Synthetic Function and Regulation of Osteoblasts: Current Knowledge and Applications

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INTRODUCTION

In 1886, the histologist Julius Wolff proposed that "every change in the function of a bone is followed by certain definite changes in internal architecture and external conformation in accordance with mathematical principles" (1). Wolff's hypothesis that "form follows function" has spurred intense research over the past century into the cellular and molecular mechanisms underlying the pathophysiological changes in bone.

Osteoblasts play a principal role in bone formation. These cells not only regulate new bone formation, but also indirectly mediate osteoclastic bone resorption. Studies examining the effects of mechanical stress and electrical energy on bone remodeling (2-5) support the notion that osteoblasts transform stimuli into chemical signals that trigger either bone formation or resorption in accordance with Wolff's law (1). In health, osteoblasts maintain an equilibrium between bone formation and bone resorption. This tightly regulated link, termed "coupling," allows the skeleton to maintain its structural and anatomical functions (6,7).

Bone is continuously remodeled throughout life via the breakdown of older osteons and the replacement with new ones. Disorders of bone remodeling can lead to skeletal problems of either too much or, more commonly, too little bone (8,9). Osteoporosis and other states of pathological bone remodeling such as osteomalacia, osteogenesis imperfecta, Paget's disease, and bone tumors currently account for significant morbidity and mortality in North America (10). Thus, considerable research efforts have been aimed at understanding the cellular and molecular steps in bone remodeling. This review article discusses current knowledge of the origin and differentiation, synthetic functions, and osteotrophic regulation of osteoblasts. Potential clinical applications in the management of bone diseases are also reviewed.

ORIGIN AND DIFFERENTIATION

Osteoblasts are of mesenchymal origin, arising primarily from stromal cells found in bone marrow. At times, the periosteum can serve as an important reservoir of osteoblasts, particularly during childhood growth, after a skeletal fracture, or with bone-forming tumors. Ontogeny occurs along a continuum, beginning with pluripotent stem cells and ending with terminally differentiated osteocytes. Pluripotent stromal stem cells
have an endless capacity for renewal but are unable to synthesize many of the proteins necessary for bone formation. Some of these stromal precursors, under the influence of growth factors and other stimuli, evolve into the more differentiated osteoblasts (11). With differentiation, new genes are activated while others are inhibited, and the cells gradually and systematically change their phenotypes. From the undifferentiated, pluripotent stromal stem cells arises a group of mesenchymal cells that have been termed "inducible osteoprogenitor cells" (IOPC's) (12,13). The IOPC's respond to bone morphogenetic protein (BMP) and other growth factors, eventually differentiating into "determined osteoprogenitor cells" (DOPC's) (12,13). The DOPC's, under the influence of a variety of systemic and bone-derived growth factors, mature into osteoblasts. Interestingly, both the IOPC and DOPC populations appear to have a limited number of cell divisions and therefore, unlike the pluripotent stem cells, are not completely self-renewing (14). Both the IOPC's and DOPC's are found within the bone marrow and give rise to orthotopic (intraskelatal) bone formation. However, IOPC's are also found in other connective tissues and therefore can be a source of heterotopic (extraskeletal) bone formation (11,12). Dysregulation of these precursor cells may manifest clinically in diseases such as fibrodyplasia ossificans progressiva, a genetic disease characterized by the ossification of skeletal muscle and other connective tissues (15), or in the periariticular heterotopic ossification that can complicate skeletal trauma (16).

Synthesis, deposition, and mineralization of the organic matrix of bone are complex processes that require the ordered expression of a number of osteoblast genes. The maturation sequence of osteoblasts has been divided into three consecutive phases: proliferation, extracellular matrix maturation, and mineralization (Fig. 1). Osteoblasts that have become encased in mineralized matrix are termed osteocytes. Morphologically, osteocytes resemble osteoblasts in every respect, having prominent nucleoli, abundant RER, and a perinuclear Golgi apparatus. Biochemically, they produce the same proteins as fully mature osteoblasts. However, as more mineral is laid down and osteocytes become encased deeper within the matrix, biochemical and morphological changes gradually occur that distinguish them from their precursors. As a result of the decreased synthetic capacity and cytoplasmic volume, the nuclei become the most prominent osteocytic cellular features (17,18).

SYNTHETIC FUNCTION

The principal role of osteoblasts is to form new bone via the synthesis of various proteins and polysaccharides. Other functions include the regulation of bone remodeling and mineral metabolism. Studies of osteoblastic cells isolated from trabecular bone, embryonic calvaria, and osteosarcoma have established a set of phenotypic markers of osteoblastic function (Fig. 1) (19,20). These include the synthesis of type I collagen, the expression of alkaline phosphatase (ALP), the secretion of osteocalcin, and the production of mineralized matrix (8,9,21,22). The phenotype is modulated by 1, 25-(OH)2 D3 (vitamin D3), parathyroid hormone (PTH), glucocorticoids, prostanoids, and a number of cytokine growth factors (23-28).

Type I Collagen

Type I collagen is the single most abundant protein in the human body and the major organic component of bone matrix. Collagen's basic structure consists of three polypeptide chains, each with a repeating primary amino acid sequence of -glycine-X-Y-.

The production of collagen by osteoblasts heralds their differentiation into a more mature phenotype (29). Type I collagen is produced abundantly during the proliferative phase of osteoblast development and is the first recognizable marker of differentiation (Fig. 1). Osteoblasts synthesize type I collagen molecules that are staggered end-to-end to form fibrils which give the characteristic cross-bandung pattern seen by electron microscopy. Current understanding of the role of type I collagen has come from in vitro studies. If the deposition of the collagenous matrix is disrupted in vitro, differentiation will be arrested (30). In
mesenchymal precursor cells, type I collagen synthesis is always linked with that of type III collagen; however, once cells are committed to the osteoblast lineage, type III collagen is no longer expressed.

Disordered type I collagen synthesis by osteoblasts becomes apparent in a variety of diseases. Errors in processing can give rise to abnormalities in collagen structure. For example, osteogenesis imperfecta (brittle bone syndrome) results from a defect in either the coding or processing of collagen and gives rise to a heterogeneous group of genetic diseases characterized by increased bone fragility (31).

The hydroxylation of collagen is necessary for interchain cross-linking, the extent of which is directly proportional to tensile strength. Defective lysyl oxidase activity, an enzyme that modulates cross-linking, can result in one variant of the Ehlers-Danlos syndrome. Marfan's syndrome is another genetic disease caused by defective collagen cross-linking. Both of these diseases are characterized by increased bone fragility and joint hypermobility (Table 1) (32).

**Alkaline Phosphatase**

The most widely measured osteoblast marker is alkaline phosphatase (ALP), a ubiquitous enzyme, which catalyzes the hydrolysis of phosphate esters at an alkaline pH (33). In humans, three isoenzymes are currently known: (i) tissue non-specific, (ii) intestinal, and (iii) placental. The tissue non-specific isoenzyme has three isoforms, from bone, liver, and kidney, which contain different carbohydrate moieties on the same polypeptide backbone (34,35). The skeletal isoform of ALP is a glycoprotein found on the cell membrane of osteoblasts (33,34,36).

Gene expression for this ectoenzyme characteristically begins immediately following the cessation of cell proliferation. It then reaches a maximum during the phase of matrix maturation and declines as matrix mineralization commences (Fig. 1) (19,30). Several studies have demonstrated the importance of ALP in bone matrix mineralization. For example, a missense mutation in the ALP gene results in the potentially lethal clinical syndrome known as hypophosphatasia, a disease characterized by abnormal mineralization of the skeleton (37). Furthermore, when the gene for ALP is transfected into ALP-negative cells, they become capable of mineralization in vitro (38). Another study has demonstrated that vesicles released from osteoblast-like cells contain ALP, which subsequently enables matrix mineralization in vitro (39). Extensive studies have been undertaken at the cellular level to gain an understanding of the expression and synthesis of ALP. The expression of ALP by osteoblasts has been shown to be regulated by a wide variety of systemic and local factors (21,22).

Total ALP activity has been recognized as a reliable indicator of osteoblast function. For example, several human osteosarcoma cell lines have been characterized as osteoblastic by their ability to express ALP in culture (39,40). In addition, plasma ALP levels have been used clinically to monitor the excess activity of osteoblasts associated with certain physiological and pathological conditions. For example, Leung et al. recently demonstrated that the plasma levels of the ALP specific to bone is a sensitive and reliable measure of the healing response following fractures (41). ALP is also used as a marker for osteoblastic activity in certain bone-forming tumors.

**Osteocalcin**

Osteocalcin is the major non-collagenous protein of bone. It is a low molecular weight vitamin K-dependent protein that is synthesized exclusively by osteoblasts and odontoblasts. When osteocalcin was first discovered, it was called bone Gla protein (BGP) because it contained three carboxyglutamic acid (Gla) residues (42). Gla is an amino acid formed by the vitamin K-dependent post-translational carboxylation of glutamate in the endoplasmic reticulum. Gla residues are also found in the vitamin K-dependent clotting factors II, VII, IX, and X, where their specific role is to bind calcium ions (43). The specific tertiary protein
The precise biological function of osteocalcin has not yet been fully elucidated. Osteocalcin is produced by mature osteoblasts during the mineralization phase and is only marginally detectable during the earlier phases of proliferation and matrix maturation (Fig. 1). Some investigators have suggested that osteocalcin may act either as a cytokine or as a chemoattractant for osteoblasts, osteoclasts, and blood monocytes (44,45). It has been shown that serum and synovial fluid osteocalcin levels represent the activity of fully differentiated non-dividing osteoblasts and correlate with new bone formation. As a result, they have been shown to be elevated in infants, children, and in patients with conditions characterized by increased bone metabolism, such as Paget's disease, osteomalacia, pathological bone resorption, and osteitis fibrosa cystica (Table 1) (46).

Several studies have established a positive modulatory role for vitamin D3 on osteocalcin biosynthesis (24,25,47,48). PTH, on the other hand, seems to have a paradoxical effect in osteoblasts, increasing their mRNA levels, while, at the same time inhibiting protein synthesis (47,48).

**Other Osteoblast Products**

There are several other non-collagenous proteins found in the organic bone matrix, including osteonectin, osteopontin, bone sialoprotein, biglycan, decorin, matrix Gla-protein, and bone acidic glycoprotein (8,9). Most of these proteins have only recently been discovered and investigations are currently in progress to determine their specific structures and functions (49). Briefly, osteonectin is produced by osteoblasts and may mediate the deposition of hydroxyapatite, bind to growth factors, and influence the cell cycle. Osteopontin, although not associated specifically with bone, may be implicated in regulating mineralization. Similarly, bone sialoprotein, which is bone specific, and bone acidic glycoprotein are also thought to play a role in mineralization. Biglycan and decorin are proteoglycans that may bind to collagen and various growth factors in the matrix. Finally, matrix Gla-protein may be involved in cartilage metabolism.

**OSTEOTROPIC REGULATION**

Many growth factors influence bone formation by direct action on osteoblasts. Chief factors include the systemic hormones vitamin D3, PTH, and growth hormone. The discovery of autocrine and paracrine growth factors, particularly transforming growth factor-beta (TGF-ß) and prostaglandin E2 (PGE2), has added further insight into the mechanism behind bone remodeling.

**Vitamin D3**

The activated molecule 1,25-(OH)2 D3 is an important physiological regulator of mineral metabolism. It is a steroid hormone synthesized in response to hypocalcemia and hypophosphatemia (50).

Several allelic variants in the gene for the vitamin D3 receptor have been described (51,52). Recent investigations by Morrison et al. have reported an association between bone mineral density and the specific allelic variants in the gene encoding the vitamin D3 receptor. This has important clinical ramifications for osteoporosis. Consequently, they have proposed a genetic screening method that may be able to predict the development of postmenopausal osteoporosis (52).

The target tissues of vitamin D3 are the intestine, bone, kidney, and parathyroid glands. As described earlier, activated vitamin D3 influences a spectrum of osteoblastic cellular functions and is an important cell differentiation factor (21). Effects on cultured osteoblasts include the inhibition of cell proliferation (26,27), the stimulation of ALP (38,39) and osteocalcin production (24,25), the modulation of type I collagen production (53), and the release of cytokines and matrix proteins (21,26,27). Vitamin D3 also has potent bone resorbing effects that are mediated through the osteoblast.
The physiological importance of its effects becomes readily apparent during states of either vitamin D3 deficiency or resistance (50,54). Rickets and osteomalacia are two examples that illustrate the physiological role of vitamin D3. Inadequate mineralization of bone is the hallmark pathological feature of both processes. Histologically, there is an increase in osteoid (unmineralized organic matrix), which is due to the failure to maintain serum calcium-phosphate ion levels sufficient for normal osteoblast-mediated mineralization (55). Rickets develops in the growing skeleton of children and adolescents, and classically develops from the combination of inadequate sun exposure and vitamin D3 deficiency (low dietary intake, malabsorption syndromes, liver disease, and genetic disorders affecting vitamin D3). While it has become rare in western countries due to the widespread supplementation of dairy products, rickets continues to remain a major public health concern worldwide. Clinical symptoms include bone pain, tetany, convulsions, and bone deformity. Histologically, apart from the increase in osteoid, the most remarkable changes are seen as widened epiphyses and costochondral junctions (rachitic rosary).

Osteomalacia, on the other hand, appears in the mature adult skeleton. In osteomalacia there is generalized bone loss with resulting cortical thinning. Associated with the same etiological factors as rickets, osteomalacia presents typically with bone pain, muscular weakness, or pathological fracture. Both conditions have been treated, in part, with vitamin D supplementation.

**Parathyroid Hormone**

PTH, an 84-amino acid peptide hormone synthesized by the parathyroid glands, is an important modulator of calcium homeostasis. While PTH secretion is stimulated by a low plasma ionic calcium concentration, other divalent cations, such as magnesium, strontium, and manganese also influence its release (56).

The principal target organs for PTH are the kidneys and bone. In bone, PTH causes an increase in osteoclastic bone resorption and bone remodeling. Bone resorption elevates the serum ionized calcium level. Meanwhile, bone remodeling results from the breakdown of bone by osteoclasts and the synthesis of a new organic matrix by osteoblasts that have infiltrated the area.

PTH mediates its effect by binding to extracellular membrane-bound receptors that activate an intracellular adenylate cyclase-dependent signal transduction system. When activated, adenylate cyclase causes an increase in intracellular cyclic adenosine monophosphate (cAMP). As osteoclasts are devoid of PTH receptors, their response to PTH is dependent on PTH binding to the membrane-bound receptors on osteoblasts, which then indirectly mediate the osteoclastic resorption through secondary coupling factors. Through this mechanism, PTH can modulate both bone resorption and bone formation (28). PTH simultaneously elevates the serum ionized calcium level by the release of calcium ions from the bone matrix and promotes bone remodeling through the activation of osteoblasts and osteoclasts (28).

The effects of excess PTH on the skeletal system illustrate the important physiological regulatory role of this hormone. Hyperparathyroidism occurs as the result of excess PTH secretion by the parathyroid gland and may be either primary or secondary. Typical laboratory findings include hypercalcemia and hypophosphatemia (56). Secondary hyperparathyroidism is a state of compensatory hypersecretion of PTH, which generally occurs in a state of chronic hypocalcemia. It often results from abnormal vitamin D3 metabolism with a subsequent decrease in serum calcium, and is associated with diseases such as renal failure, rickets, osteomalacia, and malabsorption syndromes.

Whether primary or secondary in nature, elevated PTH stimulates osteoblasts, which in turn signals osteoclasts to resorb bone. The excess resorption may produce characteristic skeletal lesions known as osteitis fibrosa cystica that can cause bone pain, pathological fractures, bone cysts, and localized bony swellings (57). Radiographic findings of excess osteoblast-mediated osteoclastic activity include subperiosteal bone resorption, generalized osteopenia, demineralization of the skull, and bone cysts or brown
tumors. These cysts show up as areas of radiolucency, particularly in the long bones.

Recent studies have shown potential therapeutic applications for PTH in the treatment of osteoporosis. In osteopenic experimental animals, PTH has been shown to increase trabecular and cortical bone mass (58,59). Currently, however, PTH administration is limited to the parenteral route as it is denatured by the acidic pH of the stomach.

**Growth Hormone**

Growth hormone (GH) is a peptide hormone released by the anterior pituitary gland. Its secretion is regulated by two hypothalamic regulatory hormones, growth hormone releasing hormone (GHRH) and somatostatin, which stimulate and inhibit its release, respectively. GH has diverse metabolic effects that may be broadly categorized as anabolic, diabetogenic, and lipolytic. Furthermore, many physiological effects of GH are mediated through the GH-induced release of insulin-like growth factor-1 (IGF-1).

Evidence from pathological conditions of either GH excess or deficiency shows that GH promotes skeletal growth by binding to membrane-bound GH receptors on the activated osteoblasts (60). In the growing skeletal system (open epiphyses), GH acts directly on the epiphyseal plate to stimulate longitudinal growth. The cytokine IGF-1, which is subsequently released from target cells, induces chondrocyte maturation.

The clinical manifestations of GH excess vary according to the age of onset. In the young, immature skeleton, gigantism may develop. In the adult skeleton, excess GH will cause acromegaly. In acromegals, the hands and feet enlarge, the skull grows in diameter, and the brow becomes more pronounced (frontal bossing). In contrast, the manifestations of GH deficiency have distinct consequences, depending on the age of onset. In the adult, there is generally little effect; however, in children, the result is either short stature or, in severe congenital cases due to complete GH absence or defective GH receptors, dwarfism (60).

It has been hypothesized that GH may play a role in the development of osteoporosis; specifically, a decreased osteoblast responsiveness to GH may contribute to postmenopausal osteoporosis. However, Kassem et al. were unable to demonstrate any difference in response between stromal osteoblasts isolated from osteoporotic women and age matched controls (61). There has also been interest in the use of exogenous GH as a possible osteoinductive agent that could be used clinically to stabilize prosthetic skeletal implants by stimulating new bone formation. Clifford et al. have recently shown that GH-loaded biomaterials actually increase osteoblastic bone formation around implants (62).

**Cytokines**

Cytokines and growth factors are important mediators of cell-to-cell communication. They can also mediate the effects of many systemic hormones locally. Acting in autocrine and paracrine ways, they can promote cell proliferation, cell differentiation, bone formation, and bone resorption (Table 2). Prostaglandins, especially PGE2, are produced by osteoblasts, stimulate osteoclastic bone resorption, and may play a central role in the skeletal response to physical stress. This is supported by the finding that cultured osteoblasts release PGE2 when physiological strains are applied (63,64).

The osteotropic factors include cytokines that can affect the recruitment and differentiation of osteoprogenitor cells and/or the function of the mature osteoblast. As discussed previously, BMP's stimulate the IOPC's to differentiate into the DOPC's (12,13). TGF-β and IGF-1 are reported to have stimulatory effects on both osteoprogenitor cells and mature osteoblasts (65). Fibroblast growth factors (FGF's), both acidic (a-FGF) and basic (b-FGF), stimulate the proliferation of osteoblasts in culture, while platelet derived growth factor (PDGF) acts as a potent mitogen for all cells of mesenchymal origin (66,67). Moreover, both BMP and TGF-β have been shown to promote osteoblastic bone formation in animals (68,69). For example, recent
experimental work has shown that TGF-β can actually hasten fracture healing in a rat model (69). A summary of the effects of cytokines on osteoblasts is included in Table 2. The potential clinical implications of cytokines, though still largely experimental, include their use as therapeutic agents for the treatment of skeletal disorders such as osteoporosis, fracture healing, and Paget's disease.

SUMMARY

Osteoblasts play a central role in the skeletal system. Derived from pluripotent mesenchymal stromal stem cells, they develop along a specific, tightly regulated pathway in which morphology and biosynthesis come to reflect their tremendous synthetic function. In response to local, systemic, and mechanical triggers, osteoblasts vigorously synthesize new extracellular matrix proteins, such as type I collagen and osteocalcin, facilitate bone mineralization by expressing alkaline phosphatase and other factors, and continuously orchestrate bone remodeling through their interaction with osteoclasts.

Because bone is a multipurpose organ that not only serves as the principal structural support for the body, but also as an important mineral depository and site for hematopoiesis, the role of osteoblasts in regulating bone remodeling is essential. In the future, a more detailed understanding of both normal and aberrant functioning of these cells promises to allow a more elegant approach to the treatment of diverse types of skeletal diseases.

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