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## MEETING REPORT

# Osteoblasts: more than we thought

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

We thought we knew all about the osteoblast; where it came from, what it did and didn't do. This year's ASBMR meeting shows that there is more to this intriguing cell than meets the eye.

### Origins

Dogma has it that osteoblasts arise from mesenchymal stem cell (MSC) precursors. However, exactly where those cells are located and how they get to the bone continues to be an area of controversy. Several interesting abstracts addressed various aspect of this issue.

The first, and perhaps most important, has been to identify appropriate markers to distinguish true MSCs from the large variety of other cells that occupy the marrow stroma. Nestin and α-smooth muscle actin (αSMA) are two recently identified proteins that selectively mark the pluripotent MSC populations. Nestin is an intermediate filament protein previously associated with other stem cell populations. Use of nestin as a marker for sorting marrow yields a cell population that is 97% positive for Sca-1, a well-recognized MSC marker.[1] Nestin$^+$ single-cell colonies were capable of osteogenic, adipogenic and chondrogenic differentiation, consistent with pluripotency. Furthermore, these cells were capable of self-renewal after passaging *in vivo* and formed complete bone ossicles after injection under the kidney capsules. The intimate relationship between the vasculature and skeletal progenitors is reinforced by tracking studies that used a αSMACreERT2; Ai9 transgenic mouse to follow the movement of progenitor cells from the microvasculature into the bone.[2] Previous work identified αSMA as a marker for skeletal progenitors.[3] These cells are associated with the microvasculature of the periosteum and marrow. Cell sorting of αSMA+ cells yields a highly enriched population of cells with adipogenic and osteogenic potential. Two days after tamoxifen induction of αSMACreERT2; Ai9 mice, fluorescently tagged cells were detected in the microvasculature and periosteum, but were completely absent from osteoblasts or osteocytes. In contrast, after 12 days, strong labeling of these two osteogenic populations was seen as cells migrated into the bone. Similarly, during fracture healing, tagged cells were present in chondrocyte and osteoblast populations of the callous.

Consistent with the concept of a vascular origin of skeletal progenitors, selective deletion of bone morphogenetic protein (BMP)2 in osteoblasts dramatically reduced blood vessels in the periosteum and trabeculae, and reduced αSMACre-marked vascular progenitor cells in this compartment.[4] The BMP defect in osteoblasts was associated with decreased *Vegf* expression, a BMP-regulated gene. On the basis of this work, the authors of this study proposed a feedback loop between vasculature and osteoblasts, whereby growing bone recruits new bone progenitors by stimulating angiogenesis.

Interestingly, the vascular compartment can also be a source of BMP. During distraction osteogenesis, smooth muscle and vascular endothelial cells lining arteries and veins were found to be the predominant source of BMP2.[5] This BMP might be involved in recruitment of osteoprogenitor cells from the vasculature, as well as assist blood vessels in patterning the Haversian units and trabeculae.

A number of reports over the past few years suggested that bone progenitor cells are present in the circulation.[6] It has also been proposed that hematopoietic stem cells may be able to trans-differentiate into osteoprogenitors, although rigorous evidence for this concept was lacking. Compelling evidence for this concept was presented at the meeting.[7] Specifically, enhanced green fluorescent protein-marked, single-cell colony-derived hematopoietic stem cells were injected into lethally irradiated mice. After multi-hematopoietic lineage engraftment was established, the tibia were fractured and the localization of the enhanced green fluorescent protein-positive cells was monitored. Interestingly, the fluorescent marker was detected in cells positive for osteoblast markers (Runx2, osteocalcin), as well as in hypertrophic chondrocytes and osteocytes.

### Mechanical Loading

Biomechanical stimulation, long appreciated by the orthopedic community as a major anabolic signal in bone, has only recently gained prominence at the American Society of Bone Marrow and Mineral Research. Several important abstracts at this year's meeting elucidated various aspects of mechanotransduction. The observation that selective deletion of osteocytes reduces the ability of bone to respond to mechanical loading highlights

the role of these cells as possible mechanosensors.[8] Less clear is how osteocytes send signals to the osteoblasts, which must be activated for new bone formation to occur. Insulin-like growth factor-1 is one likely candidate that may be released from the osteocytes in response to loading. Consistent with this concept, osteocyte-specific deletion of insulin-like growth factor-1 in osteocytes using a DMP1-Cre was shown to severely reduce the ability of mechanical loading to increase the bone area.[9] Wnt signaling has long been suspected of having a role in load-induced bone formation.[10] The importance of Wnt in this pathway is reinforced by the observed decrease in load-induced bone formation observed in mice heterozygous for β-catenin in osteocytes.[11] Although osteocytes have a prominent role as mechanosensors, other cells of the osteoblast lineage, including MSCs and osteoblast, also respond to mechanical loads. In the case of MSCs, mechanical stretching is known to suppress adipogenesis and may preferentially stimulate differentiation of cells toward an osteoblast fate. Stretching activates β-catenin-dependent transcription by activation of AKT, leading to phosphorylation and degradation of the β-catenin inhibitor, GSK3β. Unlike insulin, which blocks GSK3β through activation of the phosphoinositide-3 kinase pathway, recent evidence suggest that mechanical stretching of MSCs activates AKT through the mammalian target of rapamycin complex-2 phosphorylation at Ser473.[12] ERK/MAP kinase is another major pathway activated by mechanical loading. An interesting study showed that oscillatory fluid-flow shear-stress loading of preosteoblasts could activate gene expression via the extracellular signal-regulated kinase/mitogen-activated protein kinase phosphorylation of the RUNX2 transcription factor at two specific serine residues.[13] Serine-to-alanine mutation of these phosphorylation sites totally blocked the response to loading. Furthermore, phosphorylation of RUNX2 on the same sites was detected *in vivo* after bones were subjected to cyclical loading. Consistent with these findings, these same RUNX2 phosphorylation sites were previously shown to be necessary for extracellular matrix-induced osteoblast differentiation.[14]

### Transcriptional Controls

Many of the key factors controlling transcription of the osteoblast-related genes have been known for some time. More recent studies are focusing on the genomic distribution of these transcription factors and interactions with other nuclear components. Some highlights in this area are: (1) the introduction of chromatin immunoprecipitation/high-throughput DNA sequencing to examine the genomic distribution of RUNX2 and DLX3 transcription factor-binding sites, and (2) studies identifying new interactions between the nuclear factors. In the first area, genomic RUNX2-binding regions were identified in mesenchymal cells, primary osteoblasts and breast/prostate cancer cells induced to overexpress RUNX2, using a doxycycline-inducible system.[15] Approximately 5400 RUNX2-binding sites were identified. Of these, less than 5% were found in the proximal promoter regions of the *RUNX2* target genes, whereas 90% were either in introns or the intergenic regions. Binding sites were identified near genes encoding intermediates in Wnt, transforming growth factor-β, BMP and related pathways. Chromatin immunoprecipitation/high-throughput DNA sequencing analysis of the DLX3-binding sites identified this transcription factor in association with homeobox regions of known osteoblast target genes, such as *Twist*, *osteopontin*, *Gli3*, *Smad2*, *bone sialoprotein* and *osteocalcin*.[16] Overall, DLX3 was shown to be associated with 356 genes. Of these, 232 were active and 63 were repressed. These genomic approaches should eventually allow the discovery of gene networks controlled by each transcription factor, as well as assessment of control mechanisms that are currently only able to be analyzed one gene at a time.

Although there are already several examples of interactions between osteogenic transcription factors and other nuclear components, two new interactions, both of which affect the Wnt signaling, are noteworthy. First, in a complex gene deletion study, the suppressive effects of FOXO transcription factors on osteoblast differentiation were characterized *in vivo*.[17] This family of factors competes with β-catenin for binding to the TCF/LEF transcription factor on the promoters of the *Wnt* target genes. This study used an Osx-Cre to induce an osteoblast-specific deletion of FOXO 1, 3 and 4. The resulting mice exhibited increased bone mass and osteoblast proliferation. Effects of the FOXO deletion were not restricted to development, as deletion of these same factors in 3-month-old animals with an inducible Cre also increased the bone mass. Thus, FOXO factors can be considered suppressors of the Wnt pathway through their actions on the TCF/LEF signaling. A second new interaction was described between RUNX2 and Axin2, a component of the β-catenin degradation complex in Wnt signaling.[18] These two factors have opposite effects on cranial bone development with Runx2 haploinsufficiency causing cleidocranial dyplasia, and Axin2 deficiency causing craniosynostosis. Physical and functional interactions between these two factors were demonstrated. Furthermore, RUNX2 was shown to suppress Axin2 expression, thereby allowing increased Wnt signaling.

### MicroRNAs and Osteoblasts

As was highlighted by the Plenary Lecture by Eric Olson (Texas Southwestern Medical Center) at the Sunday morning session, micro RNAs (miRNAs) provide a means of regulating multiple mRNAs encoding proteins that are part of specific regulatory circuits. Evidence for miRNA regulation of osteogenic circuits is beginning to emerge. For example, miR218 was shown to increase during osteoblast differentiation and could selectively block inhibitors of the Wnt pathway, including SOST, sFRP, DKK2.[19] Overexpression of miR218 increased Wnt signaling and increased expression of osteoblast markers, such as RUNX2 and alkaline phosphatase. A panel of 11 miRNAs was also identified that targets RUNX2. These miRNAs are expressed in a lineage-related pattern in mesenchymal cells and generally inhibit osteoblast differentiation.[20]

### Conflict of Interest

The authors declare no conflict of interest.

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