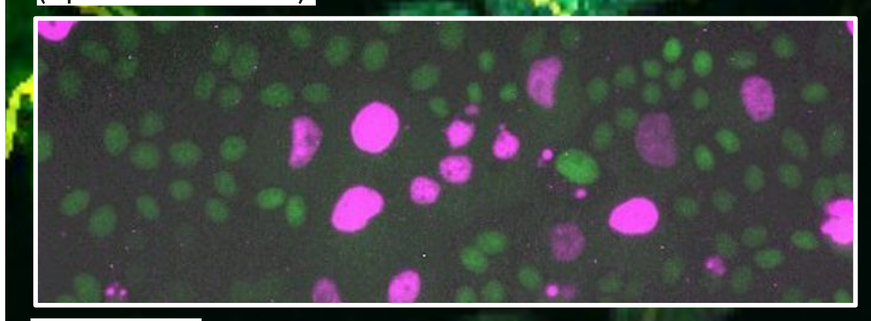
Cell competition: who makes the first move?

Wild-type cell proliferation precedes Ras^{V12} cellular extrusion, but not Scr^{KD} apoptosis.

Introduction

Cell competition is broadly defined as a quality control mechanism that results in less-fit "loser" cells being eliminated from a biological tissue. This mechanism impacts a wide variety of different physiological and pathological scenarios, from senescence to tumorigenesis. Previous studies into cell competition have typically characterised it in relation to population-level dynamics, whereas this project has a single-cell focus. By observing the individual actions of cells in a competitive scenario we aim to understand the rules that govern this mechanism. In this project, two modes of MDCK cell competition were studied with different methods of loser elimination: wild-type vs. Scr^{KD} (apoptosis) and wild-type vs. Ras^{V12} (apical extrusion).



(Left) Cell competition assay of winner $MDCK^{WT}$ H2B-GFP(green) and loser MDCK $Scrib^{KD}$ H2B-RFP (magenta)[1]

Method

Time-lapse fluorescence microscopy and deep learning image analysis allows both for the labelling of individual cells and the classification of their phase in the cell cycle. Bayesian tracking then maps the path the cell takes throughout its lifetime, allowing for the construction of a detailed spatio-temporal map of key cellular events. This is followed by a space-time K-function clustering analysis of wild-type divisions around loser cell apoptosis or extrusion. This analysis indicates whether there is any correlation between single-cell wild-type activity and loser cell elimination. The K-function equation, $\boldsymbol{K}$, is shown below, featuring a weighted indicator function, $\boldsymbol{I}$, for division events that is calculated for all distance, $\boldsymbol{d}$, and time, $\boldsymbol{t}$, coordinates around a focal elimination event. This function is then scaled by the number and rate of division events, $\boldsymbol{n}$ and $\boldsymbol{\lambda}$ respectively. P-values for all space-time coordinates can then be calculated by comparing the observed K-function to a series of null hypotheses generated from random permutations of space-time coordinates.

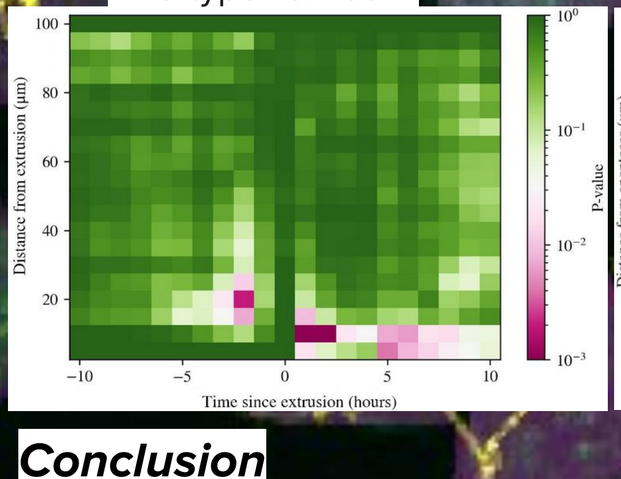
$$\hat{K}(h,\Delta) = \frac{1}{n \cdot \lambda_{st}} \sum_{i=1}^{n} \sum_{j \neq i} \frac{I_{(h,\Delta)}(d_{ij}, t_{ij})}{\omega_{ij}}$$

Results

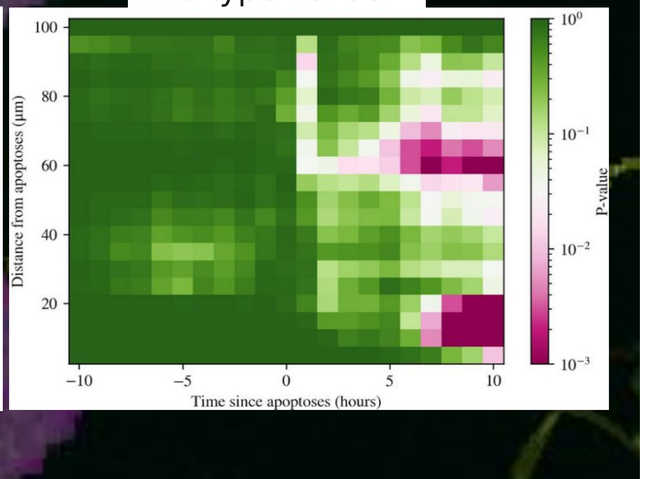
Clustering of wild-type divisions around Ras^{V12} extrusion events. There appears to be a statistically significant ($p<0.01$) peak in wild-type divisions prior to and after Ras^{V12} extrusions. This suggests that wild-type mitotic activity could play a role in expediting Ras^{V12} apical extrusion from the monolayer and subsequently be a fundamental mechanism of competitive elimination. Moreover, there appears to be more clustering of wild-type division after extrusion, indicating that there is a space-filling behaviour after the loser cell has been ejected from the wild-type monolayer.

Clustering of wild-type divisions around Scr^{KD} apoptotic events. There appears to be a statistically significant ($p<0.01$) peak in wild-type divisions many hours after the apoptoses, both locally and further away. This suggests that wild-type mitoses are not directly influencing Scr^{KD} apoptoses in this competitive scenario. There does appear to be a strong clustering many hours after apoptosis at both the local vicinity and further distances. This could be indicative of a space-filling behaviour and also a response to general tissue relaxation due to apoptosis.

Wild-type vs. Ras^{V12}



Wild-type vs. Scr^{KD}



Conclusion

A single-cell understanding of two different mechanisms of cell competition was found using a novel quantitative image analysis.

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