The Role of Paracrine Signaling between Breast Cancer and Stromal Cells on Remodeling of Tumor Microenvironment ECM

H Saini[1], K Rahmani[2], M Allam[1], C Silva[1], J Veldhuizen[1], D Truong[1], G Mouneimne[4], R Ros[2,3], M Nikkhah[1*]

[1]School of Biological and Health Systems Engineering; Arizona State University

[2] Center of Biological Physics and Department of Physics; Arizona State University

[3]Biodesign Institute; Arizona State University

[4]University of Arizona Cancer Center, Department of Cellular and Molecular Medicine

Introduction: Stromal extracellular matrix (ECM) dysregulation has been closely associated with advanced stages of breast cancer. Amongst various cell types found in the tumor microenvironment, cancer associated fibroblasts (CAFs) are known to be the major regulators of ECM remodeling. Our previous results suggested that the interaction between tumor cells and CAFs is necessary to modulate the biophysical properties of the tumor stroma. To gain further molecular insight into this cellular crosstalk, we utilized a 3D tumor-stroma model and dissected the role of paracrine signaling between tumor cells and CAFs on stromal ECM remodeling. We characterized the expression of multiple tumor secreted pro-fibrotic factors and specifically identified the role of platelet derived growth factor (PDGF-AA/-BB) on stromal fibrosis.

Materials and Methods: The soluble factor signaling between tumor cells and CAFs were studied by collecting conditioned media (CM) from monoculture and coculture groups of MDA-MB-231 and MCF7 cells, with CAFs, and subsequently exposing the monoculture of CAFs to the collected CM. ECM remodeling was characterized by measuring matrix stiffness and fiber density using atomic force microscopy and confocal imaging. Proliferation assays and qPCR were performed to study replication and gene expression changes in CAFs in presence of CM. ELISA was further utilized to assess the expression of pro-fibrotic factors in CM collected media.

Results and Discussion: Our results exhibited the crucial role of paracrine signaling between breast tumor cells and CAFs on stromal ECM remodeling (Fig. 1A, B, C). CM obtained from 3D culture of tumor cells was able to induce a desmoplastic response from CAFs, however on the contrary CM obtained from coculture of tumor cells and CAFs failed to elicit a significant change in stromal matrix properties, suggesting a unidirectional crosstalk between tumor cells and CAFs (Fig. 1A, B, C). We observed enhanced CAF proliferation when incubated with CM obtained from MCF7 cells as compared to CM collected from MCF7+CAF coculture (Fig. 1D). On the contrary, qPCR results demonstrated no significant change in gene expression of fibrotic genes including collagen I, collagen III, fibronectin, lysyl oxidase and TGF-β across all culture conditions (Fig. 1E, F). ELISA assay demonstrated significantly higher expression of PDGF-AA and PDGF-BB among other pro-fibrotic factors.

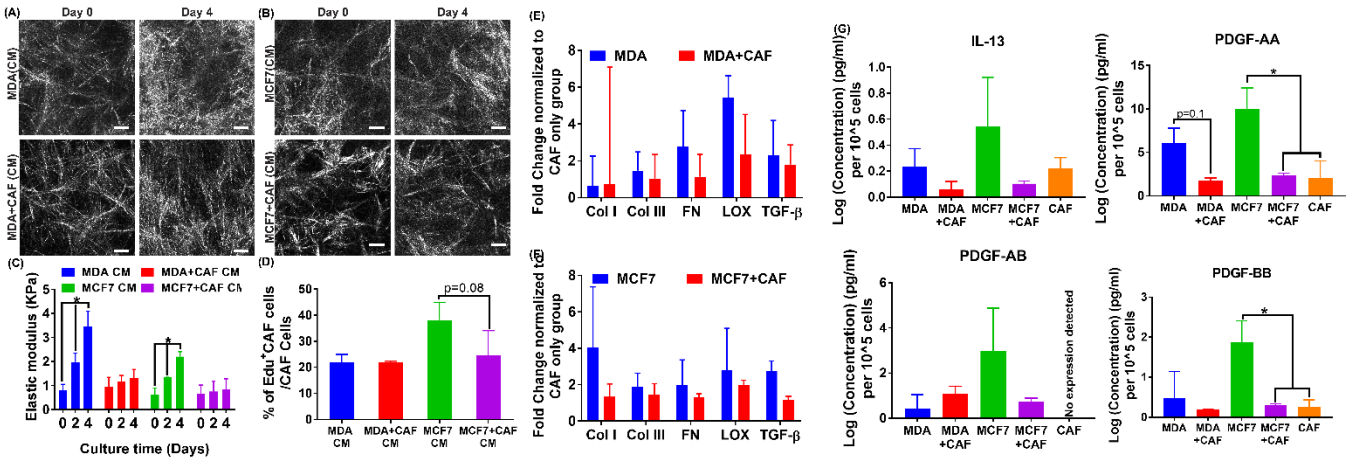

Figure 1. (A), (B) ECM fiber density for CAF mono-culture incubated with different CM. (C) Quantification of matrix stiffness (D) Quantification of proliferation of CAFs (E), (F) Fold change of fibrotic genes in CAFs. (G) Cytokine expression in various CM. Scale bar represents 10 µm.

Conclusions: We hereby present the crucial role of soluble factor signaling between tumor cells and CAFs and identify the significance of PDGF signaling on stromal ECM remodeling. Our ongoing work will include detailed assessment of matrix properties upon impairment of PDGF signaling between tumor cells and CAFs.

Acknowledgements: The authors would like to acknowledge NSF (Award# 1510700 and #1914680) for funding this project.