Engineered Organotypic Breast Tumor Model Elucidates the Role of Tumor-Stromal Interactions on Dynamic Remodeling of Tumor Microenvironment

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Statement of Purpose: Cancer-associated fibroblasts (CAFs) are the most abundant stromal cells in the tumor microenvironment.[1] CAFs interact with cancer cells mainly via paracrine signaling of soluble factors that bind with receptors on cancer cells to activate oncogenic pathways that promote tumor progression.[2] Compelling evidence suggests that treatments tailored toward cancer cells alone are often ineffective to produce a lasting response in patients. Considering the role of the stroma in cancer progression, targeting tumor-stromal interactions is critical to improve outcomes for patients.[3] Lack of physiologic human tumor models has been an obstacle to understand tumor-stromal interactions and develop therapeutic interventions. 2D and conventional 3D cell cultures and animal models do not adequately represent human tumors, especially the tumor stroma. Thus, novel human tumor models are critical to facilitate drug discovery against tumor-stromal interactions. We addressed this need by developing an organotypic breast tumor model that mimics the architecture and cellular and extracellular matrix (ECM) composition of breast tumors. Our studies establish a major role for patient-derived CAFs in remodeling of the microenvironment and promoting pro-metastatic activities in cancer cells.

Methods: The organotypic model was developed in two robotic steps. First, a mass of breast cancer cells, SUM159 and MDA-MB-231, was made using our polymeric aqueous two-phase micropatterning technology.[4] Next, the spheroid was encapsulated in a collagen hydrogel containing dispersed normal human mammary fibroblasts (HMFs) or CAFs derived from patients (CAF1 and CAF2). Incubation at 37°C resulted in a tumor model. The stiffness of hydrogels was determined using AFM. A collagen contraction assay was performed using hydrogels of different Young's moduli containing 4×10^3, 10×10^3, and 15×10^3 fibroblasts in 20 µl collagen solution in each well of 384-well plates. Contractility was quantified as the ratio of final to initial gel areas. In organotypic cultures, confocal microscopy was used to capture matrix invasion of cancer cells and flow cytometry was used to quantify proliferation of cancer cells. Proteomics was used to identify signaling mechanisms between CAFs and cancer cells. Statistical analysis was done using two-way ANOVA with Bonferroni *post hoc* test.

Results: Figure 1a shows the organotypic culture that contains key components of the tumor microenvironment: a mass of cancer cell, dispersed fibroblasts, and ECM. A large number of 3D cultures were formed using a robotic liquid handler in 384-well plates. Our AFM study resulted in elastic moduli of hydrogels with various concentrations of collagen (Figure 1b). For biological studies, we used a ~2.53 kPa hydrogel to mimic advanced-stage human breast tumors.[5]

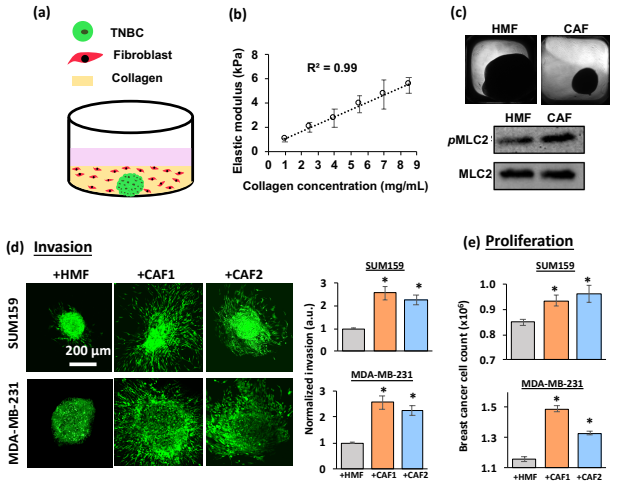

Figure 1. (a) Schematics of organotypic tumor model. (b) AFM characterization of hydrogels. (c) Matrix contractility by HMFs and CAFs over a 2-week culture. (d) Confocal images of organotypic tumors showing ECM invasion of GFP^+ breast cancer cells. Quantified results are shown for both breast cancer cells. (e) CAFs promote proliferation of breast cancer cells. *$p<0.01$.

We observed a significantly greater matrix contractility by increase in the density of fibroblasts and at a lower protein concentration (1 mg/ml vs 4 mg/ml). Importantly, CAFs contracted the ECM 34% more than HMFs did (Figure 1c). Molecular analysis validated this result and showed greater activity of RhoA/ROCK/MLC2 mechanotransduction pathway in CAFs. Confocal imaging of organotypic models showed that CAFs significantly promote matrix invasion of breast cancer cells, but HMFs suppress cell invasion (Figure 1d). Cytometry analysis showed that CAFs significantly enhance proliferation of cancer cells compared to HMFs (Figure 1e). Our molecular analysis showed that CAFs activate tumorigenic functions of cancer cells primarily through the HGF-cMET pathway. Our combinatorial drug screening directed toward this signaling axis showed significant reduction in pro-metastatic activities of cancer cells, establishing the feasibility of this approach as a novel therapy strategy.

Conclusions: We developed a scalable organotypic breast tumor model that contains key components of the tumor microenvironment and mimics its biological properties. This tumor model offers a novel tool for mechanistic studies of tumor-stromal interactions and cancer drug discovery to block stroma-mediated tumorigenic activities.

References:

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