Bone Remodeling

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ABSTRACT: The skeleton is a metabolically active organ that undergoes continuous remodeling throughout life. Bone remodeling involves the removal of mineralized bone by osteoclasts followed by the formation of bone matrix through the osteoblasts that subsequently become mineralized. The remodeling cycle consists of three consecutive phases: resorption, during which osteoclasts digest old bone; reversal, when mononuclear cells appear on the bone surface; and formation, when osteoblasts lay down new bone until the resorbed bone is completely replaced. Bone remodeling serves to adjust bone architecture to meet changing mechanical needs and it helps to repair microdamages in bone matrix preventing the accumulation of old bone. It also plays an important role in maintaining plasma calcium homeostasis. The regulation of bone remodeling is both systemic and local. The major systemic regulators include parathyroid hormone (PTH), calcitriol, and other hormones such as growth hormone, glucocorticoids, thyroid hormones, and sex hormones. Factors such as insulin-like growth factors (IGFs), prostaglandins, tumor growth factor-beta (TGF-β), bone morphogenetic proteins (BMP), and cytokines are involved as well. As far as local regulation of bone remodeling is concerned, a large number of cytokines and growth factors that affect bone cell functions have been recently identified. Furthermore, through the RANK/ receptor activator of NF-kappa B ligand (RANKL)/osteoprotegerin (OPG) system the processes of bone resorption and formation are tightly coupled allowing a wave of bone formation to follow each cycle of bone resorption, thus maintaining skeletal integrity.

KEYWORDS: bone remodeling; osteoblast; osteoclast; cytokines

INTRODUCTION

The remarkable increase in the research of bone biology over the last two decades has not only enhanced our understanding of the regulation of bone remodeling but also enabled us to address some of the major unanswered

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questions. Bone remodeling, a complex process by which old bone is continuously replaced by new tissue, requires interaction between different cell phenotypes and is regulated by a variety of biochemical and mechanical factors. This review outlines our current understanding of bone remodeling and its regulation.

Bone and cartilage constitute the skeletal system, which serves two main functions. The first, a structural function, consists of support and protection of vital internal organs and bone marrow as well as the muscle attachment for locomotion. Second, the skeleton has an essential metabolic function serving as a reserve of calcium and phosphate needed for the maintenance of serum homeostasis by contributing to buffering changes in hydrogen ion concentration. Remodeling is the process by which bone is being turned over, allowing the maintenance of the shape, quality, and size of the skeleton. This is accomplished through the repairing of microfractures and the modification of structure in response to stress and other biomechanical forces. This process is characterized by the coordinated actions of osteoclasts and osteoblasts, organized in bone multicellular units (BMU) that follow an activation-resorption-formation sequence of events.

SKELETAL DEVELOPMENT—BONE ORGANIZATION

Bone is a porous mineralized structure made up of cells, vessels, and crystals of calcium compounds (hydroxyapatite). Their proportion varies according to bone types and regions. The processes of cellular differentiation that give rise to the skeleton are regulated by genes, which first establish the pattern of skeletal structure in the form of cartilage and mesenchyme and then replace them with bone through the differentiation of osteoblasts.[1]

The structural components of bone consist of extracellular matrix (largely mineralized), collagen, and cells. Two types of bone are observed in the normal, mature human skeleton: cortical and trabecular.[2] Although macroscopically and microscopically different, the two forms are identical in their chemical composition. *Cortical bone*, which comprises 80% of the skeleton, is dense and compact, has a slow turnover rate and a high resistance to bending and torsion, and constitutes the outer part of all skeletal structures. The major part of the cortical bone is calcified and its function is to provide mechanical strength and protection, but it can also participate in metabolic responses, particularly when there is severe or prolonged mineral deficit. *Trabecular bone* represents 20% of the skeletal mass but 80% of the bone surface is found inside the long bones throughout the bodies of the vertebrae, and in the inner portions of the pelvis and other large flat bones. Trabecular bone is less dense, more elastic, and has a higher turnover rate than cortical bone exhibiting a major metabolic function. Trabecular bone contributes to mechanical support, particularly in

bones such as the vertebrae, and provides the initial supplies of mineral in acute deficiency states.

Bone Matrix

Bone matrix mainly consists of type I collagen fibers (consisting of two a1 chains and one a2 chain) and noncollagenous proteins, and represents approximately 90% of the organic composition of the whole bone tissue. Within lamellar bone, the fibers form arches that allow the highest density of collagen per unit volume of tissue. The lamellae can run parallel to each other (trabecular bone and periosteum), or be concentric surrounding a channel centered on a blood vessel (cortical bone Haversian system). Crystals of hydroxyapatite [3Ca3(PO4)2·(OH)2] are found on the collagen fibers, within them, and in the matrix, and tend to be oriented in the same direction as the collagen fibers.

The role of numerous noncollagenous proteins present in bone matrix has not been fully explained. The major noncollagenous protein produced is osteocalcin (Gla protein), which plays a role in calcium binding, stabilization of hydroxyapatite in the matrix, and regulation of bone formation.[3] Gla protein is a negative regulator of bone formation, which appears to inhibit premature or inappropriate mineralization.[4] In contrast, biglycan, a proteoglycan, is expressed in the bone matrix and positively regulates bone formation.[5]

Osteocytes

Osteoblasts that have been trapped in the osteoid are called osteocytes. Even though the metabolic activity of the osteoblast decreases once it is fully encased in bone matrix, these cells still produce matrix proteins. Osteocytes have numerous long cell processes rich in microfilaments that are organized during the formation of the matrix and before its calcification. They form a network of thin canaliculi permeating the entire bone matrix.

Osteocyte functional activity and morphology varies according to cell age. A young osteocyte has most of the structural characteristics of the osteoblast but decreased cell volume and capacity of protein synthesis. An older osteocyte, located deeper within the calcified bone, presents with a further decrease in cell volume and an accumulation of glycogen in the cytoplasm. The osteocytes are finally phagocytosed and digested during osteoclastic bone resorption.[6]

Despite the complex organization of the osteocytic network, the exact function of these cells remains obscure. It is likely that osteocytes respond to bone tissue strain and enhance bone remodeling activity by recruiting osteoclasts to sites where bone remodeling is required.[7] However, so far there is no direct evidence for osteocytes signaling to cells on the bone surface in response to bone strain or microdamage.

Osteoblast—Bone Formation

The osteoblast is responsible for the production of the bone matrix constituents. Osteoblasts do not function individually but are found in clusters along the bone surface, lining on the layer of bone matrix that they are producing. They originate from multipotent mesenchymal stem cells, which have the capacity to differentiate into osteoblasts, adipocytes, chondrocytes, myoblasts, or fibroblasts.[8] Recent gene deletion studies have shown that absence of runt-related transcription factor 2 (Runx2) or of a downstream factor, osterix, is critical for osteoblast differentiation.[9] Toward the end of the matrix-secreting period, 15% of mature osteoblasts are entrapped in the new bone matrix and differentiate into osteocytes. On the contrary, some cells remain on the bone surface, becoming flat lining cells.

Bone formation occurs in three successive phases: the production and the maturation of osteoid matrix, followed by mineralization of the matrix. In normal adult bone, these processes occur at the same rate so that the balance between matrix production and mineralization is equal. Initially, osteoblasts produce osteoid by rapidly depositing collagen. This is followed by an increase in the mineralization rate to equal that of collagen synthesis. In the final stage the rate of collagen synthesis decreases and mineralization continues until the osteoid becomes fully mineralized.

Osteoblasts produce a range of growth factors under a variety of stimuli including the insulin-like growth factors (IGF),[10] platelet-derived growth factor (PDGF),[11] basic fibroblast growth factor (bFGF),[12] transforming growth factor-beta (TGF-β),[13] and the bone morphogenetic proteins (BMP).[14] Osteoblast activity is regulated in an autocrine and paracrine manner by these growth factors, whose receptors have been found on osteoblasts. Receptors for classical hormones such as parathyroid hormone, parathyroid hormone-related protein, thyroid hormone,[15] growth hormone,[16] insulin,[17] progesterone,[18] and prolactin[19] are located in osteoblasts as well. Osteoblastic nuclear steroid hormone receptors include receptors for estrogens,[20] androgens,[21] vitamin D3,[22] and retinoids.[23]

Osteoclast–Bone Resorption

The osteoclast, a giant multinucleated cell up to 100 mm in diameter, derives from hematopoietic cells of the mononuclear lineage[24] and is the bone lining cell responsible for bone resorption. It is usually found in contact with a calcified bone surface and within a lacuna (Howship's lacunae) as a result of its own resorptive activity.

Osteoclasts have abundant Golgi complexes, mitochondria, and transport vesicles loaded with lysosomal enzymes. They present deep foldings of the plasma membrane in the area facing the bone matrix (called ruffled border)

and the surrounding zone of attachment (called sealing zone). Lysosomal enzymes such as tartrate-resistant acid phosphatase and cathepsin K are actively synthesized by the osteoclast and are secreted via the ruffled border into the bone-resorbing compartment.[25]

The process of the osteoclast attachment to the bone surface involves binding of integrins expressed in osteoclasts with specific amino acid sequences within proteins at the surface of the bone matrix.[26] After osteoclast adhesion to the bone matrix, avb3 integrin binding activates cytoskeletal reorganization within the osteoclast.[27] Attachment usually occurs via dynamic structures called podosomes. Through their continual assembly and disassembly they allow osteoclast movement across the bone surface during which bone resorption proceeds. Integrin signaling and subsequent podosome formation is dependent on a number of adhesion kinases including the proto-oncogene src.[28]

Osteoclasts resorb bone by acidification and proteolysis of the bone matrix and of the hydroxyapatite crystals encapsulated within the sealing zone. The first process during bone matrix resorption is mobilization of the hydroxyapatite crystals by digestion of their link to collagen. Then the residual collagen fibers are digested by either cathepsins or activated collagenases and the residues from this digestion are either internalized or transported across the cell and released at the basolateral domain. Osteoclast function is regulated both by locally acting cytokines and by systemic hormones. Osteoclastic receptors for calcitonin,[29] androgens,[30] thyroid hormone,[31] insulin,[32] PTH,[33] IGF-1,[34] interleukin (IL)-1,[35] CSF-1,[36] and PDGF[37] have been demonstrated.

BONE REMODELING

Bone is a living organ that undergoes remodeling throughout life. Remodeling results from the action of osteoblasts and osteoclasts, and defects such as microfractures are repaired by their coupling. In a homeostatic equilibrium resorption and formation are balanced so that old bone is continuously replaced by new tissue so that it adapts to mechanical load and strain. In 1990 Frost defined this phenomenon as bone remodeling.[38]

Osteoclasts and osteoblasts closely collaborate in the remodeling process in what is called a basic multicellular unit (BMU). The organization of the BMUs in cortical and trabecular bone differs, but these differences are mainly morphological rather than biological. In cortical bone the BMU forms a cylindrical canal about 2,000 μm long and 150–200 μm wide and gradually burrows through the bone with a speed of 20–40 μm/day. During a cycle 10 osteoclasts dig a circular tunnel in the dominant loading direction[39] and then they are followed by several thousands of osteoblasts that fill the tunnel.[40] In this manner, between 2% and 5% of cortical bone is being remodeled each year. The trabecular bone is more actively remodeled than cortical bone due to the much

larger surface to volume ratio. Osteoclasts travel across the trabecular surface with a speed of approximately 25 μm/day, digging a trench with a depth of 40–60 μm.

The remodeling cycle consists of three consecutive phases: resorption, reversal, and formation. Resorption begins with the migration of partially differentiated mononuclear preosteoclasts to the bone surface where they form multinucleated osteoclasts. After the completion of osteoclastic resorption, there is a reversal phase when mononuclear cells appear on the bone surface. These cells prepare the surface for new osteoblasts to begin bone formation and provide signals for osteoblast differentiation and migration. The formation phase follows with osteoblasts laying down bone until the resorbed bone is completely replaced by new. When this phase is complete, the surface is covered with flattened lining cells and a prolonged resting period begins until a new remodeling cycle is initiated. The stages of the remodeling cycle have different lengths. Resorption probably continues for about 2 weeks, the reversal phase may last up to 4 or 5 weeks, while formation can continue for 4 months until the new bone structural unit is completely created.

The assumption that a coupling mechanism must exist between bone formation and resorption was first reported in 1964.[41] However, the exact molecular mechanism that describes the interaction between cells of the osteoblastic and osteoclastic lineages was only recently identified.[42] The bone remodeling cycle begins with activation mediated by cells of the osteoblast lineage. Activation may involve the osteocytes, the lining cells, and the preosteoblasts in the marrow. The exact cells of the osteoblast lineage responsible have not been fully defined. These cells undergo changes in their shape, they secrete enzymes that digest proteins on the bone surface and express a 317 amino acid peptide, member of the tumor necrosis factor (TNF) superfamily, called receptor activator of NF-kappa B ligand (RANKL). RANKL interacts with a receptor on osteoclast precursors called RANK. The RANKL/RANK interaction results in activation, differentiation, and fusion of hematopoietic cells of the osteoclast lineage so that they begin the process of resorption. Furthermore, it also prolongs osteoclast survival by suppressing apoptosis.[43] This interaction indicates that bone resorption and bone formation are coupled among others through RANKL.

The effects of RANKL are blocked by osteoprotegerin (OPG), a secretory dimeric glycoprotein belonging to the TNF receptor family with a molecular weight of 120 kDa. OPG acts as a decoy receptor (a soluble receptor acting as antagonist) for RANKL and it is mainly produced by cells of the osteoblast lineage, but it can also be produced by the other cells in the bone marrow.[44,45] OPG regulates bone resorption by inhibiting the final differentiation and activation of osteoclasts and by inducing their apoptosis. OPG is not incorporated into bone matrix and its effects on bone resorption are therefore fully reversible.

REGULATION OF BONE REMODELING

The overall integrity of bone appears to be controlled by hormones and many other proteins secreted by both hemopoietic bone marrow cells and bone cells. There is both systemic and local regulation of bone cell function.

Systemic Regulation

Parathyroid hormone is the most important regulator of calcium homeostasis. It maintains serum calcium concentrations by stimulating bone resorption, increasing renal tubular calcium reabsorption and renal calcitriol production. PTH stimulates bone formation when given intermittently and bone resorption when secreted continuously.[46] *Calcitriol* is essential in enhancing intestinal calcium and phosphorus absorption, and in this way it promotes bone mineralization. In addition, vitamin D3 possesses important anabolic effects on bone, thus exerting a dual effect on bone turnover.[47] *Calcitonin*, in pharmacologic doses, mediates loss of the ruffled border, cessation of osteoclast motility, and inhibition of the secretion of proteolytic enzymes through its receptor on osteoclasts. This effect, however, is dose limited and its physiologic role is minimal in the adult skeleton. The growth hormone (GH)/IGF-1 system and IGF-2 are important for skeletal growth, especially at the cartilaginous end plates and during endochondreal bone formation. They are among the major determinants of adult bone mass through their effect on regulation of both bone formation and resorption.[48] *Glucocorticoids* exert both stimulatory and inhibitory effects on bone cells. They are essential for osteoblast maturation by promoting their differentiation from mesenchymal progenitors but they decrease osteoblast activity. Furthermore, glucocorticoids sensitize bone cells to regulators of bone remodeling and they augment osteoclast recruitment.[49] *Thyroid hormones* stimulate both bone resorption and formation. Thus, bone turnover is increased in hyperthyroidism and therefore bone loss can occur.[50] *Estrogens* decrease the responsiveness of the osteoclast progenitor cells to RANKL, thereby preventing osteoclast formation.[51] Furthermore, besides reducing osteoclast life span,[52] estrogens stimulate osteoblast proliferation and decrease their apoptosis. They affect gene coding for enzymes, bone matrix proteins, hormone receptors, transcription factors, and they also upregulate the local production of OPG, IGF I, IGF II, and TGF-β.[53] *Androgens* are essential for skeletal growth and maintenance via their effect on androgen receptor, which is present in all types of bone cells.[54]

Local Regulation

As far as the local regulation of bone cell function is concerned, after the recent discovery of the OPG/RANKL/RANK system, there is a clearer picture

regarding the control of osteoclastogenesis and bone remodeling in general. RANKL, expressed on the surface of preosteoblastic/stromal cells binds to RANK on the osteoclastic precursor cells and is critical for the differentiation, fusion into multinucleated cells, activation, and survival of osteoclastic cells.[43] OPG inhibits the entire system by blocking the effects of RANKL.[45] Macrophage colony-stimulating factor (M-CSF), which binds to its receptor, c-Fms, on preosteoclastic cells, appears to be necessary for osteoclast development because it is the primary determinant of the pool of these precursor cells.[55]

The opposite phenotypes of OPG overexpression or with RANKL deletion mice (osteopetrosis) and OPG-deficient or with RANKL overexpression (osteoporosis) have led to the hypothesis that OPG and RANKL can be the mediators for the stimulatory or inhibitory effects of a variety of systemic hormones, growth factors, and cytokines on osteoclastogenesis. This is recently referred to as "the convergence hypothesis" in that the activity of the resorptive and antiresorptive agents "converges" at the level of these two mediators, whose final ratio controls the degree of osteoclast differentiation, activation, and apoptosis.[56]

A number of cytokines such as TNF-α and IL-10 modulate this system primarily by stimulating M-CSF production and by directly increasing RANKL expression.[57] In addition, a number of other cytokines and hormones exert their effects on osteoclastogenesis by regulating cell production of OPG and RANKL (see TABLE 1). Furthermore, IL-6, a pleiotropic cytokine secreted by osteoblasts, osteoclasts, and stromal cells, appears to be an important regulator of bone remodeling by stimulating osteoclastic bone resorption[65] but also by promoting osteoblast generation in conditions of high bone turnover.[66] Recent studies have also suggested that osteoblast-derived PTHrP promotes the recruitment of osteogenic cells and prevents the apoptotic death of osteoblasts, thus being an important regulator of bone cell function.[67]

Abnormalities of bone remodeling can produce a variety of skeletal disorders. The recent advances concerning systemic and local regulation of bone remodeling have led to new approaches in the diagnosis and treatment of

TABLE 1. Effects of cytokines and hormones on bone remodeling through RANKL and OPG secretion

	RANKL	OPG
Transforming growth factor-β[58]	–	↑
Parathyroid hormone[59]	↑	↓
$1,25(OH)_2$ vitamin D_3[60]	↑	–
Glucocorticoids[61]	↑	↓
Estrogen[62]	–	↑
Basic fibroblast growth factor 2[63]	↑	↓
Prostaglandin E_2[64]	↓	↑

skeletal disorders. In particular, the newer methods in molecular and cellular biology aid to define the abnormalities in cells of the osteoblastic and osteoclastic lineages that lead to bone disease and to develop new therapeutic approaches based on a better understanding of the pathogenetic mechanisms. These involve production of recombinant molecules of cytokines and their soluble receptors, development of inhibitory peptides, and specific inhibition of key signaling pathways.

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