

BONE

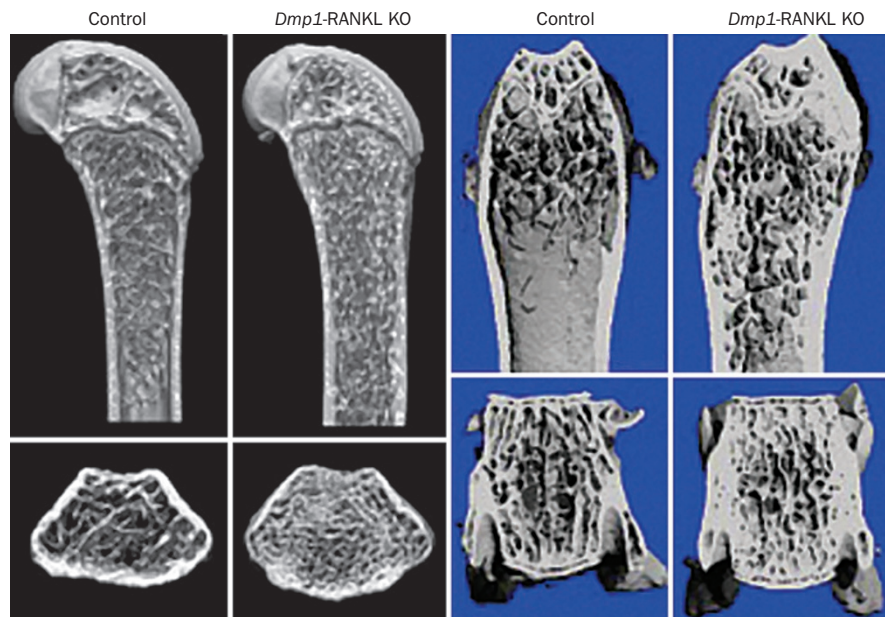
Osteocyte RANKL in bone homeostasis: a paradigm shift?

Receptor activator of nuclear factor- κ B ligand (RANKL) regulates osteoclast function, and its expression by osteoblasts is postulated to link bone resorption and formation. However, two independent studies, led by Hiroshi Takayanagi of the Tokyo Medical and Dental University and Charles O'Brien of the University of Arkansas for Medical Sciences, now challenge this paradigm by providing evidence that osteocytes are a major source of RANKL, and are essential for bone remodeling—a process that is defective in many rheumatic diseases.

“Many different cell types express RANKL and it remained unclear which ones were providing the RANKL involved in osteoclast formation *in vivo*,” states O'Brien. His group and Takayanagi's initially took different approaches to attempt to fill this gap in our knowledge; Takayanagi *et al.* purified osteoblast and osteocyte populations, analysis of which revealed that RANKL levels were considerably higher in the latter, and the isolated osteocytes were more effective at driving the differentiation of osteoclast precursors *in vitro*.

This discovery led Takayanagi *et al.* to generate knockout (KO) mice, hence forth referred to as *Dmp1*-RANKL KOs, with the gene encoding RANKL deleted specifically in osteocytes. Meanwhile, after initially finding that deletion of RANKL from all mesenchymal cells resulted in developmental defects, O'Brien *et al.* created their own *Dmp1*-RANKL KOs. In addition, O'Brien's group deleted RANKL at various genetically defined stages of osteoblast or chondrocyte development. Using these knockout mice, they showed that hypertrophic-chondrocyte-derived RANKL was essential for osteoclastogenesis during bone development, whereas no effect was seen in *Dmp1*-RANKL KOs. Though interesting, this finding did not provide much insight into the role of RANKL in non-developmental bone remodeling processes that are probably more relevant to rheumatic disease.

Takayanagi *et al.* also reported normal bone development in *Dmp1*-RANKL KOs,



Micro-CT analysis of femurs longitudinal view (top left) and axial view (bottom left) of 12 week old mice from the Takayanagi study, and femurs (top right) or L4 vertebra (bottom right) of 6 month old mice from the O'Brien study. © Nature Publishing Group.

but showed that osteocytes purified from these mice had reduced ability to support osteoclastogenesis *in vitro*, compared with those from control littermates. Interestingly, Takayanagi *et al.* found normal osteoclast numbers in *Dmp1*-RANKL KOs at birth, whereas both groups found a >70% reduction postnatally compared with controls. “It was a big surprise that cells embedded within the bone matrix control osteoclast formation,” claims O'Brien.

Both research groups went on to examine the role of osteocytes in bone homeostasis. Their combined data demonstrated that *Dmp1*-RANKL KO was associated with postnatal increases in bone mineral density (BMD) and volume compared with controls, and filling of the bone marrow cavity with bone. Additionally, markers of both bone formation and resorption were decreased in *Dmp1*-RANKL KO mice. These data suggest that, postnatally, “osteocytes are the major source of RANKL and thus regulate osteoclast formation and bone resorption,” says Takayanagi.

O'Brien *et al.* also demonstrated a role for osteocyte RANKL in pathological bone remodeling using a model of

unloading-induced bone loss; increases in RANKL mRNA and osteoclast numbers in bones of control mice were associated with changes in BMD, trabecular spacing and cortical thickness, but were not observed in *Dmp1*-RANKL KOs.

These findings are “...contrary to the pre-existing dogma that RANKL is expressed by osteoblasts or bone marrow stromal cells, but consistent with the concept that osteocytes are mechanosensing cells that regulate other bone cells,” states Takayanagi. “The concept put forward in our papers challenges a long-standing model...,” adds O'Brien. “Now it will be important to determine whether RANKL produced by osteocytes is important in situations that lead to pathological bone loss, such as loss of sex steroids, aging, or inflammation,” he continues.

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Original articles Xiong, J. *et al.* Matrix-embedded cells control osteoclast formation. *Nat. Med.* doi:10.1038/nm.2448 | Nakashima, T. *et al.* Evidence for osteocyte regulation of bone homeostasis through RANKL expression. *Nat. Med.* doi:10.1038/nm.2452.