petrotic (*54*). Given the central role of RANK in osteoclastogenesis and its use of TRAF 6 as an adapter molecule, this osteoclast-abundant phenotype is paradoxical, but it may reflect the fact that RANKL is needed not only for osteoclast differentiation but also for osteoclast activation (*55*). Finally, the absence of molecules necessary to degrade bone matrix, such as those regulating proton transport [H^+-ATPase (*27*) and carbonic anhydrase II (*56*)] or organic matrix degradation [cathepsin K (*30*)], results in morphologically normal osteoclasts that are incapable of effective resorption.

Where Are We Going?

The past decade has witnessed a renaissance in osteoclast biology, due largely to the development of gene deletion technology and the capacity to generate this cell type in vitro. We now know that this polykaryon is central to the pathogenesis of postmenopausal osteoporosis and that the successes achieved thus far in preventing this disease reflect successes in decreasing osteoclast number and activity. A number of effective anti–bone-resorptive agents, such as estrogen, selective estrogen receptor modulators, and bisphosphonates, are in hand. Given our capacity to study the osteoclast both in vitro and in vivo, which will continue to provide new insights into its origin and function, new antiresorption drug targets are certain to emerge. Together with the promise of agents that are capable of stimulating bone formation, this offers real hope that effective prevention and reversal of osteoporosis are on the horizon.

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REVIEW

Therapeutic Approaches to Bone Diseases

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The strength and integrity of our bones depends on maintaining a delicate balance between bone resorption by osteoclasts and bone formation by osteoblasts. As we age or as a result of disease, this delicate balancing act becomes tipped in favor of osteoclasts so that bone resorption exceeds bone formation, rendering bones brittle and prone to fracture. A better understanding of the biology of osteoclasts and osteoblasts is providing opportunities for developing therapeutics to treat diseases of bone. Drugs that inhibit the formation or activity of osteoclasts are valuable for treating osteoporosis, Paget's disease, and inflammation of bone associated with rheumatoid arthritis or periodontal disease. Far less attention has been paid to promoting bone formation with, for example, growth factors or hormones, an approach that would be a valuable adjunct therapy for patients receiving inhibitors of bone resorption.

To carry out its functions, bone is continuously destroyed (resorbed) and rebuilt at about 1 to 2 million microscopic sites per adult skeleton. Resorption is carried out by hematopoietically derived osteoclasts and takes about 3 weeks per site, whereas the rebuilding of lost bone by osteoblasts, derived from bone marrow stromal cells, takes about 3 to 4 months. In young adults, bone destruction and formation are balanced, and bone mass is maintained in a steady state, which is influenced by mechanical usage (*1*) and possibly by central homeostatic factors (*2*). There are a number of diseases of bone that result from an imbalance between bone resorption and formation. After age 40, bone destruction begins to exceed bone formation, leading to local or systemic bone loss called osteoporosis. Osteoporosis is a major public health problem and, although it occurs most commonly in women as a result of estrogen deficiency after menopause, it is increasingly recognized that other causes exist and that there is a high incidence of osteoporotic fractures in older men. Large increases in bone resorption and loss of calcium from bone (hypercalcemia of malignancy) are skeletal complications associated with many cancers and with bone metastases of breast and prostate tumors. A number of therapeutic strate-

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gies to treat these common conditions, as well as Paget's disease of bone and inflammatory bone disorders of rheumatoid arthritis and periodontal disease, are already in use or are under development. Efforts have been primarily concentrated on the development of drugs to block bone resorption through decreasing the formation or activity of osteoclasts. Principles guiding the development of therapeutics to treat diseases of bone (or of any other organ) include: (i) Selectivity. That is, the action of the drug must be specifically targeted to bone and to the molecule or rate-limiting process that is the cause of the disease. (ii) Therapeutic index. The developed therapy must optimize the benefit-to-risk ratio of the drug. (iii) Convenience. For example, a more optimal drug is one that can be administered orally rather than parenterally.

Bone Diseases

Osteoporosis. The reduction in bone mass and deterioration in bone architecture after age 40 that is characteristic of osteoporosis results in an increase in the fragility of bone and its susceptibility to fractures. For every 10% of bone that is lost, the risk of fracture doubles. In the United States, it is estimated that 16.8 million postmenopausal women have lost more than 10% of their peak adult bone mass, another 9.4 million have lost more than 25%, and 4.8 million have already suffered an osteoporotic fracture (*3*). In a 50-year-old Caucasian American woman, the lifetime risk for total osteoporotic fractures and hip fractures (which incur the highest morbidity, mortality, and cost) is 45% and 17.5%, respectively. About 25 to 30% of all hip fractures occur in men, and male osteoporosis is increasing as men live longer, probably due to a decrease in sex steroids and age-related bone loss.

The most common cause of osteoporosis in women is the decrease in estrogen that accompanies menopause. Estrogen loss is associated with elevated bone resorption caused by a rise in osteoclast number, which is driven by increases in the cytokines that regulate osteoclast generation as follows: RANK (receptor for activator of nuclear factor-κB) ligand; TNF-α (tumor necrosis factor–α); interleukin-1 (IL-1), IL-6, IL-11; M-CSF (macrophage–colony stimulating factor); and prostaglandin E (*4*). RANK ligand (RANKL), its receptor RANK, and a neutralizing soluble receptor that blocks RANK activity called osteoprotegerin (OPG) have been shown to fully control osteoclast formation in mice (*5*). Production of all of these cytokines is either directly or indirectly suppressed or regulated by estrogen.

Pathological conditions causing bone loss, other than estrogen or androgen deficiency, include multiple myelomatosis, hyperparathyroidism, and hyperthyroidism. Treatment of these secondary forms of osteoporosis is directed at their primary cause, such as removal of the parathyroid glands. Osteoporosis induced by glucocorticoid treatment results from suppression of osteoblast activity and decreased bone formation, with the possible contribution of an increase in bone resorption. Inhibitors of bone resorption can prevent glucocorticoid-induced bone loss.

Paget's disease. Paget's disease of bone occurs in 3% of subjects over the age of 40 in the United Kingdom and is only slightly less common in the Caucasian population of North America. It is marked by an increase in osteoclast numbers and activity and often affects multiple sites throughout the skeleton. Increased bone resorption is met by a compensatory increase in bone formation and local bone turnover, leading to woven bone, which is bulky, weak, and prone to bowing and fracture.

The discovery of viral inclusions within the nuclei of osteoclasts from Paget's disease patients suggests that the disorder might result from infection with viruses of the paramyxovirus class (which includes measles, respiratory syncitial, and canine distemper viruses) (*6*). Transfection of osteoclast precursors with retroviral vectors expressing the measles virus nucleocapsid gene (MNVP) generated hypernucleated osteoclasts that had a greater resorptive capacity, increased expression of RANK and activation of the transcription factor NF-κB, and a substantially increased sensitivity to the hormone 1,25(OH) vitamin D (*7*). Furthermore, in four families with Familial Expansile Osteolysis (FEO), an autosomal dominant juvenile variant of Paget's disease, two insertional mutations in exon 1 of the RANK gene were identified (*8*). These activating mutations resulted in enhanced expression of RANK and increased NF-κB signaling, which are known to stimulate osteoclast generation. These observations raise possibilities for therapeutic targeting of the OPG/RANKL/RANK pathway in Paget's disease, which is a valuable model for other resorptive bone diseases.

Bone diseases of cancer. Several cancers, both solid tumors and hematopoietic malignancies, have profound effects upon the skeleton, causing an increase in osteoclast formation and activity, either systemically as in humoral hypercalcemia of malignancy (HHM) or locally in bone metastases. HHM is caused most commonly by the hormonal action of parathyroid hormone-related protein (PTHrP), which greatly stimulates resorption and overrides normal calcium homeostasis (*9*).

Tumor cells need the ability to promote osteoclast formation in order to establish and grow in bone as metastases (*9*). The frequency of PTHrP production in breast cancers and especially in breast tumor–bone metastases, suggests that they promote osteoclast formation by producing PTHrP (*10*). Experimental support for this hypothesis comes from the demonstration that bone lytic lesions, produced by injection of human breast cancer cells into the left ventricle of nude mice, can be inhibited by bisphosphonates (BPs) as well as by neutralizing antibody against PTHrP (*11*). Current evidence suggests that products of breast cancer cells [PTHrP and probably others, including IL-6, IL-11, and Cyclo-oxygenase–2 (COX-2)–generated prostanoids], promote RANKL formation by acting on resident osteoblasts and/or stromal cells (*12*). Another important element in the successful seeding of bone metastases of breast cancer cells is the part played by the bone microenvironment. The release of growth factors (especially TGF-β) can influence the growth of tumor cells and their production of bone-resorbing cytokines (*13*). Prostate cancers, which metastasize very commonly to bone (almost uniformly as osteoblastic metastases), are increasingly recognized as having an accompanying osteoclast component, presumably to facilitate their establishment and expansion.

Inflammatory bone disease. Rheumatoid arthritis is characterized by destruction of articular cartilage and by excessive subchondral osteoclastic bone resorption (*14*). In the inflammatory state, macrophages (which differentiate into osteoclasts) accumulate in the rheumatoid synovial membrane (*15*). Here, there are many osteoclastogenic cytokines, including IL-1, IL-6, IL-11, IL-13, IL-17 (*16*), and PTHrP. Rheumatoid synovial fibroblasts produce RANKL (*14*), and T cells producing RANKL have been shown to promote osteoclast formation without the participation of other cells (*17*, *18*). The rheumatoid joint also houses known inhibitors of osteoclast formation, such as IL-18. This cytokine inhibits osteoclast formation by acting on T cells to increase production of granulocyte-macrophage–colony stimulating factor (GM-CSF) (*19*); it also has pro-inflammatory effects on human rheumatoid synovium in vitro and in mice with induced arthritis (*20*). Other inhibitors of osteoclasts are interferon-γ (IFN-γ) and IL-12.

There is probably no single cytokine responsible for osteoclast formation and activity and the ensuing bone erosion in rheumatoid arthritis. It is more likely that a large number of stimulators and inhibitors of osteoclast formation converge on the OPG/RANKL/RANK pathway, which makes this pathway or the downstream activated osteoclast more appropriate targets for therapeutic intervention.

One of the most common bone diseases of all is periodontal disease, in which the accumulation of bacteria that cause dental plaque results in the destruction of cellular and struc-

tural components of the periodontium (the gum). The cellular and molecular processes in periodontal disease, and therefore possible therapeutic targets, are likely to be similar to those operating in rheumatoid arthritis.

Therapies

Inhibitors of bone resorption. Most bone diseases are due to increased bone resorption, rendering its inhibition a primary therapeutic objective. Indeed, most bone therapies that are currently available belong to this category. Inhibition of bone resorption can be accomplished by reducing either osteoclast generation (for example, with estrogens) or osteoclast activity (with BPs). These processes point to rate-limiting steps in osteoclast formation and function, and offer a number of targets for therapeutic intervention.

Estrogens and selective estrogen receptor modulators (SERMs). Estrogen replacement therapy has long been considered the first line therapy for preventing osteoporosis in women. Several estrogens are currently in use, including orally administered conjugated estrogens extracted from pregnant mare urine and the synthetic human hormone 17β estradiol administered through a skin patch. Estrogens are always given with a progestin to prevent uterine cancer in women that have not undergone hysterectomy.

Treatment with estrogens clearly inhibits bone loss as well as bone turnover and increases bone mineral density (BMD). In early postmenopausal women, estrogens increase spine BMD by 3 to 4%, as well as hip BMD to an extent similar to that induced by BPs (alendronate) (*21*). In late postmenopausal women, the effect is less pronounced. The efficacy of estrogens in the prevention of fractures has not been established in large randomized prospective clinical trials, which are needed to provide compelling evidence for drug efficacy. We must await the results of the Women's Health Initiative (WHI), a 15-year megatrial with 161,000 subjects conducted by the National Institutes of Health. Retrospective epidemiological studies suggest that estrogens can reduce the risk of hip fractures by over 50% while subjects are on hormone (estrogen/progestin) replacement therapy (HRT), or if they have received HRT relatively recently (within the last 5 to 9 years, for at least 5 years) (*22*). However, besides their effects on bone, estrogens affect many other tissues including breast and uterus; undesirable side effects have limited the long-term use of estrogen in the United States and in many other countries.

Estrogen treatment is associated with a well-established increase in the risk of uterine cancer, which can be fully prevented by administering progestins simultaneously with estrogen. There is also a 20 to 50% increase in the risk of breast tumors, which deters many women from receiving HRT, especially those with a family history of breast cancer (who are currently advised not to take estrogen). Other potentially serious, but rare side effects of estrogen therapy are thromboembolic events (for which the risk increases about threefold). These negative effects are assumed to be vastly outweighed by the positive effects of estrogens, especially on the prevention of cardiovascular disease (CVD), indicated by the lower incidence of CVD in premenopausal women compared with postmenopausal women. However, the effects of estrogen on CVD, like its effects on fractures, have not been demonstrated in long-term prospective randomized trials; again, for this we must await the results of the WHI study. A recent 4-year randomized trial on the efficacy of conjugated estrogens in the secondary prevention of CVD showed no benefit (*23*). Interestingly, this study also showed no reduction in fractures, although the study was done with a population that was not at high risk. Other potential benefits of estrogens on cognition and the prevention of Alzheimer's disease are intriguing but require much more investigation.

There are, however, several important and well-documented beneficial effects of estrogens in perimenopausal women, including maintenance of the thickness and elasticity of skin, of the vagina and perineal connective tissue, and the prevention of hot flushes. However, estrogen use may be of short duration (often less than 3 years) due to vaginal bleeding, breast tenderness, and anxiety about cancer.

The molecular mechanism of action of estrogens on bone, as well as on other tissues, is not fully understood. Two estrogen receptors (ERs), α and β, have been identified, but their relative contributions to the various effects of estrogens are still under investigation. Broadly, ERα seems to be responsible for most of estrogen's effects on reproduction and reproductive organs, which are fully compromised in its absence in mice. No unique function has yet been assigned to ERβ.

The discovery that agents (historically referred to as antiestrogens) were able to exert full or partial estrogen agonist effects on various tissues initiated the development of a new class of drugs known as SERMs (Fig. 1). The first SERM identified was tamoxifene, a triphenylethylene compound that was found to prevent bone loss (*24*).

The molecular mechanisms of SERM action require that they bind with high affinity to the ER. The structural features of each SERM differ so that unique ligand-induced conformational changes take place in the ER, which are thought to be the likely basis for tissue-selective pharmacology. For example, raloxifene operates as an estrogen agonist in bone but as an antagonist in the breast and uterus. Evidence for different ER conforma-

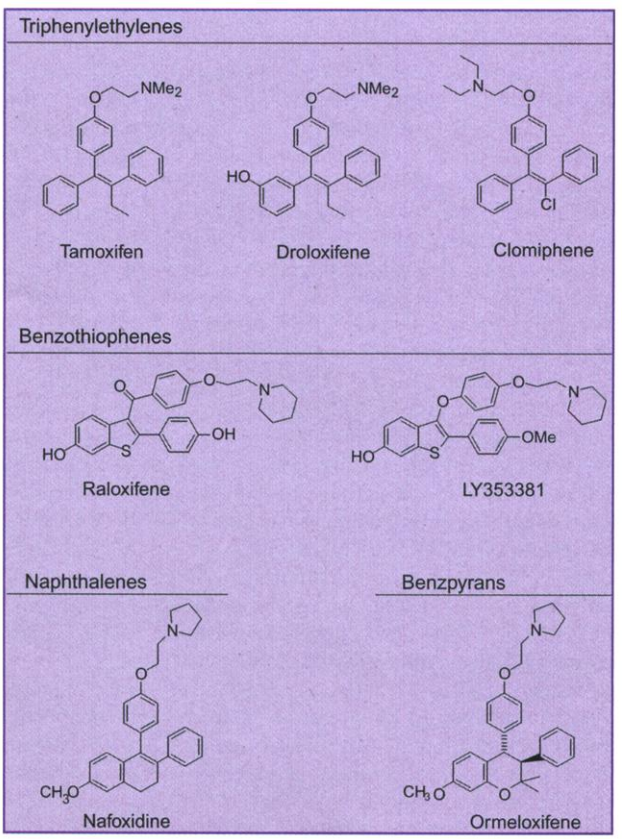

Fig. 1. Chemical structure of several SERMs.

tions with different ligands comes from in vitro protease digestion profiles for ERα complexed to several SERMS, as well as from crystallographic analysis of the structure of the ER when complexed with estrogen or raloxifene (*25*). Each unique SERM-ER complex recruits different combinations of coactivator proteins depending on the tissue, thus, explaining how the same SERM can be an agonist in one tissue but an antagonist in another.

A disadvantage of tamoxifene is its uterotrophic effect and the increased risk of uterine cancer. The next generation of SERMS includes benzothiophenes, naphthalenes, and benzpyrans (Fig. 1) (*26*). Raloxifene, considered in the early 1980s to be a possible treatment for breast cancer, was found to prevent bone loss induced by estrogen deficiency in rats and monkeys. In clinical studies of raloxifene in post-menopausal women, a 40% reduction in relative risk of vertebral fractures was achieved, despite the fact that there was only a 3 to 4% increase in bone density (*27*), and a significant reduction in new breast cancers was noted. In the animal and clinical studies, no stimulatory effects on the uterus were found.

The mechanism by which SERMs inhibit bone resorption is likely to be the same as estrogen's mechanism, that is, by blocking production of cytokines that promote osteoclast differentiation (*4*). The effect of raloxifene on bone is less pronounced than that of estrogen. This raises the question of whether a SERM could be as effective at blocking bone resorption as estrogen, could mimic the beneficial effects of estrogen in other tissues, and avoid estrogen's undesirable effects, especially on breast and uterus. The discovery of a second ER, ERβ, which can form a heterdimer (ERα/ERβ) with ERα (*28*), implies that different combinations of a SERM with ER homo- or heterodimers are likely to exist. It remains to be seen whether the perfect SERM can be developed and whether ERα and ERβ pathways can be manipulated to generate new and even better SERMs.

An important implication of the development of SERMs is that this approach should be applicable to other nuclear receptors. Development of glucocorticoid receptor agonists with sufficient antiinflammatory action but without detrimental effects on bone that often lead to osteoporosis, would indeed be a major advance. Similarly, androgen receptor ligands that are nonvirilizing and do not stimulate prostate tissue would be an advantage.

Bisphosphonates. BPs are analogs of pyrophosphate (P-O-P) in which the oxygen in P-O-P has been replaced by a carbon with various side chains (Fig. 2). They concentrate in bone and are, to date, the most effective inhibitors of bone resorption, a property discovered empirically during studies of bone mineralization. Nitrogen-containing BPs are taken up by osteoclasts, where they inhibit farnesyl diphosphate synthase, an enzyme in the mevalonate pathway of cholesterol synthesis (*29*). This leads to reduction in the levels of geranylgeranyl diphosphate, which is required for prenylation of guanosine triphosphate (GTP)–binding proteins (such as Rho, Rab, and Cdc42) that are essential for osteoclast activity and survival. Consequently, BPs inactivate osteoclasts, which then undergo apoptosis, resulting in reduced bone resorption, lower bone turnover, and a positive bone balance. The BP alendronate (ALN) was the first inhibitor of bone resorption to show a significant reduction (about 50%) in fractures of the spine and hip in large randomized prospective placebo-controlled clinical trials (*30*). It is approved for the treatment and prevention of postmenopausal osteoporosis and glucocorticoid-induced osteoporosis. Risedronate has also been shown to reduce spine and all-site fractures and was recently approved for treating these two types of osteoporosis.

Because BPs reduce elevated bone resorption regardless of cause, they are used to treat Paget's disease and tumor bone disease and, additionally, are being evaluated for treatment of inflammation-related bone loss, osteogenesis imperfecta (increased susceptibility to fractures due to mutations in type I collagen), fibrodysplasia, and immobilization-induced bone loss.

There are several new BPs under clinical development, some, such as zoledronate, acting at doses lower than a microgram per kilogram. More potent BPs may not provide a substantial advantage if side effects (such as, upper gastrointestinal distress) are mechanism-based, as suggested by recent in vitro studies. BPs are the first-line therapy for hypercalcemia of malignancy and Paget's disease and are becoming the standard of care for patients with bone metastases. A large fraction of osteoporotic patients are currently treated with BPs.

Calcitonin. Calcitonin is a polypeptide hormone that inhibits bone resorption by acutely blocking osteoclast activity. Physiologically, its role in the fine-tuning of extracellular calcium regulation may be confined to times of "stress," such as growth, pregnan-

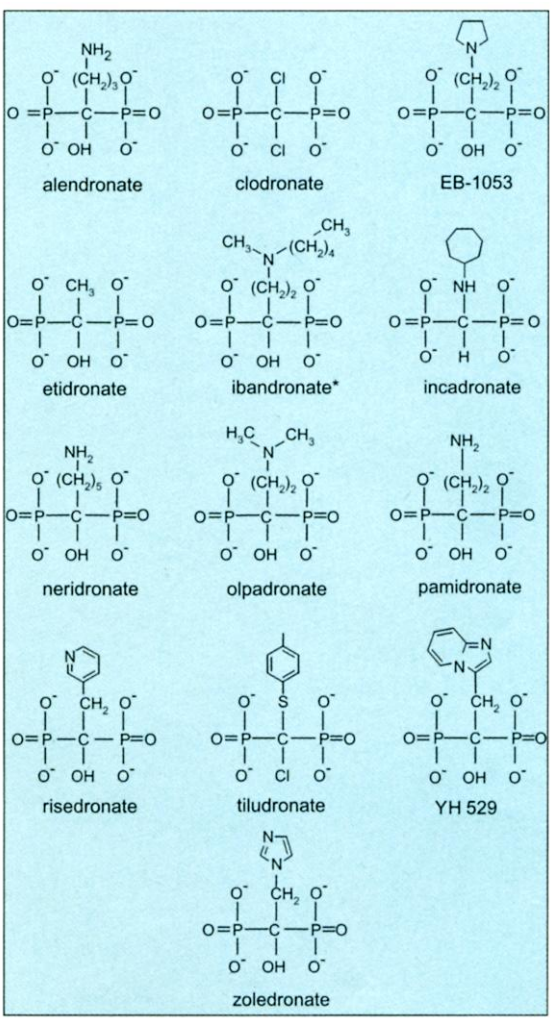

Fig. 2. Chemical structure of bisphosphonates, including compounds in clinical use and development.

cy, lactation, and high calcium intake (*31*).

Calcitonins of human, pig, salmon, and eel have been used therapeutically. The injection forms have been used for many years in the treatment of Paget's disease. In osteoporosis both injected and intranasal salmon calcitonin are approved in some countries, but are used in a relatively small proportion of osteoporotic patients. Calcitonin-induced loss of calcitonin receptors, resulting in hormone-induced resistance (analogous to β-adrenergic down-regulation), is a feature of the hormone's action that was noted many years ago both in vitro and in vivo (*32*, *33*). This facet of calcitonin's action must be borne in mind when it is used therapeutically, and it remains to be seen whether this problem can be overcome.

New Targets

TNF receptor/ligand family members and their signaling pathways. The recent discovery of RANKL, a key factor in osteoclast formation and activity, RANK, its receptor, and OPG (the inhibitory "decoy" receptor) provides a number of new therapeutic targets for osteoclast inhibition.

These members of the tumor necrosis factor (TNF) receptor and ligand families are crucial for osteoclast control and mediate the regulation of osteoclast formation and activity by cytokines and hormones [reviewed in (*5*)]. OPG, a secreted member of the TNF receptor family, is produced by osteoblastic stromal cells and inhibits osteoclast formation and activity. It does so by binding to RANKL, a type II membrane protein of the TNF ligand family, also produced by osteoblastic stromal cells, and an essential stimulator of osteoclast differentiation. The action of RANKL requires that it bind to its receptor, RANK, on hematopoietic cells, initiating processes leading to osteoclast development. Osteoclast formation in vitro is inhibited by OPG, which binds to RANKL, preventing its interaction with RANK on hematopoietic cells. Mice lacking the *OPG* gene are severely osteoporotic (as are mice engineered to overexpress RANKL) because of the resulting dysregulation of osteoclast formation (Fig. 3).

OPG injected into rats decreases blood calcium in cancer-induced hypercalcemia, prevents bone loss following removal of the ovaries (oophorectomy), and prevents bone metastases in an experimental mouse model (*34*). Furthermore, OPG blocks the periarticular bone destruction in collagen-induced arthritis in mice, without influencing the inflammation in and around the joint (*17*), as well as reducing cancer-induced bone destruction and pain in mice (*35*).

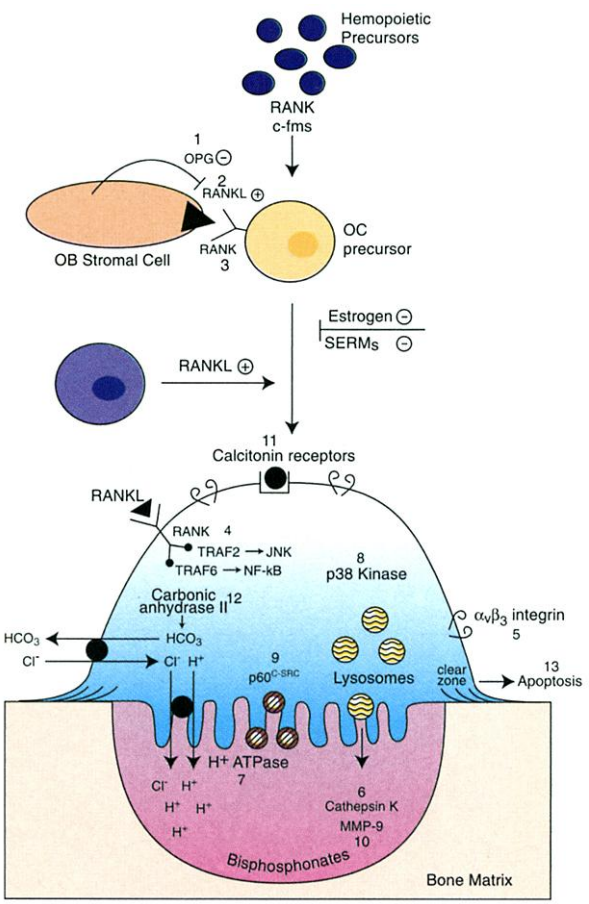

Fig. 3. Diagram of potential therapeutic targets (indicated by numbers below) for pharmacological modulation of osteoclast formation and activity. Current drug treatments include estrogens and SERMs, for suppressing osteoclast formation, and bisphosphonates and calcitonin, for suppressing osteoclast activity. Additional compounds are being developed in each category. Possible new targets for controlling osteoclast formation and activity are as follows: (1–4) blocking the action and signaling of the OPG/RANKL/RANK pathway; (5) blocking ligands of the $\alpha_v\beta_3$ integrin; (6) inhibition of cathepsin K protease; (7) inhibition of the vacuolar H^+-ATPase; (8) inhibition of the p38 kinase; (9) inhibition of $p60^{C\text{-}SRC}$ kinase or interference with $p60^{C\text{-}SRC}$ signaling via SH2/SH3 domains; (10) inhibition of matrix metalloproteinase 9 (MMP-9); (11) calcitonin receptor ligands with better bioavailability and less receptor down-regulation; (12) inhibition of CA2 (carbonic anhydrase II); and (13) stimulation of osteoclast apoptosis.

These observations identify OPG's interaction with RANKL as a target for therapeutic intervention. Should the protein itself be used for therapy? Although apparently effective, it is a large protein, needs to be given in substantial doses, and may induce immune responses and act in organs other than bone. Physiologically, OPG may accumulate to some extent in the bone matrix and is able to block osteoclast formation from here. If the physiological process requires tight local regulation of OPG production, then perhaps a logical therapeutic approach would be to search for ways to modulate OPG formation by bone cells. It is possible that OPG gene therapy could be used in the future.

Other possible therapeutic targets in the RANKL/RANK axis include (i) production of RANK ligand, (ii) interaction of RANK ligand with RANK, and (iii) RANK signaling through Jun NH_2-terminal kinase (JNK) and NF-κB pathways and through TNF receptor–associated factor (TRAF) adapter molecules (Fig. 3).

In addressing these possibilities, one should keep in mind that RANKL activates signaling through RANK to influence important events other than osteoclastogenesis. The most obvious of these is the regulation of interactions between T cells and dendritic cells, which is manifested in mice with a disrupted RANKL gene as a defect in early differentiation of T and B lymphocytes and a lack of lymph nodes (*36*).

Other targets for inhibiting osteoclast function. Cathepsin K is a lysosomal cysteine proteinase expressed selectively and at high levels in osteoclasts, with enzymatic properties suited for degrading type I collagen. Cathepsin K inhibitors with appropriate pharmaceutical properties could potentially be used to block bone resorption (*37*).

Another relatively selective osteoclast-specific structure that seems to play a rate-limiting role in osteoclast activity is the αv β3 integrin receptor, which is produced in osteoclasts as well as in budding blood vessels and leukocytes. Treatment of rats with the disintegrins, echistatin or kistrin, which bind with high affinity to αv β3, was shown to effectively inhibit bone resorption stimulated by parathyroid hormone or estrogen deficiency (*38*). Moreover, small molecular weight compounds that mimic the tripeptide RGD sequence, recognized by the integrin, were shown to have similar effects (*39*). Target selectivity of such a compound would have to be determined in toxicological and clinical studies.

An essential component of osteoclastic bone resorption is acidification of the resorption lacuna, which reduces the pH to about 4 in order to dissolve the mineral content of bone. A vacuolar H^+–adenosine triphosphatase (H^+-ATPase) in the osteoclast mem-

brane plays a key role in this process. Inhibitors of this enzyme, for example, bafilomycin, have been shown to inhibit osteoclastic bone resorption in vitro and in vivo (*40*). It has been reported that the H^+-ATPase, an enzyme present in virtually every cell and highly conserved during evolution, may have a unique 116-kD subunit in the osteoclast that could provide a target for selective inhibition of osteoclast activity (*41*). Recently, an osteoclast-selective H^+-ATPase inhibitor was shown to inhibit bone loss in ovariectomized rats (*42*).

Other enzymes involved in intracellular acidification are carbonic anhydrase II (CA2) and the sodium bicarbonate exchanger in the basolateral membrane, which help maintain the neutral pH inside the cell. Genetic mutations of CA2 in patients produce not only osteopetrosis, but also renal acidosis and mental retardation (*43*). Inhibition of CA2 has been considered for suppression of osteoclast-mediated bone resorption; however, to our knowledge, it is not being pursued at this time due to lack of tissue specificity and potential side effects.

The c-src kinase gene is ubiquitously expressed in all cells and is highly abundant in brain and platelets. When this gene was deleted in mice through homologous recombination, osteoclast inactivation was the only detectable phenotypic change (*44*). Subsequent investigations documented the role of c-src in osteoclast activation via phosphorylation of Cbl (a multiadaptor proto-oncogene product involved in tyrosine kinase signaling) (*45*) and of the αv β3 activated adhesion kinase, PYK2 (*46*). Src kinase binds to its downstream effectors via src homology domains SH2 and SH3. Interference with these interactions or with src activity using specific src kinase inhibitors could, in principle, selectively inhibit osteoclast function (*47*).

Bone resorption has many similarities to inflammation in which the p38 kinase has a rate-limiting role. p38 kinase inhibitors were reported to block osteoclast activity (*48*), offering an additional approach for developing antiresorptive agents. Kinase inhibitors raise important challenges regarding target specificity, and because p38 is not unique to osteoclasts, the selectivity is an even greater problem. The list presented here is not exhaustive and further insights into rate-limiting steps in osteoclast activation or function should provide additional targets for selective osteoclast inhibition.

Thus, although effective inhibitors of osteoclast activity are currently known and successfully used in the clinic, additional agents are likely to be developed and used if they are better suited for particular indications or provide greater efficacy or convenience.

Stimulation of bone formation. In osteoporosis, bone loss may far exceed the amount that can be restored by inhibitors of resorption, making stimulators of bone formation a highly desirable adjunct therapy. Many known growth factors stimulate osteoblast proliferation in vitro. However, there is no good correlation between osteoblast mitogenic activity in vitro and osteogenic activity in vivo (*49*). In vivo, osteogenesis is a complex process that involves cell-cell and cell-matrix interactions and depends strongly on angiogenesis (formation of new blood vessels).

Parathyroid hormone. The most interesting anabolic prospect is parathyroid hormone (PTH). Although best recognized for promoting bone resorption and elevating blood calcium, PTH was noted in the 1930s to stimulate bone formation (*50*). Interest was revived with clinical studies beginning in the 1970s. Today, there is a wealth of preclinical data showing that daily intermittent dosage of PTH increases mechanical strength and mass in trabecular and cortical bone of ovariectomized rats. Clinical studies show impressive gains in bone density of spine and femoral neck resulting from daily injections of PTH in osteoporotic women and in men (*51*).

There is evidence that one component of the cellular activity of PTH increases osteoblast numbers and activity by inducing bone-lining cells to become osteoblasts without stimulating proliferation of precursor cells (*52*) (which could be explained if PTH increased the life-span of mature osteoblasts by preventing apoptosis) (*53*). Another anabolic agent, prostaglandin E (PGE), also blocks osteoblast apoptosis (*54*). The inhibition of bone formation by glucocorticoid treatment has been ascribed to the enhanced apoptosis of osteoblasts (*55*). A persistent puzzle has been that the anabolic effect of PTH is dependent on intermittent administration, that is, attaining a peak blood level which is maintained for only a short time. For example, when PTH is infused for as little as 2 hours per day, hypercalcemia and bone loss occur as osteoclasts are stimulated to resorb bone (*56*). Thus, maintenance of elevated PTH initiates processes leading to new osteoclast formation, and the consequent resorption overrides the effects of activating genes that direct bone formation. One approach to combating this problem has been to develop PTH secretagogues (oral drugs that stimulate PTH secretion) as anabolic agents (*57*). These secretagogues will have to satisfy the pharmacokinetic requirement of transiently increasing PTH in patients to the desired level.

Pharmacological manipulation of osteogenic factors. A novel prospect for anabolic therapy arises from the discovery that the statins, inhibitors of hydroxy-methyl-glutaryl–CoA (HMG-CoA) reductase that decrease cholesterol synthesis and are used widely as drugs to lower cholesterol, can enhance bone formation in vitro and in vivo in rats (*58*). Lovastatin and simvastatin increase bone formation when injected directly over calvaria of mice, and when rats that have lost bone after oophorectomy are treated with simvastatin they show an increase in bone formation rate and trabecular bone volume. This discovery came from a high-throughput screening program seeking agents that activated the promoter of the bone morphogenetic protein–2 (BMP-2) gene, chosen as a target because osteoblast differentiation is enhanced by members of the BMP family. Those observations were followed by three recent case-control studies reporting that statins reduce the risk of fractures (*59*). There are limitations to such observational studies, as recognized by the authors and by Cummings and Bauer (*60*); only prospective, randomized trials can establish definitively whether statins have a beneficial effect on bone. If the existing statins, which are aimed at the liver HMG-CoA reductase, were to prove unsuitable as bone-active agents, this new pathway could provide attractive new drug targets.

Fluoride treatment markedly increases spinal BMD; however, it does not reduce fractures and can inhibit bone mineralization (*61*). Fluoride is not approved in the United States, although unraveling its anabolic effect might provide useful therapeutic leads.

Understanding of bone formation has lagged behind that of bone resorption. However, there have been two exciting recent advances: (i) the discovery that core binding factor–a1 (cbfa1) is a key transcription factor in osteoblast differentiation and in the maintenance of the differentiated state of the osteoblast and (ii) that leptin is a potential mediator of centrally regulated bone homeostasis (*2*, *62*). Just as the regulation of endogenous growth factor production might be used as a target for drug development, one could explore the cbfa1 and leptin pathways to seek agents that could activate them to promote bone formation.

Growth factors. The most important and effective growth factors (GF) of bone, insulin-like GF (IGF-1), transforming GF–β (TGF-β), fibroblast GFs (FGFs), and the BMPs have come under consideration as potential treatments for bone diseases, especially severe osteoporosis. Several are under investigation as possible local therapies in the healing of fractures and bone defects. As systemic therapies, however, each has major disadvantages (*63*). Their normal roles are exerted through finely controlled local events: production, storage in bone matrix, and activation at appropriate sites and times. They all have multiple effects in tissues other than bone, and systemic administration inevitably causes undesirable side effects.

Future developments might yield ways

to overcome these difficulties, for example, by confining these growth factors to bone sites through osteoblast-targeted regulation of their production or, perhaps, by gene therapy.

Genetic information and gene therapy. There is compelling evidence that osteoporosis has a strong genetic component, which accounts for over 50% of the risk for this polygenic disease (*64*). One can think of at least three ways to use genetic information for the prevention and treatment of bone diseases.

The peptide nature of growth factors, which limits the possibility for their oral administration, may lend itself to gene therapy. Expression of externally delivered genes has been proposed for stimulating bone formation. For example, the expression of BMP, administered by transfection of osteoblasts with a vector containing the BMP gene, increases local bone formation in rats (*65*). It has been suggested that systemic delivery could be accomplished by transfecting syngeneic bone marrow stromal cells or stem cells with the therapeutic gene. Further proof-of-concept and technological advances are probably needed to bring this approach to fruition.

A second application of genomic information, and potentially the earliest to be implemented, could be genetic profiling for risk assessment, for example, the likelihood of suffering a hip fracture. Even without knowing the role of specific genes in the pathophysiology of a given disease, the presence of certain genetic patterns in patients could identify individuals for preventive care. This approach, which will be technologically feasible in the near future, has substantial public health and ethical implications, which will have to be addressed.

The third potential application of genomic information is identification of genes directly involved in bone function or disease, which could be used either as drug targets or as reagents for drug development (for example, rate-limiting enzymes or receptors). One would use the protein products of these genes to develop small molecular weight activators, inhibitors or ligands, or bioavailable compounds adequate for oral therapy, which are more appealing for long-term treatment.

Recent understanding of bone cell function and of the pathophysiology of bone diseases has led to the availability of new therapeutic agents to treat these diseases. There are clearly additional opportunities for developing new inhibitors of bone resorption, as well as effective stimulators of bone formation, so far a major unmet need.

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