Citation: IBMS BoneKEy **10**, Article number: 268 (2013) | doi:10.1038/bonekey.2013.2

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MEETING REPORT

Highlights on the negative and positive regulators of the osteoclast (ASBMR 2012)

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IBMS BoneKEy **10,** Article number: 268 (2013) | doi:10.1038/bonekey.2013.2; published online 20 March 2013

Meeting report from the 34th Annual Meeting of the American Society for Bone and Mineral Research, Minneapolis, MN, USA, 12–15 October 2012

Numerous presentations shed new light into the understanding of negative regulators that restrain osteoclast (OC) formation and function. Wu *et al.*[1] from Yi-Ping Li's group identified Gna13, a member of the G protein family, as a new inhibitory factor in OC formation. Conditional deletion of Gna13 using either LysM-Cre or CathepsinK-Cre induced a striking osteoporotic phenotype in 2-month-old mice with a twofold increase in OC number, quantified by tartrate-resistant acid (TRAP)-stained histological sections of femurs. *In vitro*, Gna13 cKO bone marrow macrophage (BMM) cultured with RANKL formed larger and more numerous OCs that also harbor enhanced function, as reflected by a significant increase in resorptive pit area and carboxy-terminal collagen crosslinks released into the media when cells were cultured on bone slices. Mechanistically, Gna13 cKO enhanced AKT activation in the OC, as well as NFATc1 accumulation and nuclear translocation. Treatment with the src inhibitor PP2 reversed the enhanced resorptive phenotype in the cKO, thus suggesting that Gna13 targets the src-AKT-NFATc1 pathway to inhibit OC formation and activity.

Xiong *et al.*[2] presented novel findings that VPS35, a component of the retromer regulating endosome–Golgi recycling of membrane receptors, is required to dampen RANKL signaling cascades. While VPS35 $-/-$ mice are embryonic lethal, 3-month-old VPS35 heterozygous mice exhibit osteoporosis with reduced trabecular and cortical bone as measured by ultrasonic computed tomography. This defect was attributed to a significant increase in OC number *in vivo* and *in vitro*, and enhanced function. *In vitro*, VSP35 $+/-$ BMM displayed sustained RANKL signaling and RANK receptor maintenance up to 46 h post stimulation, indicating that VPS35 is required to restrain RANKL signaling cascades by mediating RANK trafficking and degradation.

The mitogen-activated protein kinase phosphatase Dusp1 has previously been described as a critical negative regulator of inflammatory bone loss.[3] Hirose *et al.*[4] from Sakae Tanaka's group further described the Stat5-dependent mechanism of Dusp induction to limit OC activity. Conditional deletion of Stat5a and Stat5b using the CathepsinK-Cre led to a decrease in bone mass with age. Eight-week-old cKO mice displayed a significant increase in eroded bone surface and OC surface with a corresponding elevation in serum carboxy-terminal collagen crosslinks; the number of OCs was unchanged however. Mechanistically, this hyperactivity in the OC was characterized by an increase in extracellular-signal-regulated kinase (ERK) activation and significant decrease in Dusp1 and 2 mRNA. Moreover, overexpression of Stat5 in OCs *in vitro* induced Dusp1 and 2 expression, and IL-3, the sole stimulator of Stat5 in OCs, directly activated Dusp1 and 2 upregulation in wild-type (WT) but not Stat5 cKO OCs.

TMEM178 was also defined as a novel negative regulator of OC formation *in vitro* and *in vivo*.[5] TMEM178 is induced in a PLCγ2-dependent manner during osteoclastogenesis, and deletion of TMEM178 led to accelerated OC formation *in vitro* with a striking increase in NFATc1 mRNA and protein in response to RANKL. Addition of lipopolysaccharide (LPS) to OC precursors further enhanced OC formation in TMEM178 $-/-$ cultures compared with WT, suggesting that TMEM178 may have an important role in restraining OC formation in an inflammatory setting. Indeed, TMEM178 $-/-$ mice treated with arthritogenic serum from K/BxN mice showed more severe bone erosion at the knee with a significant increase in TRAP $+$ OC on the femoral periosteum.

Several reports defined novel significance on previously understudied signaling pathways in the OC, including Wnt and BMP pathways. Broege *et al.*[6] presented new insights delineating the differential contributions of canonical and non-canonical BMP signaling during osteoclastogenesis. Conditional deletion of the BMPRII using the LysM-Cre resulted in an increase in trabecular bone volume at 3 and 6 months of age. *In vitro*, BMPRII cKO BMM displayed impaired OC formation characterized by smaller OC and low expression of OC-specific genes TRAP, NFATc1 and Cathepsin-K. Although phosphorylation of SMAD1/5/8 was not different in cKO OC, activation of p38 and ERK was decreased compared with WT, suggesting that the non-canonical BMP signaling pathway

governs OC differentiation in the BMPRII;LysM-Cre mice. Interestingly, *in vitro* signaling assays showed that p38 was phosphorylated early after treatment with RANKL, whereas SMAD1/5/8 phosphorylation was detected only in fused OC. Furthermore, inhibitors of the canonical BMP pathway were effective to block mature OC development only when given on days 3–5 of RANKL treatment. On the other hand, addition of BMP2 to OC cultures elevated p38 and ERK levels, and the BMP antagonist Noggin1 blocked OC formation when added from day 1 of RANKL. Taken together, these data showed a differential requirement for non-canonical and canonical BMP signaling during early and late stages of osteoclastogenesis, respectively.

New insights into the role of Wnt signaling in the OC were reported by several groups. Merry Jo Oursler and colleagues[7] generated mice with an OC-specific deletion of Lrp5 and 6 or β-catenin using the CathepsinK-Cre to specifically delineate the direct role of Wnt signaling in committed OCs. Both genetic models displayed an increase in OC number with decreased trabecular bone density in the femur, but only the β-cat cKO showed cortical bone loss as well as increased rates of bone formation and mineral apposition. *In vitro*, osteoclastogenesis was increased in both cKOs, while treatment with exogenous Wnt3a suppressed WT OC formation via blockade of NFATc1 nuclear translocation. Mechanistically, Wnt3a induced $Ca2+$ fluxes activating the cAMP/PKA pathway (inhibitory of NFATc1) in WT or β-cat cKO cells, but this response was abolished in Lrp5/6 cKO cells.

Canonical Wnt signaling via the Frizzled-8 receptor was also described as a potential pathway restraining osteoclastogenesis.[8] Fzd-8-deficient mice display an osteopenic phenotype owing to an increase in OC number; the opposite effect is observed *in vitro* when activation of the canonical Wnt pathway in WT BMMs blocks osteoclastogenesis, an effect shown to be independent of osteoprotegerin (OPG).

The continuing efforts to identify promising therapeutic targets for inflammatory bone loss were underscored by several presentations. Kang *et al.*[9] from Agnes Vignery's group described SK4 as a positive regulator of OC differentiation, which can be successfully targeted by specific inhibitors. SK4-deficient mice have an increase in bone mass in basal conditions owing to a decrease in OC number, as assessed by microCT and TRAP stain of histological sections. Using two models of inflammatory bone loss, SK4 $-/-$ mice are protected from inflammation and bone erosion in collagen antibody-induced arthritis as well as LPS-induced bone resorption on the calvaria. *In vitro*, SK4 $-/-$ BMM show impaired OC formation with concomitant defects in AKT activation and reduced NFAT expression and nuclear translocation. Mechanistically, these defects may be attributed to defective calcium oscillations in the absence of SK4, as measured by intracellular calcium imaging of fura-2-loaded cells. Importantly, human monocytes treated with the SK4 inhibitors TRAM-34 or ICAGEN-17043 failed to undergo cell fusion and form mature OC, suggesting that SK4 may be a promising therapeutic target in human inflammatory bone loss.

Gu *et al.*[10] provided evidence that macrophage migration inhibitory factor (MIF) is crucial to the development on inflammatory arthritis. Induction of arthritis by K/BXN serum transfer resulted in a reduced clinical score and less severe bone erosion accompanied by fewer TRAP $+$ OCs in MIF-null mice. *In vitro*, MIF $-/-$ BMM exhibited impaired osteoclastogenesis and actin ring formation, presumably due to defective ERK, nuclear factor-κB (NF-κB) and NFATc1 activation. Importantly, multiple members of the Dusp family of protein phosphatases were upregulated in MIF $-/-$ BMM, suggesting that ablation of MIF can simultaneously activate negative regulatory pathways.

New insights into the molecular mechanism underlying the inflammatory bone lesions were reported by Mukai *et al.*[11] who postulated that pro-osteoclastogenic tumor-necrosis factor (TNF) signaling is heightened by gain-of-function mutations in *SH3BP2*, the causative gene in cherubism. *In vitro*, BMMs from heterozygous cherubism mice (KI/$+$) display enhanced formation of functional OCs in response to macrophage colony-stimulating factor (MCSF) and TNF ($100\ ng\ ml^{-1}$) in the absence of RANKL. This differentiation was unaffected by treatment with OPG but blockade of either TNFRI or II inhibited osteoclastogenesis. Mechanistically, TNF-induced OC formation in KI/$+$ cells was dependent on an upregulation of NF-κB subunit p50, cFos and NFATc1 nuclear localization. *In vivo*, KI/$+$ mice treated with 1.5 mg TNF over the calvaria for 5 days developed more than double the number of OCs and eroded surface compared with WT mice, indicating that a gain-of-function mutation in SH3BP2 promotes inflammatory osteoclastogenesis and bone loss not only through hyperactive RANKL cascades but also via TNF signaling.

Bartell[12] of Stavros Manolagas's group built on her previous work describing the skeletal effects of reactive oxygen species by addressing the OC-specific function of the anti-oxidant catalase. Bartell generated mice overexpressing catalase specifically in the mitochondria of myeloid-lineage cells by crossing MitoCat-flox stop mice with LysM-Cre mice. These transgenic mice exhibited an increase in bone mineral density and BV/TV in the vertebrae and femur at 6 months of age. *In vitro*, MitoCat;LysM-Cre BMM showed a mild decrease in osteoclastogenesis and decreased survival, presumably owing to a decrease in AKT and ERK activation in response to MCSF and RANKL stimulation. Importantly, an increase in antioxidants in OC-lineage cells appears to be advantageous in a pathological setting, as MitoCat;LysM-Cre mice were protected from ovariectomy-induced bone loss.

Undercarboxylated osteocalcin is a metabolic hormone that functions to enhance insulin sensitivity, secretion and energy usage. Evidence suggests that bone resorption by OCs is required for decarboxylation of osteocalcin. The direct impact of OC activity on osteocalcin carboxylation status and consequent glucose metabolism was addressed by Ferron and Karsenty[13] of Gerard Karsenty's group using two genetic models to compare an OC-rich versus an OC-poor environment. Opg $-/-$ mice which have an increase in OC number showed improved glucose tolerance on a normal diet compared with control mice at 1 and 3 months of age. Hypoglycemic Opg $-/-$ mice also showed improved insulin sensitivity and lower fat mass compared with controls. Importantly, serum levels of undercarboxylated osteocalcin were also increased more than 10 times compared with WT mice. In the converse, Ferron generated an OC-deficient model (ΔOsc) by crossing DTA-induced stop-casette mice

with CatK-Cre mice, thereby generating progeny with conditional OC loss. These osteopetrotic mice displayed a loss in glucose tolerance, decrease in serum insulin and a three-fold decrease in pancreatic beta cell mass, as well as a two-fold decrease in serum levels of undercarboxylated osteocalcin. Transplant of ΔOsc fetal liver cells into WT mice induced glucose intolerance, reduced insulin secretion and decreased serum levels of undercarboxylated osteocalcin. Moreover, infusion of undercarboxylated osteocalcin into ΔOsc mice restored glucose tolerance responses to that of WT mice, indicating that the OC is required for active osteocalcin and consequent control of glucose metabolism.

Finally, several presentations discussed new details of cytoskeletal events governing OC formation function. Weivoda *et al.*[14] presented surprising findings that deletion of the anti-adhesive molecule podocalyxin (PODXL) in hematopoietic cells (Vav-Cre) leads to an increase OC numbers *in vivo* and *in vitro*. These OC are not functional, however, as bone volume, bone mineral density and bone formation were also higher in PODXL-Vav-Cre mice. Mechanistically, PODXL expression in OC precursors appears to be required for sufficient Rac1 activation downstream of MCSF. On the other hand, deletion of PODXL late in osteoclastogenesis using CathepsinK-cre resulted in no differences in bone density *in vivo* but more numerous and larger OCs *in vitro*. PODXL-CatKCre OC showed defects in *in vitro* bone resorption, presumably due to defective integrin signaling and Rac1 activation.

Zou *et al.*[15] of Steve Teitelbaum's group reported the dual requirement of Talin1 and Rap1 for OC bone resorption. Conditional deletion of Talin1 by CatK-Cre leads to defective macrophage colony-stimulating factor-induced inside–out integrin activation and consequent impaired bone resorption, manifested in a fivefold increase in bone mass. Talin1 is also essential for generation of functional osteclasts, as LysM-Cre-induced Talin1 ablation inhibits precursor migration and attachment, thereby arresting the development of mature resorptive OCs. Importantly, loss of talin1 late in osteoclastogenesis protects mice from ovariectomy-induced bone loss and bone erosion accompanying inflammatory arthritis. The necessity for both Talin1 and Rap1, the small GTPase which facilitates talin's interaction with β-integrin, for OC function was illustrated by the osteopetrotic phenotype of Rap1;CatK-Cre mice.

Conflict of Interest

The authors declare no conflict of interest.

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