# Altered Extracellular Matrix Properties Drive Tumor Progression

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**Statement of Purpose:** Understanding how extracellular matrix (ECM) dysregulations occur and amplify over time is critical to elucidating tumor progression. The ECM is a complex microenvironment that provides biochemical and mechanical cues to cells; therefore, any ECM dysregulation directly alter cues to cells. Fibronectin (Fn) is a fundamental extracellular matrix (ECM) protein implicated in cell signaling and behavior in both physiological (wound healing, embryogenesis) and pathological (fibrosis, cancer) conditions. Moreover, Fn has been shown to be not only upregulated in tumors, but also deposited into highly stretched and unfolded fibers by pre-adipocytes exposed to breast tumor soluble factors (Chandler EM, et al., Phys Biol (2011), 8:015008). Another and the most abundant ECM protein, collagen I (Col I), when dysregulated and crosslinked was shown to enhance tumor progression (Levental KR. Cell. 2009; 139:891-906). Past studies have shown a dependence of Col I deposition on underlying Fn matrices (Sottile J. Mol Biol Cell. 2002; 13: 3546-3559). In this study, we seek to understand how early tumor-associated ECMs alter the cellular microenvironment to promote tumor progression. Specifically, we (i) assessed tumor-associated Fn-Col I matrix deposition and topology over time, (ii) used förster resonance energy transfer (FRET) to analyze how Fn initial conformation affected the developing Fn-Col I microenvironment, and (iii) evaluated whether Fn-Col I matrix dysregulations were mediated by matrix metalloproteinases (MMPs).

**Methods:** 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) with or without Batimastat (a broad spectrum MMP inhibitor) up to 24 hours before a timepoint, at which the conditioning media was switched to low serum media containing exogenous Fn (10% FRET-labeled). At each timepoint (1, 5, 9d), the culture systems were fixed and either immunostained for nuclei, f-actin, Fn, and Col I or FRET-imaged. Confocal immunostained images were analyzed via ImageJ for Fn and Col I fiber diameter and linearity (defined as fiber short length/full length). Matlab analyses of fluorescence intensity (FRET) ratios were used to discriminate stretched and partially unfolded ECM fibers (low FRET bluish fibers), from relaxed and folded fibers (high FRET yellowish fibers).

**Results:** Our results indicate that tumor-associated cells initially deposited a Fn matrix (only) that was remodeled over time and replaced by a dense Fn-Col I matrix (Fig 1A & B), in which mature Fn and Col I fibers were thicker and more linear in tumor-associated matrices than in control samples (Fig 1C & D). At all timepoints tumor-associated Fn fibers were more unfolded than control Fn fibers (Fig 2A). Interestingly, initial Fn fibers were also more unfolded (1d) when no Col I was present than in presence of Col I (9d) (Fig 2B). Furthermore, treating tumor-associated cells with an MMP inhibitor resulted in less cell proliferation and ECM deposition (Fig 3).

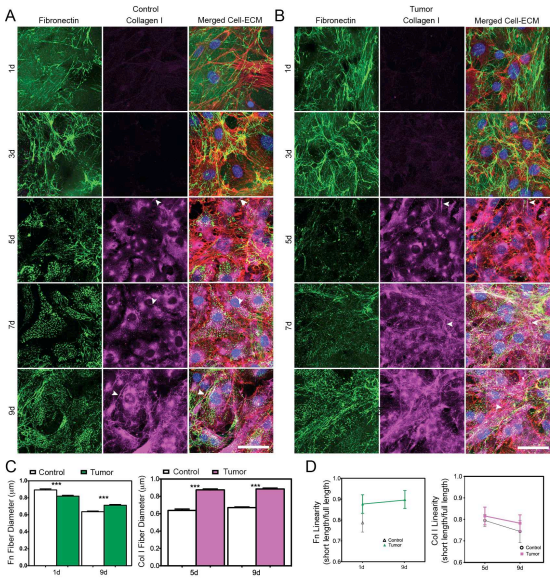


**Figure 1. ECM Composition and Topology**

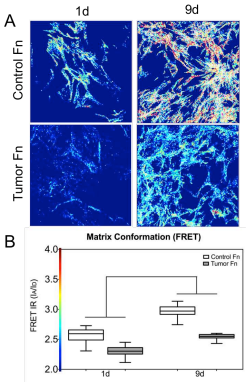


**Figure 2. Fibronectin Matrix Conformation**

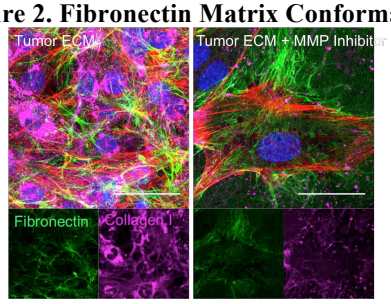


**Figure 3. Effect of MMP Inhibitor on ECM Assembly**

**Conclusions:** Our study indicates that the early deposited tumor-associated ECM comprises exclusively highly stretched (unfolded) fibronectin fibers. This initial dysregulated Fn ECM leads to the rapid, MMP-dependent assembly of a thick, unfolded, and linearized Fn-Col I matrix network. Collectively our findings suggest that early ECM dysregulations enhance tumor progression (and likely helps metastasis).