Cell geometry modulates the activation of fibroblasts in 3D tumor microenvironment

Saradha Venkatachalapathy[1], D.S. Jokhun[1] and G.V.Shivashankar[1,2]
1.Mechanobiology Institute National University of Singapore, Singapore
2.FIRC Institute of Molecular Oncology, Italy

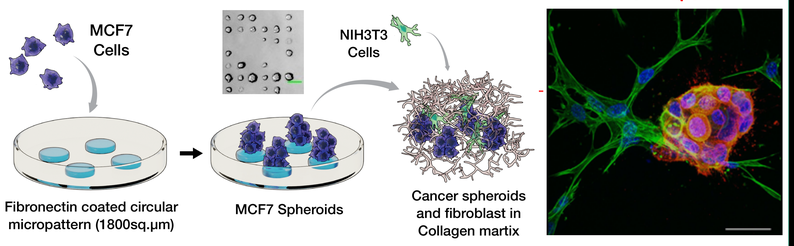
ABSTRACT

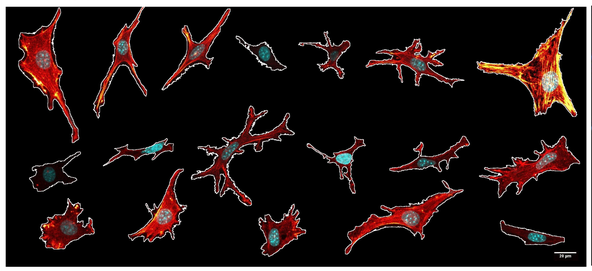
In the tumor microenvironment, the stromal fibroblast population consists of subsets of cells that are activated and promote tumor initiation and growth. The factors that contribute to such selective activation of fibroblasts are not understood. Recent studies from our lab have highlighted the importance of cell mechanics in modulating the transcriptional response to signals from the microenvironment[1,2]. In this study, we assessed the role of intrinsic cell geometric state in modulating fibroblast activation.

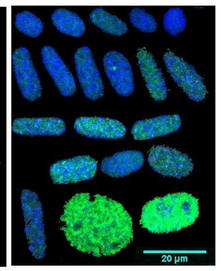
To test this, we developed an engineered 3D fibroblast tumor co-culture system and used high resolution images to quantify multiple cell geometry sensitive nuclear morphological and chromatin organizational features. These features were then mapped to activation levels as measured by the nuclear abundance of transcription cofactor, MKL and protein levels of its target, αSMA. We observe the presence of activation-"primed" cell geometries that present higher activation levels which are further enhanced in the presence of cancer cells.

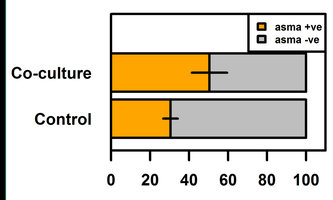
Further we show that enriching the population of activation-primed cell geometric states by either increasing matrix rigidity or micro-patterning primed cell shapes results in increased efficiency of fibroblast activation. Collectively, we present a framework for studying single cell heterogeneity and our observations highlight the importance of cell mechanics in fibroblast activation within the heterogenous tumor microenvironment.

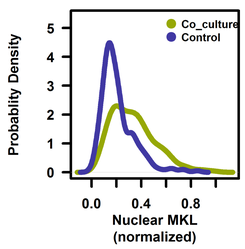
FIBROBLAST ACTIVATION IN 3D CO-CULTURE MATRIX









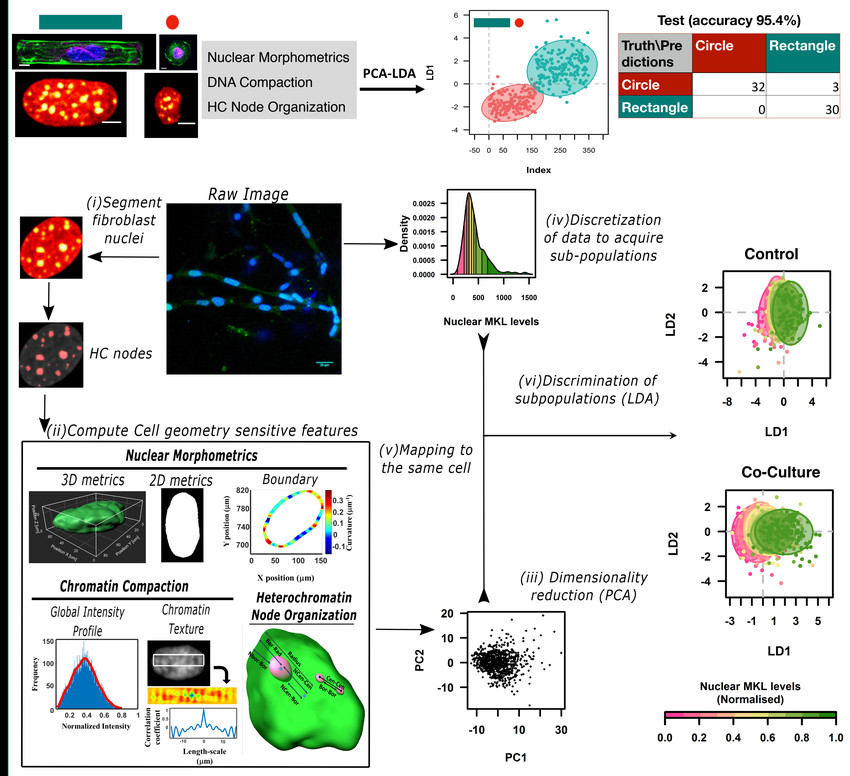


In the co-culture system there is increased TGF-beta signaling in fibroblast cells

Only subsets of the population are activated by the cancer cells

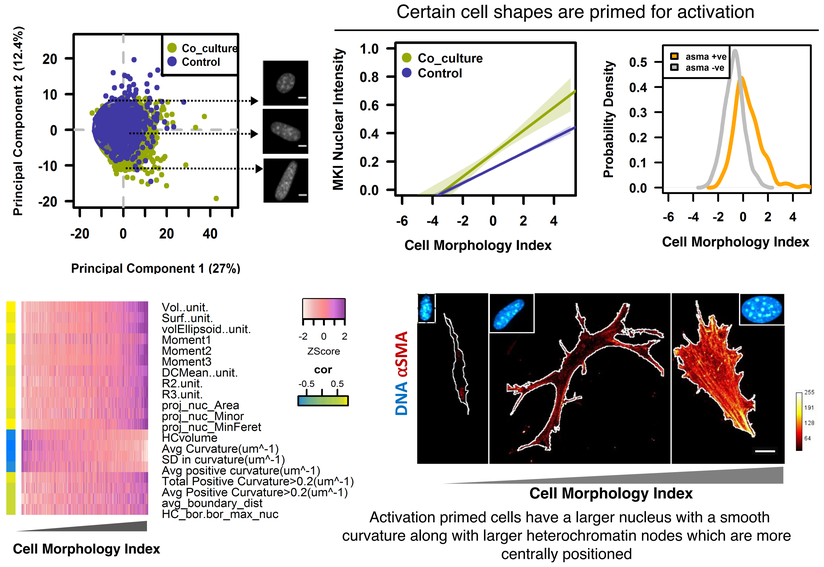
MAPPING CELL SHAPE TO ACTIVATION LEVELS

Nuclear morphometrics and internal chromatin organizational features are sensitive to and predictive of cell geometry



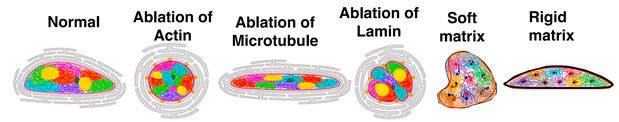
MKL NUCLEAR LEVELS ARE REFLECTIVE OF CELL SHAPE

Certain cell shapes are primed for activation



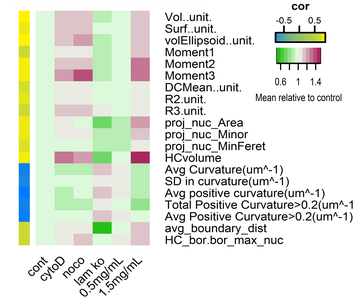
Activation primed cells have a larger nucleus with a smooth curvature along with larger heterochromatin nodes which are more centrally positioned

TUNNING ACTIVATION BY CONTROLLING CELL SHAPE

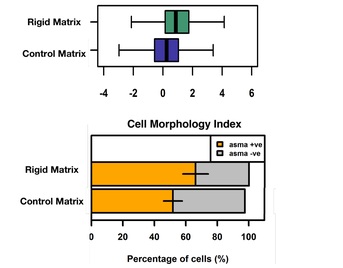


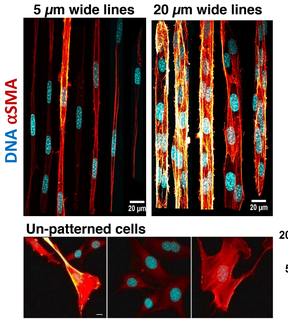
Adapted[3]

Modulators of cell shape in 3D matrix

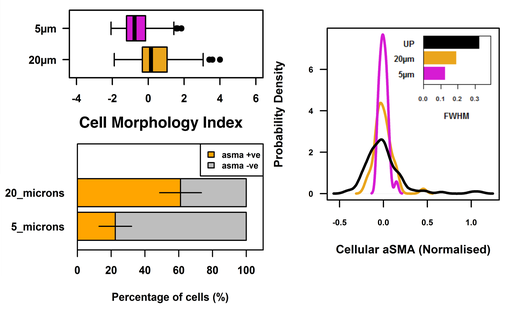


Increasing matrix rigidity enriches primed cell shapes leading to increased fibroblast activation



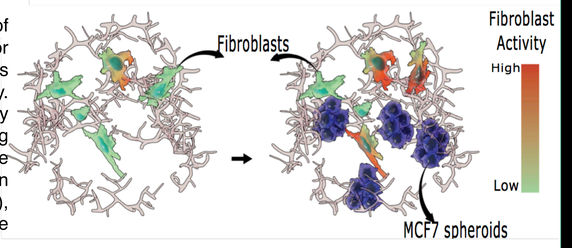


Controlling cell geometric heterogeneity allows control of fibroblast activation levels and variance



CONCLUSION

The nuclear enrichment of MKL, a TGF-beta effector transcription factor, is modulated by cell geometry. Such cell geometry dependent nuclear signaling leads to the selective activation (increased protein expression levels of aSMA), a target gene of MKL, in the presence of cancer cells.



Our study presents a framework for studying single cell heterogeneity and highlights the importance of the geometric state of fibroblasts in the interpretation of environmental signals.

REFERENCES AND FUNDING

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