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| The role of matrix stiffness on the morphology and migration of lung cancer cells  **Androutsopoulou A** 1, **Maroudi M** 1, **Pothos P** 1,2#, **Petropoulos N** 1,2, **Roupakia E** 2 and **Georgiadou M** 2\*  1 University of Ioannina, Ioannina, Greece  2 Biomedical Research Institute, FORTH, Ioannina, Greece  # Presenting author: Panagiotis Pothos, email: <panagiotis_pothos@bri.forth.gr>  \* Corresponding author: Maria Georgiadou, email: <mariageorgi@bri.forth.gr> |

abstract

Lung cancer – with non-small cell lung cancer (NSCLC) accounting for about 85% of all cases - is a leading cause of cancer mortality worldwide. Most NSCLCs present as stage IV metastatic disease associated with poor survival and high symptom burden. Compared to normal lung, lung cancer extracellular matrix (ECM) is shaped by increased stiffness, a biophysical property associated with increased tumour growth. While it is well established how normal cells sense and respond to changes in ECM stiffness, how cancer cells respond to ECM stiffness is not as clear.

Directed cell migration is a critical aspect of cancer metastasis and involves sensing and responding to signals from the environment and moving in a specified direction. The directional migration towards regions of increasing stiffness is called durotaxis and provides normal cells with stable anchorage. However, the impact of ECM stiffness to the durotactic potential of lung cancer cells remains unexplored. During metastasis, cancer cells may need to move from a stiffer to a softer environment thus cancer cells that are adurotactic - migrate to any directions regardless of stiffness levels - or undergo negative durotaxis - migrate from stiffer to softer regions - might have an advantage.

The aims of this study are (1) to assess if and how EGFR-driven NSCLC cells respond to matrix stiffness and (2) to evaluate the durotactic potential of EGFR-driven NSCLC.

Here, we used four EGFR-driven NSCLC cell lines (PC-9, HCC-827, NCI-H2279 and HCC-4006) and fabricated thin polyacrylamide (PA) hydrogels with tunable stiffness levels. We found that all four NSCLC cells are smaller and rounder and formed less filopodia (which have important role in cell migration) on soft hydrogels, representing the normal lung ECM (<1kPa), compared to stiff hydrogels (>20kPa), representing cancerous or fibrotic lung ECM.

To assess the durotactic potential of those cells we plated them on hydrogels with a stiffness gradient of 0.5-22 kPa [1] and performed live-cell imaging to record the migration patterns of individual cells. Interestingly, we found a diverse range of migration modes in response to changes in ECM stiffness. The HCC-827 and HCC-4006 cells exhibit durotaxis when they are on softer environments, but their directional movement diminishes on stiffer regions. The NCI-H2279 cells appear to be adurotactic across the entire gradient. The PC-9 cells show higher heterogeneity, employing both durotactic and adurotactic behavior, but further investigation is still needed. By measuring the migration distance and speed we found that HCC-827, NCI-H2279 and PC-9 cellsmigrate longer distances and at higher speeds as the stiffness levels increase, whereas HCC-4006 cells maintain constant migration distance and speed across the stiffness gradient.

This study underscores the importance of ECM stiffness in lung cancer cell migration and highlights the need for further research to fully understand the mechanistic underpinnings of durotaxis in cancer cells.

**REFERENCES**

[1] Barber-Perez N\*, Georgiadou M\*, Guzman C, Isomursu A, Hamidi H, Ivaska J. 2020. *J Cell Sci*, **133**(12): jcs242909.