Kidney-Bone, Bone-Kidney, and Cell-Cell Communications in Renal Osteodystrophy

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The relationship between bone and the kidney in renal osteodystrophy is a complex interplay of kidney to bone connections, bone to kidney connections, and cell to cell connections. In addition, such interactions have a profound effect on the vasculature. In this review, we discuss the role of the bone morphogenetic proteins (BMPs) in the skeleton, kidney, and vasculature. In addition, we propose that deficiencies of these BMPs seen in chronic kidney disease (CKD) result in decreased bone remodeling and a compensatory secondary hyperparathyroidism (high turnover state). Treatment of the hyperparathyroidism blocks this compensatory arm and thus decreased bone remodeling occurs (low turnover). We review animal models of CKD in which treatment with BMP-7 resulted in normalization of both high and low turnover states. Finally, we discuss vascular calcification as it relates to bone metabolism. We discuss the roles of BMP-7 and 2 other bone regulatory proteins, osteoprotegerin (OPG) and α2-HS glycoprotein (AHSG, human fetuin), in the human vasculature and their implications for vascular calcification.

NEW DISCOVERIES RELATED to substances made in the kidney regulating bone remodeling are addressed in this article along with mention of new skeletal hormones that regulate kidney function. In addition, recent progress related to the receptor activator of nuclear factor κ B ligand (RANKL)/RANK/osteoprotegerin (OPG) pathway, the bone regulatory protein α2-HS glycoprotein, and the dependency of bone remodeling on heterotypic cell-cell communications is discussed. Thus, kidney-bone, bone-kidney, and cell-cell communications are discussed as they relate to the pathogenesis of renal osteodystrophy.

BONE REMODELING

The human skeleton is remodeled continuously during life, and the rate of remodeling is stimulated during growth and fracture healing. A concept exists that skeletal growth is a modeling-only issue. This is not true because the term *modeling* refers to the function of the epiphyses of long bones where their elongation occurs owing to endochondral bone formation and the process of periosteal deposition and endosteal resorption that shape long bones. However, a critical remodeling component is present during growth in most parts of the skeleton. At remodeling sites, the rates of bone formation exceed those of bone resorption. Unappreciated until the present time are critical regulatory relationships between bone modeling, remodeling, and the hematopoietic system in the marrow space of long bones. These ties contribute to the normal function of the alternate environment. Thus, the functioning immune system is an important regulator of bone remodeling (Fig 1), and the bone mesenchymal compartment contributes to regulation of hematopoiesis. Bone is the major storehouse of calcium, phosphorus, and buffer equivalents in the body, and bone remodeling is regulated to maintain calcium homeostasis during pregnancy, lactation, and metabolic acidosis. Osteodystrophies develop from disordered bone remodeling—such is the case in renal osteodystrophy.[1-4] Regulation of bone remodeling involves both endocrine and paracrine signaling to osteoblasts and osteoclasts through hormones, cytokines, and growth factors (Fig 2). In chronic kidney disease (CKD), aberrant levels of agents regulating bone remodeling are released into the system, resulting in the lack of normal bone formation rates, inappropriate bone resorption, and defects in mineralization constituting renal osteodystrophy.

THE KIDNEY-BONE AND BONE-KIDNEY CONNECTIONS

Evidence exists to indicate that CKD produces a decrease in skeletal remodeling that leads to an attempt to maintain remodeling through increased parathyroid hormone (PTH) activity. The best clin-

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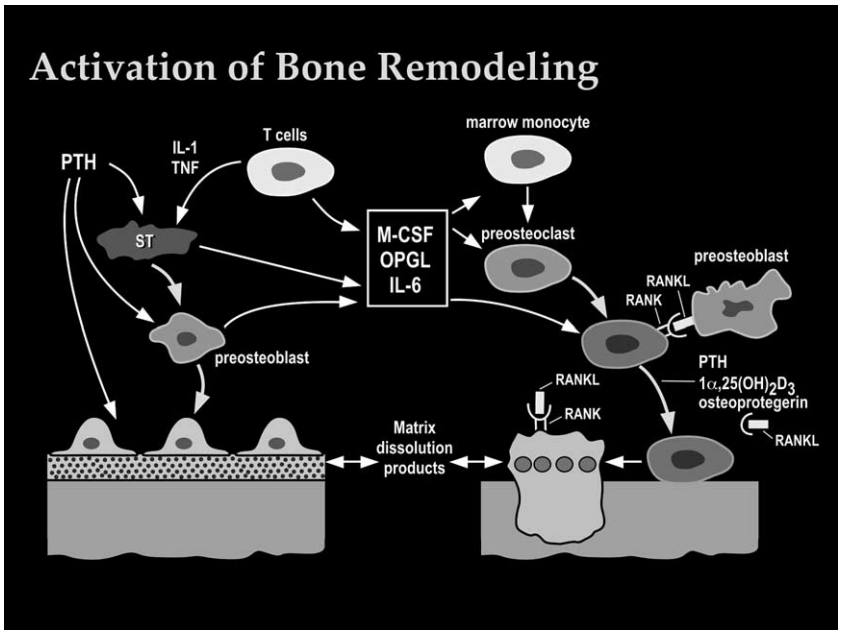

Fig 1. Interactions between systems and cell types during stimulation of bone remodeling. Remodeling can be stimulated by hormones such as PTH (interaction between the system for divalent ion homeostasis and bone remodeling), interleukin 1 and TNFα, cytokines released by T cells of the immune system. Furthermore, cytokines produced by cells of the immune system contribute macrophage colony stimulating factor (M-CSF) and interleukin 6, which support osteoclast progenitor survival and differentiation. Bone remodeling is a coupled process of bone formation and bone resorption because differentiation of bone marrow stromal cells, the progenitors of the osteoblast lineage, and preosteoblasts directly interact with cells in the osteoclast lineage through surface cell–expressed RANKL binding to its receptor, RANK, on the preosteoclasts. RANKL is required and sufficient for terminal differentiation and regulation of osteoclast function. Osteoprotegerin, a RANKL decoy receptor produced by bone cells and immune cells, decreases RANKL stimulation by decreasing availability of RANKL for binding with RANK. The effects of PTH and calcitriol ($1,25[OH]_2D_3$) on osteoclast differentiation and stimulation of bone resorption are mediated by their stimulation of RANKL production by preosteoblasts. CKD is a state of altered immune cell activation leading to increased interleukin 1, TNFα, and interleukin 6 levels in the circulation and the bone marrow environment.

ical demonstration of this is the observation that prevention of hyperparathyroidism in CKD patients leads to the adynamic bone disorder.[3] We recently have shown in an animal model of CKD that maintenance of normal phosphorous, calcium, and PTH levels through phosphate restriction and calcitriol supplementation leads to a marked decrease in osteoblast number, bone formation rates, and activation frequency similar to adynamic bone disease.[4] Based on these findings, we hypothesized that CKD inhibits osteoblast differentiation by decreased growth factors and/or increased levels of inhibitory substances blocking the action of growth factors. Two important regulatory families involved in osteoblast differentiation are the bone morphogenetic proteins (BMPs) and the Wnt family proteins. Phosphatonins are potential inhibitors of the Wnt family and are discussed in a separate article in this issue by Kumar. We focus our discussion on the BMPs and their relationship to bone, the kidney, and the vasculature.

The BMPs in Bone

BMPs are members of the transforming growth factor β (TGF-β) superfamily. BMPs were first isolated from demineralized bone, indicating that demineralized bone is a rich source for BMPs.[5,6] At least 15 BMPs have been identified, of which BMP-2, BMP-3, BMP-4, BMP-6, and BMP-7 (osteogenic protein-1) have been shown to be potent inducers of ontogenesis. BMPs interact with a complex set of type I and type II receptors of the activin receptor family to mediate their biologic function[7,8] through activation of the Smad family of transcription factors.

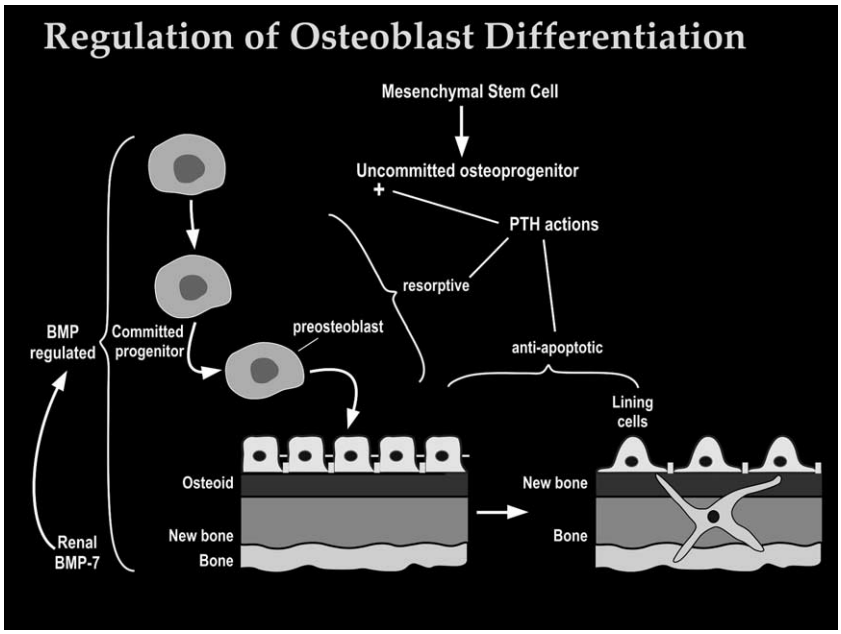

Fig 2. The physiology and pathophysiology of osteoblast differentiation. Osteoblasts derive from mesenchymal stem cells (also referred to as bone marrow stromal cells; Fig 1) through a multistep differentiation program regulated by the BMP family and the Wnt family of differentiation factors. Some of the skeletal BMP-7 burden is of hormonal origin deriving from the kidney. PTH regulates osteoblast differentiation in the presence of normal BMP levels by stimulating osteoprogenitor proliferation and inhibiting mature osteoblast and osteocytes apoptosis. The effects of PTH on the phenotype of cells in the osteoblast differentiation program are resorptive (see Fig 3). CKD affects osteoblast differentiation by impairing the BMP and Wnt physiologic system. As an adaptation, PTH levels are increased, but the phenotype of the osteoblastic cells is not normal and excess bone resorption is stimulated.

BMPs have a profound effect on osteoblastic growth and differentiation as shown by in vitro cell culture studies. BMP-2, BMP-4, and BMP-6 stimulate osteoblast differentiation of rat and mouse cells.[9-11] BMP-2 and BMP-7 stimulate a differentiation program of human bone marrow osteoprogenitors and bone-derived osteoblasts.[12-15]

This is accompanied by increased expression of alkaline phosphatase, type I collagen, osteopontin, osteocalcin, decorin, osteonectin, and bone sialoprotein. BMP-2 is a potent inducer of osteoprogenitor differentiation with a decreased effectiveness in more mature cells. BMP-3 increased alkaline phosphatase activity, type I collagen, and osteocalcin in human bone marrow stromal cells.[16] BMP-3 antagonizes the osteogenic effects of BMP-2 and TGF-β in osteoblastic cells.[17,18] Adenoviral vectors carrying BMP-2, BMP-4, BMP-6, BMP-7, and BMP-13 complementary DNAs delivered intramuscularly or subcutaneously to animals induce ectopic bone formation at the site of delivery through endochondral ossification,[19-21] showing that BMP gene therapy may have potential use in the treatment of degenerative, rheumatic, traumatic bone injury, and kidney diseases.

The expression of BMP-7 in adult rat kidney is decreased in conditions such as animal models of acute renal ischemia and diabetic nephropathy.[22-25] This suggests that part of the pathophysiology of renal osteodystrophy may in part be caused by BMP deficiency and decreased osteoblast differentiation (Fig 2). Hyperparathyroidism, an adaptive mechanism to maintain remodeling rates in the face of CKD, is a maladaptive process because PTH is not an osteoblast differentiation factor.[26-30] Increased levels of PTH stimulate an abnormal phenotype of osteoblastic cells with fibroblast-like properties that accumulate in the peritrabecular space and produce marrow fibrosis (Fig 3). Treatment with recombinant BMP-7 results in the disappearance of these cells, probably by promoting commitment to the osteoblast program.[31] BMP-7, even in the presence of high PTH levels, stimulated remodeling, increased bone formation, and decreased bone resorption. BMP-7 also reversed the adynamic bone disorder and restored bone remod-

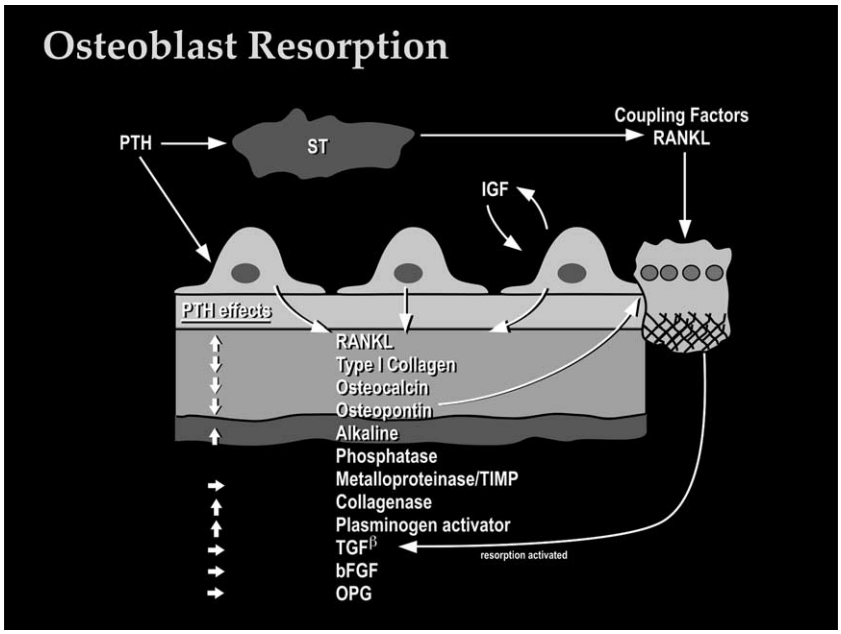

Fig 3. The relationship between secondary hyperparathyroidism and the osteodystrophy of CKD. Because of decreased osteoblast differentiation as a result of CKD, skeletal resistance to PTH actions develop, leading to changes in phosphorus and calcium homeostasis and secondary hyperparathyroidism. The increased PTH levels restore rates of bone remodeling, but an osteodystrophy develops because PTH stimulates an abnormal phenotype of osteoblastic cells. Decreased mineralization (hyperosteoidosis) and increased bone resorption result, the latter caused by PTH stimulation of excess RANKL production by preosteoblasts and stromal cells.

eling.[4] The successful treatment of renal osteodystrophy (both high and low turnover) in animal models of CKD, regardless of serum PTH, calcium, or phosphorous levels, by BMP-7, implicates BMP deficiency in the pathogenesis of renal osteodystrophy.

The Function of BMPs in the Kidney

BMPs have emerged (through genetic studies) as important regulators of kidney development.[32,33] In addition to being present during kidney development, BMP-7 messenger RNA also is expressed in the adult kidney, predominantly in the tubules of the outer medulla, podocytes, and ureter.[34] Furthermore, BMP-7 expression has been shown in cultured kidney cells including glomerular cells and distal Madin Darby kidney cell (MDKC), but not human proximal HK-2 cells.[35]

BMP-7 prevented renal failure in a variety of animal models of renal injury including acute renal ischemia, unilateral ureteral obstruction, diabetic nephropathy, and lupus nephritis.[36-40] In addition, treatment with BMP-7 was superior to treatment with enalapril in preventing tubulointerstitial fibrosis in the unilateral ureteral obstruction and lupus nephritis models. Tubulointerstitial fibrosis is a major component of several kidney diseases associated with the progression to CKD stage V. Finally, treatment with BMP-7 in the diabetic nephropathy model was more effective than enalapril in reversing proteinuria, preserving glomerular filtration rate, and preventing glomerular sclerosis. Taken together, the BMP-7 actions appear to be preservation of epithelial phenotype,[40] inhibition of epithelial-mesenchymal transdifferentiation,[37,40] and inhibition of injury-induced epithelial cell apoptosis.[37]

BMPs in the Vasculature

Vascular calcification is a common problem that correlates with increased cardiovascular mortality among patients with CKD. The process appears to be regulated by an osteoblast-like cell,[41] possibly of vascular smooth muscle cell (VSMC) origin. VSMCs share a common progenitor lineage with osteoblasts and retain sufficient pluripotentiality to transdifferentiate. VSMCs are a potential therapeutic target for BMP-7 because they have been shown to inhibit smooth muscle proliferation and to stimulate expression of markers of smooth muscle cells in vitro.[42] In low-density lipoprotein receptor−/− mice, an animal model of atherosclerosis and vascular calcification, BMP-7 prevented vascular calcification in uremic animals,[43] suggesting a possible protective role for BMP-7 in the vasculature among patients with CKD.

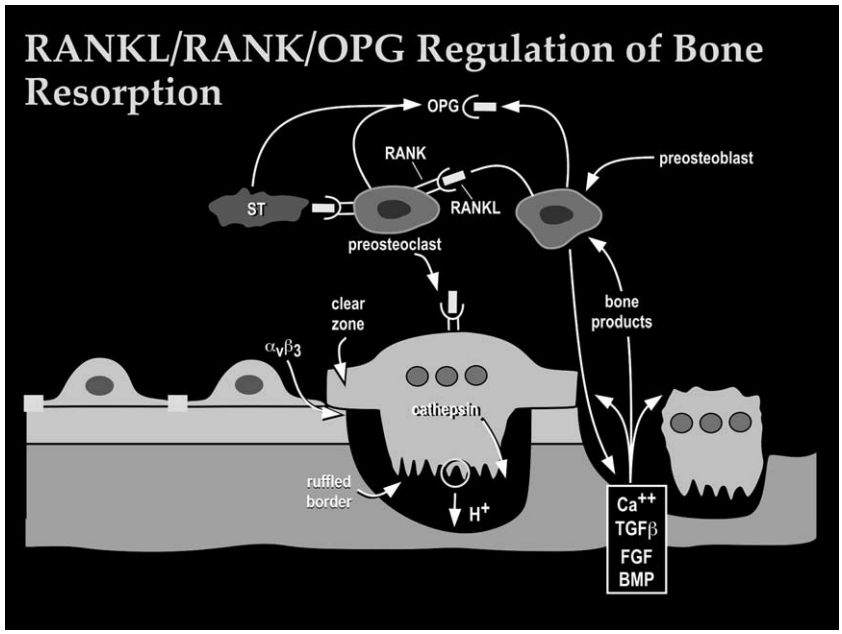

Fig 4. RANKL/RANK/OPG regulation of bone resorption. RANKL produced by bone marrow stromal cells, preosteoblasts, and cells of the immune system in either a soluble or cell membrane–anchored form interacts with the receptor RANK on cells in the osteoclast lineage supporting their terminal differentiation to multinucleated bone-resorbing cells. OPG is a secreted circulating receptor for RANKL, making it an inhibitor of osteoclast stimulation by the RANKL/RANK ligand/receptor complex. RANKL is the main regulator of mature osteoclast activity.

CELL-CELL CONNECTIONS

In states in which there is decreased mineralization of bone, there is an association with vascular calcification. Various regulatory proteins involved in bone metabolism and mineralization exist in serum and the vasculature. The role of 2 such proteins, OPG and α2-HS glycoprotein (AHSG), are discussed with regard to bone and the vasculature in patients with CKD.

OPG in Bone

With the discovery of OPG, osteoblasts have been implicated in the regulation of osteoclastogenesis (Fig 4). OPG initially was isolated as a novel member of the tumor necrosis factor (TNF) receptor superfamily made by stromal cells, preosteoblasts, and preosteoclasts. Unlike most members of the TNF receptor superfamily, which are transmembrane proteins, OPG is a secreted protein.[44] The discovery that OPG can bind to RANKL, and compete with RANK, a membrane-bound receptor found on osteoclast precursor cells, revealed a complex mechanism regulating the cell-to-cell contact–dependent process of osteoclast differentiation.

RANKL, present in a membrane-bound or soluble form, is produced by the osteoblast and binds to RANK to stimulate osteoclast formation and activity (Figs 1 and 4). However, the binding of OPG to the soluble or membrane-bound form of RANKL inhibits osteoclastogenesis through competition of binding and prevention of RANKL binding to RANK on the osteoclast precursor cell membrane. Therefore, it has been suggested that the degree of osteoclastogenesis and resulting bone erosion is dependent on the ratio of RANKL to OPG.[45]

Studies performed in animal models support the role of OPG and RANKL in osteoclast activation and differentiation. Mice genetically engineered to be OPG deficient (*opg−/opg−*) exhibit, by adolescence and adulthood, an early onset of osteoporosis.[46] Intravenous injection of recombinant OPG and transgenic overexpression of OPG in *opg−/opg−* mice effectively rescued this osteoporotic bone phenotype.[47] Overexpression of OPG in transgenic mice resulted in osteopetrosis, a state of abnormally dense bone caused by inhibition of osteoclast activity.[44] In vitro cell culture was used to determine that OPG inhibits osteoclast maturation in a dose-dependent manner.[44] In vivo, injection of OPG resulted in osteoclast apoptosis.[48] In rats, in vitro studies showed incubation of mature rat osteoclasts with RANKL stimulated them through multiple cycles of bone resorption.[49] In addition, in vitro studies indicate that RANKL is necessary but not sufficient for osteoclast survival.[48]

AHSG Glycoprotein in Bone

AHSG is a noncollagenous protein found both in the serum and in bone matrix. In mineralized human tissues, AHSG has been shown to be concentrated with respect to other plasma proteins by factors of 30 to 100.[50] However, the concentration of AHSG in bone and in serum is not constant and is age dependent. The protein appears to be in higher concentrations in children as compared with adults.[51,52] In addition, in states of high bone resorption such as renal osteodystrophy and Paget's disease, the content of AHSG in bone is higher than in normal controls.[53] However, the serum levels of AHSG in patients with CKD stages I through V and Paget's disease is lower than in normal controls.[54-56] This has led to speculation that AHSG is involved in bone metabolism. Initial studies supported this by revealing that AHSG modulates bone resorption in a concentration-dependent manner.[57] The ability to modulate bone resorption may be related in part to osteoclast precursors. Osteoclasts are derived from monocytes and AHSG has been shown to increase recruitment and enhance the function of monocytes in vitro.[58-60]

AHSG is a 2-chain protein composed of a heavy (A) and light (B) chain connected by a disulfide bond.[61] AHSG has been shown to be structurally similar to bovine fetuin, a protein that makes up the major portion of bovine fetal serum.[62,63] This led to speculation that fetuin is the bovine homologue of AHSG, designating AHSG as fetuin-A or human fetuin. Furthermore, a striking sequence homology was found between AHSG and the cystatin superfamily of cysteine proteinase inhibitors.[64,65] Further analysis of the A chain of fetuin revealed it to be composed of 5 disulfide loops arranged in a manner similar to the disulfide loops of the cystatin superfamily.[65] Because cysteine proteinases are involved in bone resorption, it was speculated that AHSG may inhibit bone resorption in this manner.[64]

The function of AHSG/fetuin has been examined in bone cell cultures. When grown in media containing calcium and phosphorous in concentrations that cause spontaneous salt precipitation, the addition of bovine fetuin to rat calvaria osteoblast cultures inhibited apatite formation.[66] Similar findings occurred when other fetuins were used including AHSG. It also was found that the concentration of fetuin necessary to prevent precipitation is much lower than the concentration of fetuin in serum. Subsequently, it also was determined that the amino acids in the cystatin-like domain 1 of the protein mediated the inhibition of apatite formation.[66]

Fetuin also has been shown to have effects on bone independent of apatite inhibition. Bovine fetuin has been found to be an antagonist to the TGF-β cytokines.[67] Fetuin binds to BMP-2 $>$ BMP-4 $>$ BMP-6 $>$ TGF-β1 $>$ TGF-β2. Fetuin and TGF-β receptor type II share sequence homology with a disulfide looped sequence termed *TRH1* that is the major cytokine binding domain.[67] The binding of fetuin to the cytokine blocks binding to the receptor and has been shown to inhibit the activity of TGF-β1 and BMP-2 in cell culture.[67] Further studies have shown that although TGF-β1 is required for osteogenesis in dexamethasone-treated rat bone marrow cells, TGF-β1 in high concentrations inhibits osteogenesis.[68] The addition of bovine fetuin to the high concentration TGF-β1 cultures restores osteogenesis. However, when added to cultures in the presence of low concentrations of TGF-β1, bovine fetuin is inhibitory. The inhibition of osteogenesis by bovine fetuin or high-concentration TGF-β1 also occurs well before bone mineralization.[68] This suggests that the relative concentrations of fetuin and TGF-β1 play an important role in regulating osteogenesis. In addition, this biphasic response also can explain how, despite being antagonists, both AHSG/fetuin and BMPs can have protective roles in vascular calcification (see later).

Studies with mice genetically engineered to be fetuin deficient (*ahsg−/ahsg−*) have had somewhat inconsistent results. One study showed that *ahsg−/ahsg−* mice were fertile and showed no gross skeletal abnormalities at day 1 or at 4 months.[69] Another study found impaired maturation of growth plate cartilage and slower femur lengthening among *ahsg−/ahsg−* mice later in life (3-18 mo).[70] Interestingly, however, bone formation was increased, manifested by greater cortical thickness, increased trabecular remodeling, and increased osteoblast numbers on bone surfaces.[70] The increased bone formation may be caused in part by the absence of the inhibitory effects of fetuin on TGF-β1 or BMPs at physiologic concentrations.

OPG in the Vasculature

Apart from bone, OPG is found in a number of tissues including the major arteries such as the abdominal aorta.[44] Furthermore, OPG is highly expressed in VSMCs, but not endothelial cells. Platelet-derived growth factor, basic fibroblast growth factor, angiotensin II, tumor necrosis factor α, and interleukin-1β all up-regulate OPG expression in VSMCs.[71] In addition to osteoporosis, mice engineered to be *opg−/opg−* exhibit medial calcification of the aorta and renal arteries.[46] These data support a link between osteoporosis and vascular calcification wherein decreased orthotopic mineralization leads to increased pressure for heterotopic mineralizaton. However, despite the fact that intravenous injection of recombinant OPG rescued the osteoporosis phenotype, it did not reverse the arterial calcification in *opg−/opg−* mice.[47] In contrast, transgenic OPG delivered from midgestation to adulthood does prevent arterial calcification in *opg−/opg−* mice,[47] suggesting that although OPG can help prevent arterial calcification, it cannot reverse it. Similarly, mice null for matrix gla-protein (MGP) develop severe medial vascular calcification and die at age 1 month from coronary artery disease and vascular aneurysms.[72] This animal model has similarities to a rat model of vascular calcification induced by warfarin.[25,73] Warfarin inhibits γ-carboxylation of the gla residues on MGP and results in aortic calcification in the rat. Interestingly, warfarin therapy of humans does not lead to vascular calcification. OPG therapy of warfarin-induced vascular calcification eliminates it.[73] In addition, other inhibitors of bone resorption such as the bisphosphonates and SB 242784, a selective inhibitor of the osteoclastic V-H-adenosine triphosphatase, have been shown to inhibit warfarin- and vitamin D–induced vascular calcification.[24,74] These findings all support a causal link with bone mineralization and medial calcification.

Although OPG normally is present in arteries, RANKL and RANK are not detected in the arterial walls of wild-type *opg+/opg+* adult mice.[47] However, RANKL and RANK transcripts are found in the calcified arteries of *opg−/opg−* mice. Furthermore, RANK expression in the calcified arteries coincides with the presence of multinucleated osteoclast-like cells. The presence of osteoclast activators and osteoclast-like cells in the calcified vessels may imply an attempt to inhibit the mineralization by stimulating bone resorption. This also further emphasizes the importance of the RANKL:OPG ratio in modulating osteoclast differentiation and bone resorption.

Studies of human medial calcification and atherosclerosis have revealed possible roles for bone regulating proteins in vascular calcification. With Monckeberg's sclerosis, calcification occurs in direct apposition to VSMCs without the presence of macrophages in lipids seen in the intimal calcification of atherosclerosis.[75] These VSMCs directly abutting the area of calcification express high levels of MGP. In contrast, as compared with normal vessels, vessels with medial calcification globally express lower levels of MGP and higher levels of markers for osteoblasts and chondrocytes such as alkaline phosphatase, bone sialoprotein, and collagen II.[75] The low levels of MGP in vessels with medial calcification may suggest a predisposition to calcification. The localized up-regulation of MGP by VSMCs may reflect a reaction to the calcification and an attempt to increase calcium clearance. This is supported by the fact that when VSMCs calcify, MGP expression is up-regulated.[76] Similarly, in a study of human calcified atherosclerotic plaques, expression of calcification inhibitors such as MGP and OPG are down-regulated whereas markers of osteoblasts and chondrocytes were up-regulated.[77] Another study of human atherosclerotic plaques also found increased expression of MGP along the boundary of mineral deposits in advanced calcific lesions similar to that seen with purely medial calcification.[78] In addition, RANKL, weakly expressed in VSMCs in normal vessels, only could have been shown in association with the extracellular matrix surrounding calcium deposits.

OPG also has been studied as a potential marker of cardiovascular disease. In one study, 490 Caucasian women of at least 65 years of age were recruited to assess whether OPG levels were associated with stroke, mortality, and cardiovascular risk factors such as diabetes mellitus. The study found that OPG levels obtained at baseline were about 30% greater in women with diabetes mellitus and that increased OPG levels were associated with an increase in all-cause mortality and cardiovascular mortality.[79] OPG levels did not correlate with C-reactive protein levels in these patients, suggesting that OPG was not a marker of inflammation. Another study evaluated whether OPG was

associated with the presence of coronary disease. A total of 201 patients who underwent coronary angiography because of chest pain had serum OPG levels measured. The study found that serum OPG levels were increased significantly in patients with significant stenoses as compared with those without stenoses. In addition, as the severity of disease increased, there also was a subsequent increase in OPG levels.[80] Another study with 522 Caucasian men undergoing coronary angiography showed that once again there was a positive correlation between serum OPG levels and the severity of coronary disease.[81] In addition, OPG levels increased with age and were increased in diabetic as compared with nondiabetic patients. Finally, the investigators of the previous study also have reported that among 346 Caucasian men undergoing coronary angiography, serum levels of soluble RANKL were significantly lower in patients with coronary disease than those without. However, there was no correlation between soluble RANKL and the severity of coronary disease.[82] This is an interesting finding because it suggests the RANKL:OPG ratio in diseased vessels is much greater than that found in serum among those with cardiovascular disease. How this applies to the pathogenesis of cardiovascular disease and vascular calcification, particularly in CKD patients in whom OPG levels accumulate in part owing to decreased clearance (see later), remains to be seen. Clearly, however, it again appears that the RANKL:OPG ratio is more important than the individual concentrations.

Why OPG is down-regulated in diseased tissues and up-regulated in the serum in vascular disease is unclear. Animal models suggest that low OPG levels predispose to vascular calcification. The presence of RANKL, RANK, and osteoclast-like cells in the calcified vessels of *opg−/opg−* mice suggests a protective mechanism against calcification by osteoclastogenesis. Perhaps the down-regulation of OPG also plays a role in this manner. However, it is possible that the role of OPG in VSMCs is independent and separate from regulation of osteoclastogenesis. TNF-related apoptosis-inducing ligand (TRAIL) plays an important role in a variety of processes including inducing cellular apoptosis. TRAIL is found in a variety of tissues throughout the body including vascular smooth muscle and has been found to be cytotoxic to both vascular endothelial cells and medial smooth muscle cells.[83] OPG has been found to bind TRAIL and inhibits TRAIL-induced apoptosis.[84] In addition, OPG expression in VSMCs has been shown to be down-regulated by ligands for the peroxisome proliferator-activated receptor-γ (PPAR-γ), part of a family of ligand-activated nuclear transcription factors. PPAR-γ has been identified as a nuclear receptor for thiazolidinediones, compounds used to treat diabetes as insulin sensitizers.[85] Ligands for PPAR-γ have been shown to inhibit VSMC proliferation[86] and thus are thought to have anti-atherogenic properties. Finally, platelet-derived growth factor and basic fibroblast growth factor, known to stimulate expression of OPG in VSMCs, also are potent stimulators of VSMC proliferation and migration. Moreover, ligands for PPAR-γ have been shown to inhibit the effects of platelet-derived growth factor and basic fibroblast growth factor on VSMCs.[87] All of these findings taken together suggest that OPG may play a regulatory role in VSMCs. Decreased OPG expression in diseased vessels may be an attempt to inhibit VSMC proliferation and migration. However, further studies need to be performed, particularly in regard to what role, if any, the increased serum OPG plays in the pathogenesis.

AHSG in the Vasculature

In addition to osteoblast cell cultures, fetuin inhibits apatite formation in vitro, suggesting a similar role for fetuin in the serum.[66] Recently, in an attempt to understand how MGP inhibits ectopic calcification, a new circulating protein mineral complex was discovered. The composition of this complex consists of mineral, fetuin, and MGP.[88] The protein mineral complex was discovered after rats were injected with etidronate, resulting in the increase of nonionic calcium, as well as phosphorous and MGP, in serum. Subsequent analysis determined the nonionic calcium, phosphorous, and MGP to be components of this protein mineral complex. Fetuin was discovered to be the predominant molecule in this complex. The source of the fetuin content was found to be derived from serum, consuming nearly half of the serum fetuin content without affecting total serum levels (50% bound to the protein mineral complex, 50% free). Moreover, the protein mineral complex is cleared rapidly from blood by 9 to 24 hours after etidronate injection. The investigators concluded that the formation of the complex occurred as a result of

inhibition of bone mineralization rather than inhibition of bone resorption. This was supported by the fact that the appearance of the complex occurs before any expected inhibition of bone resorption and by the fact that treatment with alendronate, a bisphosphonate that did not inhibit bone mineralization, did not result in the formation of the protein mineral complex.[88] In addition, etidronate injection in rats pretreated with warfarin did not result in an increase in serum MGP levels. This suggests that the increase in MGP is caused by new synthesis and the γ-carboxylation of MGP is required for its binding to the protein mineral complex.

In a second study, the formation of the fetuin mineral complex was inhibited by calcitonin, OPG, and alendronate—all inhibitors of bone resorption. The investigators suggested that this implied a bone origin of the mineral complex.[89] This was corroborated by a similar increase in calcium, phosphorous, and MGP levels after injection with etidronate among mice fed a calcium-deficient diet as compared with those fed a calcium-replete diet, suggesting bone as the origin of the increased calcium. A third study found that after the protein mineral complex was cleared, there was a nearly 50% reduction of the serum fetuin level, suggesting that clearance of the protein mineral complex also cleared the associated fetuin.[90] In addition, the third study also showed that the protein mineral complex also contained an additional protein, secreted phosphoprotein 24, a protein similar in domain structure to fetuin. Exogenous secreted phosphoprotein 24 was found to associate strongly with the protein mineral complex when added to it. A fourth study documented that the addition of calcium and phosphate to rat serum, increasing the concentration of each by 10 nmol/L, also resulted in the formation of this protein mineral complex.[91] Moreover, bovine fetuin inhibits mineral precipitation when added to solutions containing 5 nmol/L of phosphate and calcium, and this inhibition is associated with the formation of a fetuin-mineral complex. This fetuin-mineral complex also was found to bind MGP strongly.

Finally, a recent study showed, using electron microscopy and dynamic light scattering, that apatite precipitation inhibition by fetuin/AHSG is caused by the transient formation of soluble, colloid spheres containing calcium, phosphorous, and fetuin/AHSG in vitro.[92] The findings also support previous reports that the cystatin-like domain 1 was critical to precipitation inhibition. The investigators also speculated that these colloid spheres may be identical to the protein mineral complex described earlier. These findings all suggest that this circulating protein mineral complex serves as an inhibitor of mineral precipitation in serum.

Among *ahsg−/ahsg−* mice, some developed ectopic microcalcifications in soft tissues.[69] Also, the addition of BMPs to ectopic sites resulted in further bone extension in *ahsg−/ahsg−* mice as compared with *ahsg+/ahsg+* mice.[70] This further supports the concept that fetuin/AHSG acts as an inhibitor of mineralization. Among calcifications of atherosclerotic human aortic tissue, AHSG has been found in concentrations approximately 7-fold greater than that in plasma.[93]

As stated earlier, AHSG levels are lower among patients with CKD stages I through V than with normal controls. In patients with CKD stage V on hemodialysis, low concentration of AHSG is associated with increased C-reactive protein, enhanced cardiovascular mortality, and enhanced all-cause mortality.[56] In addition, the sera of these patients showed impaired ex vivo ability to inhibit apatite formation. Patients on long-term hemodialysis (>2 y) showed a trend toward increased severity of vascular calcification than patients on short-term dialysis (<1 y), however, this was not statistically significant.

Another study found that nondiabetic patients presenting with acute ST elevation myocardial infarction showed a trend toward lower serum AHSG levels than normal controls.[94] AHSG levels in these patients also trended back to normal levels on discharge. This finding suggests the long-known idea that AHSG is also a negative marker of inflammation. Previous studies have shown that AHSG has a negative correlation with acute phase reactants such as α1-antitrypsin and haptoglobin and a positive correlation with albumin.[95] In addition, interleukin 1β and interleukin 6 have been shown to down-regulate expression of AHSG and its messenger RNA in cell culture similar to albumin.[96] Because inflammation is thought to play a pivotal role in the pathogenesis of atherosclerosis, the association of low AHSG levels and cardiovascular mortality among patients with CKD stage V and nondiabetic patients presenting with ST elevation myocardial infarction, may in part be related to inflammation.

OPG in Patients With CKD

Two recent reports indicate that levels of OPG are increased in patients with CKD stages I through V.[97,98] One of these reports[98] determined that OPG levels increase with age in a healthy population as well as in patients with CKD stage V on hemodialysis. The levels in the latter, however, were significantly higher independent of age. In patients with CKD stages I through V not on hemodialysis, serum levels of OPG correlated with serum creatinine levels and had a reciprocal relationship to creatinine clearance over a 24-hour period. Thus, the kidney was reported to be the major site for clearance of OPG. In addition, it was determined that OPG found in serum of patients with CKD was capable of binding to RANKL in vitro, showing putative bioactivity in vivo.

Recent studies have looked at serum OPG as a possible predictor of bone turnover rate in renal osteodystrophy. One study examined 26 patients with CKD stage V just before kidney transplantation.[99] These patients had been on maintenance hemodialysis from 9 to 67 months at the time of sampling. Determination of bone mineral density and bone histomorphometry allowed the correlation of bone morphology to markers of bone metabolism. The patients were divided into type II (normal or low turnover) and III (high turnover) renal osteodystrophy based on the histomorphometric results. In corroboration of previous studies it was determined that significantly high intact parathyroid hormone (iPTH) levels correlated with histomorphometric indices of high bone turnover. Examination of OPG indicated increased levels in all dialysis patients, but with lower amounts in type III as compared with type II disease state. Finally, it was reported that the combination of iPTH and OPG levels correctly classified types II and III in 72% and 88% of the patients, respectively. Thus concluding that measuring circulating OPG and iPTH levels might be a valid noninvasive means of determining bone turnover rate in renal osteodystrophy.

A second study also examined bone biopsy specimens along with markers of bone turnover in 39 patients with CKD stage V.[100] The average time on dialysis was 60.9 $\pm$ 83.4 months. Patients were described as having adynamic bone disease, osteomalacia (low turnover states), predominant hyperparathyroidism, or mixed osteodystrophy (high turnover states). Results similar to those reported earlier indicated that OPG levels were increased markedly over the normal range in both the low and high turnover conditions. Correlation of disease state with OPG levels in conjunction with the markers of bone remodeling indicated significant relationships. Similar to what was reported earlier, diagnosis of high turnover osteodystrophy, and PTH levels less than 1,000 pg/mL, OPG was correlated inversely to iPTH, total PTH, and parameters of bone resorption. In addition, negative correlations were reported between OPG and parameters of bone formation. However, when parameters of patients with iPTH levels less than 300 pg/mL were examined, those with adynamic bone disease were found to have lower OPG levels than those diagnosed with either hyperparathyroidism or mixed osteodystrophy.

The discrepancy between the 2 studies described earlier, in which attempts were made to use OPG levels to predict degree of renal osteodystrophy, could be owing to the small number of patients examined. This incongruity also could reflect that other factors, as noted earlier, are involved in the osteoblast regulation of osteoclastogenesis. The ratio of RANKL to OPG is actually the important parameter in determining the progress of osteoclast development and activation in the bone, thus measuring the serum levels of OPG rather than determining ratios of RANKL to OPG, a more difficult feat, appears to have significant shortcomings.

AHSG in Patients With CKD

As stated earlier, AHSG levels appear to be lower among patients with CKD stages I through V as compared with normal controls. In addition, AHSG appears to have increased expression in calcified vessels. This is in contrast to OPG, which has increased serum expression and decreased expression in calcified vessels. Possible causes for the decreased serum AHSG levels in patients with CKD include chronic inflammation and protein malnutrition. Current theories regarding vascular calcification suggest that decreased bone mineralization leads to heterotopic mineralization. In rats, decreased mineralization leads to the formation of a protein mineral complex containing fetuin, the rat homologue of AHSG. The purpose of this protein mineral complex is thought to be inhibition of apatite precipitation at ectopic sites. Low levels of AHSG in these patients also predicted an increased

cardiovascular risk. It certainly is conceivable that among patients with CKD, low levels of AHSG result in a reduced ability to inhibit apatite precipitation. Indeed, it was documented that patients with CKD stage V on hemodialysis had such an impaired inhibition. In addition, fetuin has been shown to modulate osteogenesis. What role decreased levels of AHSG in CKD patients plays with regard to osteogenesis, either in bone or in VSMCs, remains to be seen.

CONCLUSION

The relationship between bone and the kidney is a complex interplay of kidney-bone connections, bone-kidney connections, and cell-cell connections. Bone is in a constant state of remodeling. Alterations in remodeling rates lead to adaptive processes to correct it. In CKD, deficiency of BMP-7 leads to decreased osteoblast differentiation and decreased bone remodeling. As an adaptive mechanism, PTH levels increase in an attempt to restore normal bone remodeling. However, PTH is not an osteoblastic differentiation factor. Instead, increased levels of PTH stimulate osteoblast progenitors to become fibroblast-like cells, resulting in marrow fibrosis. In addition, PTH stimulation leads to increased RANKL expression and decreased OPG, resulting in osteoclast formation and bone resorption. Treatment of this maladaptive process results in quiescent bone or the adynamic bone disorder. We have shown that treatment with BMP-7 in animal models of CKD effectively reverses both of these high and low turnover states.

Second, vascular calcification is an important cause of morbidity and mortality in CKD. The pathogenesis of medial calcification likely involves an osteoblast-like cell within the vessel wall. The likely candidate for this is the VSMC because it shares common progenitors with osteoblasts and retains the ability to transdifferentiate. Evidence has shown that decreased bone mineralization leads to increased pressure toward heterotopic mineralization. BMP-7 has been shown to induce the phenotype of VSMCs. In addition, by restoring normal bone mineralization, BMP-7, in theory, also decreases the pressure toward heterotopic mineralization. Indeed, we have shown that treatment with BMP-7 in animal models of atherosclerosis and vascular disease in CKD reverses vascular calcification. Thus, it appears that BMP-7 is a potential therapy for renal osteodystrophy and vascular calcification.

Finally, 2 bone regulating proteins found in bone, serum, and the vasculature also play a role in bone remodeling and vascular calcification. OPG and AHSG, the human homologue of fetuin, have varying roles in these processes. Indeed, mice engineered to be OPG deficient show severe osteoporosis and vascular calcification, whereas those engineered to be fetuin deficient have increased bone formation and an increased susceptibility to ectopic calcification. Among patients with CKD, serum OPG levels appear higher than in normal controls whereas serum AHSG levels are lower than normal in controls. Higher serum levels of OPG correlate with increased cardiovascular mortality, diabetes, and coronary disease whereas low AHSG levels correlate with increased cardiovascular mortality. AHSG modulates osteogenesis and inhibits apatite formation by forming soluble mineral complexes, indicating a protective role of this substance against vascular calcification. The overall role of OPG in vascular calcification is less clear, and likely the ratio of RANKL and OPG plays a more important role.

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