THE TENSION-GENERATING ABILITY AND APPEARANCE OF MYOFIBROBLAST TENSION PHENOTYPE BY PRECANCEROUS CELLS, KER-CT-RAS

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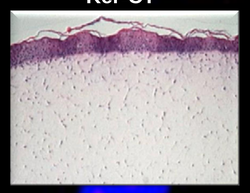
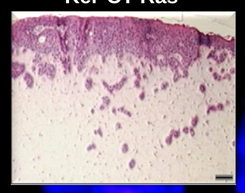
Abstract

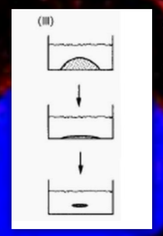
Recent research activity has focused on the tumor stroma. Tumor stroma are typically connective tissues containing fibroblasts and myofibroblasts. These cells are required for the wound healing processes of the body. There is evidence that myofibroblast presence in tumor stroma leads to poor prognosis. Mechanical tension, one of three key factors, enhances differentiation of myofibroblasts.
In vitro carcinomas can form through a pathway which involves the up-regulation of the ras protein. Precancerous keratinocytes lead to two types of carcinomas. They take on properties of fibroblasts and metastasize, spreading into the dermis. Fibroblasts generate tension in the dermis during the wound healing process. Our experiment focuses on keratinocytes and their journey into the dermis, which we model using an in vitro dermal equivalent.
Specifically, we used Grinnell's stress-relaxation collagen matrix model, a model that acts like a wounded dermis; that is, it provides the necessary microenvironment for myofibroblasts. The model was originally used to investigate the properties of fibroblasts, cells native to the dermis. Our research has taken to using it in the research of invasive epithelial, precancerous cells called Ker-CT-Ras. Previously, we set up Ker-CT-Ras lattices void of fibroblasts. In this year's poster, additional data will be presented on the comparative tension-generating ability of fibroblast lattices (DP-147-H-Tert) and co-culture lattices comprised of the two. Also, we will present preliminary data from a monolayer (coverslip) model to describe the structural properties of the myofibroblasts phenotype.

Ker-CT



Ker-CT-Ras





Grinnell, F. The Journal of Cell Biology, Volume 124, 1994

Epithelial to mesenchymal transition (EMT) (Vaughan et. al., PloS ONE 2009)

Methods

The stress-relaxation collagen matrix (figure above right) allows *in vitro* contraction of fibroblast in a synthetic wounded dermis, due to the characteristic fibroblast contraction that is associated with wound healing. Invasive Ker-CT-Ras previously demonstrated the ability to contract the matrix void of fibroblasts. We used Goat anti-mouse (GAM) rhodamine in indirect immunoflorescence staining to detect alpha smooth muscle actin (α-sm-actin) fibers, stress fibers characteristic of myofibroblasts. All lattices are measured according to the greatest diameter during a best-fit area measurement taken with Image J. Lattices, the 3D model, were either stained or released. Coverslips, the 2D model, have no release method but provided more defined qualitative data than the lattices. Transforming growth factor β (TGF-β) was added to the media surrounding the experimental coverslips and lattices prior to staining or release.

Acknowledgements

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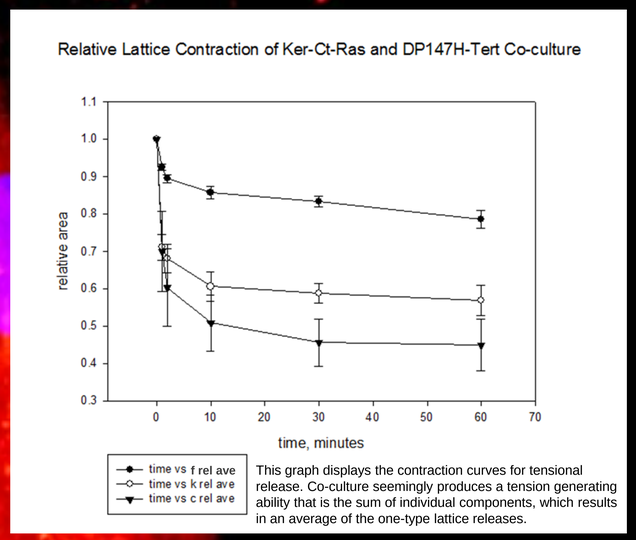
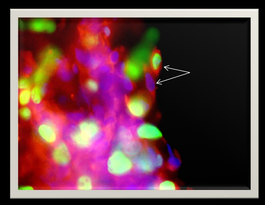
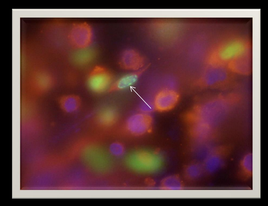
Lattice Results



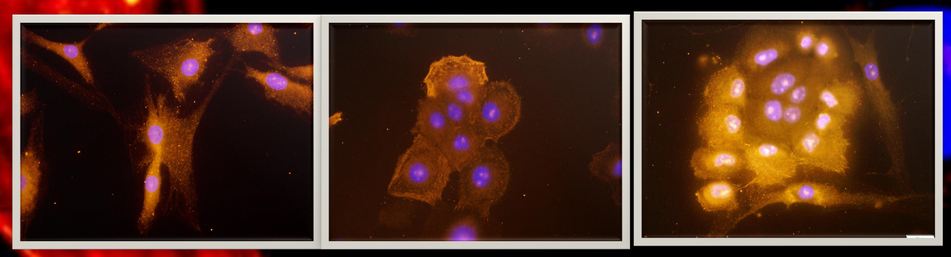
This graph displays the contraction curves for tensional release. Co-culture seemingly produces a tension generating ability that is the sum of individual components, which results in an average of the one-type lattice releases.



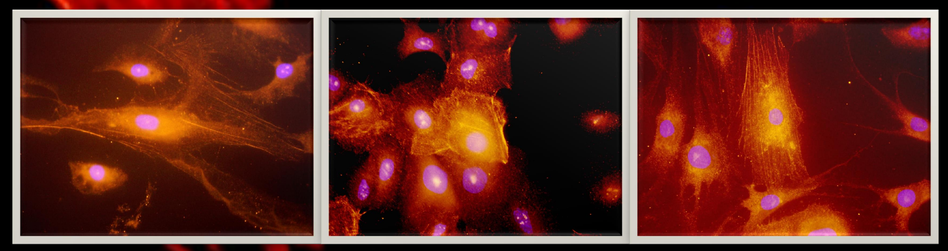


Ker-CT-Ras controls at 60x (top), 100x (bottom) elongation gives some evidence of myofibroblast phenotype. Blue nuclei due to DAPI, green from "EDU" stains.

Coverslip Results

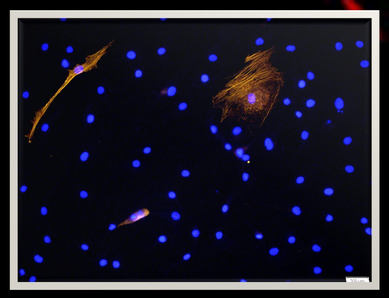


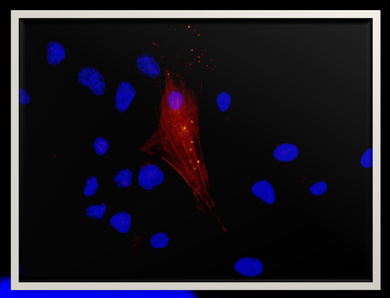
DP147-H-Tert control 60x | **Ker-CT-Ras control 60x** | **Co-Culture control 60x**



DP147-H-Tert and TGF-beta 60x | **Ker-CT-Ras and TGF-beta 60x** | **Co-culture and TGF-beta 60x**

Above. DP147-H-Tert fibroblasts, Ker-CT-Ras, and the co-culture. Both cell types are not known for expression of the myofibroblast phenotype, and the fibroblasts are not known for differentiating into myofibroblasts.





Coverslips: Differentiated Myofibroblast(s) from fibroblast* (20x, left) and co-culture (60x, right)

Discussion

TGF- β is known for increasing the up-regulation of stress fibers produced by fibroblasts. Because of this up-regulation, the fibroblasts release more EDA-fibronectin, which acts as a surfactant and makes the lattices or coverslips more "sticky", an effect that increases the resultant tension generated. Ker-CT-Ras, like fibroblasts, increase tension generation when treated with tgf-β. Ker-CT-Ras are shown to be using this α-sm-actin. This find provides new insight to the myofibroblast-like qualities of Ker-CT-Ras.

Conclusion

Our study shows that Ker-CT-Ras contracts in a collagen lattice and assembles contractile stress fibers. Coupled with previously demonstrated EMT, these effects show that the Ker-CT-Ras demonstrate the myofibroblast tension-generating phenotype. Also, through a co-culture of Ker-CT-Ras and DP147-H-Tert, cells not known for differentiating into myofibroblasts, we were able to induce a strong myofibroblast phenotype.

Future Goals

At Oklahoma Research Day 2014, we intend to present on the correlation between transformation and size of cell nuclei, reduce the spread of the error bars on co-culture lattice release, and determine whether Ker-Ct-Ras lattices are EDA-fibronectin dependent or independent.

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