Nanoscale properties of hydroxyapatite influence osteolytic activity of metastatic breast cancer

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Statement of Purpose: Breast cancer frequently metastasizes to bone leading to secondary tumor growth, osteolytic bone degradation, and a poor clinical prognosis. Microenvironmental cues have been implicated in osteolytic metastasis, but how material properties of hydroxyapatite (HA) affect cancer biology remains unclear, despite evidence indicating that HA properties change as a function of age and disease. Interleukin-8 (IL-8) is a key tumor-derived soluble factor that mediates osteolysis and tumorigenesis and has been shown to be regulated by the global presence of HA. This study combines a biomaterial-based tumor engineering approach with a new hydrothermal method for synthesizing HA with tightly controlled size and crystallinity to investigate the hypothesis that local nanoscale properties of HA in the bone microenvironment influence and promote the metastatic and osteolytic character of breast cancer through regulation of IL-8.

Methods: HA nanoparticles of varying size and crystallinity were synthesized using a hydrothermal method with different aging schedules and incorporated into poly(lactide-co-glycolide) scaffolds using a gas foaming/particulate leaching method. HA nanoparticles were characterized using TEM to determine particle size along with XRD and FTIR to assess crystal structure and relative crystallinity. HA-incorporating scaffolds were analyzed with TEM, SEM, and light microscopy to observe particle orientation and presentation within the polymer. To examine protein adsorption as a function of HA nanoparticle characteristics, scaffolds were incubated with fibronectin (FN), and analyzed using a BCA assay and immunochemical staining. For cellular studies, MDA-MB231 cells were seeded into scaffolds and maintained under dynamic culture conditions for up to 5 days. The resulting cell polymer constructs were assayed for DNA content by PicoGreen assay as well as expression and secretion of IL-8 by ELISA and Real Time RT-PCR, respectively. In all experiments, non-mineralized scaffolds were used as controls.

Results: TEM images (Fig. 1. left) and XRD and FTIR analysis confirmed that variation of the hydrothermal aging time enabled synthesis of monodisperse populations of small poorly crystalline (A) and large highly crystalline (B) HA nanoparticles, relative to commercial preparations (SIG). Imaging of scaffolds by SEM and TEM confirmed that HA was present at the surface of the scaffold pores to allow direct interaction between mineral and cells or biomolecules. Protein analysis via BCA and immunochemical staining indicated that serum proteins such as FN preferentially adsorbed onto mineralized scaffolds with smaller and less crystalline HA nanoparticles. Growth of MDA-MB231 breast cancer cells was enhanced on mineralized scaffolds relative to non-mineralized controls. This effect was even more pronounced on the scaffolds with smaller and less crystalline HA (Fig. 1, right). Conversely, tumor cell secretion of IL-8 was increased on scaffolds with larger HA crystals (Fig. 1, right). These changes were due to altered expression rather than solely secretion because larger HA crystals caused increased expression of IL-8 mRNA by MDA-MB231 cells relative to smaller, less crystalline HA and non-mineralized controls. Variations in IL-8 expression were linked to the differences in adsorption of RGD-containing proteins, as inhibiting integrin-engagement through the use of a monoclonal antibody against integrin $\alpha_v\beta_3$ suppressed mineral-mediated upregulation of IL-8.

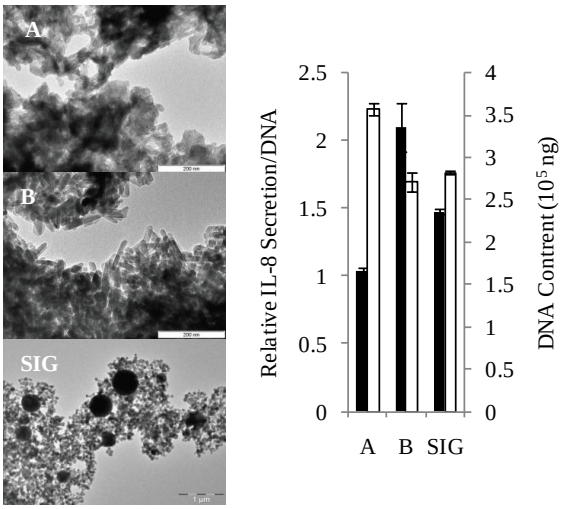

Figure 1: TEM images of hydrothermally aged (A, B) and commercial (SIG) HA and corresponding changes in IL-8 (black bars) and cell growth (white bars). Scale bars: 200 nm (A, B) and 1 μm (SIG).

Conclusions: By incorporating hydrothermally aged HA nanoparticles of varying size into polymeric scaffolds, we were able to simulate bone microenvironmental conditions and evaluate how nanoscale HA properties affect bone metastatic breast cancer cell behavior. We found that HA particle size and crystallinity are crucial to regulating tumor growth and expression of IL-8, two key aspects of osteolytic breast cancer metastasis. These effects are mediated in part by adsorption of RGD-containing proteins such as FN, which varies with both crystal size and crystallinity and initiates integrin signaling. These findings are pathologically relevant, as we have previously shown that tumor-derived IL-8 enhances osteoclast differentiation and activity leading to the formation of osteolytic lesions. Overall, our studies suggest that the nanoscale material properties of the bone mineral HA represent a key regulator of osteolytic metastasis and that hydrothermally aged HA can be incorporated into scaffold systems to create mineralized tumor models for the effective study of breast cancer biology.