Current Concepts Review

A Review of Osteocyte Function and the Emerging Importance of Sclerostin

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Osteocytes, derived from osteoblasts, reside within bone and communicate extensively with other bone cell populations to regulate bone metabolism. The mature osteocyte expresses the protein sclerostin, a negative regulator of bone mass.

In normal physiologic states, the protein sclerostin acts on osteoblasts at the surface of bone and is differentially expressed in response to mechanical loading, inflammatory molecules such as prostaglandin E2, and hormones such as parathyroid hormone and estrogen.

Pathologically, sclerostin dysregulation has been observed in osteoporosis-related fractures, failure of implant osseous integration, metastatic bone disease, and select genetic diseases of bone mass.

An antibody that targets sclerostin, decreasing endogenous levels of sclerostin while increasing bone mineral density, is currently in phase-III clinical trials.

The osteocyte has emerged as a versatile, indispensable bone cell. Its location within bone, extensive dendritic network, and close communication with systemic circulation and other bone cells produce many opportunities to treat a variety of orthopaedic conditions.

Bone is the major component of the skeletal system that provides locomotion, muscle attachment, protection of internal organs, and calcium homeostasis. In addition, bone provides a unique microenvironment in which osteoblasts, osteocytes, osteoclasts, hematopoietic cells, mesenchymal cells, and immune cells interact (Fig. 1). Monocytes differentiate and fuse to form bone-resorbing osteoclasts. Osteoblasts are derived from mesenchymal stem cells and primarily deposit new bone osteoid. Osteoblast, osteocytes, and T cells produce a key osteoclastogenic protein called receptor activator of nuclear factor kappa-β ligand (RANKL). To balance osteoclastogenesis and bone resorption, osteocytes and osteoblasts also produce osteoprotegerin (OPG) to interfere with RANKL signaling. There is increasing evidence that bone cells act on other systems such as the central nervous system, energy metabolism, serum glucose regulation, and gonadal function. One example is osteocalcin, a protein secreted by osteoblasts that modulates pancreatic insulin secretion and gonadal function.

The osteocyte is the master signal sensor, integrator, and transducer of the skeleton. Osteocytes, osteoblasts, and osteoclasts are the major bone cells that orchestrate growth, maintenance, and healing of bone. Sclerostin, a glycoprotein secreted

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predominantly by osteocytes under physiologic conditions, is an important negative regulator of bone mass through the inhibition of bone formation by osteoblasts. Bone cells are highly metabolically active components of bone. Mineralized bone matrix comprises hydroxyapatite, calcium, and other ions important for homeostasis. Bone matrix is a reservoir for many proteins such as collagen, osteocalcin, osteopontin, transforming growth factor, and bone morphogenetic protein (BMP). On the bone surface, osteoblasts produce new matrix while osteoclasts resorb and remodel bone. Osteocytes, which make up >95% of bone cells, are differentiated osteoblasts encased in the bone matrix. The myriad functions of osteocytes have not been unraveled until recently.

Osteocytes form a living network within the mineralized matrix of bone, appearing quiescent to observational researchers of the past. From within a 15 to 20-μm lacunar space, the osteocyte cell body communicates via dendrites that extend through tubular canaliculi (Fig. 2). These dendrites contact other osteocytes, the marrow, and the osteoblast layer. The distribution of osteocytes within bone is a highly organized three-dimensional matrix designed to enhance adaptation. The vast network of osteocytes forms a large “bone membrane” and cell-matrix interface. Osteocytes are bathed in a unique canalicular fluid that delivers nutrients, oxygen, and information from the systemic circulation. Canalicular fluid carries hormones, exchanges circulating factors, conducts mechanical signals, and provides access to potential therapeutic drugs.

The signaling pathways underlying the osteoblastic differentiation into osteocytes are under investigation. Several key proteins that identify the maturing osteocyte, such as E11, alkaline phosphatase, Pi-regulating endopeptidase on chromosome X (PHEx), matrix extracellular phosphoglycoprotein (MEPE), sclerostin, and fibroblast growth factor-23 (FGF-23),...
are differentially expressed (Figs. 1 and 3). Only after completion of differentiation does the osteocyte produce sclerostin and FGF-23, which are important factors in osteocyte-osteoblast communication. FGF-23 acts locally within the bone and also acts on the kidneys to influence phosphate homeostasis; however, discussion of osteocyte expression of this intriguing protein is beyond the scope of this review.

Since its initial description in 1958, sclerostin has emerged as an important osteocytic secretion with implications for a variety of bone density diseases. Although osteocytes are the best-researched producers of sclerostin, mRNA (messenger RNA) has also been detected in chondrocytes, kidney, lung, vasculature, and heart. Sclerostin is a SOST gene product under nuanced regulatory control. It is integral to osteocyte function as a signal to damp the action of osteoblast bone deposition and to control bone metabolism. Its anti-anabolic effect on bone has rendered it an important molecule in fracture-healing, osteoporosis, metastatic disease, and a variety of other disorders. Development of sclerostin antibodies has shown promising results and wide applicability to a multitude of orthopaedic conditions.

Physiologic Roles of Sclerostin

Sclerostin expression is regulated by a wide variety of factors, including mechanosensation, local cytokines, and endocrine factors.

Osteocytes are integral mechanosensors of bone, and osteocytes regulate bone mass in response to mechanical loading. The lacunae and the canalicular network among osteocytes may act as mechanical strain amplifiers, in order to increase osteocyte sensitivity to mechanical loading. The exact location of signal detection is not known, and may reside within the osteocyte cell body or dendrites, or both. Sclerostin is differentially regulated depending on skeletal mechanical loading. Areas of concentrated strain in the skeleton show decreased levels of sclerostin. In microgravity and decreased mechanical loading experiments, both upregulation of sclerostin and decreased bone mineral density occur. Clinically, the serum sclerostin level is increased in healthy adult males during bed rest. In cases of chronic unloading, such as spinal cord injury, levels of sclerostin also increased and correlated with osteoporosis progression.

Downregulation of sclerostin is associated with increased osteogenesis and bone mass. Sclerostin is thought to act by inhibition of the Wnt/β-catenin pathway in osteoblasts, binding at the LRP (low-density lipoprotein receptor-related protein) 5/6 receptor on the osteoblast cell membrane. Therapeutically, antibodies against sclerostin protein (AMG 785; Amgen, Thousand Oaks, California) reduce levels of sclerostin and restore bone mineral density. In this pathway, it has been hypothesized that sclerostin travels through the canalicular network to the bone surface to reach target osteoblasts at the bone surface and activate the Wnt pathway. Subsequently, sclerostin promotes osteoblast apoptosis to ultimately decrease osteoid deposition.

In response to mechanical loading, osteocytes release prostaglandins. In vitro studies have shown that prostaglandin E2 rapidly inhibits sclerostin through the EP4 receptor. This release serves to regulate osteoblast proliferation and can also interact with the Wnt/β-catenin pathway. It has also been suggested by in vitro data that hypoxia inhibits sclerostin
expression in osteocytes through the Wnt/β-catenin pathway\textsuperscript{49}. Many other inflammatory molecules, such as tumor necrosis factor, oncostatin M, cardiotrophin-1, and leukemia inhibitory factor, rapidly downregulate sclerostin expression in osteocyte cell lines\textsuperscript{50-53}.

Osteocytes are sensitive to a variety of systemic hormones that change bone mass by altering the expression of sclerostin. Studies have shown rapid, efficient communication between osteocytes and systemic circulation\textsuperscript{19}.

For example, parathyroid hormone (PTH) is a resorptive signal released into the bloodstream by the parathyroid glands to act on bone in response to low levels of circulating calcium. PTH acts on osteocytes by altering sclerostin expression. Constitutively active PTH receptor-1 (PTHR1) in osteocytes is sufficient to increase bone modeling and remodeling due to the inhibition of sclerostin expression\textsuperscript{54,55}. In a study of twenty-seven postmenopausal women treated with intermittent PTH, levels of sclerostin in the peripheral blood and bone-marrow plasma were decreased significantly\textsuperscript{56}. Evidence has shown that PTH causes increased proteasomal degradation of runt-related transcription factor-2 (RUNX2) protein, which is a direct up-regulator of SOST expression\textsuperscript{57-59}. One possible pathway for the anabolic effect of intermittent PTH is inhibition of sclerostin expression in osteocytes.

Sex steroids are important for bone growth and maintenance. Estrogen signaling affects osteocyte regulation of bone density. Deletion of the estrogen receptor-α results in decreased sensitivity to mechanical loading, and estrogen withdrawal induces osteocyte apoptosis\textsuperscript{59,60}. In men with idiopathic osteoporosis, circulating sclerostin levels correlate with estrogen exposure, possibly reflecting lower osteocyte cell mass or number\textsuperscript{61,62}. In elderly men treated with gonadotropin-releasing hormone (GnRH) and 17β-estradiol, serum sclerostin levels were significantly reduced, while men treated with GnRH agonist and testosterone showed increased circulating sclerostin\textsuperscript{63}. Studies on circulating sclerostin levels and sex hormones have not, however, clearly demonstrated that sclerostin levels are due to osteocyte expression. At this stage, much research is based primarily on circulating levels of sclerostin and is largely preliminary.

It is well known that patients with type-II diabetes mellitus have associated low bone mass and poor fracture-healing. In experimental rats with type-II diabetes mellitus, treatment with sclerostin antibody increases bone mass and bone strength and improves bone defect regeneration\textsuperscript{63}. It has been proposed that bone defect regeneration may be dysregulated in patients with type-II diabetes mellitus because of premature callus resorption before adequate ossification occurs\textsuperscript{64,65}.

The Role of Sclerostin and Osteocytes in Orthopaedic Disorders

Osteocytes and their secretory products are under investigation in a variety of known bone diseases. Osteoporosis, fracture-healing, implant osseous integration, metastatic bone disease, and genetic bone disease each involve and affect osteocyte biology (Fig. 4). Sclerostin, secreted by mature osteocytes, is a subject of much interest as it pertains to bone disorders.
Fracture-Healing
Understanding and improving fracture-healing is a major area of research. Optimization of this system would decrease morbidity and mortality due to traumatic and pathologic fractures. Initial disruption of the vasculature at a fracture site induces cell necrosis; however, it has been suggested that surviving osteocytes are important for robust cellular recruitment and OPG release in initial fracture-healing stages. Sclerostin expression is important during later callus remodeling by down-regulating callus formation and maturation.

Rodent models suggest downregulation of sclerostin is beneficial in fracture-healing. In mice lacking the sclerostin gene, healing was significantly hastened as evidenced by increased bone area, decreased cartilage area, and osseous bridging in the callus as early as fourteen days (no wild-type mice displayed osseous bridging at this time). Sclerostin antibody, when administered twice weekly after fracture, showed increased bone formation, bone mineral density, and bone strength as early as two weeks after fracture. At six weeks, the maximum failure load was increased by 68%. Sclerostin antibody administered to rats with a critical-sized femoral defect and mice with a non-critical-sized femoral defect showed earlier healing, complete union, and physiologic maturation of the defect. Similar conclusions can be drawn from the rat model of experimental periodontitis, in which sclerostin antibody treatment increased alveolar bone regeneration and filled substantial oral bone defects.

Contrastingly, in a study of seventy-five human patients with long-bone fractures, fracture hematoma and serum sclerostin levels were elevated, evidencing a local and systemic role for sclerostin in fracture-healing. Modulation of the Wnt pathway may be necessary for proper fracture-healing. While sclerostin activates the Wnt pathway, dickkopf-related protein 1 (Dkk-1), a protein that also acts at LRP6 to inhibit Wnt signaling, inhibits fracture-healing. In a head-to-head clinical trial comparing zoledronic acid and denosumab treatment with Dkk-1 and sclerostin serum levels in postmenopausal women, it appeared that zoledronic acid and denosumab exert opposite effects on the Wnt pathway to ultimately decrease bone resorption.

In postmenopausal women with osteoporosis, sclerostin levels may predict the risk of fracture: an increased level of serum sclerostin, independent of bone mineral density, age, and other confounding factors, strongly correlates with fracture risk. Fragility fractures are a major complication of osteoporosis and are difficult to surgically stabilize because of inadequate bone strength to hold metallic implants used for internal fixation, with a high rate of delayed fracture union and nonunion. In the first few days after fracture, inflammatory molecules such as interleukin-6 are expressed at the fracture site, which may induce bone-remodeling gene expression and sclerostin downregulation after the fourth day. Ovariectomized rats treated with sclerostin antibody showed enhanced, accelerated bone repair in proximal tibial defects after one week. Sclerostin antibody not only improves bone-healing but also has anabolic effects on the entire skeleton. Sclerostin antibody may reduce the risk of nonunion after surgical stabilization due to fixation failure.

These data taken together paint an interesting potential role for sclerostin in fracture-healing. Although neutralization of sclerostin in rodent models enhances bone formation, human patients demonstrate increased levels of sclerostin during physiologic fracture-healing. Furthermore, levels of sclerostin are altered in an osteoporotic state. These data suggest the regulation of bone mineralization, callus ossification, and remodeling are closely related to sclerostin expression. Osteocytic secretion of sclerostin at specific time points after skeletal injury most likely plays a critical regulatory role in bone-healing.

Implant Osseous Integration
Orthopaedic implants in joint arthroplasty are most likely to fail because of late aseptic loosening. After surgery, all prostheses inevitably generate wear particles that embed in adjacent tissues. Implant failure often is accompanied by peri-implant bone resorption and osteocyte death.

Studies have shown that wear particles generated from implants significantly altered osteocyte function in vitro resulting in Akt inactivation and cell apoptosis, providing a link between macroscopic findings and molecular biology. To increase bone formation around implants, Liu et al. and Virdi et al. treated experimental rat implant models with sclerostin antibodies. Sclerostin antibody in those studies prevented the negative effect of wear particles and increased bone volume. Osteocyte viability and maintenance of bone mass are critical to reduce the complication rate of joint arthroplasty; revision rates for total hip and total knee replacements are projected to increase to 137% and 601%, respectively, by 2030.

Metastatic Bone Cancer and Cancer-Induced Bone Loss
Metastatic and primary cancers that involve osseous lesions may interact closely with osteocyte protein products. Sclerostin may be a key mediator of cancer-induced bone disease and represents a promising therapeutic avenue for bone metastasis in breast cancer and myeloma-related bone disease. Other targets include OPG, RANK/RANKL, cathepsin K, and other molecular pathways as potential emerging targets for metastatic bone pain. In multiple myeloma, myeloma cells override osteocyte regulation of bone cells. Myeloma cells release RANKL and inhibit production of as well as induce degradation of OPG. Additional studies have revealed that myeloma cells also suppress osteoblasts by secreting sclerostin. A positive correlation between severity of bone disease and circulating levels of serum sclerostin suggests multiple myeloma cells utilize sclerostin in the formation of osteolytic lesions.

In some disease states, such as Paget disease of bone and prostate cancer, there is a substantially increased number of osteoblasts and osteoblastic activity. In addition to increased bone mineralization and osteoblastic lesions, respectively, these patients demonstrate levels of sclerostin that correlate with the rate of bone turnover. Perhaps these data illustrate compensatory sclerostin release from osteocytes to downregulate an overwhelming level of osteoblast activity. Alternatively, sclerostin may induce osteocytic RANKL production, upregulating osteoclast activity and bone resorption.
Breast cancer metastatic cells also modulate bone cell activity. In osteolytic metastatic breast cancers, RUNX2 and co-activator core-binding factor beta (CBFB) modulate both osteoclast and osteoblast function. Specifically, osteopontin secretion by breast cancer metastatic cells activates osteoclasts, while osteoblasts may be inhibited by RUNX2/CBFB-dependent sclerostin expression in osteoblasts. Sclerostin domain containing 1 (SOSTDC1), a gene from the sclerostin family, is active in the BMP and Wnt signaling pathways and may be downregulated in breast cancer. Higher levels of SOSTDC1 mRNA in patients correlate with metastasis-free survival rates, while primary breast tumors exhibit decreased levels of SOSTDC1 signaling. These data, taken together, suggest a complex role for sclerostin in breast cancer metastatic disease.

**Genetic Bone Diseases**
A number of bone diseases are due to genetic aberrations that specifically implicate osteocytes. Whether osteocyte dysfunction or dysfunction of osteocyte gene products is culpable in each disease is often uncertain. However, specific dysregulation of sclerostin results in characteristic bone abnormalities.

Sclerostin dysregulation is most specifically seen in sclerosteosis and van Buchem disease. In sclerosteosis, patients have increased bone mineral density and syndactyly due to sclerostin loss-of-function mutations. Only six mutations have been identified in sclerosteosis, and they involve both the sclerostin gene and LRP5. Van Buchem disease is a similar, less severe disease of generalized osteosclerosis. Increased osteoblast activity is due to a regulatory domain deletion downstream of the SOST gene. In human teeth and growth plates from patients with van Buchem disease, osteocytes did not produce appreciable levels of sclerostin. Clinically, levels of serum sclerostin in van Buchem disease show a gene-dose effect of the deletion (as measured by serum sclerostin levels) with relation to the phenotypic severity of disease.

Although sclerostin is clearly implicated in van Buchem disease and sclerosteosis, it may also play a relevant role in osteogenesis imperfecta. Osteogenesis imperfecta, classically due to a defect in type-I collagen, is characterized by osteopenia and brittle bones prone to fracture. In a mouse model of osteogenesis imperfecta, short-term sclerostin-neutralizing antibody improved bone mass and reduced fractures.

Other genetic diseases have shown extensive bone involvement and may be related to osteocytic dysfunction. For example, ankylosing spondylitis is a disease of ectopic bone formation that causes a bamboo-like appearance of the spine with limited mobility and lower back pain. Altered OPG levels in ankylosing spondylitis favor osteoblastic activity in active disease states and ectopic bone formation. Osteoporosis due to thalassemia also shows high bone turnover and increased Dkk-1 serum concentrations. High sclerostin in these patients was not reduced after zoledronic acid administration, suggesting high osteocyte activity.

**Emerging Osteocyte and Sclerostin-Targeted Therapeutic Options**
Sclerostin is emerging as a key molecule in governing bone health. As the source of sclerostin, osteocytes are master regulators of chemical and mechanical signals that affect profound changes within the human body. The intimate access of the vascular system through the canalicular fluid and osteocytic network provides an ideal delivery system for a variety of small molecules. Understanding the systems that regulate osteocyte function and expression of sclerostin are important facets of ongoing research in bone health. Additional applications of sclerostin action are also emerging, as in chondrocyte expression of sclerostin in osteoarthritis. To manage sclerostin levels is to protect the delicate balance among the osteocyte, the osteoblast, and the osteoclast.

An antibody against sclerostin, as discussed above, has been developed specifically for the treatment of osteoporosis. The sclerostin antibody (AMG 785; Amgen) decreases the endogenous levels of sclerostin, allowing for osteogenesis by improved osteoblast survival. Many exciting new applications for sclerostin antibody have been discussed in this article, including sclerosteosis, van Buchem disease, fracture-healing, and osteoporosis. Phase-I and II clinical trials of AMG 785 are ongoing to assess the effectiveness of antisclerostin antibody for the treatment of osteoporosis. A phase-I trial in 2011 showed single doses of AMG 785 were generally well tolerated. Phase-II clinical trials enrolled women with femoral neck T-scores between −2.0 and −3.5 treated with five subcutaneous dosing regimens. The results showed increased bone mineral density at the lumbar spine and proximal part of the femur at twelve months after treatment with the five dosing regimens compared with other available therapies. Additionally, Amgen performed phase-II trials to assess AMG 785 fracture-healing efficacy in patients who were fifty-five to ninety-five years old after hip fracture and surgical fixation. In February 2013, Amgen and collaborator UCB SA halted patient testing mid-stage because AMG 785 treatment did not improve the time of healing of the fractures, as assessed on radiographs, compared with a placebo. Phase-III trials are currently under way for the treatment of osteoporosis in postmenopausal women. Despite an unremarkable toxicity profile in these trials, it appears sclerostin has other important functions in the bone-marrow environment on immune cell support; studies of sclerostin dysregulation have shown B-cell apoptosis and decreases in B-cell populations.

Many studies have identified diseases in which osteocytes have been abolished or decreased in number. In multiple myeloma, osteocyte apoptosis is markedly increased compared with that in normal patients and patients with monoclonal gammopathy of undetermined significance (MGUS). Osteocytes lose control of sclerostin secretion in this clinical scenario. Patients with Crohn disease with major mineral deficiencies due to gastrointestinal damage have also demonstrated deleterious effects on osteocytes and decreased bone remodeling. However, animal models of Crohn disease treated with sclerostin antibody have dramatic improvements in bone density. Additionally, bisphosphonates, calcitonin, and QVDPH, a pan-caspase inhibitor, each show promise in maintaining osteocytes in disease states.

The network formed by osteocyte dendrites has also been implicated in disease and may represent aberrant communication...
between osteocytes and the osteoblasts on the bone surface. For example, in osteoporosis, osteocyte dendrites demonstrate a decreased number of connections, and the carefully laid three-dimensional network is disturbed\(^1\). Conversely, in osteomalacia, there is an increased number of dendrites and connectivity within the network\(^1\). In osteomalacia, the extracellular osseous matrix is hypomineralized; recent studies have shown that hypomineralization of osteocyte lacunae is associated with other disease processes, such as osteoarthritis\(^2\). Changes in the morphology of osteocytes and their extracellular environment is intimately affected by and related to disease process and bone density.

Systemic diseases are also related to osteocyte and bone cell activity. Hyperparathyroidism, in which PTH is increased, promotes pathologic osteclast activity and, in turn, results in destruction of the bone. However, intermittent PTH administration decreases sclerostin levels and increases bone mineral density\(^3\). Thus, PTH function as related to bone mass is directly related to temporal administration. This novel effect of intermittent PTH administration yielded the development of teriparatide (Eli Lilly, Indianapolis, Indiana), which has efficacious therapeutic effects in the treatment of osteoporosis and has been shown to decrease sclerostin expression and increase osteocyte density\(^4\).

### Overview and Future Directions

Treatments to increase bone mass are undergoing rapid changes. In addition to AMG 785, bisphosphonates denosumab (Amgen) and teriparatide (Eli Lilly) became available for clinical applications with a high level of enthusiasm and expanding indications. (Denosumab was approved by the U.S. Food and Drug Administration [FDA] for use in the treatment of osteoporosis and skeleton-related events in bone metastases from solid tumors; bisphosphonates were approved by the FDA for use in the prevention and treatment of osteoporosis and other bone diseases, such as Paget disease; and teriparatide was FDA approved for osteoporosis treatment in men and postmenopausal women at risk of fracture.) However, early studies have indicated several adverse side effects associated with denosumab, alendronate, and teriparatide\(^5,7\). Therefore, there is a constant need for advancement of clinical bone biology and for the development of new therapeutics.

Currently available data regarding osteocyte function have already given rise to a variety of therapeutic measures to alter osteocyte function. Osteocytes, the predominant resident cells of bone, and osteocyte-derived proteins such as sclerostin provide ample opportunities to develop novel strategies to treat many orthopaedic conditions.

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