Extracellular Matrix Properties regulate Osteolytic Potential of Human Breast Cancer Cells

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Statement of Purpose: When breast cancer cells metastasize to bone, patient survival drops from 98 to 27%.[2] At this point, the cancer cells change their gene expression patterns and express PTHrP, a key component in the vicious cycle of cancer-induced bone resorption.[1] The reason for this change is unknown. Breast cancer cells alone cannot degrade mineralized matrix to invade bone, and thus require the secretion of PTHrP to recruit osteoclasts. Previous studies have shown that microenvironment plays a vital role in the differentiation and morphology of various cell types by modulating cytoskeletal tension.[3-5] In particular, abnormal morphogenesis of mammary epithelial cells has been attributed to an increase in cytoskeletal tension caused by a stiffer substrate.[4] Likewise, breast cancer cell aggression has been linked to the cell's ability to invade the surrounding microenvirontment, which depends both on the stiffness and proteolytic degradability of the substrate[5]. Thus, we hypothesized that expression of osteoclastogenic factors by the tumor cells (such as PTHrP) is upregulated when the cells encounter an increased resistance to invasion in the bone microenvironment resulting from the stiff, mineralized bone matrix. **Methods:** To test effects of substrate stiffness, model soft and hard polyurethane (PUR) substrates were synthesized with elastic moduli from 0.03 GPa to 1.2 GPa, to mimic the estimated moduli of soft and hard tissue. Briefly, a lysine diisocyanate prepolymer was mixed with a polyester polyol, cast in the wells of a 6-well tissue culture plate, and cured at 37°C for 24h. To assess effects of proteolytic degradability, cross-linked gelatin[7] and polyacrylamide (PAA)[8] gels were synthesized following previously described methods to give degradable and non-degradable substrates of equal modulus (~5kPa). Substrate modulus was measured by nanoindentation or rheology (data not shown). MDA-MB-231 human breast cancer cells were cultured on all substrates for 48 hours. Tissue culture polystyrene (TCPS, ~2 GPa), which has a modulus near the low end range for cortical bone, served as a control substrate. While longstanding clinical data show an increase in circulating PTHrP in patients with bone metastases as compared to those with only primary breast tumors[1,6], we needed to confirm the levels of PTHrP in the microenvironment for breast and bone metastases *in vivo*. Athymic nude mice received intracardiac (IC), intratibial (IT) and mammary fat pad (MFP) inoculations of MDA231 cells and tumor growth was verified weekly via Faxitron and GFP imaging. Animals were sac-ed at 4 weeks and tumor cells were isolated with FACS. Expression of PTHrP was measured by RT-PCR for mRNA and immunoradiometric assays (IRMA) for secreted protein in the blood and supernatant. **Results:** RT-PCR and IRMA showed a significant increase of PTHrP in MDA231 cells responding to a stiff substrate (~1.5 GPa) as compared to a soft substrate (~20 MPa) *in vitro* (Fig 1). There was also a significant decrease in PTHrP secretion on the proteolytically degradable gelatin as compared to the non-degradable PAA (Fig 2). Figure 3 shows increased PTHrP expression on the mRNA level in stiff, proteolysis resistant microenvironments (IT) *in vivo* as compared to softer, proteolysis permissive microenvironments (MFP) (Though bone metastases were present in the IC group, the number of tumor cells isolated was insufficient for RT-PCR analysis). Likewise, secreted PTHrP levels were much higher in the IC and IT groups as compared to the MFP group (due to low blood volume, samples from each treatment group were pooled, so standard error could not be calculated).

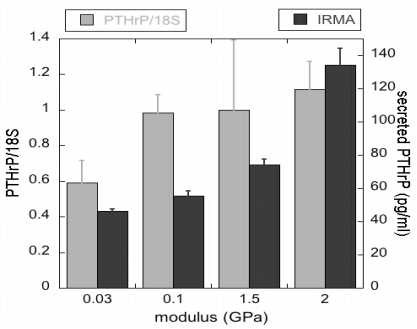

Figure 1. PTHrP expression and secretion as a function of substrate stiffness *in vitro*

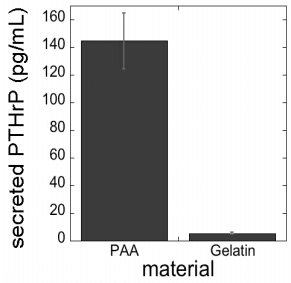

Figure 2. PTHrP secreted on proteolysis resistant (PAA) and proteolysis susceptible (gelatin) substrates *in vitro*

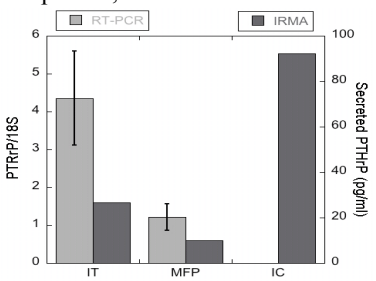

Figure 3. PTHrP expression and secretion *in vivo*

Conclusions: MDA231 human breast cancer cells respond to stiffer, proteolysis resistant substrates by increasing PTHrP expression. These effects further our understanding of the initiation of cancer induced bone resorption and could lead to potential therapies in the prevention of bone metastases, hypercalcemia and increased fracture risk. **References.** 1.Southby J, Cancer Res 1990;50. 2.ACS 3.Engler AJ, Cell 2006;12. 4.Paszek MJ,Cancer Cell 2005;8. 5.Alexander NR, Curr Biol 2008 6.Powell GJ, Cancer Res 1991;51. 7.Martucci JF, MSciEng 2006;435:. 8.Pelham RJ, PNAS 1997;94