Cancer-activated adipocytes and their role in ECM remodeling and angiogenesis

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Statement of Purpose: Adipose tissue (AT), a main component of mammary tissue, regulates the pathogenesis of breast cancer. Historically, varied endocrine functions have been attributed to the tumor-promoting capability of AT[1)]. However, changes in extracellular matrix (ECM) remodeling may be similarly important, but have been largely overlooked. Our previous studies indicate that adipose-derived stem cells (ASCs) in mammary tissue enhance cancer progression due in part to fibrotic remodeling and stiffening of the breast tumor microenvironment[2)]. Nevertheless, little is known how mature adipocytes modulate mammary stroma composition and architecture in breast tumors. Here, we investigated whether or not adipocytes are also actively engaged in tumor-dependent ECM remodeling, and if this possible change increases breast tumor progression. Specifically, we hypothesize that tumor-derived soluble factors cause trans-differentiation of adipocytes into myofibroblasts and that this phenotypic change leads to increased contractility and deposition of matrix-stiffening ECM components including fibronectin and collagen ultimately favoring tumor angiogenesis.

Methods: To investigate the influence of tumor-derived soluble factors on mature adipocytes, 3T3-L1 mouse preadipocytes (ATCC) and human ASCs (Lonza) were subjected to adipogenic differentiation. Subsequently, these cells were cultured in tumor-conditioned media (TCM) collected from MDA-MB231 human breast cancer cells or control media for up to 4 weeks in 2D plates or 3D collagen scaffolds. Adipocyte phenotypic changes were analyzed via Oil Red O staining and glycerol-3-phosphate dehydrogenase (GPDH) activity for loss of adipogenic features. Additionally, myofibroblastic markers α-smooth muscle actin (α-SMA) and ECM components (fibronectin and collagen I) were analyzed by Western Blot analysis, immunofluorescence, and qRT-PCR. To evaluate tumor-mediated changes in cell contractility, adipocyte-embedded circular 3D collagen scaffolds were fabricated and their diameter was measured before and after treatment with TCM. Also, the stiffness of these different matrices was assessed by dynamic mechanical thermal analysis (DMTA). Changes in pro-angiogenic factor secretion were determined via ELISA of vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8). Furthermore, to confirm the effect of cancer-activated adipocytes on endothelial growth and invasion, endothelial cells were seeded on top of adipocyte-embedded 3D collagen scaffolds following TCM treatment. Endothelial cell growth and invasion through the 3D collagen matrices were analyzed by confocal image analysis. Lastly, human and mouse mammary fat and tumor specimens were assessed for adipocyte size, fibronectin (Fn) and collagen deposition and CD31 blood vessel density to confirm our *in vitro* findings with clinical samples.

Results: Oil Red O staining and GPDH activity analysis indicate that adipocytes cultured in TCM loose their adipogenic properties in both 2D and 3D culture. Simultaneously, TCM-treated adipocytes developed into α-SMA positive myofibroblastic cells and deposited thicker, fibronectin and collagen-enriched matrices as compared to their control counterparts. Moreover, when cultured in TCM, the size of adipocyte-embedded collagen scaffold significantly decreased relative to controls suggesting increased cell contractility under these conditions. Accordingly, DMTA analysis of the different collagen matrices suggests that enhanced ECM deposition and contraction by TCM-treated adipocytes collectively regulate substrate stiffness. In addition to altered ECM remodeling, TCM-treated adipocytes secreted higher levels of VEGF and IL-8 as compared to their control counterparts. Correspondingly, endothelial cell growth and invasion increased when cultured on top of collagen matrices embedded with TCM-treated rather than control adipocytes. Finally, analysis of mouse and human mammary tissues confirmed our *in vitro* findings by showing (i) decreased size of adipocytes, (ii) increased quantities of Fn and collagen, and (iii) greater blood vessel density in tumor-associated relative to control tissues.

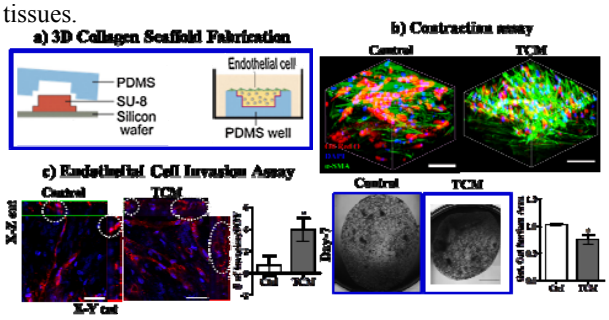

Figure 1. a) Illustration of our 3D collagen scaffold fabrication b) Contraction assay of adipocyte-embedded collagen scaffolds c) Endothelial cell invasion assay on the top of adipocyte-embedded collagen scaffold matrices

Conclusions: Our findings suggest that tumor-derived soluble factors can contribute to the conversion of mature adipocytes into myofibroblastic cells. These phenotypic changes lead to active remodeling of the ECM that stimulates increased pro-angiogenic factor secretion collectively promoting tumor angiogenesis.

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