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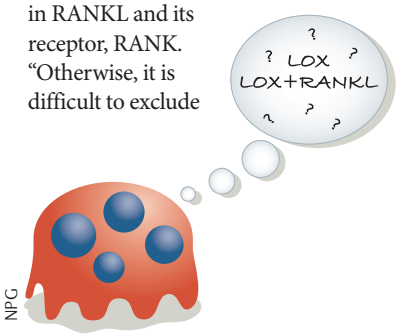
BONE

The concept of RANKL-independent osteoclastogenesis refuted

> LOX fails to induce osteoclast differentiation in the absence of RANKL

Receptor activator of nuclear factor κB ligand (RANKL, also known as TNFSF11) has long been considered essential for osteoclast differentiation, until the recent identification of lysyl oxidase (LOX) as a RANKL-independent stimulator of osteoclastogenesis by Cox and colleagues. Tsukasaki *et al.* now rebut these findings and show that LOX fails to induce osteoclast differentiation in the absence of RANKL.

Tsukasaki *et al.* contend that assessment of RANKL independence requires the use of models deficient in RANKL and its receptor, RANK. "Otherwise, it is difficult to exclude the involvement of RANKL produced by contaminating cells present in the culture system," claims Hiroshi Takayanagi, corresponding author on the latest paper. Treatment with recombinant LOX (rLOX) could not induce osteoclastogenesis in RANKL and RANK deficient conditions *in vitro* and *in vivo*, confirming that LOX cannot substitute for RANKL.



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Interestingly, LOX is able to enhance the effect of RANKL. Indeed, co-treatment of mouse bone marrow cells with RANKL and rLOX led to a higher number of differentiated osteoclasts than RANKL treatment alone. Induction of RANKL expression by osteoblast lineage cells might explain this synergistic effect.

This notion is supported by several lines of evidence. First, the use of *Rankl*-deficient cells abrogates the synergistic effect of RANKL and rLOX. Second, no synergy was observed when expansion of the contaminating stromal cells was minimized by shortening the culture period. Third, rLOX treatment induced *Rankl* expression in cultured stromal cells and primary calvarial osteoblasts.

The involvement of stromal cells might explain the apparent discrepancy between the outcome of this study, in which rLOX treatment did not support osteoclastogenesis, and the Cox *et al.* study: despite the use of similar culture conditions, the level of stromal cell contamination and/or expansion might have differed.

"We challenged the concept of RANKL-independent osteoclastogenesis," concludes Takayanagi. However, he also notes that LOX has the capacity to potentiate osteoclastogenesis in the presence of RANKL and might have an important role in specific conditions, such as arthritis and bone metastasis, in which it could serve as a therapeutic target.

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ORIGINAL ARTICLE Tsukasaki, M. *et al.* LOX fails to substitute for RANKL in osteoclastogenesis. *J. Bone Miner. Res.* http://dx.doi.org/10.1002/jbmr.2990 (2016)
FURTHER READING Cox, T. R. *et al.* The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. *Nature* **522**, 106–110 (2015)