FUNCTION AND REGULATION OF MAMMALIAN OSTEOCLASTS

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INTRODUCTION: Osteoclasts are the multi-nucleated cells responsible for resorption of mineralised tissues during skeletal development and remodelling. In addition, these cells mediate the excessive bone resorption that occurs in osteoporosis, rheumatoid arthritis and periprosthetic osteolysis[1]. The process of osteoclastic resorption is complex and involves control at various levels, including osteoclast formation, migration, resorptive activity and cell death. Osteoclasts arise by the fusion of precursors from the monocyte-macrophage lineage. The actively resorbing osteoclast forms a tight contact between the cell periphery and the matrix, giving rise to a confined compartment termed the resorption lacuna. The resorption lacuna is actively acidified, leading to dissolution of bone mineral, while secreted enzymes degrade organic components of the matrix.

METHODS: Since osteoclasts are difficult to isolate in large numbers and do not proliferate in culture, we use disaggregated mammalian osteoclasts (Fig.1). Cells are studied using patch-clamp electrophysiology to monitor membrane currents, single-cell fluorescence to monitor cytosolic Ca^{2+}, time-lapse video microscopy to observe cell morphology and motility, and RT-PCR to study gene expression.

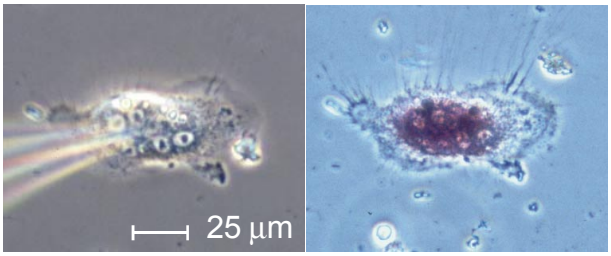

Fig. 1: Phase-contrast photomicrograph of isolated rabbit osteoclast with patch-clamp microelectrode (left). Following recording, cell was stained for tartrate-resistant acid phosphatase (purple), an osteoclast marker (right).

RESULTS: We have characterized chemotactic responses of osteoclasts to the cytokines M-CSF[2] and TGF-β. Migration in response to these cytokines was found to depend on αvβ3 and α2β1 integrins, respectively. We have also characterized signalling by extracellular nucleotides[3,4]. Nucleotides bind to cell surface P2 receptors in many tissues. Osteoclasts possess both P2Y receptor subtypes, which are coupled through G proteins to mobilization of intracellular Ca^{2+}, and P2X receptor subtypes, which are intrinsic ion channels that mediate influx of cations and depolarisation (Fig. 2).

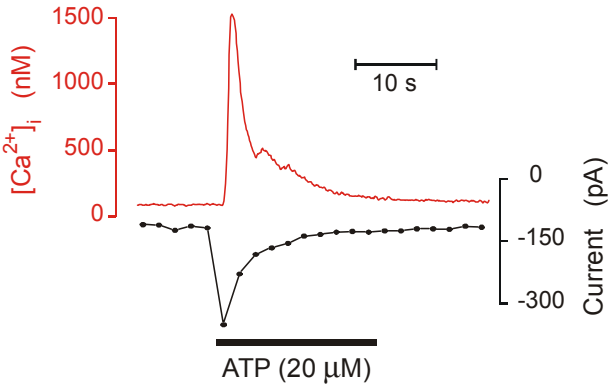

Fig. 2: Combined Ca^{2+} fluorescence and whole-cell patch-clamp recording from an isolated rat osteoclast. Application of ATP first elicits inward current due to activation of $P2X_4$ receptors. ATP also induces large transient elevation in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) due to activation of P2Y receptors and release of Ca^{2+} from stores.

DISCUSSION & CONCLUSIONS: Under physiological conditions, nucleotides act as autocrine/paracrine signalling molecules that may transduce cellular responses to mechanical stimuli. Knowledge of osteoclast regulation and function is essential for the rational development of therapies directed at arresting the loss of bone in inflammatory and metabolic diseases as well as in tumour-induced and periprosthetic osteolysis.

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