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# Peering from the outside in: Viscoelastic Properties of the Extracellular Matrix Dictate Spatial Organization and Apoptosis Resistance in Mammary Epithelial Cells

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*Abstract* – **The compliance of the extracellular matrix (ECM) differs between tissues and is altered in tumors. We examined the consequence of modifying the viscoelastic properties of the ECM on mammary epithelial cell (MEC) morphogenesis and apoptosis regulation. Results showed that the elastic modulus of the ECM exerts a profound effect on MEC tissue organization and gene expression that correlates with changes in actin organization and apoptosis resistance. Altering the rigidity of the ECM directly influences integrin expression and additionally modifies integrin-induced gene expression in association with actin reorganization. These data suggest that the compliance of the ECM may cooperatively regulate cell behavior by altering integrin function. Studies are now underway to investigate the possibility that these effects are mediated via changes in integrin-actin cytoskeletal dynamics.**
***Keywords*** – **Extracellular matrix, apoptosis, mechanics**

## INTRODUCTION

Apoptosis is essential for normal tissue homeostasis, immune surveillance and the efficacy of tumor therapy [1]. Although much is known about the mechanism whereby apoptosis is executed at the molecular level, factors that modify apoptotic decisions on a cellular and tissue level remain poorly understood.

The ECM regulates cell growth, differentiation and survival via integrins [2]. Cell-ECM interactions and integrins can also protect cells from apoptosis induction [3]. Recently we showed that the ability of cell-ECM interactions and integrins to inhibit apoptosis induction is significantly enhanced when cells form three-dimensional (3D) polarized tissue-like structures within a malleable ECM [4]. How integrins mediate apoptosis resistance and whether the enhanced resistance phenotype observed in the malleable ECM is linked to differences in the mechanical properties of the ECM and/or the altered spatial organization of the cells is not known.

The ECM has both biochemical and mechanical properties [2]. Biochemically the ECM regulates cell phenotype by activating signaling pathways and inducing changes in cytoskeletal organization that lead to modulation of gene expression. Yet the compliance of the ECM also profoundly influences cell growth and survival [5]. Because viscoelastic properties of the ECM can induce changes in cytoskeletal organization [4] we hypothesized that the compliance of the ECM modifies cell phenotype and enhances apoptosis resistance by altering integrin mediated effects on cytoskeletal organization and gene expression.

To test this possibility we employed two model systems and systemically altered the viscoelastic properties of the ECM in both two dimensions (2D) and 3D. We used the HMT-3522 human MEC tumor progression model [6] and NIH 3T3 fibroblasts in conjunction with mechanically rigid and pliable 2D and 3D collagen I and reconstituted basement membrane (rBM) assays. We found that the elastic modulus of the ECM exerts a profound effect on tissue organization and gene expression that correlate with alterations in actin organization and apoptosis resistance. We also determined that changing the rigidity of the ECM directly alters integrin expression in association with actin reorganization. Studies are now in progress to investigate whether the compliance of the ECM influences cell phenotype by altering integrin cytoskeletal dynamics and to identify the gene circuits regulated by this pathway.

## MATERIALS & METHODS

Commercial EHS matrix (Matrigel, Collaborative Research) was used for the rBM assays; Vitrogen (Vitrogen 100, Celtrix Laboratories; bovine skin collagen I, 3mg/ml), for collagen I coating of dishes; and Cellagen Solution AC-5, for 3D collagen I embedding studies. Polyacrylamide (PA) gels were prepared as described [7], and the dynamic shear storage modulus (G') was measured using a dual plate rheometer (Rheometrics Fluid Spectrometer II, Rheometrics Inc.). Antibodies included: $\alpha 5$ integrin, polyclonal; and $\beta 4$ integrin, clone 3E1 (Chemicon International); Laminin 5, clone BM165 (gift from P. Marinkovich); and HRP and FITC conjugated secondaries (Jackson Laboratories). Actin was visualized using FITC Phalloidin (Sigma) or by expression of GFP actin.

The HMT-3522 MECs were grown and manipulated in 2D and 3D and immunofluorescence was conducted as described previously [8]. Apoptosis was induced by TNFá (100 ng/ml) followed by secondary Ab-mediated clustering. Apoptosis was detected using either the Live/Dead Assay (Molecular Probes) or by assaying for internucleosomal DNA fragmentation in intact fixed cells using a commercially available *in situ* apoptosis TUNEL kit (Boehringer Mannheim). RNA from 2D and 3D cultures was prepared and microarray analysis was conducted using Affymetrix U95A arrays containing 12,500 identified probes, as previously described [4]. One expression profile array image data file from each of the 6 different experimental conditions was imported into Rosetta Resolver System 3.0 (Rosetta Inparmatics, Inc.) for expression analysis and analyzed in a 2-D agglomerative clustering using only genes with a p-value cutoff of $\leq 0.01$ in 6 of 6.

## RESULTS

We first investigated whether altering the elastic modulus of the ECM influences tissue organization and/or apoptosis sensitivity. We grew nonmalignant S-1 MECs on tissue culture plastic coated with a thin coat of rBM (a mechanically rigid rBM, G' > 50,000 Pa) or within a malleable rBM (mechanically pliable rBM, G' > 200 Pa) and assayed for effects on tissue organization and apoptosis sensitivity. Despite ligation of BM integrins, MECs grown on a rigid rBM failed to form 3D polarized acini and retained their sensitivity to apoptosis induced by TNFá. In contrast, MECs embedded within a malleable rBM formed polarized 3D acini that were extremely apoptotically resistant. MECs interacting with collagen I remained exquisitely sensitive to apoptosis induction regardless of the mechanical properties of the ECM. Thus ligation of BM integrins is necessary for apoptosis resistance in MECs but the mechanical properties of the BM modifies this effect.

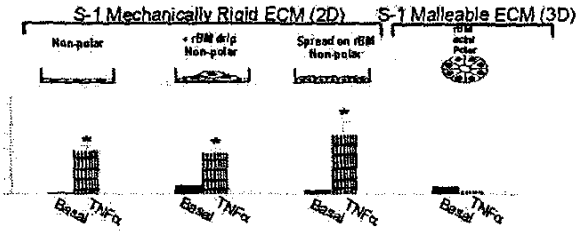


Fig. 1. Apoptotic labeling indices (mean ± SEM of 3-6 expts.)

To understand the mechanism whereby mechanical properties of the BM influence MEC behavior we used Affymetrix gene expression arrays to profile gene expression patterns and BM responsiveness in MECs grown in pliable as compared to rigid ECM microenvironments before and after BM ligation. 2D agglomerative clustering analysis across 6 different experimental conditions demonstrated that ECM compliance significantly alters gene expression and that a malleable ECM permits the BM to induce a program of genes that is associated with tissue polarity and apoptosis resistance (Data not shown).

We next investigated whether ECM compliance induced these effects on cell phenotype and gene expression by directly altering integrin expression and/or function. We used NIH 3T3 fibroblasts and mechanically modified ECM gels and assayed for changes in cell spreading, actin organization and integrin expression. Data showed that 3T3 fibroblasts grown on rigid PA gels (G' = 17,200 Pa) spread rapidly, and exhibited pronounced actin stress fibers (Data not shown) in association with increased expression of α5 integrin (Fig. 2). In contrast, 3T3s grown on pliable gels (G' = 1200 Pa) failed to spread appreciably, did not form actin stress fibers (Data not shown), and expressed low levels of α5 integrin (Fig. 2). These results illustrate that the elastic modulus of the substrata influences integrin expression and actin organization to influence cell behavior.

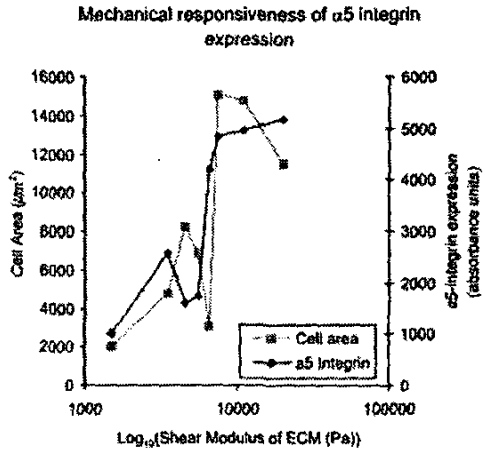


Fig. 2. Quantification of cell spreading and á5 integrin expression as a function of increasing the elastic modulus of the ECM.

## DISCUSSION & CONCLUSION

The data described here demonstrate that the mechanical and biochemical properties of the ECM cooperatively regulate cell phenotype via integrins. Specifically, we showed that the viscoelastic properties of the ECM modify BM-integrin induced MEC polarity and apoptosis resistance and alter the pattern of expressed genes. The data suggest that ECM compliance mediates these effects on cell phenotype by altering integrin expression and actin organization. These results could explain the clinical observation that multidrug and immune resistant tumors often occur in metastatic tissues with low elastic moduli such as in pleural effusions. Indeed tumors that metastasize to the bone, a mechanically rigid microenvironment, are often more treatable.

## ACKNOWLEDGMENT

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