Fibronectin and Type I Collagen Synergy in Tumor Progression

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Statement of Purpose: Understanding tumor progression requires an understanding of how the extracellular matrix (ECM) is altered and remodeled. As a mammary tumor progresses from normal to malignant, there is a significant increase in the Young's elastic moduli of these mammary tumors compared with that of a normal mammary duct (Lopez JI. Integr Biol. 2011; 3:910-921). Fibronectin (Fn), a major ECM protein that is upregulated in tumors, has been utilized as a molecular tool to study ECM conformation and was used to show that cells treated with tumor soluble factors deposit an altered, unfolded matrix (Chandler EM. Phys Biol. 2011; 8: 015008). Another major and the most abundant ECM protein, type I collagen (Col I), has been shown to promote tumor progression when dysregulated and highly crosslinked (Levental KR. Cell. 2009; 139:891-906). Previous work has shown that early matrices require the deposition of Fn in order for fibrillar collagen to be assembled (Sottile J. Mol Biol Cell. 2002; 13: 3546-3559). We seek to understand how early dysregulated matrices deposited by cells treated with tumor soluble factors present a microenvironment that would promote tumor progression. Specifically, we (i) utilize intramolecular förster resonance energy transfer (FRET) and the surface forces apparatus (SFA) technique to correlate unfolded, highly strained Fn matrices to an altered mechanical microenvironment, and (ii) show how the presence or absence of this early dysregulated Fn matrix would affect Col I assembly over time.

Methods: Murine 3T3-L1 pre-adipocyte cells were pre-conditioned for 3 days with Control (Ctrl) and Tumor Conditioned Media (TCM). Preconditioned cells were seeded with reduced serum (1% fetal bovine serum) and exogenous Fn (10% FRET-labeled Fn, 90% unlabeled Fn). After 24 hours in culture, culture systems were either: (i) fixed with formaldehyde, (ii) decellularized with Triton X-100 and deoxycholic acid and left unfixed, or (iii) decellularized and fixed. These samples were imaged through confocal microscopy. Matlab analyses of z-stack images of fluorescent intensity ratios were used to assess FRET in the ECM, and to discriminate stretched and partially unfolded fibers (low FRET) from relaxed and folded fibers (high FRET).

SFA studies were performed in parallel by seeding cells on top of one of a pair of cylindrical silica disks covered with atomically smooth silvered mica sheets, the second disk was used for indentation measurements. Normalized force curves were generated as a function of ECM thickness, and, E was extracted using Hertz contact mechanics modeling and fitting of the following force-indentation expression:

$$\frac{F}{R} = \pi E \frac{\delta^2}{D}$$

where F, is the applied load; R, the radius of curvature of the discs; D, the thickness of the relaxed ECM; δ, the indentation, and E, the effective compressive modulus.

Preconditioned cells were seeded initially at low density and maintained in conditioning media (Ctrl or TCM) up to 24 hours before a timepoint (1, 3, 5, 7, 9 days). 24 hours before a timepoint, the conditioning media was switched to low serum (1% fetal bovine serum) medium containing exogenous unlabeled Fn (+Fn), or regular low serum media (-Fn). At each timepoint, the culture system was washed in PBS, fixed for an hour, and washed again with PBS. Samples were permeabilized with PBS-X, blocked with SuperBlock Blocking Buffer for an hour, and incubated at 4°C with mouse anti-Fn and rabbit anti-Col I overnight. After washing with PBS-X, samples were incubated with Alexa Fluor 488 goat anti-mouse, Alexa Fluor 633 goat anti-rabbit, Alexa Fluor 546 Phalloidin, and DAPI for an hour at room temperature, and washed again with PBS-X. These samples were left in PBS and imaged through confocal microscopy.

Results: Our results depict highly strained and unfolded matrices deposited by cells treated with tumor soluble factors even after decellularization (Fig 1 left). This altered matrix without the presence of cells correlates to a stiffer matrix, with a loss in mechanical information after fixation (Fig 1 right). Furthermore, we show that as the early dysregulated Fn matrix (Fig 2 left) is remodeled, by day 9, a dense type I collagen matrix is assembled (Fig 2 right). Interestingly, even without exogenous Fn added, both Ctrl and TCM samples are able to assemble a type I collagen matrix.

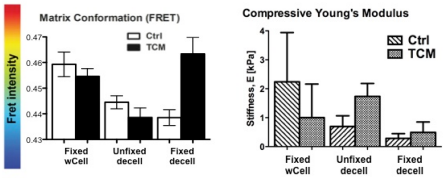

Figure 1. Matrix Conformation and Mechanics

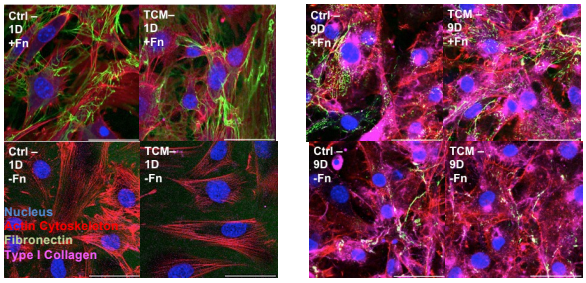

Figure 2. Immunostained Cytoskeletal and Matrix Components

Conclusions: Our study suggests that tumor soluble factors are at the origin of an altered, stiffer ECM made of highly stretched Fn fibers likely exposing cryptic sites that further disrupt biochemical signaling (i) between the cell and its ECM, and (ii) between ECM components, to further tumor progression and eventual metastasis.