Heterogeneity in cell geometric states regulate the selective activation of fibroblasts in engineered 3D tumor microenvironments

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INTRODUCTION

- Cancer associated fibroblasts are important regulators of tumor initiation and growth. Such activated fibroblasts often express high levels of alpha-Smooth Muscle Actin (aSMA).
- Only a subset of fibroblasts in the stromal microenvironment are activated by the cancer cells and the mechanisms that regulate such selective activation are not understood.
- Fibroblasts are morphologically and functionally heterogeneous in the stromal microenvironment. Recent studies have suggested that the cellular perception of environmental signals are modulated by cell geometry[1-2].

OBJECTIVE

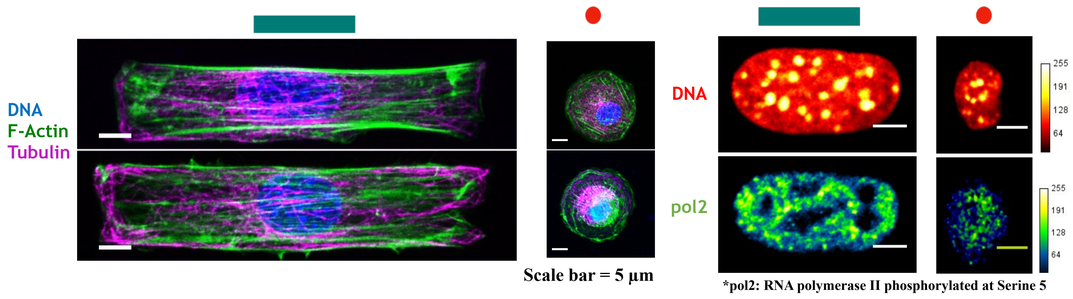
To study the contribution of the intrinsic cell geometric heterogeneity in modulating the nuclear mechanotransduction of signals from the microenvironment, resulting in their selective activation of fibroblasts in the presence of cancer cells.

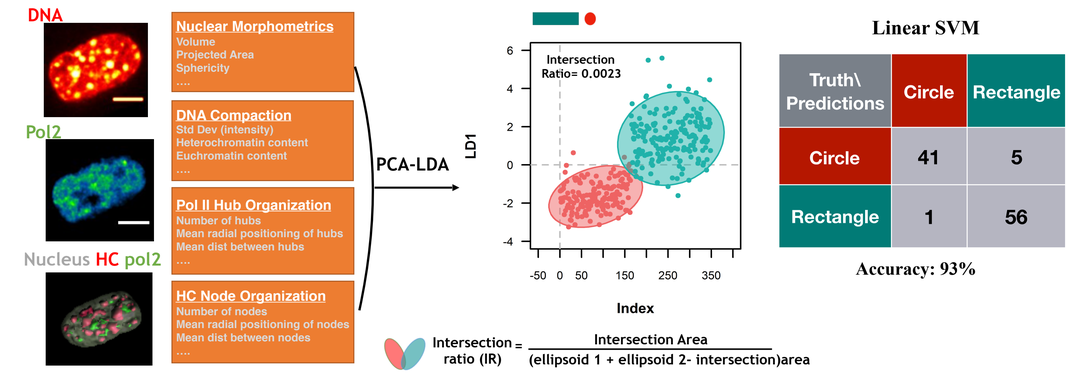
APPROACH

I. Use high resolution imaging to obtain cell geometry sensitive multi-parametric single cell measurements of nuclear morphometric and chromatin organization as well as nuclear enrichment of transcription factor of the same cells.
II. Dimension reduction using Principle component Analysis (PCA) and maximize inter-class separation using Linear Discriminant Analysis (LDA).
III. Test the predictive potential of the features by employing linear SVM for classification and linear regression for continuous variable.

Nuclear morphology and chromatin organization can be used as markers to delineate different cell geometric states in a heterogeneous cell population.

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



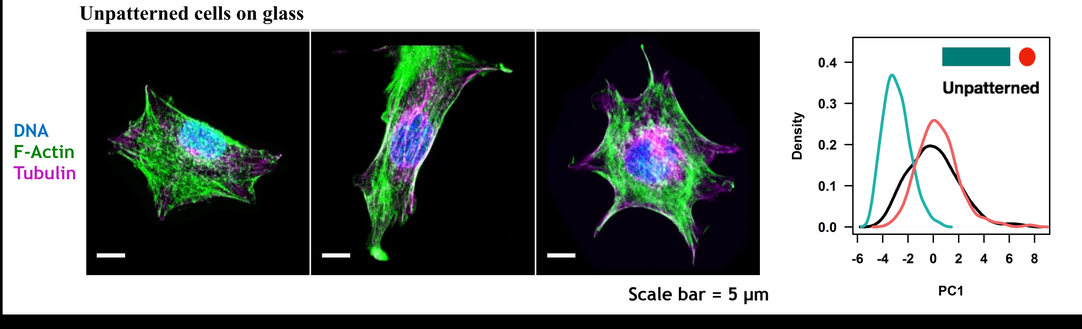


Linear SVM

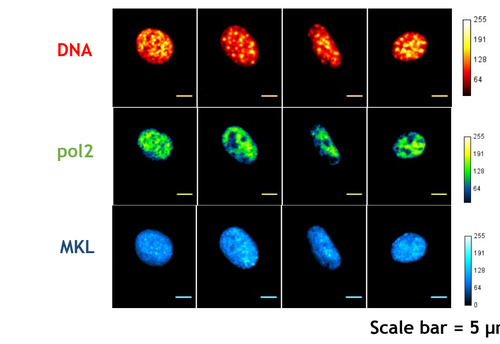
Truth\ Predictions	Circle	Rectangle
Circle	41	5
Rectangle	1	56

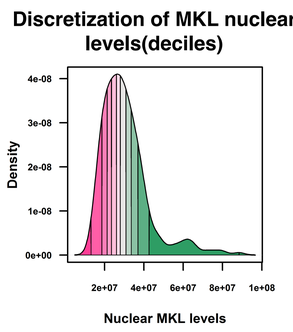
Accuracy: 93%

Rectangular and circular cells are subsets of the heterogeneous un-patterned cells.

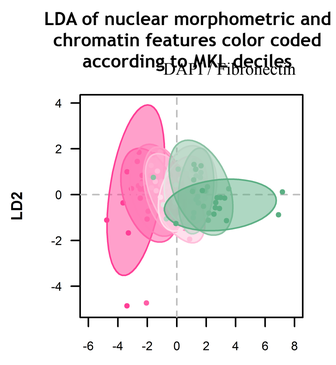


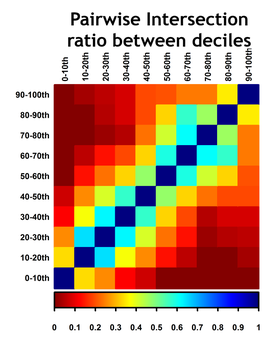
MKL NUCLEAR ABUNDANCE IS REFLECTIVE OF CELL GEOMETRY

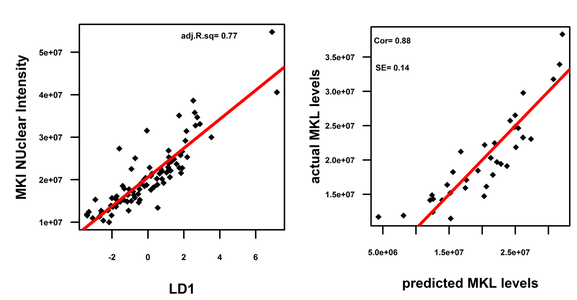




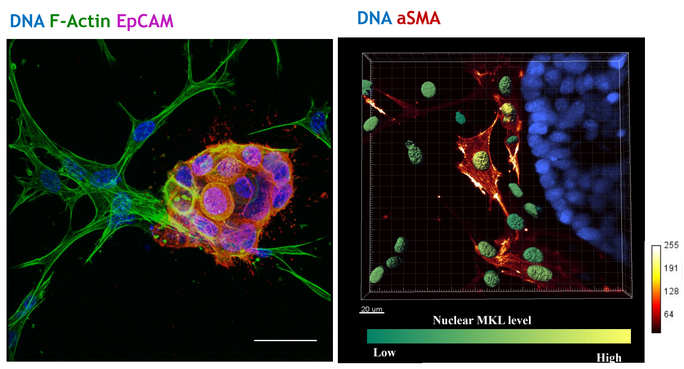
Nuclear levels of MKL are heterogeneous in un-patterned cells grown on glass surfaces



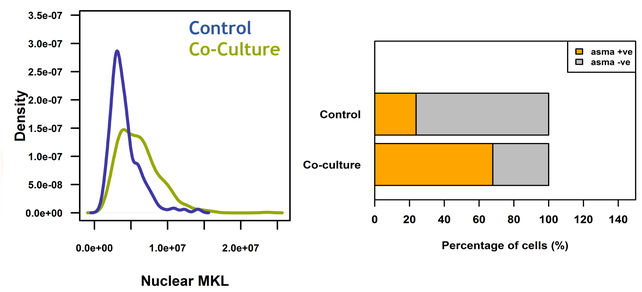




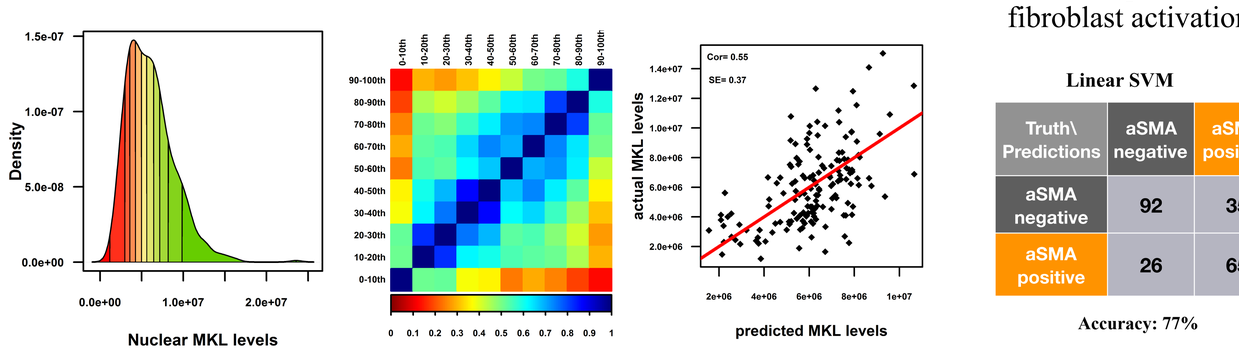
FIBROBLAST ACTIVATION IS COUPLED TO CELL GEOMETRY



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



Nuclear MKL levels are reflective of the cell geometric state



Nuclear and chromatin features are predictive of fibroblast activation

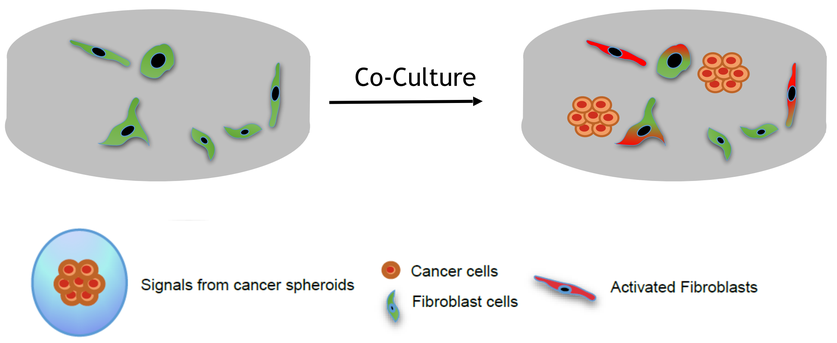
Linear SVM

Truth\ Predictions	aSMA negative	aSMA positive
aSMA negative	92	35
aSMA positive	26	65

Accuracy: 77%

CONCLUSION

The nuclear enrichment of MKL, a TGF-beta effector transcription factor, is modulated by cell geometry in 2D and 3D systems. 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

1. Mitra, A., Venkatachalapathy, S., Ratna, P., Wang, Y., Jokhun, D. S., & Shivashankar, G. V. (2017). Cell geometry dictates TNFα-induced genome response. Proceedings of the National Academy of Sciences, 114(20), E3882-E3891.
2. Damodaran, K., Venkatachalapathy, S., Alisafaei, F., Radhakrishnan, A. V., Sharma Jokhun, D., Shenoy, V. B., & Shivashankar, G. V. (2018). Compressive force induces reversible chromatin condensation and cell geometry dependent transcriptional response. Molecular biology of the cell, mbc-E18.cv

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