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Growth Factor Control of Bone Mass

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ABSTRACT

Bone formation is determined by the number and function of osteoblasts. Cell number is governed by factors that regulate the replication and differentiation of pre-osteoblasts and factors that regulate osteoblastic cell death. Cell function is controlled by signals acting on the mature osteoblast. Platelet-derived and fibroblast growth factors are bone cell mitogens. Bone morphogenetic proteins (BMPs) and Wnt induce the differentiation of mesenchymal cells toward osteoblasts, and insulin-like growth factor (IGF)-I stimulates the function of mature osteoblasts and prevents their death. The activity of BMP, Wnt, and IGF-I is modulated by extracellular antagonists or binding proteins. Changes in growth factor synthesis and activity may play a role in the pathogenesis of selected forms of osteoporosis, and alterations in the expression or binding of the extracellular antagonists can be associated with changes in bone mass. Current approaches to bone anabolic therapies for osteoporosis include the administration of a growth factor, such as IGF-I, or the neutralization of an antagonist. Ideally, the targeting of an anabolic agent should be specific to bone to preclude non-skeletal unwanted side effects. Clinical trials are needed to determine the long-term effectiveness and safety of novel anabolic agents for the management of osteoporosis. J. Cell. Biochem. 108: 769–777, 2009.

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Bone remodeling is a tightly regulated process resulting in the coordinated resorption and formation of skeletal tissue, carried out in basic multicellular units (BMUs). There, osteoclasts resorb bone and when resorption is completed, a reversal period follows after which osteoblasts fill the cavity with new collagenous matrix. Following a resting phase, the matrix is mineralized. Osteoclasts are multinucleated cells derived from pluripotential hematopoietic cells, and osteoblasts are mononuclear cells derived from mesenchymal cells [Canalis, 2005]. Signals that determine the replication, differentiation, function, and death of cells of both lineages will dictate bone remodeling and determine whether bone tissue is gained, lost, or in balance. Bone remodeling is necessary to maintain calcium homeostasis and to remove bone, preventing the accumulation of aged or weakened bone. In postmenopausal osteoporosis, bone resorption exceeds bone formation leading to a negative skeletal balance. Consequently, anti-resorptive agents, such as bisphosphonates, can restore the balance and are frequently used in the treatment of the disease. However, established severe osteoporosis may require the use of a bone forming or anabolic agent. In the United States, the only anabolic agent approved for the treatment of osteoporosis is a 34 amino acid fragment of recombinant human parathyroid hormone [PTH(1–34)] or teriparatide. The target cell of an anabolic agent is a cell of the osteoblastic lineage, and an increase in bone formation can be achieved by increasing the number or the activity of these bone forming cells (Table I). Signals that determine the replication and differentiation of pre-osteoblastic cells, those that determine the function of mature osteoblasts, as well as the death of these cells are central to the processes that govern bone formation. Anabolic agents can act on these signals increasing the osteoblast cellular pool or the function of the mature cell.

Growth factors can regulate the replication, differentiation, and function of bone cells. There are no growth factors specifically synthesized by skeletal cells, and growth factors are expressed in a variety of tissues. However, skeletal growth factor synthesis or activity can be targeted to bone tissue by agents that act on skeletal cells, such as PTH. Growth factors synthesized by skeletal cells may be present in the systemic circulation and act both as local and systemic regulators of bone remodeling. Some growth factors, such as platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF), display primarily mitogenic activity for cells of the osteoblastic lineage [Canalis, 2007]. Other factors, such as bone

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TABLE I. Possible Mechanisms to Increase Bone Formation

Increase the osteoblast cell pool
Increase cell replication
Induce differentiation
Inhibit cell death
Increase osteoblast function

morphogenetic proteins (BMPs) and Wnt, induce the differentiation of cells of the osteoblastic lineage into mature osteoblasts playing a fundamental role in osteoblastogenesis [Canalis et al., 2003; Krishnan et al., 2006]. Factors, such as insulin-like growth factor (IGF), target the mature osteoblast enhancing its differentiated function [Gazzerro and Canalis, 2006]. It is noteworthy that the activity of BMPs, Wnt, and IGF-I is controlled by specific extracellular binding proteins or antagonists and intracellular regulatory proteins. Consequently, the anabolic activity of these factors could be altered by modifying the expression or activity of an antagonist or binding protein.

GROWTH FACTORS WITH MITOGENIC PROPERTIES FOR SKELETAL CELLS

PDGF has mitogenic properties for cells of the osteoblastic lineage and has the capacity to increase the osteoblastic cell pool, but PDGF does not enhance the differentiated function of the osteoblast [Canalis, 2007]. Skeletal cells express three *pdgf* genes, *pdgfa*, *pdgfb*, and *pdgfc*, indicating that PDGF may act as an autocrine regulator of skeletal cell function [Canalis, 2007]. However, the principal source of PDGF is the systemic circulation, and skeletal cells are likely to become exposed to significant concentrations of PDGF following platelet aggregation. The release of PDGF following platelet aggregation could play a role in fracture repair, since PDGF could rapidly increase a population of bone forming cells. However, additional signals are required to induce the differentiation of these cells toward mature osteoblasts.

FGF-2 is the member of the FGF family more thoroughly examined for its activities in bone. FGF-2 has mitogenic activity for skeletal and non-skeletal cells and has potent angiogenic properties. The actions of FGF-2 bear similarities with those of PDGF. FGF-2 increases a population of cells of osteoblast precursors, which eventually can differentiate into osteoblasts [Canalis et al., 1988]. However, FGF-2, like other potent mitogens, does not appear to enhance the differentiated function of the osteoblast and alternate signals are necessary to induce cell differentiation and function. FGF-2 inhibits the synthesis of classic markers of osteoblastic function, such as alkaline phosphatase activity, type I collagen, osteocalcin and osteopontin, independently of its stimulatory effects on osteoblastic cell growth [Canalis et al., 1988]. Similar effects are observed in vivo, and transgenic mice overexpressing FGF-2 are osteopenic, although *fgf2* null mutants exhibit impaired bone formation indicating that FGF-2 is required for this process [Canalis, 2007]. FGF-2 inhibits osteoblast differentiation by inducing the transcription factor Sox 2 and inhibiting Wnt signaling, which is essential for osteoblastogenesis [Mansukhani et al., 2005]. FGF-2 also suppresses IGF-I synthesis, and this may contribute to the inhibitory effect of FGF-2 on osteoblastic function, since IGF-I plays a critical role on the function of the mature osteoblast [Canalis et al., 1993; Gazzerro and Canalis, 2006; Canalis, 2007]. FGF-2, like PDGF, accelerates fracture healing, but neither factor seems to have a definitive anabolic function in the skeleton.

BONE MORPHOGENETIC PROTEINS

BMPs are members of the transforming growth factor (TGF) β superfamily of polypeptides and were identified because of their ability to induce endochondral bone formation [Canalis et al., 2003]. BMP-1 is a protease unrelated to other BMPs and BMP-3 or osteogenin inhibits osteogenesis [Daluiski et al., 2001]. BMP synthesis is not limited to bone, and BMPs are expressed by a variety of extraskeletal tissues, where they play a critical role in organ development and cell function. BMP-2, -4, and -6 are the most readily detectable BMPs in osteoblasts, where they play an autocrine role in osteoblastic cell differentiation and function [Canalis et al., 2003]. BMPs interact with type IA or activin receptor like kinase (ALK)-3 and type IB or ALK-6, and BMP type II receptors. Upon ligand binding and activation of the type I receptor, dimers of the type I and type II receptor initiate a signal transduction cascade activating the signaling mothers against decapentaplegic (Smad) or the mitogen-activated protein (MAP) kinase signaling pathways [Miyazono, 1999]. Following receptor activation by BMPs, Smad 1, -5, and -8 are phosphorylated at serine residues and translocated into the nucleus following heterodimerization with Smad 4 to regulate transcription. MAP kinase signaling results in P38 MAP kinase or extracellular-regulated kinase (ERK) activation by BMPs. The pathway utilized is dependent on the cell type being examined and on the state of dimerization of the BMP receptors.

BMPs induce endochondral ossification and chondrogenesis [Canalis et al., 2003]. BMPs stimulate chondrocyte maturation and function, enhancing the expression of type II and type X collagens. In cells of the osteoblastic lineage, the primary function of BMPs is to induce the maturation of osteoblasts. The genesis and differentiation of bone forming osteoblasts and bone resorbing osteoclasts are coordinated events. Receptor activator of nuclear factor-κ B-ligand (RANK-L) and colony stimulating factor 1 are osteoblast products and are major determinants of osteoclastogenesis [Teitelbaum, 2000]. By inducing osteoblast maturation, BMPs increase RANK-L and induce osteoclastogenesis [Kaneko et al., 2000]. Therefore, BMPs can enhance bone remodeling. BMPs also favor osteoclast survival and induce the transcription of osteoprotegerin, a decoy receptor that binds RANK-L to temper its effects on osteoclastogenesis.

BONE MORPHOGENETIC PROTEIN ANTAGONISTS

The effects of BMPs are regulated by an extensive family of extracellular proteins, the BMP antagonists (Table II). Classic extracellular BMP antagonists prevent BMP signaling by binding BMPs. Often, the synthesis of these BMP antagonists is induced by BMPs themselves, suggesting the existence of local feedback

TABLE II. Secreted BMP Antagonists

Noggin
Follistatin and follistatin related gene
Chordin family
Chordin, Chordin-like, or Neuralin
CR rich motor neuron (CRIM1)
BMP-binding endothelial regulator (BMPER) or Crossveinless, Kielin
Amnionless Nel
Twisted gastrulation
Dan/Cerberus family
Dan, Cerberus, Cer1, Wnt modulator in surface ectoderm (WISE), Coco, Gremlin/drm
Protein related to dan/Cerberus, Caronte, Dante
Sclerostin

mechanisms necessary to modulate BMP activity. Of the many BMP antagonists described, noggin, gremlin, and twisted gastrulation have been studied in detail for their effects on skeletal tissue. Noggin is a classic BMP antagonist, whose sole function is the binding of BMP-2 and -4. Noggin, a glycoprotein, was discovered as a component of the *Spemann organizer*, an organ with a fundamental role in early development and patterning [Zimmerman et al., 1996]. Gremlin, also a glycoprotein, is a member of the *''Differential screening–selected gene aberrative in neuroblastoma'' (Dan)* family of genes. Gremlin is co-expressed with BMPs and binds BMP-2, -4, and -7, but it also has cellular effects independent of its BMP-binding activity. Gremlin expression is clearly detectable in the skeletal environment, making it a possible therapeutic target for osteoporosis, and its conditional inactivation in skeletal tissue results in increased bone formation. This does not appear to be the case for noggin, since its basal level of expression in bone is modest. Therefore, the inactivation of noggin in the skeleton may not result in greater availability of BMPs and an anabolic response. Since BMPs are critical for the differentiation and function of many cellular systems, besides the skeleton, the global inactivation of the BMP antagonists often is lethal and it results in severe developmental abnormalities. In accordance with the mechanism of noggin and gremlin action, transgenic mice overexpressing either protein under the control of the osteoblast-specific osteocalcin promoter develop marked osteopenia and fractures [Devlin et al., 2003; Gazzerro et al., 2005]. Accordingly, the conditional deletion of gremlin in the skeletal environment results in increased bone mass [Gazzerro et al., 2007]. These observations suggest that the removal of a BMP antagonist present in significant concentrations in the bone environment, such as gremlin, could lead to an anabolic response in bone. Gremlin expression is induced by high glucose concentrations, and gremlin is highly expressed in kidneys of experimental models of diabetes mellitus and in biopsy specimens of patients with diabetic nephropathy [Zhang and Zhang, 2009]. Gremlin could play a role in the skeletal manifestations of diabetes either directly by blocking skeletal BMPs or indirectly by contributing to the renal disease. If this were the case, gremlin could serve as a therapeutic target in diabetic patients with nephropathy or skeletal disease. Twisted gastrulation can act as a BMP agonist or antagonist, but its overexpression impairs osteoblastic differentiation in vitro. Twisted gastrulation is dispensable for postnatal skeletal formation [Gazzerro et al., 2006].

ACTIVIN

Activin, a BMP-related protein is constituted by homo- and heterodimers of inhibin β_A and β_B subunits. Activin stimulates the release of follicle-stimulating hormone by pituitary cells and regulates cell replication and differentiation. Activin has mitogenic properties for cells of the osteoblastic lineage, favors osteoblastogenesis, stimulates collagen synthesis in osteoblast cultures and enhances osteoclastogenesis [Centrella et al., 1991]. Activin promotes endochondral bone formation and fracture healing, but some studies have suggested inhibitory effects on bone formation. Follistatin is an activin-binding protein that opposes many of the biological effects of activin, and, like activin, it is expressed by osteoblasts and chondrocytes. Activin signaling follows its binding to cell surface receptors, which are expressed by osteoblasts [Centrella et al., 1991]. Four activin receptors have been described, two type I (ActRIA or ALK-3 and ActRIB or ALK-6) and two type II (ActRIIA and IIB) receptors. Activin binds to the type II receptor, which phosphorylates and activates the type I receptor to initiate signal transduction. ActRIB does not induce the phosphorylation of Smad 1/5, suggesting that activin receptors do not mediate BMP-induced osteoblastogenesis. It is important to note that BMP-3 or osteogenin is an inhibitor of bone formation that binds to activin receptors [Daluiski et al., 2001]. Recent studies have demonstrated that a soluble activin receptor type II fused to IgG-Fc (RAP011) increases bone volume in ovariectomized rodents by decreasing bone resorption [Pearsall et al., 2008]. The soluble receptor acting as a decoy receptor would prevent activin effects on osteoclastogenesis. In addition, the soluble activin IIA receptor increases bone formation and bone mass in intact mice. The mechanism of the anabolic effect is not clear. It is possible that under certain circumstances activin has inhibitory activity on bone formation or that RAP011 by binding the inhibitory BMP-3 causes an anabolic response.

Wnt

The Wnt/β-catenin signaling pathway plays a critical role in osteoblastic cell differentiation and bone formation. Wnt and BMPs have similar and overlapping effects. Mutations in Wnt receptors leading to alterations in Wnt signaling result in profound changes in bone mass [Westendorf et al., 2004]. Wnt can use various signaling pathways, but in skeletal cells the canonical Wnt/β-catenin signaling pathway operates [Westendorf et al., 2004]. In this pathway, when Wnt receptor-binding interactions are absent, β-catenin is phosphorylated by glycogen-synthase kinase-3β (GSK-3β), leading to the degradation of β-catenin in the proteasome. Following the binding of Wnt to frizzled receptors, and to the low-density lipoprotein receptor-related protein (LRP) co-receptors 5 and -6, the activity of GSK-3β is inhibited by disheveled, leading to the stabilization of β-catenin and its translocation to the nucleus. There, it associates with T-cell factor (TCF) 4 or lymphoid enhancer binding factor (LEF) 1 to regulate transcription and gene expression. Deletions of *wnt* or *β-catenin* result in absent osteogenesis and increased osteoclastogenesis, whereas expression of a stable form of

TABLE III. Selected Skeletal Diseases With Wnt Involvement

Osteoporosis pseudoglioma
High bone mass disorders
Sclerosteosis
van Buchem disease
Glucocorticoid-induced osteoporosis
Multiple myeloma

β-catenin, shielded from degradation, in the skeleton results in osteopetrosis because of enhanced osteogenesis and impaired osteoclastogenesis and bone resorption [Glass et al., 2005; Holmen et al., 2005]. The importance of the Wnt/β-catenin signaling pathway is documented by a number of skeletal and non-skeletal disorders that occur when this pathway is perturbed (Table III) [Westendorf et al., 2004].

Wnt ANTAGONISTS

Wnt activity is modulated by various antagonists, including secreted molecules, transmembrane modulators, or intracellular signals [Kawano and Kypta, 2003]. Secreted Wnt antagonists can act by binding Wnt or by preventing its interactions with its receptor frizzled or its co-receptors LRP-5/6 (Table IV). Secreted Wnt antagonists that interact with Wnt include Wnt inhibitory factor (WIF 1), secreted frizzled related proteins (sFRP), and Cerberus. Secreted Wnt antagonists that interact with Wnt co-receptors LRP-5/6 include sclerostin and Dickkopf (Dkk-1). Extracellular and intracellular antagonists interfere with the Wnt/β-catenin signaling pathway. Transmembrane modulators also can modify Wnt signaling. Interestingly, Wnt antagonists, such as Dkk-1 and sclerostin, have dual inhibitory actions on BMP and Wnt signaling. This is not surprising in view of the closely related and often overlapping functions of BMPs and Wnt. Furthermore, there is evidence suggesting intracellular interactions between BMP/Smad and Wnt/β-catenin signaling pathways.

Sclerostin, Dkk-1, secreted frizzled related protein, and WIF are among the Wnt antagonists examined in greater detail for their effects on cells of the osteoblastic lineage and bone formation. Sclerostin, the product of the *sost* gene, is a cysteine knot-containing protein, expressed by osteoblasts, osteocytes, and osteoclasts. Sclerostin has Wnt and BMP antagonistic properties, but its ligand specificity seems to be different from that of classic BMP antagonists since it binds and inhibits BMP-6 and -7, but not BMP-2 or -4 [Winkler et al., 2005]. Sclerostin binds to the Wnt co-receptors LRP-5/6, preventing Wnt signaling. The suppression of BMP activity by sclerostin may be secondary to its ability to bind to selected BMPs or a consequence of the suppression of Wnt signaling. In osteoblasts, sclerostin expression is downregulated by PTH, and upregulated by BMPs [Bellido et al., 2005]. The downregulation of sclerostin expression in osteocytes and osteoblasts by PTH could play a role in the anabolic effects of PTH in bone.

TABLE IV. Secreted Wnt Antagonists

Interact with Wnt
Secreted frizzled related proteins (sFRP)
sFRP 1, 2, and 5
sFRP 3 or FrzB and sFRP 4
Sizzled 1, 2 and crescent (not in mammals)
Wnt inhibitory factor 1 (WIF 1)
Cerberus
Interact with Wnt co-receptors
Dickkopf 1
Sclerostin
Wnt modulator in surface ectoderm (WISE) or ectodin

The Dickkopf family is composed of five members: Dkk-1, -2, -3, -4, and a unique Dkk-3-related protein product of *soggy* [Kawano and Kypta, 2003]. Dkks, like other BMP/Wnt antagonists, contain cysteine rich domains. Dkk-1, -3, and -4 bind to the Wnt co-receptor LRP-5/6, inactivating the Wnt canonical signaling pathway. Dkk-1 also interacts with the transmembrane molecule Kremen to promote endocytosis and internalization of the Wnt co-receptor LRP-5/6, contributing to the disruption of Wnt signaling [Kawano and Kypta, 2003]. Dkk-2 has unique activities and is necessary for terminal osteoblast differentiation.

Inactivating mutations of the Wnt co-receptor LRP-5 result in decreased bone mass. Conversely, gain of function mutations of LRP-5 that impair interactions of Dkk-1 or sclerostin with LRP-5 cause increased bone mass [Westendorf et al., 2004]. In line with these observations, transgenic overexpression of Dkk-1 in the skeletal environment inhibits Wnt signaling and causes osteopenia. Conversely, inactivation of either *secreted frizzled related protein-1* or *sost* or *dkk-1* haplo-insufficiency in the mouse result in increased bone volume, osteoblast number, and bone formation [Bodine et al., 2004; Morvan et al., 2006; Li et al., 2008]. These observations document an inhibitory effect of secreted fizzled related protein, sclerostin, and Dkk-1 on bone formation in vivo and offer clues to potential ways by which Wnt signaling can be modified to produce an anabolic activity on the skeleton. Alterations in the synthesis or activity of Wnt antagonists can explain selected skeletal disorders. For example, glucocorticoids increase Dkk-1 expression and through this mechanism impair Wnt/β-catenin signaling. Glucocorticoids also interfere with the phosphorylation and degradation of GSK-3β, and as a consequence they increase the pool of GSK-3β available to phosphorylate and degrade β-catenin. As a result, glucocorticoids impair osteoblastogenesis. This effect is to an extent responsible for the decrease in the osteoblastic cell population observed in glucocorticoid-induced osteoporosis [Canalis et al., 2007]. A potential therapeutic target for glucocorticoid-induced osteoporosis could be the Wnt signaling pathway directly or through Wnt antagonists as a way to reverse the inhibitory effects of glucocorticoids on osteoblastic cell differentiation. Dkk-1 is overexpressed in plasma cells of multiple myeloma, contributing to the bone loss observed in the disease and to the lack of a bone forming response to the osteolytic lesions caused by myeloma cells [Tian et al., 2003]. Reversal of these alterations has been attempted in experimental models of multiple myeloma, where enhancement of Wnt signaling causes an increase in bone mass.

Wnt AND OSTEOPOROSIS

Sclerostin, the product of the *sost* gene, inhibits osteoblastogenesis, and gain of function mutations of LRP-5 that prevent sclerostin-LRP-5 interactions cause increased bone mass. Mutations of *sost* are

responsible for sclerosteosis and van Buchem disease. Both skeletal dysplasias are characterized by increased bone mass [Balemans et al., 2001; Loots et al., 2005]. Sclerosteosis is caused by a mutation of *sost* near the amino terminus of the coding region, leading to the creation of a stop codon and absent sclerostin expression. Sclerosteosis is an autosomal recessive condition found in the Afrikaner population and characterized by hyperostosis, syndactyly, facial palsy, deafness, and absent nails [Balemans et al., 2001]. van Buchem disease is caused by a non-coding 52 kb deletion of an enhancer element 35 kb downstream of the coding region of *sost*. van Buchem disease is found in Dutch families and is characterized by endosteal hyperostosis of skull and long bones, protruding chin, high forehead, and facial nerve palsy [Loots et al., 2005]. It is noteworthy that individuals with sclerosteosis, as well as heterozygous gene carriers, have increased bone mineral density (BMD). These clinical observations, which are replicated in *sost* null mice, indicate that inactivation or neutralization of sclerostin could be utilized as an approach to obtain an anabolic response in bone [Li et al., 2008]. Humanized monoclonal antibodies to sclerostin cause enhanced Wnt signaling and an increase in bone mass in rodents and non-human primates [Li et al., 2009]. Sclerostin antibodies reverse the bone loss in an ovariectomized rat model of osteoporosis, increasing trabecular bone volume, and the structural properties of the skeleton in this rodent model of osteoporosis, demonstrating that blocking an inhibitor of Wnt can be used to achieve an anabolic response in bone [Li et al., 2009]. This study was followed by a phase I trial in human subjects, demonstrating that sclerostin antibodies can increase BMD and biochemical markers of bone formation in humans.

Gain of function mutations of *lrp5* that impair Dkk-1 interactions with LRP-5 cause increased bone mass [Westendorf et al., 2004]. Dkk-1 levels are increased by glucocorticoids and also in myeloma cells, explaining in part the suppression of bone formation observed in glucocorticoid-induced osteoporosis and multiple myeloma. These clinical observations, as well as those in mouse models of Dkk-1 misexpression, have established the function of Dkk-1 as an inhibitor of Wnt signaling and potential therapeutic target for osteoporosis. This led to the development and testing of Dkk-1 antibodies in female rats. Dkk-1 neutralization causes an increase in BMD, trabecular bone volume, osteoblast surface and bone formation in rats, suggesting that Dkk-1 neutralization could be pursued as an anabolic approach in the treatment of osteoporosis [Grisanti et al., 2006]. Recently, a small molecular antagonist of secreted frizzled receptor-1 was reported to enhance Wnt signaling and bone formation offering a different approach in the development of skeletal anabolic agents [Bodine et al., 2009].

Although the inhibition of Wnt inhibitors is a novel and plausible approach to the development of bone anabolic agents, it is not without potential shortcomings and concerns. An indiscriminate Wnt activation could result in unwanted side effects and even tumorigenicity in non-skeletal tissues. This concern could be minimized by the relatively specific expression of sclerostin and Dkk-1 in skeletal cells. It is reassuring that patients with high bone mass syndrome and sclerosteosis live reasonably normal lives and have not been reported to have a higher incidence of malignancies. However, it is important to note that the inactivation of the Wnt antagonist WIF pre-disposes to osteosarcoma in mice. Furthermore, the WIF promoter is hypermethylated and epigenetically silenced in osteosarcoma, and WIF is not expressed in 75% of human osteosarcomas, which also express increased β-catenin levels [Kansara et al., 2009]. These observations and findings from an experimental model of multiple myeloma, where activation of Wnt signaling rescues the skeletal disease but favors the soft tissue invasion by myeloma cells, are concerns that may temper the enthusiasm for this anabolic approach [Edwards et al., 2008]. A potential solution is the neutralization of Wnt antagonists specifically to skeletal cells and their use for defined and limited periods of time.

INSULIN-LIKE GROWTH FACTOR

IGF-I and IGF-II are the most abundant growth factors present in skeletal tissue. IGF-I can act as a systemic and local regulator of osteoblastic function. Systemic IGF-I is synthesized by the liver, where its synthesis is growth hormone (GH) dependent [Gazzerro and Canalis, 2006]. IGF-I circulates as part of a 150 kDa complex formed by IGF-I, IGF-binding protein (IGFBP)-3, the predominant circulating binding protein, or IGFBP-5 and the acid labile subunit (ALS). IGFBPs are in a concentration that is in excess that of IGF-I; therefore, IGF-I circulates mostly bound to the complex, and $\leq$1% of total serum IGF-I circulates as a free hormone. In addition to its function as a systemic hormone, IGF-I plays an important role in the autocrine and paracrine regulation of cell metabolism in a variety of tissues, including cartilage and bone. Locally, the availability and activity of IGF-I is regulated by six IGF-binding proteins (IGFBPs). IGF-II shares biochemical and biological properties with IGF-I, it is important in skeletal development, but its function in the adult skeleton is not proven. In vitro, IGF-I is more potent than IGF-II.

Upon ligand binding, the IGF-I receptor dimerizes and undergoes autophosphorylation, leading to the activation of the insulin receptor substrate (IRS)-1 and IRS-2, which mediate the effects of IGF-I in osteoblasts [Gazzerro and Canalis, 2006]. IGF-I utilizes the phosphatidylinositol-3 kinase pathway, which induces the activation of Akt, and the MAPK pathway, which activates p38, Jun-N-terminal kinases, and ERK 1/2. IGF-I has modest mitogenic properties for cells of the osteoblastic lineage and stimulates osteoblastic function and bone formation [Canalis, 1980]. By upregulating type I collagen transcription and downregulating the expression of collagenase 3, IGF-I maintains appropriate levels of bone matrix. IGF-I does not direct the differentiation of stromal cells toward mature osteoblasts. Indirectly, IGF-I may favor osteoblastogenesis by stabilizing β-catenin, enhancing Wnt-dependent activity [Playford et al., 2000]. IGF-I stabilizes β-catenin by inducing phosphatidylinositol-3 kinase and activating Akt, which phosphorylates and degrades GSK-3β, the enzyme that phosphorylates β-catenin prior to its degradation by ubiquitination [Playford et al., 2000; Krishnan et al., 2006]. This mechanism is opposite to the one utilized by glucocorticoids to inhibit Wnt signaling. The phosphatidylinositol-3 kinase/Akt pathway also is utilized by IGF-I to decrease osteoblast apoptosis. This effect, associated with modest mitogenic properties, causes an increase in the number of

osteoblasts. A decline in IGF-I expression is necessary for the death of cells of the osteoblastic lineage to occur and to allow for the terminal differentiation of osteoblasts. Less clear is the function of IGF-I on bone resorption than on bone formation. IGF-I induces RANK-L synthesis, and as a consequence osteoclastogenesis, and enhances osteoclast function [Mochizuki et al., 1992]. Vascular endothelial growth factor (VEGF) is required for blood vessel formation and vessel invasion into cartilage during the process of endochondral bone formation and for chondrocyte survival during skeletal growth. IGF-I induces *vegfa* expression in skeletal cells, and VEGF may serve to couple angiogenesis with endochondral bone formation and with osteoblastic differentiation and function [Akeno et al., 2002].

Transgenic mice expressing IGF-I under the control of the osteoblastic-specific osteocalcin promoter exhibit increases in trabecular bone secondary to an increase in bone formation [Zhao et al., 2000]. Inactivation of *igf1*, *igf2*, *igf1r*, or *irs1* in mice causes growth arrest because of failed chondrocyte proliferation and maturation [Liu et al., 1993]. *Igf1* null mutants exhibit severe developmental abnormalities and lethality. Mice that survive exhibit reduced cortical but not trabecular bone, possibly due to a compensatory increase in GH secretion or due to a decrease in trabecular bone resorption. Mice carrying mutations of the *gh releasing hormone receptor* (lit/lit mouse) or the *gh receptor* have absent GH secretion or action, and consequently low levels of systemic IGF-I [Beamer and Eicher, 1976; Sims et al., 2000]. These mutants display osteopenia and reduced cortical bone, but normal trabecular bone. The contribution of systemic IGF-I to cortical bone integrity is confirmed in mice carrying a liver-specific *igf1* deletion singly or in combination with an *als* deletion. These mice, which display reductions in serum IGF-I, have decreased cortical bone [Yakar et al., 2009]. These observations confirm the contribution of systemic IGF-I to cortical bone integrity and to a lesser extent to trabecular bone. In contrast, the locally produced skeletal IGF-I plays a more significant role in trabecular bone integrity. This is demonstrated in transgenic mice expressing IGF-I in osteoblasts and in conditional *igf1 receptor* null mice, which display decreased osteoblast number and function, causing reduced bone formation and trabecular bone volume [Zhao et al., 2000; Zhang et al., 2002]. Therefore, systemic IGF-I maintains cortical bone structure, whereas skeletal IGF-I serves to maintain trabecular bone. The function of IGF-I in skeletal homeostasis is confirmed in *irs1* or *irs2* null mutants, which also exhibit osteopenia [Gazzerro and Canalis, 2006].

The *igf1* gene consists of six exons and has alternate promoters in exons 1 and 2. The exon 1 promoter has four transcription initiation sites, and is responsible for the regulation of IGF-I expression in most extra-hepatic tissues including bone [Adamo et al., 1991]. The IGF-I exon 2 promoter has two transcription initiation sites and is responsible for the transcriptional regulation of IGF-I by GH in the liver [Adamo et al., 1991]. IGF-I exon 2 is minimally expressed by osteoblasts, and GH is not a major inducer of IGF-I in these cells. PTH and other inducers of cyclic AMP increase IGF-I expression in osteoblasts, and IGF-I mediates selected anabolic actions of PTH in bone in vitro and in vivo [Canalis et al., 1989]. Glucocorticoids decrease IGF-I transcription in osteoblasts and oppose IGF-I signaling, and the inhibitory effects of glucocorticoids on the function of the mature osteoblast can be explained in part by reduced IGF-I levels in the bone microenvironment [Delany et al., 2001]. The inhibitory effects of glucocorticoids on osteoblastogenesis can be explained by their inhibition of Wnt/β-catenin signaling [Canalis et al., 2007]. Reversal of the inhibitory effects of glucocorticoids on IGF-I expression and induction of Wnt/β-catenin signaling would be reasonable therapeutic approaches in the reversal of glucocorticoid-induced osteoporosis. This could possibly be achieved by the use of PTH, which induces IGF-I transcription and inhibits sclerostin expression in cells of the osteoblastic lineage.

INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS

IGFBPs are a family of six related proteins that modulate the cellular effects of IGFs [Jones and Clemmons, 1995]. By binding IGFs, IGFBPs may sequester the growth factor and preclude its interactions with cell surface receptors; but when IGFBPs are associated with the extracellular matrix, there may be an increase in the effective concentrations of IGF-I, resulting in enhanced IGF-I effects. Consequently, IGFBPs have been reported to have stimulatory and inhibitory effects on cell replication and function. The six IGFBPs are expressed by osteoblasts although IGFBP-1 and -6 do not have a defined function in skeletal cells. IGFBP-2 is important to transport IGFs, but its overexpression in vivo and in vitro oppose IGF-I activity and causes impaired growth and decreased osteoblastic function [Eckstein et al., 2002]. The actions of IGFBP-2 are complex, and *igfbp2* null mice exhibit gender-specific changes in bone turnover. Female *igfbp2* null mice have increased cortical bone, whereas male null mice display decreased cortical and trabecular bone secondary to decreased bone formation [Demambro et al., 2008]. These observations suggest that IGFBP-2 is required for normal bone formation in male mice and are in agreement with clinical observations indicating a correlation between IGFBP-2 levels and bone remodeling. IGFBP-3 is a major component of the circulating IGF complex and its concentrations are GH dependent [Jones and Clemmons, 1995; Gazzerro and Canalis, 2006]. In vitro, IGFBP-3 can inhibit or stimulate IGF activity. However, the constitutive overexpression of IGFBP-3 in vivo causes osteopenia [Silha et al., 2003]. Similarly, transgenic mice expressing either IGFBP-4 or IGFBP-5 under the control of the osteocalcin promoter exhibit osteopenia secondary to decreased bone formation [Devlin et al., 2002; Zhang et al., 2003]. These effects were confirmed in vitro since the constitutive overexpression of IGFBP-5 in cells of the osteoblastic lineage inhibits osteoblastic cell function. Under certain experimental conditions, IGFBPs can simulate bone cell function, independently of their interactions with IGFs, but it appears that their fundamental role is to bind and serve as transporters of IGF-I. Although in excess IGFBPs inhibit IGF-I action, the triple inactivation of *igfbp3*, *4*, and *5* demonstrated that IGFBPs are necessary to maintain appropriate levels of systemic IGF-I and adequate postnatal growth [Ning et al., 2006].

IGFBP synthesis is regulated at the transcriptional, post-transcriptional, and post-translational level. IGFBPs can be cleaved by metalloproteinases and serine proteases. These are important regulatory mechanisms. For example, by cleaving the inhibitory IGFBP-4, pregnancy-associated plasma protein-A (PAPP-A) increases the bioavailability and activity of IGF-I. *Pappa* null mice exhibit low bone turnover osteopenia due to a decrease in bioavailable IGF-I in the bone environment secondary to excessive IGFBP-4 [Tanner et al., 2008].

INSULIN-LIKE GROWTH FACTOR I AND OSTEOPOROSIS

GH and IGF-I play an important role in the acquisition of bone mass during adolescence, in the achievement of peak bone mass and in the maintenance of skeletal architecture during adult life [Giustina et al., 2008]. A decline in GH and IGF-I secretion may play a role in the pathogenesis of osteoporosis. There is a correlation between serum IGF-I levels and BMD in postmenopausal women, and *igf1* promoter polymorphisms have been linked to bone mass [Langlois et al., 1998]. The content of IGF-I in human cortical bone decreases with age, a decline that parallels the one observed in serum concentrations of IGF-I, suggesting that it is due to a decrease in skeletal IGF-I accumulation from the systemic circulation rather than in suppression of skeletal IGF-I synthesis [Canalis, 1994]. Decreased skeletal IGF-I can explain the decrease in osteoblast survival and function observed in glucocorticoid-induced osteoporosis and leading to suppressed bone formation.

Administration of IGF-I to normal individuals or to patients affected by IGF-I deficiency causes a skeletal anabolic response and an increase in bone remodeling [Grinspoon et al., 1996]. Recombinant human IGF-I is available for the treatment of severe growth retardation caused by IGF-I deficiency secondary to mutations of the *gh receptor* or the *igf1* gene. Human studies to define the effects of IGF-I on bone turnover are limited. At high doses, IGF-I increases biochemical markers of bone remodeling, whereas at low doses it increases markers of bone formation, such as osteocalcin and type I procollagen carboxy-terminal peptide, without an effect on markers of bone resorption, such as pyridinoline excretion [Grinspoon et al., 1996]. These observations parallel the effects of IGF-I in skeletal cells, and would suggest that at low doses IGF-I can increase osteoblast function without an effect on bone resorption.

Anorexia nervosa, a severe eating disorder that leads to progressive malnutrition, is associated with severe bone loss and decreased serum levels of IGF-I, which in association with hypogonadism, contribute to bone loss [Misra and Klibanski, 2006]. Although patients with anorexia nervosa exhibit hypogonadism, estrogen replacement alone does not reverse the osteopenia. Administration of IGF-I, at doses that normalize serum IGF-I, in combination with estrogen replacement therapy, increases BMD in anorexia nervosa [Grinspoon et al., 2002]. Notwithstanding these encouraging results, the long-term efficacy and safety of IGF-I for the treatment of osteoporosis, in the context or not of anorexia nervosa, remain to be determined. Potential side effects and the lack of tissue specificity are concerns for the chronic systemic administration of IGF-I to humans. Other limitations are related to the fact that systemic IGF-I might have a predominant role in cortical, but not trabecular, bone integrity and that the dose needs to be carefully monitored to avoid deleterious effects secondary to increased bone resorption. Other limitations are the expense and need for parental administration.

CONCLUSIONS

During the past few years, we have made significant progress in the understanding of cellular events that regulate bone formation. BMPs, Wnt and IGF-I are major factors regulating the fate and function of osteoblasts, and their activities are modulated by extracellular and intracellular proteins. The administration of systemic growth factors for the management of osteoporosis is limited by a lack of skeletal specificity and potential side effects. The possibility of modifying growth factor signals specifically in the skeletal environment could offer a novel therapeutic approach for the treatment of osteoporosis. Clinical trials are needed to demonstrate the effectiveness and safety of these novel anabolic therapies for the management of osteoporosis.

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