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Original Article

Hylonome

Non-invasive imaging of osteoclast activity via near-infrared cathepsin-K activatable optical probe

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The creation of accurate, non-invasive techniques for monitoring skeletal metabolism would allow for sensitive screening of changes in metabolic activity associated with events including bone metastasis, hormone withdrawal, joint destruction, and altered mechanical load. Current indicators of bone metabolism such as the monitoring of collagen degradation products or alkaline phosphatase activity provide sensitive monitoring of global changes in bone turnover, but cannot discriminate local alterations reflecting site-specific pathologies. Longitudinal *in vivo* imaging such as CT or DEXA reflects net gain or loss of bone over time, but is not a direct indicator of cellular activity at the time of imaging. Bone scintigraphy can reveal local changes in formation, as indicated by focal uptake of radiolabeled bisphosphonates; but is nonetheless insensitive to alterations in resorptive events. *In vivo* monitoring of cell-specific protein interactions is the basis for the burgeoning field of molecular imaging. Here, we demonstrate for the first time, *in vivo* non-invasive imaging of osteoclast activity via a cathepsin-K activated near-infrared optical imaging probe.

Ten-week-old female Balb/c mice were subjected to ovariectomy (OVX), ovariectomy with daily pamidronate (OVX-Pam; 0.5 mg/kg/day i.p.), or sham. Seven days post-surgery, mice were administered a cathepsin-K activatable probe i.v. (n=5/group). Cleavage of a cathepsin-K sensitive backbone activates probe fluorescence by releasing side groups whose optical activity is otherwise quenched in the non-cleaved form. Two mice per group were given an additional injection of the optical analogue of the bone scan agent ^{99m}Tc-MDP, consisting of a fluorochrome coupled to a bisphosphonate backbone which can be viewed in an optical channel separate from the cathepsin-K probe. All mice were imaged on day 8 by non-invasive fluorescence molecular tomography (FMT), allowing 3-D visualization of probe distribution in the mouse. Following FMT, mice were euthanized and tissues were dissected for *ex vivo* imaging of probe distribution.

Ovariectomy induced a 38% increase in proximal tibial fluorescence over sham controls ($p<0.05$), an increase which was entirely blocked by daily administration of pamidronate ($p<0.001$). Activation was over 6-fold higher than a similar probe containing a non-cleavable d-form of the backbone chain ($p<0.001$), demonstrating strong cleavage specificity toward the cathepsin-K sequence. Mice double-labeled with fluorescent bisphosphonate showed a strong correlation ($R^2=0.88$) between both signals, indicating tight correlation between osteoblast and osteoclast activity. *Ex vivo* fluorescence imaging suggests a spatial de-coupling of osteoblast and osteoclast activity, with a shift toward enhanced resorption in the OVX group.

Monocyte/macrophage cells isolated from mice were seeded onto dentin substrates and induced to form osteoclasts with RANKL and M-CSF. Confocal laser scanning microscopy reveals intracellular accumulation of cathepsin-K probe in discrete inclusions within the cells. Real time imaging demonstrates trafficking of probe within cells and osteoclast motility over time.

These studies demonstrate a means to non-invasively visualize and measure osteoclast activity *in vivo*. Probe activation is sensitive to resorption changes and correlates to bone formation in the increased turnover ovariectomy model. Molecular imaging of bone metabolism, specifically osteoclast function, has great potential for monitoring changes associated with genome, disease, or environmental effects.

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