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A Novel 3D Model to Study the Link Between Hormonal Exposure and Mammographic Density in Breast Cancer

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Highlights

- Lower type 1 collagen concentration (0.5 mg/ml) increases elongation of structures compared to 1 mg/ml.
- Fibroblasts enhances elongation of structures in 1 mg/ml, but not 0.5 mg/ml.
- In 1 mg/ml collagen, elongated epithelial structures had lumen formation when co-cultured with fibroblasts.
- Hormones in co-cultures of T47D + fibroblasts, significantly altered the phenotype in terms of elongation.

Introduction

- Ovarian and pituitary hormones are regulators of normal breast development as well as risk factors for breast carcinogenesis [1].
- Mammographic density, which correlates with high stromal content, including cells (stromal fibroblasts) and collagen is an additional risk factor for breast carcinogenesis [2].
- A link between hormones and mammographic density has yet to be fully explored and understood.

Role of fibroblasts in stromal density:

- Stromal fibroblasts (RMF) exert contractile forces that remodel type 1 collagen fibers, which in turn modulate cell behavior in an iterative fashion [3].
- RMF secrete several factors that can determine the density and rigidity of the stroma [3].
- In addition, RMF have been shown to accelerate the formation of epithelial ducts by organizing collagen fibers around these ducts [4].

Hormonal regulation of the stroma:

- The quality of the stroma changes during puberty and pregnancy under the influence of mammotropic hormones, estradiol (E2), progesterone (P) and prolactin (Prl) [5].
- In our hormone-sensitive three-dimensional (3D) model, E2, P and Prl respectively induce ductal elongation, branching and budding by remodeling the extracellular matrix (ECM) [6].

Hypotheses

- **Higher stromal density and rigidity favors neoplastic organization of epithelial cells.**
- **Hormones acting on stromal fibroblasts alter epithelial phenotype.**

References

1. Kelsey JL, Gammon MD, John EM: Reproductive factors and breast cancer. Epidemiologic Reviews 1993; 15: 36-47
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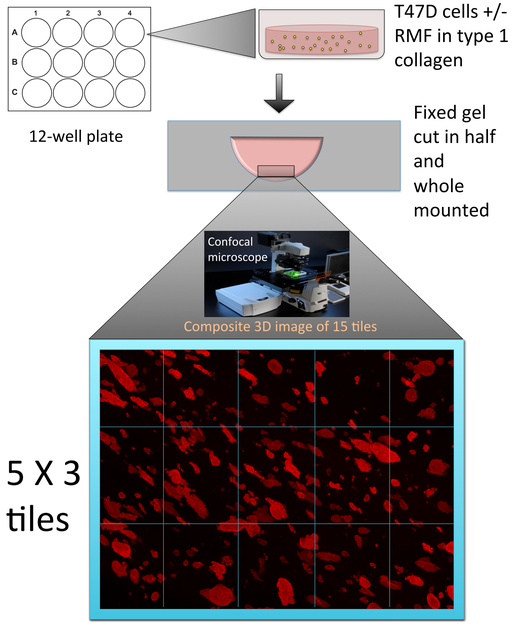
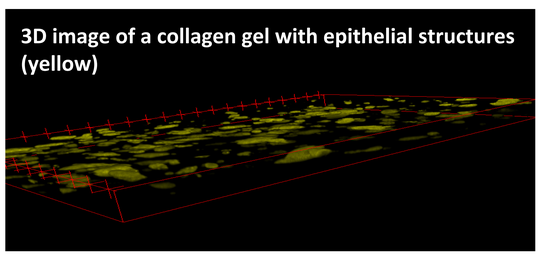
3. Grinnell F: Fibroblast biology in three-dimensional collagen matrices. Trends in Cell Biology 2003; 13: 264-9
4. Dhimolea E, Maffini MV, Soto AM, Sonnenschein C: The role of collagen reorganization on mammary epithelial morphogenesis in a 3D culture model. Biomaterials 2010; 31: 3622-30
5. Schedin P, Mitrenga T, McDaniel S, Kaeck M: Mammary ECM composition and function are altered by reproductive state. Molecular Carcinogenesis 2004; 41: 207-20
6. Speroni L, Whitt GS, Xylas J, Quinn KP, Jondeau-Cabaton A, Georgakoudi I, Sonnenschein C, Soto AM: Hormonal regulation of epithelial organization in a 3D breast tissue culture model. Tissue Engineering 2013

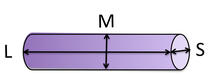
Methods



Software for **A**utomated **M**orphological **A**nalysis
(for details see Poster # SAT 0067)



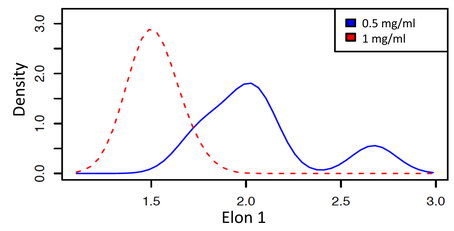
Measured Parameter – Elongation (Elon1): Ratio of the long axis to the middle axis (L/M) i.e. measure of elongation



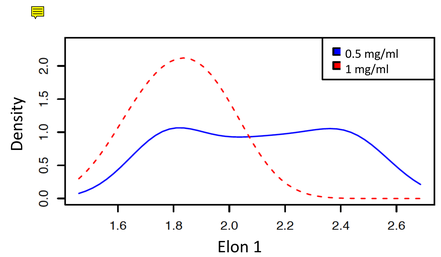
Results

1. Effect of stromal fibroblasts (RMF) and the density of collagen on mammary epithelial cells

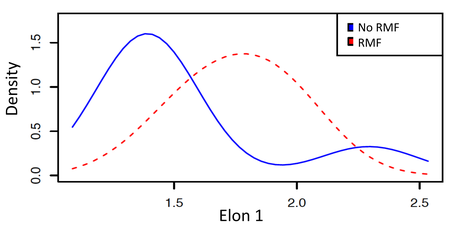
A. Higher elongation of structures in 0.5 mg/ml than 1 mg/ml collagen: T47D cells alone



B. No differences in elongation of structures in 0.5 mg/ml and 1 mg/ml collagen gels: T47D cells with RMF



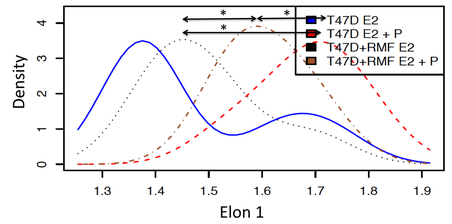
C. Higher elongation of structures with lumen formation in 1 mg/ml collagen: T47D cells +/- RMF



Graphs represent mean distribution of elon 1 of structures in 3D cultures of T47D cells. Data obtained from 6 gels run in duplicate in 3 separate experiments; p<0.05.
T47D cells (150,000 cells/well) +/- RMF (50,000 cells/well) were suspended in 0.5 or 1 mg/ml type 1 collagen.
1.5 ml of cells + collagen suspension were poured into the wells and allowed to congeal for 30 minutes. 1.5 ml of media (DMEM + 5% FBS) was poured on the congealed gels. The gels were detached from the sides of the wells.

2. Effect of stromal fibroblasts (RMF) and hormones on mammary epithelial cells

Higher elongation of structures with hormones in co-culture (T47D + RMF) than mono-culture (T47D cells alone)



Graph represents mean distribution of elon 1 of structures in 3D gels of 1mg/ml collagen. Data obtained from 6 gels run in duplicate in 3 separate experiments; *p<0.05
T47D cells (150,000 cells/well) +/- RMF (50,000 cells/well) were suspended in 0.5 or 1 mg/ml type 1 collagen.
Media: Phenol-red free DMEM-F12 + 7.5% charcoal-dextran stripped FBS.
Hormones used: E2 (10^{-10} M) and P (10^{-10} M).

Conclusions

- RMF may require a certain threshold of collagen concentration to alter epithelial phenotype. This suggests that RMF exerts physical forces and alterations in biomechanical properties of the collagen fibers, which is hindered when there are not enough collagen fibers available.
- Hormones may be acting on epithelial cells and RMFs, which then affects epithelial phenotype. The question remains whether RMF alters the biomechanical properties of collagen as well as collagen density around the elongated structures.

Significance

- A unique feature of our 3D model is its responsiveness to hormones in ways similar to the mammary gland *in situ.*
- Moreover, stroma can be manipulated by altering the properties of the ECM. In this 3D culture model, hormones affect epithelial cells that, in turn, alter collagen organization [6].
- This knowledge will explain how two of the main risk factors in breast cancer (mammotropic hormonal influence and tissue biomechanics) operate, thus paving the way for potential novel targets for breast cancer prevention, prognosis and cure.

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