nature portfolio

https://doi.org/10.1038/s41596-020-00473-2

Supplementary information

Intravital microscopy of dynamic single-cell behavior in mouse mammary tissue

In the format provided by the authors and unedited

Modelling cap cell dynamics

Event rates from the first 5 hours of imaging were calculated as the percentage of each population undergoing an event per day. We calculated that 64.8% of cap cells migrate into the TEB body per day, a higher rate than the 39% percent that we derived from previous rate estimations from static images[1]. 85% of these rapidly underwent apoptosis with a half-life of 2.67 hours (One phase decay non-linear fit, plateau 84.2; Figure 6c), however, this reached a plateau with 15% surviving the duration of these experiments. To generate the estimated number of cap-like cells in the body (37.7) from the estimated number of cap cells[1] (115.7), the half-life of the remaining 15% of cells would need to be 61.9 hours. This was calculated as follows:

> From a total of 75.0 cap cells entering the TEB body per day (64.8% of 115.7), 84.2% (63.2 cells) die rapidly with the observed half-life of 2.67 hours, giving a constant number of 7.03 cap-like cells in the body (number of cells with a short half-life entering the TEB per day x half-life in days; 63.2 x 2.67/24).
>
> The constant number of slowly dying cap-like cells in the body is thus 30.7 (37.7 – 7.03) from 11.9 entering each day (15.8% of 75.0), requiring a half-life of 61.9 hours (30.7/11.9 x 24).

Therefore, we hypothesise that a small proportion of cap cells that migrate into the TEB body survive there long-term. In further support of this, cap-like body cells present at the beginning of the experiment had a lower rate of death with a plateau at 26.9%. This may be due to enrichment of long-term surviving cap-like cells in the TEB body, since the rapidly dying cells would not contribute to this population.. Our observations may have been affected by an increased rate of cap cell death after 5 hours of imaging, which was probably an artifact of IVM, as cap cells do not normally undergo apoptosis[1]. Cap cells seem to be particularly

sensitive to surgery and IVM compared to mature myoepithelial cells, which were not affected (Figure 6a).

References

1. Paine, I. *et al.* A Geometrically-Constrained Mathematical Model of Mammary Gland Ductal Elongation Reveals Novel Cellular Dynamics within the Terminal End Bud. *PLoS computational biology* **12**, e1004839 (2016).