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Investigation of cell stiffness and cytoskeletal remodeling in response to inflammatory mediators using atomic force microscopy (AFM)

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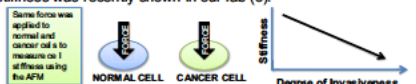
ABSTRACT

Atomic force microscopy (AFM) is a novel technology with emerging potential for cancer detection based on cell stiffness measurements. Studies have shown that cancerous cells were recognized to be less stiff than normal epithelial cells. However, the mechanisms through which their biophysical properties are altered have not been fully elucidated. In this study, we investigated the role of transforming growth factor-β (TGF-β) as a potential mediator involved in altering the biophysical properties such as cell stiffness of mammary epithelial cells (MCF10A). **We hypothesized that TGF-β will promote decreased cell stiffness through the disruption of f-actin using the AFM.** To date, we determined that disrupting f-actin of MCF10A decreased measured cell stiffness. However, disrupting microtubules using colchicine did not alter cell stiffness. Additionally, our AFM measurements revealed that MCF10A treatment with TGF-β reduced the measured cell stiffness 3-fold, down to the level measured for MDA-MB-231 cancer cells in our previous studies.

BACKGROUND

- The atomic force microscopy is a novel technology that can be used to measure cell stiffness under physiological conditions.
- Cancer cells have been shown to be less stiff than normal cells [1,2]. A correlation between the degree of invasiveness and stiffness was recently shown in our lab (3).



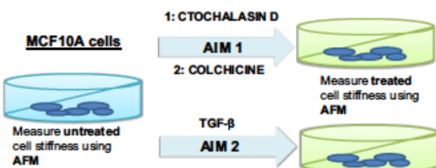
- This finding has important implications for early detection of cancer based on cell stiffness of circulating tumor cells.

OBJECTIVE

To use AFM to determine the effect of TGF-β on the measured cell stiffness of MCF10A mammary epithelial cells.

METHODS

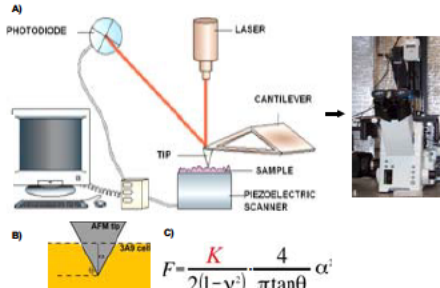
- **Treated MCF10A cells with known cytoskeleton disruptors**



- **Measure Cell Stiffness using AFM**
  - Measurements were acquired at 37deg. °C in liquid.
  - Data analysis were done using Igor Pro software.
  - Data was fitted to the Hertz model to calculate Young's modulus, a measure of cell stiffness.

METHODS

Atomic Force Microscope (AFM)

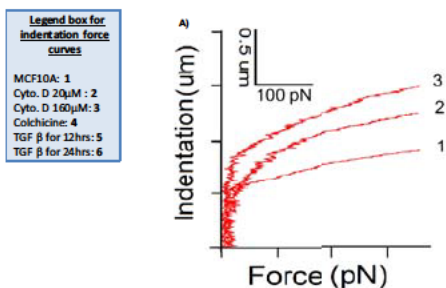


$$F = \frac{K}{2(1-\nu^2)} \cdot \frac{4}{\pi \tan\theta} \alpha^2$$

Figure 1: The technique of Measuring Cell Stiffness using the AFM A) (Left) schematic representation of the AFM (Right) Actual representation of the AFM In our lab, we used the MFP 3D Bio AFM B) Estimates of Young's modulus are made on the assumptions that the AFM tip is a rigid cone and the cell is an isotropic elastic solid C) Young's modulus equation the force indentation relation is a function of Young's modulus to measure cell stiffness, K; the angle created by the indenter and the plane of the surface, θ Poisson ratio, v, are approximately 55° and 0 5, respectively The indentation is α and F is force

PRELIMINARY RESULTS

Cytochalasin D reduced stiffness of MCF10A in a concentration-dependent manner



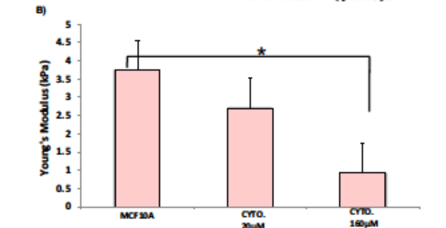


Figure 2. AFM measurements on MCF10A cells treated with cytochalasin D. A) Indentation force curves for MCF10A, cells treated with cytochalasin D 20μM, and cells treated cytochalasin D 160μM B) Average Young's modulus measurements acquired for MCF10A cells (n≥25) treated with 20μM of cytochalasin D Average Young's modulus measurements acquired for MCF10A treated with 160μM of cytochalasin D (n value is 24) Bar indicates standard error, asterisk * indicates significant differences (p<0 05) between MCF10A cells and MCF10A cells treated with cytochalasin D 160μM

PRELIMINARY RESULTS

Colchicine did not affect stiffness of MCF10A

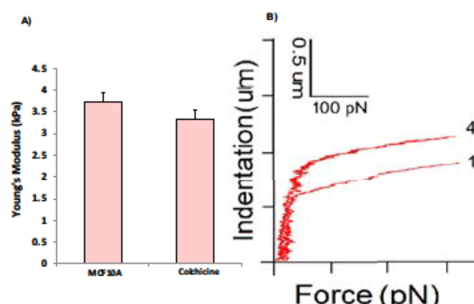


Figure 3. AFM measurements on MCF10A treated with colchicine. A) Indentation force curves for MCF10A and cells treated with colchicine B) Average Young's modulus measurements acquired for MCF10A cells and MCF10A cells treated with colchicine (4) Error bar indicates standard error (n≥25)

TGF-β showed a decrease of MCF10A cell stiffness

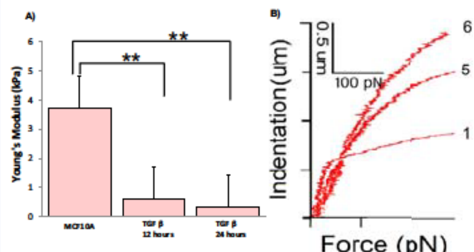


Figure 4. AFM measurements on normal mammary epithelial cells (MCF10A) treated with TGF β 12 hours of stimulation. A) Average Young's modulus measurements acquired for MCF10A cells and MCF10A cells treated with TGF β for 12 hrs, and cells treated with TGF β for 24 hrs B) Indentation force curves for MCF10A cells, cells treated with TGF β for 12 hrs, and cells treated with TGF β for 24 hrs Bar indicates standard error, two asterisks (**) indicates extremely significant differences (p<0 01) (n≥25)

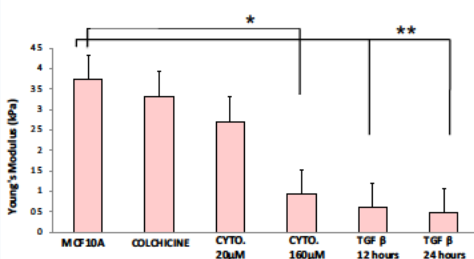


Figure 5. Summary of AFM measurements on treated MCF10A cells. Average Young's modulus acquired for MCF10A cells (n≥25) that were treated with colchicine (n≥25), cytochalasin D 20μM (n≥25) , cytochalasin D 160μM (n value is 24), and TGF β (n≥25) (4) Bar indicates standard error, asterisk (*) indicates significant differences (p<0 05) Two asterisks (**) indicates extremely significant differences (p<0 01)

DISCUSSION

- Disruption of f-actin using cytochalasin D decreased the measured cell stiffness down to the level previously measured for invasive mammary cancer cells, MDA-MB-231 [3].
- MCF10A cells treated with cytochalasin D showed significant decrease in cell stiffness that was found to be concentration-dependent. A decrease in cell stiffness of 33% was observed following treatment with 20 μM cytochalasin D and 81% with160 μM cytochalasin D.
- Disruption of MCF10A microtubules using colchicine did not affect the measured cell stiffness.
- TGF-β has been shown to disrupt f-actin [6]. MCF10A treatment with TGF-β for 12 hours resulted in a 70% decrease in cell stiffness, with a 88% reduction of cell stiffness measured for 24 hours of treatment. This result is likely mediated through the disruption of f-actin.

CONCLUSIONS

- The cell cytoskeleton allows a normal cell to maintain its stiffness. This study revealed that disrupting MCF10A f-actin, but not microtubules, greatly reduces the measured cell stiffness.
- Cancer cells have been shown to be less stiff than normal cells [1,2]. The mechanism through which a normal cell's biophysical properties are modulated during prometastatic progression remains largely unknown. This study shows, for the first time, shows that TGF-β likely plays an important role in this process by modulating the actin cytoskeleton and reducing the measured cell stiffness.

FUTURE DIRECTIONS

- Immunocytochemistry will be conducted to visualize changes in the cytoskeleton in response to treatment with inflammatory mediators including TGF-β.
- The effects TGF-β and other inflammatory mediators on cell adhesion will be investigated using AFM.

ACKNOWLEDGEMENTS

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CITATIONS

1) Lekka M, Laidler P, Gil D, Lekki J, Stachura Z, Hrynkiewicz AZ Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy *Eur Biophys J* 1999;28(4):312 316
2) Cross, S , Y Jin, et al (2007) "Nanomechanical analysis of cells from cancer patients" Nat Nanotechnol 2(12): 780 783
3) Jaczewska, J , Qureshi, A , Wojcikiewicz, E P (2011): Atomic Force Microscopy studies of the mechanochemical changes occurring during the epithelial to mesenchymal transition *2011 Proceedings of the American Association for Cancer Research*
4) Rotsch, C and M Radmacher (2000) "Drug induced changes of cytoskeletal structure and mechanics in fibroblasts: an atomic force microscopy study" Biophys J 78(1): 520 535
5) Available http //mntl i llinois edu/equipment/Images/FullScale/AsylumAFM pg Last accessed 12 Feb 2013
6) Moustakas, A and Stournaras, C (1999) Regulation of actin organisation by TGF β in H ras transformed fibroblasts Journal of Cell Science 112 (0), 1169 1179