Bimodal skin progenitors—a matter of place and time

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The skin carries out specialist functions such as barrier formation and thermoregulation. The mode of cellular replenishment of the surface epithelium that forms the outermost layer during steady-state homeostasis is a subject of intensive investigations. A detailed analysis of the interfollicular epidermis reported in this issue of *The EMBO Journal* (Roy *et al*, 2016) reveals that hair follicles in their growth phase influence cellular behaviour of epithelial cells in their immediate proximity.

See also: E Roy *et al* (December 2016)

The interfollicular epidermis (IFE) constitutes the outer layer of the skin, which forms the water-impermeable barrier that protects from excessive loss of moisture and exposure to infections and other environmental factors. Associated structures, such as the hair follicle (HF), provide adnexal functions such as a coat of hair and sebum production. The epidermis is a multi-layered stratified epithelium with proliferating cells confined to the basal layer, where they attach to the basement membrane. As cells detach from the basement membrane, they lose their proliferative potential and initiate a terminal differentiation programme as they move through the upper layers of the skin and eventually end up as squames in the heavily cross-linked epithelium that forms the water-impermeable barrier. Due to the constant and lifelong physical demands on the surface of the epithelium and the incessant loss of dead squames, the IFE is continuously replenished by proliferating cells in the basal layer (Schepeler *et al*, 2014). In order to maintain steady-state tissue

homeostasis, the production of new cells and the loss of differentiated progeny must be carefully balanced. The mode of cellular replenishment has therefore been the subject of intensive research in order to understand how it is possible to balance cell fate on a population basis. To date, numerous studies have provided various models of tissue replenishment in the mouse IFE converging on a model best described by population asymmetry, where individual cell divisions in an unpredictable manner give rise to either two daughter cells with identical fate (either both proliferative or committed to differentiate) or diverging fate (Clayton *et al*, 2007; Doupé *et al*, 2010; Mascré *et al*, 2012; Lim *et al*, 2013; Sada *et al*, 2016; Sánchez-Danés *et al*, 2016). The cellular output from cells within the IFE has been shown to vary between different cell populations. It does, however, remain an open question whether the observed differences in cell behaviour reflect differences in cellular potential within an apparently homogeneous epithelium or that cell fate and behaviour are dictated by differences in the local microenvironment. The epidermis constitutes an attractive organ to address this fundamental question and provide insight into tissue replenishment in other organs.

As with a landscape, one has to take into account the topographic features of a tissue, when describing its composition. The IFE is as such not a uniform epithelium and will, depending on species and organismal site, present local variation such as distance to hair follicles. In mouse, studies have focused on back and tail skin. Whereas the IFE in dorsal skin is seemingly homogenous with evenly dispersed HFs, tail IFE is divided into pigmented scale (parakeratotic) and

non-pigmented interscale (orthokeratotic) regions characterised by distinct differentiation programmes arranged between unique arrangements of triplet HFs (Gomez *et al*, 2013). Using mathematical modelling of quantitative fate-mapping data, a set of ground rules for tissue maintenance has been devised for mouse IFE. Firstly, the vast majority of cells in the basal layer represent an equipotent committed progenitor population, which collectively carries out the bulk of tissue replenishment via careful balancing cell gain with cell loss (Clayton *et al*, 2007; Doupé *et al*, 2010). Secondly, rare cells contribute in an imbalanced manner and will on a population basis give rise to more progeny than is lost by cell death and differentiation (Sánchez-Danés *et al*, 2016). Thirdly, the IFE is maintained without contribution from the surrounding hair follicles (Page *et al*, 2013; Schepeler *et al*, 2014). Interestingly, studies of cell cycling behaviour have suggested cellular heterogeneity within the IFE, although evidence for a distinct bimodal distribution of cell populations is lacking (Sada *et al*, 2016). Many of these ground rules have been devised from studies of the compartmentalised tail epidermis. It remains an open question whether the same rules apply to tissue replenishment in back skin IFE, which has a more uniform structure, and if a simpler structure will enable novel insights into how topographic elements might influence tissue maintenance.

In the study by Khosrotehrani and colleagues published in this issue of *The EMBO Journal* (Roy *et al*, 2016), the authors establish a comprehensive working model for tissue homeostasis in the IFE. In mouse back skin IFE, the authors observe unappreciated

intra-regional differences amongst cells located in the basal layer owing to the proximity to hair follicles in their growth phase (anagen). Roy *et al* (2016) perform detailed fate-mapping studies using an inducible Cre line that targets essentially all cells in the basal layer (K14 driven) in combination with the *Rainbow3* reporter model (Tabansky *et al*, 2012). This combination allows stochastic yet efficient labelling of cells with one of five possible fluorescent proteins, whilst still being able to identify clones derived from single labelled basal cells. Previous studies of the mouse IFE have analysed rare clonal events to ascertain their single cell origin; however, under such conditions a small subset of the total population is assessed. Although often assessed experimentally it is generally assumed that the labelled cells are representative of the entire population expressing the particular Cre driver used for labelling. The dense labelling approach employed by Roy *et al* (2016) enabled the authors to analyse hundreds of clones and thereby provide a model, which is capable

of detecting differences in cell behaviour represented by a subset of cells. This is particularly useful when analysing a tissue with a large surface area such as the IFE, which is only interrupted by interspersed HF's.

Long-term observations of cells labelled with different fluorescent proteins highlight different clonal behaviours depending on spatial arrangement within the IFE suggesting that topographic elements influence tissue replenishment either by differences in proliferation rates or via imbalanced fates of the progeny within distinct regions. Although all labelled cells within the basal layer expand into clones unless they are lost, approximately 35% of clones increase more rapidly in size when compared to the rest. Analysis of the clones with the two distinct behaviours indicates a strong correlation between size and distance to the nearest hair follicle. While larger clones were observed immediately adjacent to hair follicles, smaller clones were located much further away. Further characterisation revealed that expansion of clones located adjacent to HF's

is influenced by the cycling nature of the hair with larger proportion of cells undergoing replication, when the hair is in its growth phase (anagen) rather than in its resting phase (telogen). In line with these observations, the size of clones is highly influenced by the number of hair cycles HF's undergo, suggesting that part of tissue replenishment of the IFE is highly linked with other elements in the epithelium (Fig 1). Moreover, this also highlights the differences in clonal dynamics of the IFE. These observations could be explained either by the coexistence of two distinct cell populations with intrinsic differences, as has been observed in tail epidermis (Mascre *et al*, 2012; Sada *et al*, 2016; Sánchez-Danés *et al*, 2016), where a hierarchy between less proliferative stem cells and committed progenitor cells ensure long-term tissue maintenance, or by the inductive capacity of the surrounding microenvironment, which makes an otherwise homogeneous cell population behave in different manners. Using computational modelling, the authors propose that a yet-unknown extrinsic factor

Figure 1. Schematic representation of the intra-regional differences in clonal growth due to relative location with respect to hair follicles.

Clones located in the zone of influence have a higher proliferation rate during the hair follicle anagen leading to more rapid expansion of clone size in this area. In contrast, clones located outside the zone of influence expand at a considerably slower rate. The size of the clones in the zone of influence positively correlates with the number of anagen cycles of the corresponding hair follicle.

influences cell proliferation rates within a region (the influence zone; Fig 1) encompassing 10–30 cells immediately surrounding the hair follicle.

This study sheds new light on the seemingly complex mechanisms of cellular maintenance in back skin. Similar to recent discoveries in tail epidermis, this highlights the important influence of the local micro-environment on cell behaviour such as proliferation and cell fate decisions. It does, however, beg the question whether all cells within the epidermis are committed progenitors as first proposed by Phil Jones and co-workers (Clayton *et al*, 2007) and that the surrounding signals dictate how these cells behave. One can imagine a number of different factors that could trigger heterogeneity locally such as cytokine secretion, mechanical stress and minor injuries generated by the growing hair, or indeed a combination of these factors. Further research is required to ascertain the exact mechanism that dictates local cell behaviour. It will be exciting to follow whether seemingly heterogeneous populations of progenitors cells observed in other tissues such as the hematopoietic system and intestine are

merely a reflection of reversible changes incurred by temporal microenvironmental stimuli.

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