Bone tissue: hormonal regulating systems, growth factors and remodeling as a target for therapeutic agents in osteoporosis

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Summary
Bone regulating systems have received growing attention in recent years. Regulation of skeletal pathophysiology and modulation of osteoblast, osteoclast and osteocyte activity by hormones, cytokines, and growth factors are not only important features of bone biology, but also a target for old and new osteoporosis therapies. Molecules such as selective estrogen receptor modulators (SERMs, e.g. raloxifene and bazedoxifene), denosumab, or bisphosphonates may exert their effects through modulation of estrogen receptor, RANKL and osteoclast activity, respectively. Knowledge of the regulating systems is also the basis for developing future therapy. This review shows regulating systems as a basis for current and future therapies in the field of osteoporosis.

Key words: osteoblast, osteoclast, osteocyte, osteoporosis, RANKL, Wnt.

Introduction

Osteoporosis is a systemic, skeletal disease leading to an increased risk of fracture with severe consequences in terms of morbidity and mortality (1,2). Nowadays, there exist cost-effective pharmacological agents for treating this condition which have been shown to statistically significantly reduce the associated fracture risk (Table 1) (3). Some of the current treatments exploit physiological mechanism(s) of bone biology; this is true of estrogens, tibolone, and the selective estrogen receptor modulators (SERMs) raloxifene and bazedoxifene, which exert their effects by binding to the estrogen receptor (ER) (3); interestingly, agents that bind to the ER yield bone tissue of normal quality while obtaining bone mineral density (BMD) increases at all skeletal sites (4-8). Estrogens, tibolone, and SERMs can significantly reduce osteoporosis-related fracture risk (9-12).
Bisphosphonates (BPs) are well-known, potent anti-resorptive agents which act by blocking osteoclast activity (3), so that bone formation is greater than bone resorption. In this way, BPs can increase bone mass and strength, decreasing fracture risk (3,13-16). Parathyroid hormone (PTH) and teriparatide (fraction 1-34 of the recombinant molecule of PTH) (17) exert a potent anabolic effect, with important gains in BMD; strontium ranelate seems to have a dual mechanism of action, decreasing bone resorption and increasing formation (18,19). All these agents lead to an increase in bone mass and a decrease in vertebral and (albeit with differences in terms of efficacy between the various agents) non-vertebral and hip fracture risk.
Despite important progress in osteoporosis management, this condition still seems to be under-diagnosed and under-treated. The percentage of patients still taking medication for osteoporosis one year after it was prescribed is about 50% (20). On the other hand, even when a treatment is taken properly, according to the dosing regimen, adherence can be poor as a result of problems of efficacy (e.g. certain molecules have been shown to significantly reduce hip fracture risk only in the context of a post-hoc

Table 1. Drugs available for preventing/treating osteoporosis

- hormone replacement therapy (HRT)	- parathyroid hormone (PTH)
- tibolone	- teriparatide (PTH 1-34)
- selective estrogen receptor modulators (SERMs): raloxifene, bazedoxifene	- strontium ranelate - denosumab - bisphosphonates (alendronate, risedronate, ibandronate, zoledronate)

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analysis in a high-risk subgroup), duration of intake (e.g. administration of teriparatide is not recommended for more than 24 months), or safety in long-term use (e.g. there is a risk, albeit slight, of atypical fracture of the femur or osteonecrosis of the jaw with long-term use of BPs).

For these reasons, research into more selective agents is actively being pursued. In this context, growing knowledge of the pathophysiology of osteoporosis and regulating mechanisms of bone remodeling has allowed the development of new, more selective, treatments targeting specific cell types, growth factors and factors regulating cellular activity, or enzymes.

For example, knowledge of the receptor activator of NF-kB (RANK)-receptor activator of NF-kB ligand (RANKL)-osteoprotegerin (OPG) system, the most important regulating system of osteoclastic activity, led to the development of denosumab, a fully human, monoclonal antibody against RANKL, which significantly reduces osteoclastic recruitment, survival, and activity.

Bone remodeling is the process by which the structural integrity and biomechanical efficiency of the skeleton is maintained, once it has reached adult size and structure. Bone remodeling is the continuous replacement of "old bone", which presents microcracks and areas of altered mineralization, with new bone, of better quality and biomechanical strength, and it involves the maintenance of a balance, under normal conditions, between the processes of bone resorption and bone formation, which are mediated, respectively, by osteoclasts (OCs) and osteoblasts (OBs). Even though macroscopically the skeleton appears to be inert tissue, from a microscopic/biochemical point of view, skeletal tissue is actually highly dynamic. To sustain the body loads to which the skeleton is subjected daily, its biomechanical properties must be maintained through a continuous process of remodeling and repair of the microcracks that form in trabecular and cortical bone.

The classic concept of the bone multicellular unit (BMU) has changed in recent years (21). Today, this evolving concept is based on increased knowledge about the role of the cells [OBs, OCs, and osteocytes (OTs)] involved in bone remodeling (22-24) and about hormonal regulation systems and growth factors that, together modulate bone physiology (21). Qualitative and/or quantitative alterations of these regulating systems constitute the pathophysiological basis of various skeletal diseases, primarily postmenopausal osteoporosis. Deeper knowledge of the communication/regulation systems driven by OTs (25,26), even though these cells are entrapped in bone matrix, has led to the recognition that they play a more important role than was previously thought. OTs, which are terminally differentiated OBs, seem to be able to give the starting signal for a bone remodeling cycle, and do so on perceiving that a certain area of bone tissue needs to be reorganized. From this perspective, OTs are now thought of as mechanoceptors (27-29) able to communicate with OCs and OBs in order to start a bone resorption/formation cycle. OTs lie in newly formed bone matrix where they reside for long periods of time. They are spatially isolated from one another; however, they have long filipodial extensions by which they are connected with each other as well as with bone lining cells and OBs on the bone surface. When damage occurs, OTs sense changes in pressure within the matrix and undergo apoptosis while releasing signaling molecules, such as prostaglandins or cytokines, which induce cells on the bone surface to initiate resorption (30).

Bone multicellular unit cells are surrounded by quiescent bone surface lining cells; these surface lining cells are in communication with OTs entrapped in the bone matrix. One of the most important evolving concepts of recent years is the idea that BMUs (together with OTs, OCs, and OBs) are not the only actors in bone remodeling; indeed, an important role is now attributed to lining cells and capillaries entering bone and providing nutrients and cells, i.e. OCs and OBs, in sufficient quantities for each single BMU. The BMU + capillary + lining cells together form a complex called the bone remodeling compartment (BRC) (22). The OT, in this context, acts as a cellular element detecting microcracks and biomechanical stimuli and responding to hormonal changes in bone (for example, estrogen deficiency or abnormal production of PTH). Thus, OTs control bone remodeling, perhaps through communication with lining cells (23).

Lining cells could give the signal to start the bone resorption process, attracting OCs and starting to form a line surrounding the BRC. Capillaries entering the BRC provide both blood supply and the population of OCs and OBs. It is known that OBs and OCs interact, mainly through various molecules and systems mediating different mechanisms. Among these, RANK-RANKL-OPG is a well-known system regulating OC recruitment, activation, function and survival (31).

Receptor activator of NF-kB (RANK) is a transmembrane protein, whereas RANKL is its ligand. RANKL is expressed by OBs, T cells and endothelial cells, and plays a crucial role in OC formation by binding to the RANK present on the cell surface of mononuclear hemopoietic OC precursors to trigger OC formation. Another factor produced by OBs and critical for OC formation is the macrophage colony stimulating factor (M-CSF). While RANKL acts to promote OC precursor fusion, M-CSF is secreted by OBs and promotes OC precursor proliferation as well as RANK expression by these precursors (32).

Stimulated with M-CSF and RANKL, the pre-OCs fuse to form multinucleated cells which begin to express specific OC markers such as cathepsin K and the calcitonin receptor. The binding of RANKL to its receptor activates trimerization of both the ligand and the receptor, triggering an intracellular signaling cascade resulting in NFkB translocation into the nucleus, which ultimately leads to specific OC gene transcription. Notably, RANKL activity can be antagonized by OPG, which is able to sequester RANKL, thus impairing OC differentiation. OPG is a decoy receptor, a soluble molecule that belongs to the TNF receptor superfamily. Like RANKL, OPG is produced by OBs, which shows that OBs play a key role in controlling the process of bone remodeling. Indeed, through modulation of RANKL and OPG expression, OBs can precisely regulate the formation of OCs (30).

As mentioned, knowledge about the RANK-RANKL-OPG system has led to the development of a fully human monoclonal antibody called denosumab (Dmab). Dmab, 60

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mg administered subcutaneously twice a year, showed a significant reduction of vertebral, non-vertebral, and hip fracture relative risk in postmenopausal women with osteoporosis (FREEDOM Study) (33). A resorption phase is followed by an OB-mediated phase, due to release of factors also from OCs (34,35).
Thus, some cell types involved in BRC activity (and their relative biochemical modulating systems) could be important therapeutic targets, as also the vascular component. For example, vascular endothelial growth factor production by OBs shows an anabolic effect on bone tissue, probably linked to increases in vascularization and OB supply to the area of bone formation (36). Among the paracrine, autocrine and endocrine factors modulating OB differentiation and activity, the following are particularly important: bone morphogenetic proteins (BMPs), PTH, fibroblast growth factor, insulin-like growth factor, endothelin-1, prostaglandins, and estrogen. Interestingly, BMP2 is now used as a means of controlling bone fusion at vertebral level and BMP7 for treating traumatic long bone fractures that are not surgically treatable (37).
One of the most important signaling pathways regulating bone formation in OBs is the Wnt/ -catenin signaling pathway. The Wnt proteins are a family of molecules that, at embryogenetic level, modulate the development of different cell types in various tissues, including bone. Wnt signaling, a system increasing OB activity, starts with binding between Wnt and the low-density lipoprotein-related protein 5/6 frizzled receptor; this is an OB activity up-regulation mechanism, which thus aims to increase bone mass. In the absence of Wnt binding to its receptor, -catenin is degraded via the proteasome pathway through the involvement of GSK3- , which phosphorylates and ubiquitinates this protein. When Wnt binds its ligand, GSK-3- is inhibited and -catenin is not degraded, and is instead able to translocate to the nucleus and activate gene transcription. Physiologically, there are a number of inhibitors of this pathway, in particular the glycoprotein sclerostin (SOST, Scl) and Dickoff-1 (38-42).
The differentiation of OBs is started by the activation of Wnt signaling which thus constitutes the trigger of the OB phase (new bone formation phase) of the bone remodeling cycle. Once osteoid matrix production by OB is sufficient, newly formed OTs (derived from OBs entrapped in newly produced bone tissue) produce SOST, which, after travelling along the OT canalicular system, blocks the Wnt pathway, through LRP5 receptor binding, thus preventing Wnt signaling (43).
It is thus clear to see how OTs are able to play a main role in bone remodeling: first, they perceive the problem of microcracks or mechanical stimuli and start the remodeling process and then, when this process has achieved its aim (i.e. a result that is, from a qualitative and quantitative point of view, biomechanically adequate), they give the signal to stop the remodeling work. From a clinical point of view, the inhibition of SOST, through the development of the monoclonal antibody anti-SOST, can increase, in a dose-dependent manner, bone formation markers (44). This monoclonal antibody is under evaluation for use in postmenopausal women with osteoporosis or in the fracture healing process. Anti-SOST might also find a use in "disuse osteoporosis" (45).
One aspect of the biology of bone remodeling that has been studied with growing interest in recent years is the role of cathepsin K, an osteoclastic enzyme that degrades bone matrix during the resorption phase. The use of a specific cathepsin K inhibitor, called odanacatib (ODN), led to a significant decrease in bone resorption markers associated with a less pronounced change in bone formation markers and a significant increase in BMD at various skeletal sites (lumbar spine, total femur, femoral neck) (46). These effects are reversible by stopping the ODN administration. The current dosage is 50 mg a week. The gain in BMD is comparable to that obtained with other potent pharmacological agents, such as zoledronate or Dmab. Interestingly, ODN does not cause OC apoptotis (47), unlike other treatments.
A new and very interesting area from the perspective of the development of new therapeutic agents for osteoporosis is that of microRNA (miRNA) control of bone formation and homeostasis. miRNAs are the class of non-coding, single-stranded, RNA molecules that are composed of approximately 20-24 nucleotides and able to exert a sophisticated level of gene regulation by repressing protein levels in the cell. They thus represent a key epigenetic mechanism for the control of expressed genes (48). It has been estimated that miRNAs regulate ~60% of the human genome.
miRNAs contribute to every step of osteogenesis from embryonic bone development to maintenance of adult bone tissue, by regulating the growth, differentiation and functional activity of the cells that constitute bone tissue (49). Hence it is not surprising that impaired miRNA production/function is associated with many bone diseases. In particular, three polymorphisms predicted in miRNA targeting sites residing in the 3' untranslated region of FGF2 mRNA have been shown to be associated with low BMD in patients with osteoporosis (50), while another study identified miRNA mutations contributing to juvenile osteoporosis in two related adolescents (51). Although studies have yet to address the issue of miRNA-mediated contributions to the progression of osteoporosis in the general population, emerging evidence indicates that miRNAs may represent an attractive therapeutic target in the regulation of the signaling cascade involved in bone remodeling

Concluding remarks

In recent years, knowledge of the mechanisms underlying the recruitment, activation and survival of BMU cells (OCs, OBs, OTs) and the interactions existing between these cells and the other elements of the BRC (lining cells, capillaries), and, ultimately, the discovery of the regulating factors of bone remodeling, have made it possible to develop research strategies geared at finding more selective agents, which work through more physiological mechanisms to protect bone tissue against osteoporosis and related fractures. Some of these agents are already marketed for clinical use (Dmab) and some will become available in the near future (ODN); others (anti-SOST antibody) will be available later.
In any case, clinicians will, within in a relatively short

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pace of time, have other potent agents at their disposal, besides those already available in everyday practice. This will allow them to personalize, more accurately and more effectively, therapeutic strategies to prevent the development and harmful consequences of osteoporosis.

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