Adipose progenitor cells regulate fibronectin matrix assembly and stiffness in breast tumors

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Statement of Purpose: The extracellular matrix (ECM) of tumors is known to have altered mechanical properties, however, the mechanisms responsible for this, and how it further affects local cells remains unclear. Vascularization (angiogenesis) is particularly critical in tumor growth and we have previously found that stiff matrices (similar to malignant rather than healthy breast tissue) increase adipose progenitor cell proangiogenic secretions. In this work we have verified the relevance of those findings by investigating cell-deposited fibronectin (FN) matrices. Although collagen remodeling is often studied, FN is also a key component of the ECM and is in fact necessary for collagen deposition and stabilization.

Methods: For these studies, we have tested the ability of MDA-MB231, human breast cancer cells, to alter the ECM remodeling ability of the well-characterized adipose progenitor cell line 3T3-L1. To this end, we have used a Fluorescence Resonance Energy Transfer (FRET) imaging technique to monitor *in-situ* both FN molecular conformation and rigidity[1] in the ECM deposited by 3T3-L1s, when pre-treated either with MDA-MB231-conditioned media or with TGF-β as a mimic of tumor cell secretion.

Results: The deposition of FRET-labeled FN into newly developed fibers was monitored via confocal imaging in order to determine the conformation, stiffness, and thickness of FN matrices deposited by control and tumor-associated 3T3-L1s in 2-D culture. Our data indicate that tumor-preconditioned 3T3-L1 deposit a 1.4fold thicker Fn matrix than control cells and that this matrix is characterized by enhanced stretching and unfolding of individual FN fibers (lower FRET), which directly correlates with increased fiber rigidity[1,2] (Fig. 1). Specifically, FRET mapping of FN fiber populations showed that tumor-associated cells generate mainly highly-strained (220% in average) and stiff (Young modulus, E = 0.5-0.6 MPa[2]) FN fibers, while control cells deposit a broader population of more relaxed and compliant FN fibers (120% average strain, E = 0.1-0.2 MPa[2]). These E-values reflect the *mesoscopic* regime of deformations, mainly determined by stretching of single fibers. They are higher than *macroscopic* E-values of matrices (typically in the kPa range), which are measured when deformation is distributed over a network of disordered and connected fibers that respond collectively (by ordering/aligning) under strain. Pre-conditioning of 3T3-L1s with TGF-β as a mimic of tumor cell secretion resulted in similar changes (Fig. 1b, upper panel), while tumor-preconditioning of 3T3-L1 in the presence of a TGF-β neutralizing antibody normalized Fn matrix assembly (Fig. 1b, lower panel).

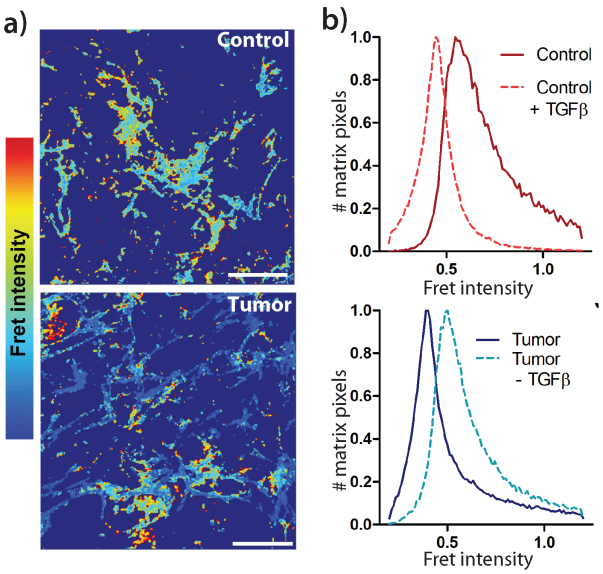

Fig. 1. 3T3-L1s pre-treated with MDA-MB231-conditioned media (tumor) assemble Fn matrices with more extended (i.e., stiffer) fibers than control cells as indicated by lower FRET (i.e., dark-blue fibers) (a). Tumor-preconditioned 3T3-L1s produce a more homogenous and stiffer matrix relative to control cells as indicated by a narrower and left-shifted FRET distribution (b, solid lines upper and lower panel). Pre-conditioning with TGF-β (control+TGFβ) mimicked tumor-mediated changes in FN deposition, while administration of a TGF-β neutralizing antibody to tumor-conditioned media (Tumor–TGFβ) inhibited these effects (b). Scale bars represent 50 μm.

Conclusions: These data indicate that chemical cues from tumor cells cause local progenitor cells to remodel the tumor microenvironment, increasing the ECM stiffness (specifically by stretching FN fibers) and that TGF-β is a critical regulator of this process. This may promote both vascularization and growth of breast tumors.

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