

## MEETING REPORTS

### Osteoclasts: Meeting Report from the 32nd Annual Meeting of the American Society for Bone and Mineral Research

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At the 2010 ASBMR Annual Meeting, highlights of the osteoclast sessions included several studies investigating the mechanism coupling bone resorption to bone formation, as well as research clarifying understanding of the epigenetic control of osteoclast differentiation. The osteoclast-related abstracts on these and other topics from this year's meeting are summarized below.

#### **IGF-1 Released During Osteoclastic Bone Resorption Induces Osteoblast Differentiation from Bone Marrow Stromal Cells**

Homeostasis of skeletal tissues is maintained by a well-balanced regulation of bone formation and bone resorption (1;2). This process is called remodeling, in which osteoblasts are involved in the bone formation process and osteoclasts are essential for bone resorption. During the remodeling process, bone resorption by osteoclasts acts as a trigger to stimulate bone formation by osteoblasts, a step called coupling (3). However, the factor(s) that mediate bone formation signals from osteoclasts to osteoblasts are not well-understood. Several factors, such as TGF- $\beta$ , have been reported as possible candidates (4), but no direct evidence exists thus far.

A possible role for IGF-1 released from bone matrix during osteoclastic bone resorption for osteoblast differentiation was reported (5). IGF-1 is one of the most abundant factors deposited in bone matrix. Conditioned medium (CM) released during osteoclastic bone resorption promoted osteoblast differentiation from bone marrow stromal cells (BMSCs). CM contained high levels of IGF-1, and an anti-IGF-1

neutralizing antibody suppressed the osteoblastogenic effect of CM, indicating an important role for IGF-1. Osteoprogenitor-specific IGF-1-deficient mice (*IGF-IR(-/-)* mice) exhibited smaller size with low trabecular bone volume. Osteoblast number and area were significantly reduced in the knockout (KO) mice relative to their wild-type (WT) littermates while osteoclast number and area did not change. *In vitro* analysis demonstrated that the osteogenic potential of BMSCs was reduced in *IGF-IR(-/-)* mice. In addition, when BMSCs from KO mice were transferred into *Rag2(-/-)* mice, no *IGF-IR(-/-)* BMSCs were found in the trabecular bone four weeks after transfer. From these observations, the authors speculated that IGF-1 released during bone resorption stimulates osteoblast differentiation from BMSCs, which were recruited at bone-resorptive sites by TGF $\beta$ 1.

#### **Identification of "Couplin" as a Possible Coupling Factor**

Through comprehensive gene expression analysis during osteoclastogenesis, "Couplin" was identified as a candidate for the coupling factor (6). Couplin was produced and secreted by mature osteoclasts actively engaged in bone resorption, and promoted osteoblast differentiation from BMSCs. The expression of Couplin in bone was decreased with aging and alendronate treatment, and increased by PTH treatment. Conditional deletion of the *couplin* gene in osteoclasts resulted in low bone mass in mice due to impaired bone formation followed by bone resorption, while transgenic mice overexpressing *couplin* were protected from age-dependent bone loss. These results suggest that Couplin

relays signals from bone-resorbing osteoclasts to bone-forming osteoblasts, and may act as a coupling factor.

### **Regulation of Osteoclast Recruitment by Osteocyte Apoptosis**

Osteocyte apoptosis in areas of microdamage was shown to precede increased local osteoclast formation and activity, initiating targeted bone remodeling *in vivo*. Several studies investigated the role of osteocyte apoptosis on osteoclastogenesis (7;8). Osteoclast precursors are transported from the bone marrow to the remodeling site via the circulation. However, the specific mechanisms that regulate the recruitment of osteoclast precursors to the adjacent bone lining surface are not clearly elucidated. CM from osteocyte cultures undergoing apoptosis by TNF- $\alpha$  treatment promoted endothelial cell proliferation, migration and complex tubule network formation with longer and more branches. This angiogenic effect may be caused by VEGF since apoptotic osteocyte CM contained more VEGF than non-apoptotic osteocyte CM and blocking VEGF with a neutralizing antibody abolished tubule formation effects. Interestingly, endothelial cells treated with apoptotic osteocyte CM exhibited greater osteoclast precursor adhesion than endothelial cells treated with non-apoptotic osteocyte CM. These results suggest that osteocyte apoptosis assists in osteoclast precursor recruitment by promoting angiogenesis and enhancing osteoclast precursor adhesion to the endothelium (7).

It was also reported that both apoptotic and non-apoptotic MLO-Y4 osteocyte-like cell CM exhibited elevated soluble RANKL and VEGF concentrations. Moreover, healthy osteocytes treated with apoptotic osteocyte CM confined osteoclasts to the apoptosis area, thereby creating a "halo" around the microdamage. These findings provide a mechanism linking osteocyte apoptosis with bone microdamage, and suggest that osteocyte apoptosis both directly and indirectly regulates the initiation of targeted bone remodeling (8).

### **Epigenetic Regulation of Osteoclast Differentiation**

An essential role for epigenetic regulation in osteoclastogenesis was reported (9). Osteoclastogenesis is tightly regulated by various transcriptional factors, which play pivotal roles by reorganizing gene networks (10). Recently, epigenetic regulations, such as histone modifications and chromatin remodeling, have been revealed as essential for the regulation of gene expression by transcription factors (11). In particular, reversibly regulated histone methylation is regarded as the most important storage of epigenetic information that defines cell fate and identity.

An essential role for Jumonji C (JmjC)-domain-containing proteins that are known as belonging to the histone demethylase family was shown. Among JmjC-domain-containing proteins, the expression levels of JmjC-domain-containing protein 5 (Jmjd5) were reduced in the course of RANKL-induced osteoclastogenesis. Down-regulation of Jmjd5 expression by constitutively expressing shRNAs against Jmjd5 resulted in promotion of osteoclast formation with up-regulated expression of osteoclast-specific genes encoding for DC-STAMP (*Dcst*) and cathepsin K (*Ctsk*). In addition, osteoclast-specific Jmjd5 KO mice were generated by crossing *Jmjd5-flox* mice with *Ctsk-Cre* mice; reduced bone mass in the femurs of these KO mice (16-week-old) was observed, as compared with WT mice. These results clearly indicate that epigenetic regulations, at least methylation and/or demethylation of histone tails, have an important role in osteoclastogenesis and bone metabolism.

### **Autophagy-Related Proteins Mediate Osteoclast Function *In Vitro* and *In Vivo***

Autophagy is the process whereby cells degrade intracellular materials within lysosomes (12), and recent human genome-wide association study (GWAS) data indicated a link between the autophagy pathway and bone density (13). The role of autophagy in the osteoclast was reported at this year's meeting (14). Activation of the autophagy pathway results in lipidation of

the protein LC3, which localizes to autophagosomes to promote their development and fusion with lysosomes. LC3 appears in punctate cytoplasmic structures in bone-residing osteoclasts, but also localizes to the ruffled border. Furthermore, inhibition of the autophagy protein Atg4, which mediates lipidation of LC3, prevents LC3 transport to the ruffled border. In addition, LC3-G120A mutants, which cannot be lipidated, also failed to be targeted to the resorptive organelle. Osteoclast-specific KO of Atg5, an essential autophagy protein, revealed that Atg5 is also necessary for LC3 localization to the ruffled border and for efficient cathepsin K accumulation within the resorptive microenvironment. Interestingly, Atg5 deficiency induced impaired ruffled border formation and a failure to degrade bone slices *in vitro*, and resulted in diminished bone loss following ovariectomy *in vivo*. Thus, autophagy proteins target lysosomal vesicles to the ruffled border membrane and facilitate bone resorption both *in vitro* and *in vivo*.

### **TNF- $\alpha$ -Induced Osteoclastogenesis and Inflammatory Bone Resorption Are Negatively Regulated by the Notch-RBP-J Pathway**

Recent studies have revealed that Notch receptors (Notch1, 2 and 3) are implicated in the regulation of RANKL-induced osteoclastogenesis *in vitro* although the mechanisms of regulation are not clear (15). The role of the Notch-RBP-J signaling pathway in pathologic inflammatory bone resorption was examined (16). TNF- $\alpha$  did not induce osteoclastogenesis in WT bone marrow macrophages (BMMs). However, deletion of RBP-J, the transcription factor downstream of Notch signaling, in hematopoietic cells resulted in a dramatic induction of osteoclastogenesis in the absence of RANKL. RBP-J deficiency had a much more modest augmenting effect on RANKL-induced osteoclastogenesis. TNF- $\alpha$  dramatically induced NFATc1, a key transcription factor for osteoclast differentiation, as well as osteoclast-specific genes encoding for TRAP, cathepsin K, and integrin  $\beta$ 3 in RBP-J-deficient cells. RBP-J suppressed NFATc1 induction probably by

decreasing c-Fos recruitment to the *NFATc1* promoter and increasing expression of IRF8 and Blimp1, transcriptional repressors that suppress osteoclastogenesis. Mice with a myeloid-specific deletion of RBP-J (*RBP-J<sup>fllox/fllox</sup> LysM-Cre*) did not exhibit a bone phenotype under physiological conditions. However, osteoclast formation was dramatically increased when the KO mice were treated with TNF- $\alpha$ , and severe bone destruction and increased serum TRAP levels relative to littermate controls were observed. Deletion of the RBP-J gene induced osteoclastogenesis by TNF- $\alpha$  even in RANK KO mice. These findings identify a key role for the Notch-RBP-J axis in suppressing inflammatory TNF- $\alpha$ -induced osteoclastogenesis. Notch-RBP-J signaling plays a more prominent role in inhibiting osteoclastogenesis in inflammatory settings than under physiological conditions.

### **Conclusion**

Overall, a number of intriguing findings were reported at the 2010 ASBMR Annual Meeting in Toronto. The molecular mechanisms regulating osteoclastogenesis were further investigated, as was epigenetic regulation of osteoclast differentiation that will be one of the major fields of osteoclast research in the near future. In addition, there is increasing interest in the relationship between osteoclasts and other types of cells such as osteoblasts and osteocytes, and in the molecules regulating these interactions.

**Conflict of Interest:** None reported.

**Peer Review:** This article has been peer-reviewed.

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