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MEETING REPORT

Preclinical studies in orthopedics and bone repair

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Meeting Report from the 33rd Annual Meeting of the American Society for Bone and Mineral Research, San Diego, CA, United States, 16–20 September, 2011

The 2011 annual ASBMR meeting featured several important themes. These included an emerging understanding of the role of the osteocyte in bone homeostasis as well as an increased focus on the crosstalk between vascular cells and osteoprogenitors. These areas have the potential to dramatically impact our understanding of mechanisms underlying bone healing.

Osteocytes are descendants of osteoblasts that become completely encased within the bone matrix, and have been shown to have a key role in bone homeostasis.[1] Osteocytes control bone mass via the production of negative regulators of the Wnt/β-catenin pathway such as Sclerostin and Dkk1. These factors are also important for mechanotransduction and regulating the response to loading.[2] Interestingly, *Sost*$^{-/-}$*, Ctnnb1*$^{loxP/loxP}$*; Dmp1-cre* double knockout mice were used to demonstrate that the bone loss induced by the lack of osteocyte β-catenin is dependent on autocrine signaling by Sclerostin.[3]

Sclerostin, an inhibitor of osteoblast activity with a highly restricted pattern of expression, has emerged as a therapeutic target for low bone mass. In preclinical models, anti-Sclerostin antibody has been used to treat ovariectomy-induced bone loss in rats,[4] and data were presented to show that it could prophylactically attenuate bone loss following ovariectomy.[5] Similarly, Dkk1 inhibition was also found to promote bone formation in growing rats.[6] An antibody to Sclerostin is currently in clinical trials for the treatment of postmenopausal osteoporosis.[7]

A number of investigators have examined the potential for Sclerostin-based therapies to augment orthopedic regeneration and repair. In a rat distraction model, continuous anti-Sclerostin antibody treatment led to significant increases in regenerate bone mineral content of 67% and 83% by 2 and 6 weeks, respectively.[8] In a rat spine fusion model, where fusion was induced by bone graft, anti-Sclerostin antibody treatment did not improve overall fusion rates, but did lead to a 86% increase in bone volume in the fusion mass.[9] In a femoral titanium rod insertion model, peri-implant bone formation was similarly reported to be increased in both ovx and sham-ovx when treated with anti-Sclerostin antibody.[10] However, mechanical measures of implant fixation were not reported. In contrast, previous reports have suggested that anti-Sclerostin antibody could not consistently resolve a 6-mm critical defect rat non-union model.[11] A 3-mm critical defect model showed only minimal improvement in union with anti-Sclerostin antibody treatment, although an increased bone formation rate was seen.[12]

Therapies that target Sclerostin produce an anabolic response by de-repressing an inhibitory signal from osteocytes. Thus, it would be anticipated that these therapies would be most effective in situations where the original bone is in close proximity, such as closed fractures, distraction osteogenesis or spine fusion. Where the original bone is more removed from the region of required healing, such as in a critical defect model or an open fracture, it would be anticipated that Sclerostin inhibition might be less effective. Reassuringly, these theoretical concepts are being supported by the current experimental findings.

In addition to the anti-anabolic Wnt/β-catenin modulators secreted by osteocytes, a key study in *Nature Medicine* has revealed that osteocytes can also regulate bone catabolism via receptor activator for nuclear factor κB ligand (RANKL) secretion.[13] In this paper a *Tnfsf11*$^{loxP/loxP}$*; Dmp1-cre* mouse model was used to show that conditional deletion of RANKL in osteocytes led to severe osteopetrosis. It is proposed that osteocyte apoptosis may directly or indirectly lead to RANKL secretion to induce bone loss. Tantalizingly, human Sclerostin treatment was found to not only produce anti-anabolic but also pro-catabolic effects on cultured osteoblasts and pre-osteocytes.[14] This was further explored using co-cultures of the MLO-Y4 osteocyte-like cell line and hematopoietic osteoclast precursors. RANKL/OPG mRNA ratio was increased in response to human Sclerostin, and osteoclast resorption in co-cultures was increased sevenfold. Thus, Sclerostin may have autocrine and/or paracrine effects on osteocytes to promote catabolism via RANKL. On the basis of these data it suggests that outcome measures examining bone resorption should be a key component of analyzing orthopedic models treated with anti-Sclerostin antibody.

Vascularity is important for bone repair and it has been experimentally shown that inhibition of angiogenesis is detrimental to[15] and promotion of angiogenesis is beneficial for[16] bone healing. Blood supply can deliver oxygen and nutrients to the site of repair, and also repress hypoxic factors that support chondrogenesis as opposed to osteogenesis. However, there is emerging evidence that vascular endothelial cells may have a more important signaling role in bone repair.

Results from a cell culture model, where human endothelial cells were co-cultured with marrow stromal cells, indicated that factors produced by the vascular endothelium can have profound effects on osteogenic differentiation. Co-culture enhanced early differentiation markers such as Osxterix, but impaired matrix mineralization and expression of terminal osteocyte markers.[17] Similarly, it was found that vascular tissues are a major source of endogenous BMP2 expression during distraction osteogenesis.[18] Using a BMP2 transgenic reporter mouse it was shown that BMP2 was expressed in vascular endothelial cells and vascular smooth muscle, as well as hypertrophic chondrocytes and osteocytes in the developing bone. It has been previously established that BMP2 is required for initiation of fracture repair, and the vascular expression of BMP2 in healing bones has yet to be described using this system.[19]

In addition to signaling roles, it has been previously reported that vascular endothelial cells themselves can give rise to heterotopic bone.[20] In cases of ALK2 mutation, a mechanism involving endothelial–mesenchymal transition has been comprehensively demonstrated.[21] Similarly, pericytes have been proposed as an alternative pool of osteoprogenitors in the context of fracture healing.[22] New data from the same group using a transgenic reporter mouse under the control of an inducible smooth muscle α-actin promoter was able to label a population of mesenchymal progenitors in the primary spongiosa or within the periosteum 2 days after tamoxifen activation.[23] More controversially, it was reported that hematopoietic stem cells can significantly contribute to the chondrocytes, osteoblasts and osteocytes involved in fracture healing.[24] This model system involved fluorescent hematopoietic stem cell *ex vivo* culture and transplantation into an irradiated recipient mouse that was subsequently fractured. Conflict with other studies and issues associated with nonspecific staining made this a contentious paper.

Aside of these major themes there were some individual preclinical studies of notable relevance to bone repair. Researchers presented a new BMP2 delivery system, where cells encapsulated within polymer hydrogel microspheres could be used, to deliver an anabolic stimulus to the fracture site.[25] This group also proposed a new mechanism for trauma-associated heterotopic ossification.[26] BMP2 can stimulate neuroinflammatory release of calcitonin gene-related peptide and substance P. In *Trpv1*$^{-/-}$ mice, impaired release of substance P and calcitonin gene-related peptide was associated with reduced heterotopic ossification. When mast cells (the putative targets of neuroinflammatory recruitment) were inhibited with cromolyn, heterotopic bone was also reduced. These data suggest a potentially important new molecular and cellular interaction involved with BMP2-induced bone formation.

Another noteworthy study examined the *Hox* (homeobox) genes, which are involved in early embryonic patterning. These genes have a key developmental role, but their function in bone repair has been poorly explored. An unexpected lack of *Hox11* gene expression was noted in the skeletal elements of the developing limb barring the outer perichondrium.[27] However, *Hox11* is expressed in limb tissues postnatally and is upregulated during fracturing healing. Preliminary conditional knockout data suggest a key role for *Hox* genes in bone repair.

Finally, data were presented on fracture healing in Cathepsin K null mice, lacking the primary cysteine protease responsible for degradation of osteoid.[28] These mice show slight increases in bone mass that are less significant than human pycnodysostosis. These mice show no delay in time to union, but larger callus size and slower hard callus remodeling. As Cathepsin K inhibitors are increasingly being developed for the treatment of osteoporosis,[29] studies on their effects on bone healing are highly relevant.

Conflict of Interest

Dr Schindeler received research support from Novartis, Acceleron, Celgene and N8, and advisory fees on an ad-hoc basis from Celgene.

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