig. 1). However, only few of them appear to be suitable and safe for long-term systemic therapy due to low specificity, non-skeletal effects, potential side effects, and cost.

Statin-like molecules

Statins are inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is involved in the biosynthesis of cholesterol and is used clinically to treat hypercholesterolemia. Statins have been identified as enhancers of the BMP2 gene expression and bone formation in vivo during drug screen for bone anabolic agents (65). Further studies have demonstrated that statins can stimulate vascular endothelial growth factor (VEGF) expression in osteoblasts via reduced protein prenylation, thus promoting osteoblastic differentiation (66). Statins can also enhance fracture healing in animal models (67). The clinical effects of statins on bone in humans have been studied in a number of case–control studies. In some studies, use of statins was associated with increased bone mass and decreased fracture risk in postmenopausal women (68, 69), whereas in others, these effects

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<tr>
<th>Statin-like molecules</th>
<th>Osteoblast number</th>
<th>Bone formation</th>
<th>Bone volume</th>
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<td>Fluoride</td>
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<td>Statins</td>
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<td>Antiscerostatin</td>
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++, indicates a huge positive effect; +, indicates a positive effect; ±, indicates a variable effect; -, indicates no detectable effect; ?, indicates unknown effect. The reader is referred to the text for more details.
were not reproducible (70, 71). A limitation of the use of available statins as bone forming agents is that the dose needed to enhance bone formation is much higher than that needed to reduce blood cholesterol levels. Thus, there is a need for developing more potent and preferably bone-specific statin-related molecules.

**Growth factors**

During the recent years, several growth factors with positive effects on osteoblastic cells and bone mass have been identified. Although in theory growth factors are possible candidates to increase bone formation, their clinical use is limited due to their systemic non-skeletal effects. Among these factors, BMPs have long been recognized for their ability to enhance differentiation of skeletal stem cells into osteoblastic cells. In vivo, BMP induce formation of significant amount of bone and cartilage when implanted subcutaneously or intramuscularly and to increase both endosteal and periosteal bone formation (72, 73). Among the BMPs, recombinant human BMP2 and BMP7 are approved for clinical use in orthopedic surgery for cases of long bone nonunion fractures and acute tibial fractures treated with intramedullary fixation. However, several off-label uses have been reported including spinal fusion in place of iliac crest bone graft. The clinical use of BMP for treating systemic diseases like osteoporosis is more complex. Only one BMP member, BMP6, has been demonstrated to promote bone formation and to restore bone mass in aged OVX rats (74). Following systemic injections, BMPs have a short half-life and the dose required is expensive. Thus, BMPs may have limited use as systemic anabolic therapies and small molecules inducers of BMP such as statins (see above) may be a more appropriate approach.

Another bone growth factor that is a member of BMP family is transforming growth factor β (TGFβ or TGFβ1) that promotes osteoblastic cell proliferation, function, and survival (75). In vivo, TGFβ does not prevent bone loss induced by ovariectomy (76) but prevents immobilization-related bone loss caused by decreased osteoblastogenesis (77, 78). Inhibins (Inh), activins, and myostatins (GDF8) are other members of the TGFβ superfamily that control bone metabolism (79). InhA overexpression was found to prevent gonadectomy-induced bone loss (80). Activin modulates osteogenic differentiation via binding to activin receptors (80, 81). Interestingly, a soluble form of activin receptor type IIA, which acts as an activin antagonist, was shown to increase bone formation, bone mass, and strength in OVX mice and non-human primates (82, 83). Furthermore, mice lacking GDF8, which binds to activin receptor type IIB and antagonizes osteogenic differentiation, show increased bone mineral density, suggesting that the development of GDF8 antagonists may promote bone formation in vivo (84, 85). Other growth factors may be candidates for use as bone anabolic factors. Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) are potent mitogenic factors for osteoblast progenitor cells in vitro (86, 87) and in vivo. In normal rats, EGF administration promotes periosteal bone formation (88). In OVX rats, PDGF administration increases osteoblast number, resulting in increased bone mass and strength (89). Fibroblast growth factors (FGFs) are other positive regulators of osteoblastogenesis (90), and systemic administration of FGF2 was found to increase bone formation and bone strength in OVX rats (91, 92). VEGF can also promote bone formation in vivo (93). However, all the above-mentioned growth factors, despite their potent anabolic effects on bone formation, exert systemic pleiotropic effects and thus may not be suitable in their current forms as systemic therapeutics.

Both GH and IGF1 are considered potential anabolic agents because they play physiological roles in bone mass acquisition and maintenance (94, 95). The levels of GH, IGF1, and some IGF binding proteins (IGFBPs) that regulate IGF activity decrease with age in elderly (96, 97) and in osteoporotic subjects (98, 99). A role of IGF/IGFBP signaling in age-related bone loss is supported by the finding that high levels of IGFBP2 correlate with increased bone turnover in aged men and women (100). Although GH promotes osteoblastic cell proliferation and differentiation (101), its effects on osteoblasts are mainly mediated by IGF1 (102). Genetic models in mice indicate that endogenous IGF1 increases bone formation and bone mass, which is more significant at the cortical than at the trabecular bone level (103, 104). Consistently, reduction in serum IGF1 levels results in decreased subperiosteal expansion and bone strength (105). In vivo treatment with IGF1 stimulates bone formation (106). However, IGF1 administration only partially restores the deficit in the expression of osteoblast markers in aging animals (107), possibly because aging induces receptor-mediated skeletal resistance to IGF1 (108). In animal models of osteoporosis, systemic administration of
IGF1 or IGF2/IGFBP2 promotes bone formation and prevents bone loss (109–111). Clinical trials revealed an increase in bone mineral density in some elderly normal and osteoporotic patients treated with low doses of GH or IGF1 (112–114). The potential use of GH or IGF1 as therapy for age-related bone loss is, however, uncertain given their stimulatory effect on bone resorption, which may compromise their positive effect on bone formation (115). Moreover, GH therapy cannot be easily used in patients with osteoporosis because of possible side effects and potential stimulation of growth of latent cancer.

**Other potential targets for anabolic therapies**

There are multiple other potential alternative approaches for increasing bone formation (Fig. 1). A theoretical approach is to inhibit marrow adipogenesis with the aim of concomitantly increasing osteoblastogenesis and bone formation. Several pharmacological agents act on bone marrow MSC to favor osteoblastogenesis and decrease adipogenesis, but these agents may also impact on other tissues (116). Non-pharmacological ways to positively influence MSC differentiation toward osteoblasts include the use of low-magnitude mechanical signals (117) and targeting signals that control MSC plasticity (118, 119). For example, inactivation of the master adipocyte transcription factor Pparγ (Pparg) in mice results in inhibition of adipogenesis and increased bone formation and bone mass (120). Another possible target is the cannabinoid receptor type 1 (CB1 or CNR1) that contributes to age-related bone loss through its effects on MSC differentiation. CB1 deficiency in aging mice leads to increased adipogenesis and defective bone formation, suggesting that CB1 agonists may promote bone formation and prevent age-related bone loss (121).

An alternative approach to promote bone formation is to antagonize molecules that inhibit bone formation. Aging is associated with increased serum tumor necrosis factor α (TNFα) levels (122). This cytokine inhibits bone formation in part by inducing osteoblast apoptosis (123). Consistently, TNFα antagonists were found to reverse the age-related deficit in bone formation (124). Another example is inhibition of the proline-rich tyrosine kinase 2 (PYK2 or PTK2B), a non-receptor tyrosine kinase expressed in bone cells. PYK2-deficient mice show high bone mass and increased MSC differentiation and bone formation, and inhibition of PYK2 was shown to prevent bone loss in OVX rats (125). It can also be considered to inhibit midkine, a heparin-binding growth factor, because midkine deficiency results in increased trabecular bone formation in mice (126).

An emerging potential therapy is to target the osteoblast proteasome. This approach is based on the observation that the proteasome inhibitor bortezomib had bone forming effects in multiple myeloma patients (127). Proteasome inhibitors can promote osteoblast differentiation in MSC via increased BMP2 expression and stabilization of RUNX2 and proteasome inhibition (128–130). In support of this effect, treatment with the proteasome inhibitor bortezomib increased osteoblast differentiation, trabecular bone formation, and bone mass in normal and OVX mice (129). Other approaches can be used to promote BMP2 expression in osteoblastic cells. For example, oxytocin was reported to upregulate BMP2 expression, which in turns promotes bone formation (131). An oxytocin analog was found to promote bone formation and to reverse bone loss in OVX mice, suggesting that oxytocin may be a potential therapeutic agent (132). Despite the potential interest of these various approaches, further basic and clinical studies are needed to determine whether these strategies can lead to effective and safe therapies for preventing or treating the defective bone formation and bone loss associated with aging (Fig. 1).

Finally, we need to consider that the aging process per se involves a number of pathophysiological mechanisms that lead to the deterioration of osteoblastic functions and impaired bone formation (3, 4, 133). This parallels the changes in many aspects in other cellular compartments in the aging organism. Thus, other medications that target the basic mechanisms of cellular aging may possibly be relevant for osteoporosis therapy (Fig. 1). Resveratrol is a polyphenol identified as activator of sirtuin 1 (SIRT1), a member of a family of NAD⁺-dependent deacetylases and ADP-ribosyltransferases that underlies some of the antiaging effects of dietary restriction in mammals (134). Resveratrol-fed-old mice show a reduction in aging features including prevention of age-related decreased bone mass (135). The positive effects of resveratrol on bone include enhanced in vitro osteoblast differentiation (136) and inhibition of adipocyte differentiation (137). Currently, SIRT1 agonists with much higher potency than resveratrol are being tested in clinical trials against type II diabetes. Depending on the initial results from clinical trials, this class of drugs may possibly have a role for preventing age-related bone loss in addition to other beneficial effects on the aging organism.

**Conclusion**

Aging is associated with impaired bone formation that is a principal pathogenetic mechanism mediating bone fragility in osteoporosis. Until recently, a limited number of approved therapeutic molecules capable of activating bone formation and increasing bone mass and strength has been available. Current promising approaches focus on agonists/antagonists of osteoblastic Wnt signaling pathways. Several other strategies, including therapeutics that target skeletal stem cells and osteoblastic cell functions, are being explored and may prove to be useful in promoting bone formation. It is hoped that providing more options for developing efficient therapeutic strategies targeting bone formation will allow prevention and restoration of age-related bone strength.
Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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