Tumor Microenvironmental Cues Signal Host Tissue Progenitor Cells to Promote Tumorigenesis

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Statement of Purpose: Breast tumors exhibit increased stiffness and altered soluble factor signaling as compared to normal mammary epithelial tissues. However, the effect of these differential microenvironmental cues on surrounding host tissue stem cells remains unclear. We have developed alginate-based culture systems and co-culture approaches to test whether mechanical and chemical stimuli in the mammary tumor microenvironment, respectively, alter the behavior of adipose-derived stem cells (ADSCs). Our results indicate that the integrated interplay of tumor-inherent physical and chemical cues regulates the proliferation and differentiation capacity of ADSCs and that these changes are critical to tumor progression.

Methods: To study the effect of tumor stiffening on ADSCs, alginate hydrogels were designed to mimic stiffnesses of normal and cancerous breast tissue via ionic and photo-crosslinking procedures. Using these matrices the proliferation, differentiation, and angiogenic potential of 3T3-L1 (ATCC) and primary human ADSCs (Lonza) were determined by cell counting, analysis of GPDH activity, and ELISA of vascular endothelial growth factor (VEGF) secretion. Accordingly, the impact of tumor-derived soluble factors was evaluated in the presence of conditioned media. To this end, media were collected from MDA-MB231 and MCF-7 human breast cancer cells (ATCC) and normalized to the same cell number. While MDA-MB231 are representative of an aggressive stage of cancer ('malignant'), MCF-7 exhibit a more differentiated phenotype ('moderate'). The effect of altered angiogenic potential of ADSCs on endothelial cells was assessed by measuring the proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVECs, Lonza) in response to conditioned media via cell counting, trans-well, and Matrigel assays. Orthotopic mouse models were used to confirm *in vitro* data. MDA-MB231 cells and ADSCs were implanted individually or together into the cleared mammary fat pads of 3 week old SCID mice. Tumors were retrieved after 5 weeks and analyzed for size and CD31+ blood vessel density.

Results: RGD-modified alginate hydrogels were crosslinked to produce compliant, moderate, and stiff gels mimicking the compliance of normal adipose tissue, moderately diseased, and tumor tissue (Samani, A. Phys Med Biol. 2003; 48: 2183-98) (Fig. 1a). In order to vary gel moduli over the appropriate range, varying concentrations of RGD-alginate or photoinitiator were used when employing Ca- or UV-crosslinking methods, respectively. Increased matrix stiffness significantly enhanced 3T3-L1and primary human ADSC proliferation, while decreasing the differentiation of these cells (Fig. 1b and d). Similar trends were also detected for ADSCs cultured in the presence of tumor-conditioned media (Fig. 1 b and d). Additionally, ADSCs upregulated VEGF

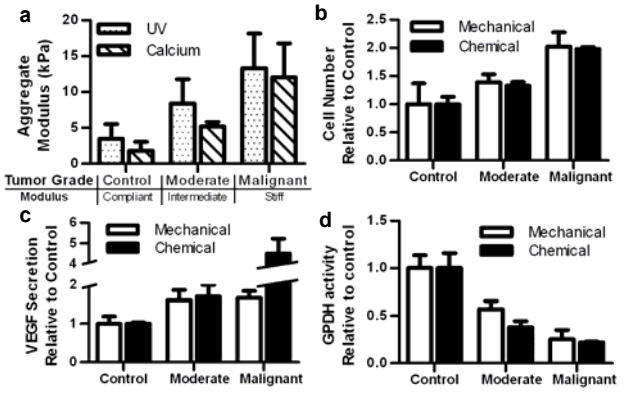

Figure 1: Ca-crosslinking of RGD-alginate of varying concentrations yields matrices mimicking normal (control), intermediate (moderate), and tumor tissue (malignant). UV-crosslinking recreates similar stiffnesses, while maintaining RGD density (a). Both mechanical and chemical cues increase ADSC proliferation (b) and VEGF secretion (c) while decreasing ADSC differentiation (d) with increasing tumor malignancy (i.e., stiffness or cell aggressiveness). Data only shown for 3T3-L1.

secretion (Fig. 1c) with exposure to mechanical and chemical tumor mimicking cues. This change in pro-angiogenic capability increased HUVEC proliferation, migration, and formation of tubular structures (data not shown). Interestingly, similar changes in ADSC proliferation, differentiation, and pro-angiogenic behavior were noted for the Ca- and UV-crosslinked gels indicating that matrix stiffness rather than RGD density mediate the altered cell behavior (data not shown). The observed changes in ADSC behavior are biologically relevant as co-implantation of MDA-MB231 and human primary ADSCs resulted in the formation of larger and more vascularized tumors as compared to implantation of MDA-MB231 alone (Fig. 2).

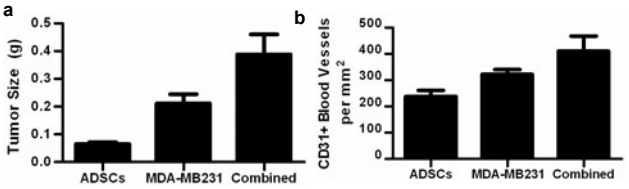

Figure 2: Implantation of MDA-MB231 in combination with human primary ADSCs yields larger (a) and more vascularized (b) tumors as compared to implantation of MDA-MB231 alone.

Conclusions: Physicochemical cues within the breast tumor microenvironment modulate the behavior of local progenitor cells. These changes promote angiogenesis ultimately increasing tumor growth and metastatic ability.

Acknowledgements: Alexander Nikitin's lab (Cornell University) for support with the *in vivo* experiments. The National Science Foundation and the NY State Center for Advanced Technology for funding.