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Perspective Article

Summary - Osteocyte control of bone formation via Sost/sclerostin

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Osteocyte regulation of the function of the executive cells of bone remodeling: the Sost/sclerostin paradigm

It has been long postulated that osteocytes initiate the adaptive response of bone to mechanical stimuli. Osteoblasts and osteoclasts are present on bone only transiently, in low number, and in variable locations. Osteocytes, on the other hand, are the most abundant resident cells (compared to lining and periosteal cells) and are present in the entire bone volume. In addition, osteocytes form a syncytium among themselves and with cells on the bone surface via cytoplasmic processes that radiate from their bodies and travel along canaliculi excavated in the mineralized matrix. This network is ideally suited to sense and respond not only to mechanical but also to systemic stimuli by generating signals that affect osteoblasts, osteoclasts, and their progenitors in the bone marrow[1]. However, in spite of significant progress in our knowledge about osteocytes in recent years, the mechanisms by which these cells control the function of osteoblasts and osteoclasts are just starting to emerge.

Osteocytes perceive changes in the level of both physical stimuli as well as circulating factors as evidenced by studies on the regulation of their life span. Thus, increased prevalence of osteocyte apoptosis accompanies the bone fragility syndrome that characterizes glucocorticoid excess and estrogen withdrawal[2-7]. Furthermore, inhibition of osteocyte apoptosis by bisphosphonates might explain at least part of the effects of these agents on fracture prevention, which is not accounted for by changes in BMD[8]. Osteocyte apoptosis is also regulated by mechanical forces as evidenced by increased prevalence of apoptotic osteocytes in bones exposed to high levels of mechanical stimulation[9] or in unloaded bones[10]. Notably, in both cases osteocyte apoptosis precedes temporally, and is spatially associated with, increased osteoclast-mediated resorption and subsequent loss of bone mineral and strength[9-12]. This evidence strongly suggests that, irrespective of the mechanism by which osteocytes are induced to die, osteocyte apoptosis might initiate a cascade of events to replace bone in the same location, namely targeted remodeling. This phenomenon represents an example of communication between osteocytes and osteoclasts; although the identity of the osteocyte-derived molecules responsible for initiating the osteoclastogenic response remains unknown.

The most compelling paradigm by which osteocytes influence the function and number of the executive cells of remodeling is symbolized by Sost/sclerostin. Osteocytes, but no other cells in bone, express sclerostin – the product of the Sost gene[13,14]. As expected for an osteocyte-derived secreted protein, high levels of sclerostin are detected in the lacunar-canaliculi system[15]. Sclerostin potently antagonizes several members of the bone morphogenetic protein (BMP) family of proteins[13,14], and also binds to LRP5/LRP6 preventing canonical Wnt signaling[16,17]. Both BMPs and Wnts are critical for osteoblastogenesis as they provide the initial and essential stimulus for commitment of multipotential mesenchymal progenitors to the osteoblast lineage[18,19]. Loss of Sost in humans causes the high bone mass disorders Van Buchem disease[20] and sclerosteosis[21]. In addition, administration of an anti-sclerostin antibody increases bone formation and restores the bone lost upon ovariectomy in rodents[22,23]. Conversely, transgenic mice overexpressing Sost exhibit low bone mass[13,24]. Taken together, these lines of evidence have led to the conclusion that sclerostin derived from osteocytes – the ultimate progeny of the osteoblast differentiation pathway – exerts a negative feedback control at the earliest step of mesenchymal stem cell differentiation toward the osteoblast lineage[13,14].

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Goals of the session – outstanding questions answered

The overall goals of this session were to bring us up to date on this rapidly growing field of study and to take advantage of the Sost paradigm to brainstorm about the control of skeletal homeostasis by osteocytes. Four speakers introduced studies that are milestones in the Sost/sclerostin area.

Will Van Hul focused on the human genetics of Sost. He recapitulated the findings that associate the Sost gene with the human diseases of skeletal overgrowth, Van Buchem disease and sclerosteosis[20,21]. Both conditions are inherited in an autosomal recessive mode. Eleven Van Buchem patients were found in a small ethnic isolate in The Netherlands allowing gene localization by homozygosity mapping. This led to the assignment of the disease causing gene to chromosome 17q12-q21[25]. The study of two families with several sclerosteosis patients and several inbred populations permitted to determine that the gene underlying this condition is located in the same chromosomal region[26]. This corroborated the hypothesis, based on clinical and radiological similarities, that both Van Buchem disease and sclerosteosis are mutations of the same gene. As a final result of a large genomic cloning and sequencing effort of the candidate region, a previously unknown gene was identified as the disease causing gene, currently known as the *Sost* gene[20,21]. Mutations in the Sost gene leading to decreased expression of sclerostin have been found in patients with sclerosteosis. However, the Sost gene is not mutated in Van Buchem patients. Instead, it was found that a 52-kb region downstream of Sost gene containing an enhancer responsible for bone-specific expression of the gene is deleted in these patients[24,27,28]. Absence of this enhancer results in complete lack of sclerostin protein in bone biopsies of Van Buchem patients. Therefore, although both Van Buchem disease and sclerosteosis are allelic disorders resulting in the loss of sclerostin, the molecular mechanisms leading to loss of sclerostin expression are different.

Clemens Lowik discussed the latest findings on the molecular mechanism of the action of sclerostin. Sclerostin is a member of the Dan family of glycoproteins, which includes proteins that antagonize the activity of BMPs and Wnts. *In vitro* and *in vivo* approaches showed that sclerostin inhibits BMP-stimulated osteoblast differentiation and bone formation. However, the mechanism responsible for this action is unclear. Transcriptional profiling analysis of cultured osteoblasts indicated that sclerostin specifically affects BMP and Wnt signaling out of many other signaling pathways. Sclerostin, however, did not inhibit stimulation of BMP target genes; and, therefore, the effect of sclerostin on BMP signaling appears to be indirect. Indeed, unlike classical BMP antagonists such as noggin or soluble BMP receptors, sclerostin does not antagonize directly BMP-induced Smad phosphorylation or Smad-driven transcriptional activation. Recently, sclerostin was found to antagonize canonical Wnt signaling by binding to the Wnt co-receptors LRP5 and LRP6. This Wnt antagonistic activity of sclerostin may explain the inhibitory effect of sclerostin on BMP-responses, since Wnts co-operate with BMPs in stimulating bone formation. Constitutively active BMP receptors, activation of Wnt reporters by BMPs, and more importantly, activation of Wnt reporters by Wnts are all antagonized by sclerostin. These findings confirm that sclerostin inhibits BMPs and Wnts through a direct effect on Wnt signaling and strongly suggest that the high bone mass observed in sclerosteosis and Van Buchem disease results from increased Wnt signaling.

Teresita Bellido presented evidence for hormonal regulation of sclerostin expression and for a crucial role of osteocytes in the skeletal effects of PTH. Continuous elevation of the hormone potently inhibits sclerostin expression in mice. This effect results from direct action on osteocytes since it is reproduced *in vitro* using osteocytic cell lines and primary cultures enriched in osteocytes. PTHrP, the other ligand of the PTH/PTHrP receptor (PTHR1), also decreased Sost expression *in vitro*. To determine whether activation of the PTHR1 in osteocytes is sufficient for Sost inhibition, transgenic mice expressing a constitutively active PTHR1 specifically in osteocytes were generated. This was accomplished by placing one of the constitutively active PTH receptors described in Jansen's metaphyseal chondrodysplasia (caPTHR1)[29,30] under the control of the promoter of the dentin matrix protein 1 (DMP1) gene, which confers osteocyte-specific expression of genes in transgenic mice[31]. DMP1-caPTH1R transgenic mice express significantly reduced levels of Sost mRNA in vertebral and tibial bone as compared to wild type littermates. On the other hand, the expression of Axin 2 and SMAD 6 – Wnt and BMP target genes, respectively – and the osteoblast-specific genes osteocalcin and collagen1a1 was elevated in DMP1-caPTH1R mice. Strikingly, DMP1-caPTHR1 mice exhibit a remarkable increase in BMD, as measured by Dexa (Piximus) and micro-CT, and strength in both the axial and appendicular skeleton. These findings demonstrate that PTHR1 signaling in osteocytes is sufficient for inhibition of Sost expression and leads to a concomitant increase in bone mass.

David Ke summarized animal experimentation demonstrating bone anabolism by administration of an anti-sclerostin antibody. He also showed that, similar to the human conditions lacking the protein sclerostin, Sost knock-out mice have increased bone mineral density throughout their skeleton but are otherwise essentially normal. To explore the therapeutic potential of sclerostin as a target for the treatment of postmenopausal osteoporosis, 6-month-old female rats were ovariectomized (OVX), allowed to lose bone for 1 year, and were then treated with a sclerostin neutralizing monoclonal antibody (Mab). Bone mineral density (BMD) was determined *in vivo* by DXA and *ex vivo* by Micro-CT. Bone loss, at 1 year post-OVX, was similar at various skeletal sites with a ~12% decrease in BMD compared to age-matched, sham-operated controls. Five weeks of Mab treatment led to statistically significant increases in BMD in lumbar vertebrae (26%), whole leg (16%), femoral metaphysis (28%) and

femoral diaphysis (9%) compared to vehicle-treated controls[23]. These increases in BMD were similar to the increases observed in a positive control group consisting of animals receiving daily injections of PTH. Micro-CT analysis of the femur revealed significant increases in both trabecular and cortical bone thickness for the Mab-treated group. There was also significantly increased osteoblast surface and decreased osteoclast surface over bone surface, as well as an increase in bone formation rate and serum osteocalcin. Additionally, osteoid was increased and there was a trend for a decrease in mineralization lag time, indicating that normal mineralization was taking place in the sclerostin antibody-treated animals. Gross observation, serum clinical chemistry, hematological analysis, and histopathological analysis revealed no abnormalities associated with pharmacological inhibition of sclerostin. In summary, robust increases in BMD were achieved in a rat model of postmenopausal osteoporosis after five weeks of treatment with a sclerostin-neutralizing Mab. These data suggest that inhibition of sclerostin may be useful in humans for building bone, even in clinical conditions where significant bone loss has already occurred[22,23].

Future directions

Several unanswered questions warrant extensive research in the osteocyte-sclerostin field in the future. Is the expression of other genes present in the same chromosome that Sost affected in sclerosteosis and Van Buchem disease? Are Sost mutations associated with mutations in other genes that induce high bone mass, such as LRP5? It is also unknown whether changes in sclerostin levels resulting from polymorphisms in the Sost gene are responsible for the inherited levels of bone mineral in the general population.

The regulation of Sost expression by PTH raises the possibility that other bone active hormones, local factors as well as mechanical stimuli exert their actions through regulation of Sost or other yet to be discovered osteocyte-specific genes. In fact, the report at this meeting of inhibition of sclerostin expression by loading *in vivo* suggests that downregulation of Sost expression in osteocytes is a convergence point for the action of chemical and physical cues known to regulate osteoblast number. Whether the PTH receptor also participates in downregulation of sclerostin by mechanical forces, may be via increases in PTHrP, is an intriguing possibility that remains to be investigated. Whether the molecular mechanism by which the Sost gene is so potently inhibited by PTH or loading involves inhibition of transcription or changes in mRNA stability is another important issue that calls for future investigation. Furthermore, it is likely that Sost inhibition is only one of the changes that results from activation of the PTHR in osteocytes. Therefore, future studies are needed to confirm a cause-effect relationship between decreased Sost expression and increased bone mass in the DMP1-caPTHR1 mice and to uncover additional effects of triggering signaling through the PTHR exclusively in osteocytes.

Further studies are also needed to determine whether the efficacy of the anti-sclerostin antibody is solely due to increased bone formation, or whether the observed decreased bone resorption contributes to this effect.

In closing, intensive research is envisioned in the area of Sost/sclerostin regulation and on the mechanisms by which osteocytes control skeletal homeostasis.

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