A cellular sense of touch

Cellular Mechanotransduction A/Prof Kate Poole

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When cancers develop there are multiple changes in the mechanical properties of both the cancer cells themselves and their surrounding microenvironment. These changes in mechanics can influence disease progression, regulating multiple hallmarks of cancer including tumour cell invasion and the formation of metastases. Our lab is interested in the force sensing molecules that enable the cancer cells to convert different types of mechanical input into biochemical signals that influence cellular function. We are specifically investigating a set of molecular force sensors known as **mechanically activated ion channels.** We use **live cell microscopy, electrophysiology**, **microfabrication, mechanical stimulation** and **gene editing** to investigate how these molecules are activated and regulated and to determine what happens to cells when this signalling is disrupted. Our goal is to understand the links between cancer progression and mechanical sensing. See below for potential projects and a statement on lab ethos.

Lab ethos:

The research program of the Cellular Mechanotransduction team represents fundamental investigation into the molecular mechanisms that underpin the role of forces and mechanics in human health and disease. Our goal is to generate mechanistic insight that will shift our understanding of disease processes. We value communication and team work, a passion for investigation and the joy of discovery. We are committed to providing detailed feedback and developing the skills of young scientists in an open, supportive and interactive environment.

Research topic 1: Mechanically activated ion channels and cancer

We have discovered that mechanically activated ion channels are important in regulating melanoma cell migration and invasion. In 2020 we identified a molecule, ELKIN1, that mediates mechanical sensing in melanoma cells and changes aspects of melanoma cell signalling, migration, invasion (Fig 1) and mechanics. We have numerous sub-projects available investigating these phenomena in melanoma cells and are currently extending these studies to breast cancer cells. General project outlines and the techniques involved are below:

1) Visualizing the activation of mechanically activated ion channels in freely migrating cells?

Techniques: CRISPR/Cas9 gene editing, Live cell imaging, TIRF microscopy

2) How does disruption of mechanically activated ion channels change cell mechanics and interaction forces?

Techniques: Atomic Force Microscopy, Real time deformability cytometry

3) Which mechanically activated ion channels are functional in breast cancer cells?

Techniques: Patch clamp electrophysiology, live cell imaging, CRISPR/Cas9 gene editing

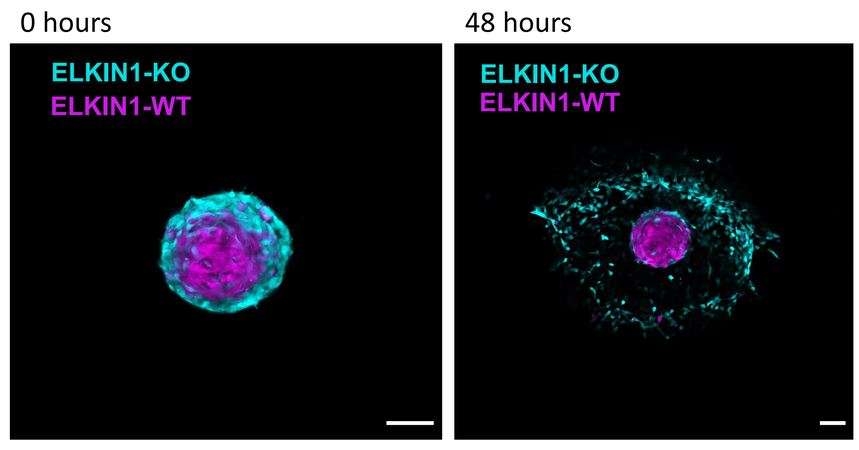

Figure 1: deletion of ELKIN1 drives partitioning of cells in multicellular spheroids that mimic tumours. The ELKIN1 KO cells are more invasive. Future work will seek to understand why. How does deleting the mechanically activated ion channels lead to this result?

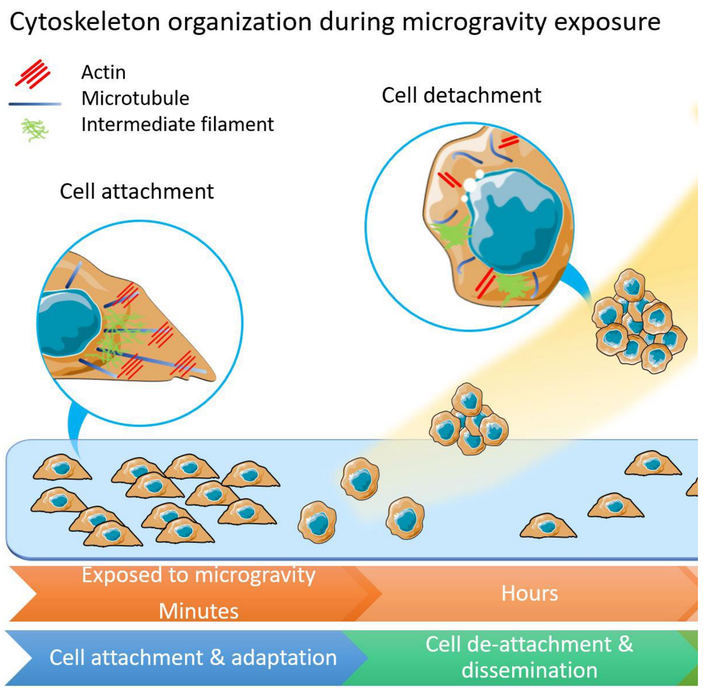

Figure 2: Exposure of cells to simulated microgravity for just a few hours is known to drive changes in cell morphology and attachment. What is not known is how the cells sense this change in the gravitational vector. This project asks whether mechanically activated ion channels might be involved

Research topic 2: Identifying the molecular force sensors that signal changes in gravity

There has been much research into how cells sense applied forces, but less is known about which force sensing molecules are involved in cellular responses to mechanical unloading, or a reduction in the forces applied to a cell. Investigating this question is important in order to understand the molecular mechanisms that underpin the physiological changes that occur in microgravity, such as during space travel. We have an instrument that can simulate microgravity, known as a random positioning machine (RPM). We have recently started investigating whether mechanically activated ion channels are involved in sensing changes in the gravitational vector.

This project is a new endeavour in the lab and subprojects will establish appropriate protocols for this work, and investigate whether cells lacking mechanically activated ion channels respond differentially to the microgravity state. To date, studies have only looked at changes in morphology (Fig 2) but not the force sensing molecules that underpin these changes.

Techniques: cell culture, simulated microgravity measurements, light microscopy, molecular biology

Research topic 3: Investigating mechanical activation of the newly identified ELKIN1

We have identified a molecule that we hypothesise is a mechanically activated ion channel: ELKIN1. We are currently characterizing how the force sensitivity of this molecule is regulated and are investigating point mutations within the molecule that change its mechanical activation profile. To investigate the mechanical activation of this protein we use a specialized technique developed by us in order to probe cellular force sensing at regions of the cell that are in contact with the substrate (Fig 3). This technique mimics the kinds of forces that cells experience in vivo and allows us to quantitatively assess changes in force sensitivity. General project outlines below:

1) Characterising point mutations in ELKIN1 that affect the flow of ions when ELKIN1 is activated

Techniques: Patch clamp electrophysiology, microfabricated pillar arrays, cloning

2) Characterising regions of ELKIN1 that interact with other proteins in the cell

Techniques: Patch clamp electrophysiology, microfabricated pillar arrays, cloning, biochemisty

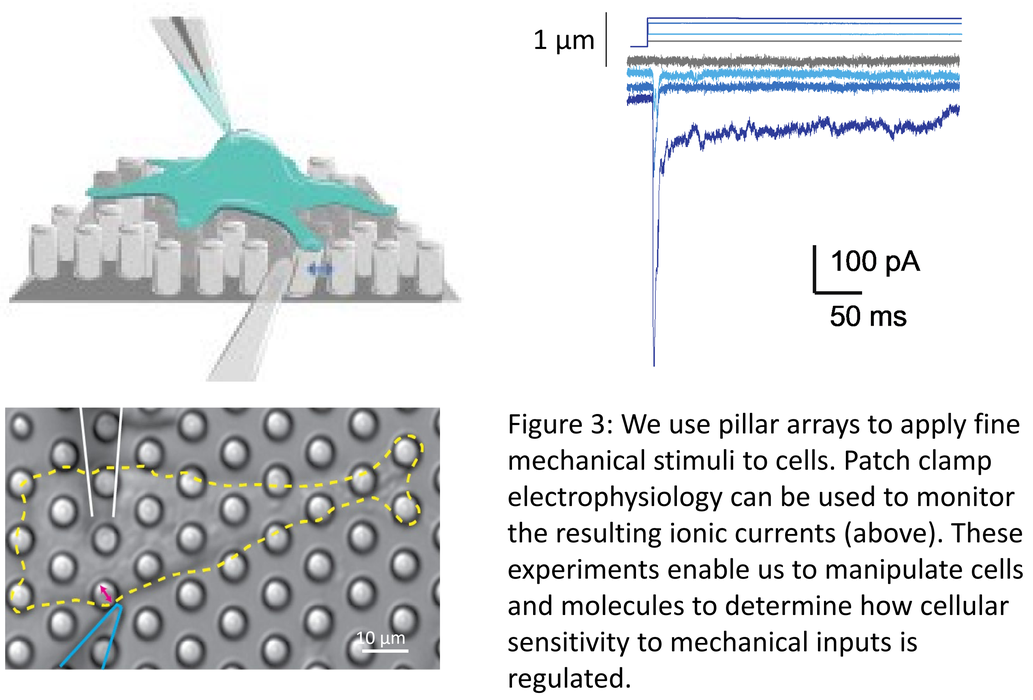

Figure 3: We use pillar arrays to apply fine mechanical stimuli to cells. Patch clamp electrophysiology can be used to monitor the resulting ionic currents (above). These experiments enable us to manipulate cells and molecules to determine how cellular sensitivity to mechanical inputs is regulated.