Dynamically Stiffening Hydrogels Differentially Modulates Malignant Transformation of Mammary Epithelial Cells

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Statement of Purpose: Mammary epithelial cells, e.g. MCF10A, are known to respond to differences in static ECM stiffness and resemble a mesenchymal, more malignant state on stiffer ECM lacking any polarized acinar structure. While this is akin to the stiff mammary tumors that one can detect with manual palpation, breast cancer fibrosis is a dynamic process that results in matrix stiffening from soft (150 Pascal; Pa) to stiff (1500 Pa) over months to years as a result of enhanced collagen expression and lysyloxidase crosslinking.

Methods: Dynamic methacrylated hyaluronic acid (MeHA) hydrogels were synthesized using a dual crosslinking scheme similar to one described by Guvendiren and Burdick[1]. Briefly, a free radical photopolymerization method using UV light and Irgacure is used to initially crosslink the hydrogel to approximately 150 Pa. Collagen is linked to the hydrogel using NHS-EDC chemistry to allow for MCF10A cells attachment. To more appropriately mimic temporal stiffening *in vivo*, the photopolymerization step is repeated using a short UV exposure of 2 minutes to create a linear pseudo-time dependent stiffening with negligible negative effect on the living cells .

Results: To more accurately mimic the onset of tumor-associated fibrosis, MCF10A mammary epithelial cells were cultured on dynamic methacrylated-hyaluronic acid (MeHA) hydrogels, whose stiffness that can be modulated by a two step polymerization process. MCF10A cells cultured on soft MeHA hydrogels form and remain polarized acini until the matrix is stiffened when they decompose and resemble a mesenchymal morphology. However, the degree of matrix stiffening and culture time prior to stiffening plays a large role in acini transformation; delayed onset of matrix stiffening from 6 to 10 days prevented malignant transformation in 35% of acini. To ensure that early stiffening was not density dependent, acini of differing cell density were transplanted onto stiffening MeHA hydrogels where we found that transformation was independent of acinus size and cell density. These data suggest that an individual acinus responds to the stiffness of the underlying substrate but that their epigenetic state and structure can protect a subset of acini from stiffening (Figure 1).

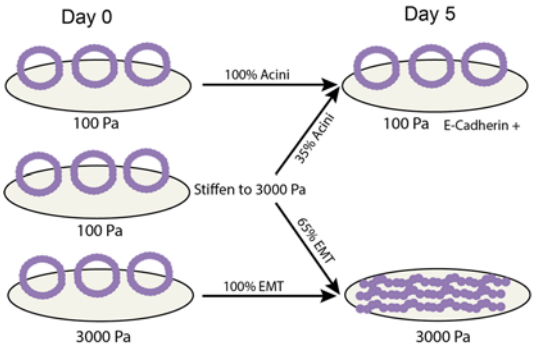

Figure 1. Schematic of acinar structures plated on stiffening MeHA hydrogels. Stiffened hydrogels develop a mixed population of EMT transformed cells as well as acinar structures that remain.

Western blotting and immunofluorescence indicated decreased E-cadherin expression and cell-cell junctional localization in acini that decomposed into mesenchymal cells, suggesting that the subset of acini that remained insensitive to stiffening could maintain epithelial markers. Mass spectrometry analyses further clarify this change in sensitivity to stiffening.

Conclusions: This data indicate a more complex interplay of intrinsic ECM cues and acinar structure in regulating signaling that results in epithelial-to-mesenchymal transition and the onset of a malignant phenotype.

References: 1. Guvendiren, M, Burdick JA. Nat Comm; 2012; 3:792

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