Fibronectin and Type I Collagen Dysregulations Drive Tumor Progression

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Statement of Purpose: The extracellular matrix (ECM) is a complex microenvironment that provides physical, biochemical, and mechanical cues to cells. Any ECM dysregulation thus directly alter cues to cells. Understanding how these dysregulations occur and amplify over time is essential to elucidating tumor progression. Fibronectin (Fn), a major ECM protein, displayed upregulation and unfolding in tumors (Chandler EM. Phys Biol. 2011; 8: 015008). Type I collagen (Col I), the most abundant ECM protein, when dysregulated and crosslinked, enhanced tumor progression (Levental KR. Cell. 2009; 139:891-906). Past studies have shown a dependence of Col I deposition on initial Fn matrices (Sottile J. Mol Biol Cell. 2002; 13: 3546-3559). We seek to understand how early tumor-associated ECMs alter cell microenvironment and promote tumor progression. Specifically, we (i) combined intramolecular förster resonance energy transfer (FRET) with the surface forces apparatus (SFA) technique to correlate structural (Fn unfolding) and mechanical properties of the tumor-asscated ECM, (ii) analyzed how the presence of this dysregulated ECM (only Fn initially) affects downstream Fn-Col I matrix deposition and (iii) quantified cell pro-angiogenic behavior in response to ECM dysregulation.

Methods: FRET & SFA studies were carried out on cylindrical silica surfaces covered with atomically smooth mica. Pre-conditioned pre-adipocytes deposited control or tumor-associated matrices in low serum media supplemented with exogenous Fn (with 10% labeled-Fn for FRET experiments or 100% unlabeled Fn for SFA experiments). Culture systems were then decellularized to measure exclusively ECM properties. Parallel samples were either FRET imaged or indented in the SFA. Matlab analyses of fluorescent intensity ratios were used to discriminate stretched and partially unfolded ECM fibers (low FRET), from relaxed and folded fibers (high FRET). Mechanical SFA data acquired in compression were fitted to extract the Young's moduli of the various ECM.

For long-term studies assessing tumor-associated ECM development, pre-conditioned cells were seeded at low density in Lab-Tek™ wells. The cells were maintained in conditioning media (control or tumor-associated) up to 24 hours before a timepoint, at which the conditioning media was switched to low serum media containing exogenous unlabeled Fn. At each timepoint (1, 5, 9d), after the media was collected, the culture systems were fixed, immunostained for nuclei, f-actin, Fn, and Col I, and imaged under a confocal microscope. The collected media were analyzed for vascular endothelial growth factor (VEGF) secretion with a mouse VEGF Quantikine ELISA kit. Furthermore, additional long-term studies were carried out with Batimastat treatment, a broad spectrum matrix metalloproteinase (MMP) inhibitor, to assess if MMPs mediated any of the tumor-associated dysregulations.

Results: Our results indicate that tumor-associated cells initially deposited a highly strained, unfolded Fn matrix (Fig 1A, B) comprised of thick Fn fibers (Fig 1C), which was also stiffer than its control counterpart (Fig 1D). Furthermore, we show that this initial Fn matrix was remodeled over time and replaced by a dense Fn-Col I matrix (Fig 2A), in which both Fn and Col I fibers were thicker in tumor-associated matrices than in control samples (Fig 2B, C). Additionally, tumor-associated VEGF secretion was measured to be higher at 1d and 5d, and to decrease upon Batimastat treatment (Fig 2D).

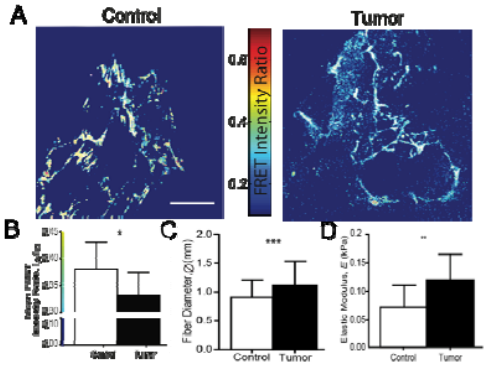

Figure 1. Structural & Mechanical ECM Properties

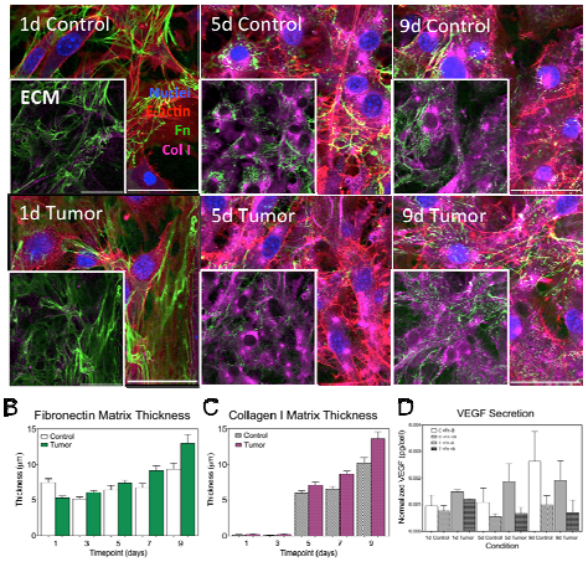

Figure 2. Tumor-associated ECM & VEGF Secretion

Conclusions: Our study indicates that the early deposited tumor-associated matrix is exclusively comprised of highly stretched (unfolded) and thick fibronectin fibers which overall lead to stiffening of the matrix. Both structural and mechanical matrix properties further disrupt downstream signaling pathways such as Col I deposition and pro-angiogenic secretion. These results suggest that early ECM dysregulation enhances tumor progression and tumor angiogenesis (for eventual metastasis).