Introduction: Osteocyte apoptosis has been shown to be key to the activation and local control of remodeling of bone microdamage\(^1\). Generalized increases in osteocyte apoptosis have also been reported after estrogen withdrawal, glucocorticoid use, and disuse\(^2\). Recent studies by Emerton\(^1\) reveal that the apoptosis following estrogen loss is not uniform, as widely thought. Rather, osteocytes consistently and predictably undergo apoptosis beneath surfaces to which osteoclasts will subsequently be recruited by this hormonal challenge. These spatial and temporal relationships between osteocyte apoptosis and bone resorption after estrogen loss resemble those observed in microdamage-induced remodeling; leading us to hypothesize that osteocyte apoptosis plays a comparable controlling role in the activation or targeting of osteoclastic resorption in both circumstances. We tested this hypothesis by pharmacologically inhibiting osteocyte apoptosis post-ovariectomy, and assessing subsequent bone resorption activity.

Materials and Methods: Under IACUC-approval, adult female C57BL/6j mice (17 weeks old, Jackson Labs) underwent either bilateral surgical ovariectomy (OVX) to induce endocortical bone resorption, or sham surgery (SHAM). To test whether osteocyte apoptosis plays a regulatory role in the initiation of bone resorption after estrogen loss, one group of OVX mice (n=6) received the pan-caspase inhibitor, QVD-OPh (MP Biomedicals, Livermore, CA, 20mg/kg/day for 14 days) to inhibit osteocyte apoptosis (OVX+QVD). Previous studies showed this dosing regimen prevented fatigued-induced osteocyte apoptosis, while other studies had established that pan-caspase inhibitors have no direct effect on osteoclast recruitment or differentiation\(^2\). Remaining experimental groups (n=6 mice/group) received either QVD or DMSO Vehicle as follows: SHAM+QVD, SHAM+Veh, OVX+Veh. Mice were euthanized at 14 days post-OVX, the peak resorption period in this model\(^6\).

Osteocyte Apoptosis: Mid-diaphyseal 5 μm cross-sections were cut from decalcified, paraffin embedded femora. Osteocyte apoptosis was assessed immunohistochemically to detect cleaved caspase-3 (Cell Signaling Technology), an effector caspase needed for apoptosis, and γH2AX (Chemicon), a caspase-independent apoptosis marker. Non-immune serum negative controls and growth plate sections as positive staining controls were included. Caspase- or H2AX-positive osteocytes (as % of total osteocytes) were measured over the entire cortical width along the principal anatomical axes (anterior, posterior, medial, and lateral).

Bone Histomorphometry: Left femora were embedded undecalcified in PMMA and 50 μm thick mid-diaphyseal cross-sections were prepared as described previously\(^6\). Osteoclastic activity was assessed from eroded perimeter (Er.Pm) at the endocortical surface.

Statistical Analyses: Differences in apoptotic osteocytes and bone resorption activity were tested using one-way ANOVA with Tukey’s Multiple Comparison Test used for post-hoc testing. Data are shown as mean±SD and significance is reported at p<0.01.

Results: Osteocyte Apoptosis: Following ovariectomy, apoptosis was increased dramatically (OVX+Veh) by more than 3-fold over control and SHAM levels. Treatment with QVD in OVX animals suppressed this osteocyte apoptosis, with levels in QVD-treated samples equivalent to baseline. Figure 1 shows casp-3 positive cells; data for H2AX staining is not shown. Figure 2 shows that caspase inhibitors do not directly affect osteoclast differentiation or function, the results indicate that osteocyte apoptosis is required to activate osteoclastic resorption subsequent to estrogen loss.

Bone resorption after estrogen loss has been widely considered a stochastic remodeling process since it lacks a defined targeting focus, as previously demonstrated with microdamage\(^1,2\). In microdamage, however, the targeting focus for resorption is not the damage itself but rather signals emanating from apoptotic cells and their surviving neighbors near damage sites. Since blocking osteocyte apoptosis prevented OVX-induced bone resorption as it previously did for microdamage remodeling, it suggests that both processes are considered a stochastic remodeling process since it lacks a defined targeting focus, as previously demonstrated with microdamage\(^1,2\). In microdamage, however, the targeting focus for resorption is not the damage itself but rather signals emanating from apoptotic cells and their surviving neighbors near damage sites. Since blocking osteocyte apoptosis prevented OVX-induced bone resorption as it previously did for microdamage remodeling, it suggests that both processes are targeted, and that osteocyte apoptosis may initiate a common pathway for activating and targeting resorption in response to diverse stimuli.

Discussion: In the current studies, prevention of osteocyte apoptosis by a pan-caspase inhibitor completely blocked the activation of bone resorption in response to OVX-induced estrogen withdrawal. Since pan-caspase inhibitors do not directly affect osteoclast differentiation or function, the results indicate that osteocyte apoptosis is required to activate osteoclastic resorption subsequent to estrogen loss.

References:

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