Bone Biology and the Role of the RANK Ligand Pathway

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Bone renewal is essential for bone strength. During childhood and early adulthood, bone formation prevails over bone resorption, as bones increase in size and strength. Peak bone mass is achieved during the third decade in life, with a higher peak bone mass being protective against osteoporosis later in life.[1] Bone loss is most prominent in women at menopause due to the effects of a natural decline in estrogen levels. However, bone mass begins to decrease with age, and bone loss is most prominent in women at menopause due to the effects of a natural decline in estrogen levels.[2]

Normal bone remodeling is dependent on osteoclasts and critical molecular mediators, including RANK ligand, a key stimulator of bone resorption, and osteoprotegerin (OPG), a key inhibitor of bone resorption. Bone renewal is successful when resorption of bone by osteoclasts is perfectly coupled with formation of bone by osteoblasts. The goals of this article are to highlight the RANK ligand pathway and its involvement in healthy bone remodeling, bone metastasis, cancer treatment–induced bone loss, and multiple myeloma. Now and in the future, this topic will become even more important as novel therapies targeted at reducing skeletal complications emerge.

Bone Remodeling

FIGURE 1

Bone Remodeling Cycle

The bone remodeling process replaces approximately 20% of bone tissue annually; this process occurs continuously, with each cycle lasting 4 to 8 months. In healthy adults, bone remodeling occurs in a balanced, highly regulated manner in five phases: activation, resorption, reversal, formation, and quiescence (Figure 1). In the first phase, osteoclast precursors are attracted to the remodeling sites. Once they have matured, osteoclasts release osteoclastic enzymes to form a resorption pit in spongy bone and burrow a tunnel in compact bone, and calcium and phosphorous ions are released into the bloodstream. Resorption, from the time of osteoclast adherence to bone until ion release into the bloodstream, is controlled by hormones and thus affected by hormonal changes that occur during life. FIGURE 2

Bone Resorption and Bone Formation
During reversal, the next stage, precursors to osteoblasts appear at the resorption site, where they proliferate and differentiate. Mature osteoblasts release osteoid to form a new soft matrix, which is then mineralized with calcium salts and phosphorous that precipitate from the blood. At the conclusion of the cycle, resting cells line the area and remain dormant until the next cycle.[3, 4]

Under both normal bone remodeling and pathologic conditions (such as metastasis), bone resorption is mediated by osteoclasts, which are large, multinucleated cells with abundant cytoplasm, Golgi apparatus, and mitochondria. They originate from myeloid stem cells that differentiate into CD14 monocytes, which are then recruited to the bone surface to undergo differentiation into multinucleated osteoclasts. The process of osteoclast formation and activation occurs over 5 to 8 days and requires two important factors that are produced by bone marrow stromal cells and osteoblasts: macrophage colony-stimulating factor (M-CSF) and RANK ligand. Activation of osteoclasts is dependent on cell-to-cell interactions between osteoblasts and osteoclast precursors; RANK ligand binds to the RANK receptor on osteoclasts and promotes osteoclast activity (Figure 2).

### TABLE 1

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<th>Regulation of Bone Renewal</th>
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<td>Other systemic factors such as parathyroid hormone (PTH), 1,25 dihydroxyvitamin D3, and prostaglandins induce osteoclast activity indirectly by increasing expression of RANK ligand (Table 1). PTH stimulates bone resorption and regulates serum calcium concentration, while 1,25-dihydroxyvitamin D3 exerts its major effect in the intestinal tract, where it promotes the absorption of calcium and phosphorous. Before osteoclastic resorption of bone occurs, osteoblasts secrete collagenase to remove osteoid. Osteoblasts are cuboidal, mononuclear cells with abundant endoplasmic reticulum and Golgi apparatus that lay down bone matrix.[3] Osteoblasts produce interleukin-6, interleukin-1, prostaglandins, and macrophage colony-stimulating factors (M-CSF) which induce formation of osteoclasts. After osteoclastic activity, osteoblasts produce and mineralize the bone matrix, eventually forming osteocytes. T cells produce cytokines (interleukin-4, interleukin-18, and interferon-gamma), which inhibit the formation of osteoclasts. Bone matrix also serves as a major source of growth factors (Figure 2).[4]</td>
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### FIGURE 3

Radiographic Signs of Osteopenia and Osteopetrosis in Mice

Several members of the tumor necrosis factor (TNF) superfamily of proteins— RANK, RANK ligand, and OPG—are key to enabling osteoclasts to resorb bone and maintain balanced bone remodeling. The importance of RANK ligand has been studied in experimental systems, in which knockout mice lack RANK ligand or the RANK gene; these mice have no osteoclasts and develop osteopetrosis (Figure 3).[5, 6] The interaction between the RANK receptor on osteoclast precursors and the RANK ligand expressed by immature osteoblasts and bone marrow stromal cells is critical for osteoclasts to form, function, and survive.[6] RANK ligand binds to the RANK receptor on osteoclast precursors, promoting their differentiation into mature osteoclasts capable of osteoclastic activity. OPG is produced by osteoblasts to maintain balance between bone resorption and formation. It is a
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soluble member of the TNF receptor superfamily and serves as a decoy receptor for RANK ligand. OPG binds to the RANK ligand and prevents it from binding to its intended receptor, RANK, on the surface of osteoclasts, thus inhibiting bone resorption (Figure 4). In experimental systems, bones of OPG-deficient mice are osteopenic. Bone resorption is thus intricately linked to the ratio between RANK ligand and OPG; osteolysis in patients with multiple myeloma and bone metastasis in patients with solid tumors often occur due to an altered ratio between RANK ligand and OPG.

FIGURE 4
Role of Osteoprotegerin (OPG) and RANK Ligand in Cancer-Bone Interactions

Experimental therapeutics targeting RANK ligand are increasingly being studied and have shown promise for decreasing tumor-related bone disease.[7] RANK ligand also has a role in cancer cell migration, whereas OPG has been shown to prevent bone metastases in experimental models.[8,9] A disparity in RANK ligand expression and OPG expression plays a key role in bone diseases that develop secondary to increased bone resorption. For instance, in cancer-induced bone disease, RANK ligand overwhelms the effects of OPG, leading to imbalanced bone remodeling and the “vicious cycle” of metastatic disease.[3]

Bone Metastases

Bone metastases are a frequent complication of cancer and may lead to devastating consequences—severe pain, pathologic fractures, life-threatening hypercalcemia, and spinal cord compression.[10] They are often seen in patients with advanced breast and prostate cancer. In metastatic disease, RANK ligand plays a key role in a continuous cycle of bone destruction and tumor growth. In this cycle, reciprocal feedback between tumor cells and the bone microenvironment leads to the release of growth factors from the bone matrix. The subsequent tumor growth causes further release of factors from the tumor itself, and bone destruction ensues.[3] Metastases can be osteoblastic, osteolytic, or mixed; they result from increased osteoclastic activity due to an imbalance between RANK ligand expression and OPG expression. In breast cancer, osteolytic lesions are most common, whereas in prostate cancer, osteoblastic lesions predominate. Purely lytic lesions develop in multiple myeloma, and suppression of osteoblastic activity also has been implicated in the development of such lesions.

For a number of reasons, bone is fertile ground for tumor metastasis. Blood flow is highest in the red marrow.[4] In addition, bone itself holds a number of growth factors that are released and/or activated during bone resorption. When tumor cells invade bone, the released factors induce RANK ligand expression in osteoblasts. Increased expression of RANK ligand in the tumor microenvironment enables osteoclasts to differentiate and survive. Greater osteoclast activity leads to more resorption and bone destruction, which in turn leads to the release of bone-derived growth factors and calcium, fueling further tumor growth.[3]
The “Vicious Cycle” of Bone Metastasis and Tumor Growth

The vicious cycle of metastatic bone disease has been further studied in breast cancer. Breast cancer cells, like cells of many other solid tumors, produce PTH-related peptide (PTHRP). Tumor cells can also produce other factors—interleukin-6, prostaglandin E2, TNF, and M-CSF. PTHrP binds the same receptor (PTHR1) as PTH and mimics its effects, leading to increased osteoclast activity. Expression of RANK ligand is increased, and osteoclasts are formed. Bone resorption leads to the release of factors such as transforming growth factor-beta (TGF-), insulin-like growth factors, fibroblast growth factors, platelet-derived growth factors, and bone morphogenetic proteins. These increase production of PTHrP and other factors from tumor cells. PTHrP is a major mediator in the cycle of bone metastasis in breast cancer and other solid tumors (Figure 5).[3]

Cancer Treatment–Induced Bone Loss

Other regulators of the bone remodeling process include growth hormone, glucocorticoids, thyroid hormone, and estrogen. Estrogen is critical for bone turnover; it promotes new bone formation and inhibits bone resorption.[11] It inhibits RANK ligand/M-CSF–induced activator protein-1–dependent transcription by regulating c-Jun activity and directly downregulating the RANK ligand and M-CSF–induced differentiation of osteoclasts.[12] Estrogen also upregulates OPG and TGF-β, which in turn increases expression of OPG by osteoblasts and stromal cells. TGF-β also inhibits bone resorption by increasing osteoclast apoptosis (Table 1).[13] Estrogen essentially suppresses expression of osteoclastogenic cytokines, downregulates osteoclast bone resorption, and decreases osteoclast lifespan. Subsequently, with estrogen withdrawal, osteoclast formation increases secondary to overexpression of osteoclastogenic cytokines, increased differentiation of osteoclast precursors, and inhibition of osteoclast apoptosis.[12] In the natural estrogen-deficient state that occurs with menopause, bone resorption occurs at a greater rate than bone formation, thus decreasing bone mass and leading to osteopenia and/or osteoporosis.[14, 15] In patients with breast cancer or prostate cancer who are undergoing endocrine treatment with aromatase inhibitors or androgen deprivation therapy, respectively, the bone resorption to formation ratio is even further imbalanced, leading to greater morbidity and decreased quality of life. Accordingly, patients with breast or prostate cancer who are receiving treatment with sex hormone ablative therapies have increased osteoclastic activity that results in treatment-induced bone loss. In essence, the estrogen-deficient state that naturally occurs in postmenopausal women is further exacerbated. During normal postmenopausal bone loss, the bone loss initially occurs at a rate of 2% per year and then decreases to 1% per year.[16] In contrast, cancer treatment–associated bone loss (ie, oophorectomy, chemotherapy, and endocrine therapy) occurs at a faster rate and is more abrupt than natural estrogen deprivation.[17] After peak bone mass is achieved at age 25, adults in general start to lose bone mass with further aging; however, women in particular note an enhanced rate of loss during their menopausal years secondary to estrogen deficiency.[16]

A selective estrogen-receptor modulator, is used in early and advanced breast cancer and has tissue-specific estrogen-agonist effects. In premenopausal women, it causes some bone loss; however, during the postmenopausal years, it is actually beneficial to bone health, improving bone density.[17] In contrast, aromatase inhibitors are appropriate only for treatment in the postmenopausal breast cancer population and are associated with increased bone loss and fracture risk. In the postmenopausal state, with the ovaries no longer functioning, estrogen production is limited and occurs only in distant sites—adipose tissue, adrenal glands, smooth muscle, and
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bone.[18] Aromatase inhibitors target the aromatase enzyme, which converts adrenally derived androgens to estrogens in peripheral tissues. The conversion of androgens to estrogens via aromatase is the main source of endogenous estrogens in postmenopausal women. When this pathway is blocked, estrogen production is limited, leading to the known sequelae of the estrogen-deficient state.[17]

The American Society of Clinical Oncology (ASCO) recognizes that postmenopausal breast cancer patients who are treated with aromatase inhibitors are at higher risk for osteoporosis and fracture. Frequent monitoring of bone mineral density in this treatment population is recommended, and baseline dual-energy x-ray absorptiometry (DEXA) scan to evaluate bone health is recommended in all patients receiving aromatase inhibitor therapy. Also, ASCO clinical guidelines recommend that bisphosphonate therapy be initiated in all patients on aromatase inhibitor therapy who have documented osteoporosis (T score less than or equal to -2.5 on DEXA scan).[19]

The guidelines recommend reviewing and limiting other risk factors that may affect bone health, such as prolonged corticosteroid use, smoking, poor calcium intake, excessive alcohol intake (more than 2 drinks per day), and limited physical activity. Patients should be counseled to avoid alcohol and tobacco use while on aromatase inhibitor therapy. Current recommendations state that all patients initiating aromatase inhibitor therapy should be on calcium and vitamin D supplementation. Additionally, bisphosphonate therapy is advised for patients initiating or receiving AI therapy who are at high risk. The high risk group is defined as patients with two or more of the following risk factors: T-score < -1.5; age > 65 years; low BMI (< 20 kg/m2); family history of hip fracture; personal history of fragility fracture after age 50; oral corticosteroid use > 6 months; and smoking.[19A]

Androgen deprivation therapy, in the form of gonadotropin-releasing hormone agonists or bilateral orchiectomy, is standard therapy in metastatic prostate cancer, and also has been used as adjuvant therapy for limited-stage prostate cancer. Although androgen deprivation therapy has been shown to improve disease-free and overall survival,[20] it reduces bone mineral density and increases the risk of fractures. Just as estrogen deficiency shifts the bone remodeling equilibrium towards more resorption, testosterone deficiency results in a similar imbalanced state. When testosterone is deficient, less estrogen is produced because less testosterone is available to be aromatized. Thus, osteoclast apoptosis is inhibited, and osteoblast apoptosis is increased.[20]

Studies have shown that rapid loss of bone mineral density occurs within the first 6 to 12 months of androgen deprivation therapy. Importantly, fracture development is associated with worse survival in prostate cancer.[21] Bisphosphonate therapy has been shown to reduce skeletal-related events and has an important effect on bone health when instituted with androgen deprivation therapy in prostate cancer.[22]

Multiple Myeloma

Finally, multiple myeloma is one of the most common hematological malignancies in the United States, with approximately 16,000 new cases per year and 11,000 deaths per year.[23] It is a B cell malignancy characterized by overgrowth of plasma cells and catastrophic lytic bone disease. All of the following factors have been implicated in the pathogenesis of bone destruction in multiple myeloma: interleukin-1, interleukin-6, macrophage inflammatory protein 1 alpha and, most importantly, RANK ligand.[4] Myeloma cells express RANK ligand, upregulate RANK ligand expression by bone marrow cells, and downregulate expression of OPG.[24] When plasma cells invade the bone marrow, they induce secretion of osteoclast-activating factors such as interleukin-6, interleukin-1, and TNF-B, which in turn signal stromal cells and osteoblasts to secrete RANK ligand. Myeloma cells secrete a molecule called syndecan-1, which inactivates OPG, the decoy receptor that normally restraints osteoclastic activity.[25] Thus, in multiple myeloma, the RANK ligand to OPG ratio is imbalanced, resulting in uncontrolled osteoclastic activity that leads to characteristic bone destruction.

Although osteoclastic bone destruction is increased in both multiple myeloma and lytic metastatic disease from other tumors, the lytic bone disease of multiple myeloma differs in a significant way, leading to even greater morbidity and poorer quality of life. Specifically, in multiple myeloma, when the tumor burden exceeds 50% in a particular area, osteoblast activity becomes suppressed or absent. Myeloma cells can produce TNF-alpha, which interrupts osteoblastic growth and differentiation; however, this is not the proven mechanism of osteoblast suppression in multiple myeloma.[26] The actual mechanism of osteoblast suppression is still unclear. Because of this lack of osteoblast activity, bone lesions in multiple myeloma are purely lytic. Secondly, in multiple myeloma, plasma cells downregulate OPG.[24] Without this regulation, tumor burden increases, and greater
Bone destruction ensues, exacerbating the vicious cycle of metastatic disease described above. In summary, in multiple myeloma, increased osteoclastic activity, suppression of osteoblastic activity, and uncoupled bone remodeling lead to significant disease morbidity. Patients suffer from pathologic fractures, hypercalcemia, and significant bone pain. The lytic lesions usually affect the vertebrae, skull, sternum, ribs, pelvis, and proximal long bones.[25] Within the first year of diagnosis, 45% of patients with multiple myeloma suffer from a fracture, and 65% of patients will have a fracture sometime during their disease course. [23] As in breast cancer or prostate cancer patients with cancer treatment--induced bone loss, the use of intravenous bisphosphonates has a palliative role in the multiple myeloma population as well. Antiresorptive therapy has been shown to significantly reduce the number of skeletal-related events (fractures, hypercalcemia, bone surgery, and bone radiation) by inhibiting the formation of osteoclasts and by inducing their apoptosis.[25]. This palliative treatment has been shown to reduce significantly the number of skeletal-related events (fractures, hypercalcemia, surgery to bone, and radiation to bone) by inhibiting formation of osteoclasts and by inducing apoptosis of osteoclasts.[23]

Conclusion

Skeletal remodeling is key to bone health and normally occurs in a balanced manner. Key players include osteoclasts, osteoblasts, the bone matrix, hormones, systemic factors, local factors and, most importantly, members of the TNF superfamily of proteins—RANK receptor, RANK ligand, and OPG. When imbalanced bone remodeling occurs in the setting of metastasis, cancer treatment–induced bone loss, and/or multiple myeloma, the RANK ligand pathway has been implicated, and understanding its key role will help to reduce morbidity and improve quality of life in patients who suffer from the resulting illnesses.

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