STEM CELLS — OPINION

Epidermal homeostasis: do committed progenitors work while stem cells sleep?

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Abstract | Tracking the fate of cells in murine epidermis *in vivo* has revealed that a committed progenitor cell population can maintain normal adult tissue in the long term without support from a long-lived, self-renewing population of stem cells. Here, we argue that these results challenge the dogma that stem-cell proliferation is required for the cellular homeostasis of the epidermis and other adult tissues, with important implications for tissue physiology and disease.

It has long been held that homeostasis of adult tissues is maintained by two populations of proliferating cells: a population of long-lived, self-renewing stem cells that support a second population of progenitor cells, which are committed to terminal differentiation[1]. This model has been used to interpret the results of several studies both on the homeostasis of normal tissue and on the development of diseases such as cancer[2–4]. The existence of a subpopulation of stem cells that can regenerate differentiated cell types has been established in most adult tissues using transplantation assays or following injury. However, far less is known about how stem cells and their progeny maintain normal adult tissue homeostasis *in vivo*. Recently, we reported studies of cell fate in murine tail epidermis that revealed a mechanism of tissue homeostasis that is independent of stem cells.

The epidermis is ideally suited for studying the behaviour of progenitor cells because proliferation and differentiation occur within clearly defined regions (BOX 1). The tissue consists of a multi-layered sheet of keratinocytes interspersed by hair follicles[3]. Proliferating cells are found within the hair follicle and in the basal layer of the interfollicular epidermis. As cells in the basal layer differentiate, they detach from the underlying basement membrane and migrate through the suprabasal cell layers to the epidermal surface where they are shed. To maintain epidermal homeostasis, new cells must be generated in the basal layer to replenish those that are lost.

> “...if the bulge does not support interfollicular epidermis, how is this compartment maintained?”

The requirement for cell replenishment in the epidermis has led to an extensive search for epidermal stem cells. There is a strong body of evidence supporting the existence of stem cells in the bulge region of the hair follicle[5–8]. When transplanted, these cells generate hair follicles, sebaceous glands and interfollicular epidermis, raising the possibility that they maintain the entire epidermis. However, the behaviour of a stem cell in transplantation assays may not reflect its function within a normal tissue. Indeed, genetic labelling studies indicate that whereas bulge stem cells support hair follicles, they do not maintain adult interfollicular epidermis[9–11]. Furthermore, deletion of bulge cells by transgenic expression of a suicide gene results in the loss of hair follicles but not of interfollicular epidermis[12]. So, if the bulge does not support interfollicular epidermis, how is this compartment maintained?

The epidermal proliferative unit hypothesis

It has long been argued that mammalian interfollicular epidermis contains long-lived stem cells, which generate a short-lived population of transit-amplifying (TA) cells. TA cells differentiate into post-mitotic keratinocytes after several rounds of cell division[4]. Within this stem–TA-cell model, it has been further proposed that mouse epidermis is organized into columns of geometric clonal units known as epidermal proliferative units (EPUs). The EPU hypothesis was based on the observation that differentiated cells in the outermost cornified layers of the epidermis are stacked in regular columns. If one assumes that cells can only migrate vertically upwards from the basal layer, each column must be maintained by the basal cells that lie beneath it. It was thus argued that the epidermis is organized into discrete EPUs of a constant size, the boundaries of which coincide with those of the stacks of cornified cells. Following the stem–TA-cell model, it is natural to envisage the epidermis as a mosaic of clonal units (or EPUs), each consisting of a single slow-cycling stem cell that supports a surrounding cluster of TA cells, which in turn maintain the overlying column of suprabasal cells[13,14] (FIG. 1a–c).

Evidence for the EPU? Although the EPU hypothesis has been widely accepted[2,3,15], evidence for its validity is open to question. Each EPU is predicted to have a slowly cycling basal layer stem cell at its centre, surrounded by more rapidly proliferating TA cells. The frequency of mitoses in basal layer cells in the centre of EPUs is indeed lower than in the surrounding cells[16]. Assays in which a high proportion of cells in neonatal mouse epidermis are labelled and analysed once the mice have reached adulthood reveal slow-cycling label-retaining cells (LRCs) — interpreted to be stem cells — that are present at the centre of EPUs in dorsal but not in tail epidermis[17,18].

However, the interpretation of this cell kinetic data is open for debate. First, the distribution of mitotic basal cells is complicated by the presence of slowly cycling Langerhans cells of the immune system in the basal layer of the epidermis[19–22]. These cells lie in

a patterned array, which coincides with the centre of the cornified cell stacks several cell layers above, offering an alternative explanation for the reduced frequency of mitoses in the centre of the hypothesized EPU[13,16,23]. Second, the keratinocytes in the centre of an EPU are just as likely to be in S-phase as the surrounding cells, an observation that conflicts with the EPU hypothesis[13]. Finally, whereas LRC assays detect cells that have dropped out of the cell cycle at some point during the rapid epidermal expansion that occurs between early neonatal and adult-stage mice, it has not been demonstrated that these are adult interfollicular epidermal stem cells.

Further evidence of epidermal homeostasis comes from studies of chimeric mice that are mosaic for two alleles of the major histocompatibility complex, which results in large patches of epidermis expressing one allele or the other[24]. A strong prediction of the EPU hypothesis is that because the single stem cell that supports each EPU must be of either one genotype or the other, the boundaries of the mosaic patches should run along the borders of EPUs. However, no such pattern was found: the borders of mosaic patches did not translate to the boundaries of the stacks of cornified cells[24]. Recently, conditional genetic labelling experiments have only succeeded in labelling a small number of cells. Whereas some studies report clones that conform to the EPU hypothesis[12,25], others describe larger clones that cross EPU boundaries[9,26–28]. Crucially, these experiments reveal that basal cells migrate laterally as well as vertically through the suprabasal cell layers, challenging the key assumption behind the EPU model that cells of the cornified layer are derived from the basal cells that lie directly below them. Thus, although interfollicular epidermis contains progenitor cells that support long-lived clusters of cells, these clusters do not always resemble classical EPUs[13,14].

These observations leave open the possibility that the interfollicular epidermis is maintained by a slowly cycling stem-cell population that supports 'irregular' clonal units of cells[18,23]. All clones that persist for months within a tissue are assumed to derive from stem cells. To account for the wide range of sizes and shapes of labelled clones, it has been suggested that the progeny of one stem cell may migrate into an adjacent EPU following stem-cell death, senescence or tissue injury[28].

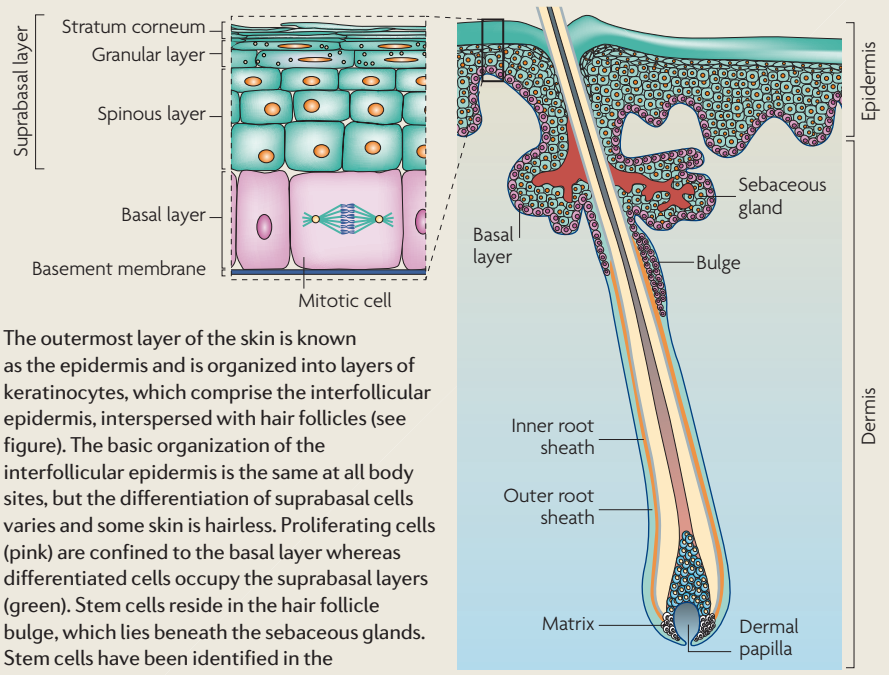
Support for the existence of interfollicular stem cells comes from studies of human epidermis, which contains a subpopulation of basal cells with a high proliferative potential and the ability to reconstitute epidermis *in vitro* and *in vivo*[29–31]. Such cells can be identified by a range of markers, such as β1 integrin, the Notch ligand Delta, the cell-surface proteoglycan MSPG, the desmosomal protein DSG3 and the transcription factor LRIG1 (REFS 29,30,32–36). Strikingly, immunostaining of human epidermis reveals that stem cells lie clustered together in a patterned distribution; stem cells within the clusters are found to cycle infrequently, whereas the proliferating and differentiating basal cells lie between the clusters[30,37]. Definitive proof that these cells are indeed stem cells would require cell-lineage tracing in humans, which is not feasible. However, lentiviral labelling of cells in hairless neonatal epidermis grafted onto nude mice revealed labelled cell clusters that vary in shape and size[38]. However, whether these clones are the progeny of individual long-lived stem cells remains unclear.

In summary, the available evidence supports the existence of a discrete population of interfollicular stem cells in human epidermis and cannot exclude their presence in the mouse. However, the spatial organization implied by the EPU hypothesis seems inconsistent with both cell kinetic and genetic labelling experiments. Rather than being maintained by an elegant, regular array of slowly cycling stem and differentiating TA cells, the distribution of proliferating and differentiating cells in murine epidermis is random, and the staining of murine epidermis for markers of human epidermal stem cells fails to reveal any evidence of patterning[18]. Equally, there is no pattern in the proliferation and differentiation of basal cells that lie between stem-cell clusters in human epidermis[37].

Tracking epidermal cell fate *in vivo*

Recently, the results of a new genetic labelling system, which relies on an inducible Cre recombinase, have offered a new perspective on cell fate in murine epidermis. These experiments have enabled the fate of a large representative sample of interfollicular cells to be tracked over a one-year time course at single-cell resolution *in vivo*[39]. By scoring clones that contain at least one basal layer cell, the rules of cell fate may be inferred from the evolving clone size distributions. Of the basal layer cells labelled at induction, a significant but ever-diminishing fraction persists, developing into cohesive, irregularly shaped clones of variable size (FIG. 2). Strikingly, the average

Box 1 | Architecture of the epidermis



The outermost layer of the skin is known as the epidermis and is organized into layers of keratinocytes, which comprise the interfollicular epidermis, interspersed with hair follicles (see figure). The basic organization of the interfollicular epidermis is the same at all body sites, but the differentiation of suprabasal cells varies and some skin is hairless. Proliferating cells (pink) are confined to the basal layer whereas differentiated cells occupy the suprabasal layers (green). Stem cells reside in the hair follicle bulge, which lies beneath the sebaceous glands. Stem cells have been identified in the interfollicular epidermis, but evidence for the existence of interfollicular stem cells in mice is limited[30,42,50]. As they differentiate, cells leave the basal layer and migrate towards the epidermal surface, changing shape until they enter the outermost cell layer as flattened cornified cells (stratum corneum). Finally, these cells are shed from the epidermal surface. In the mouse, the outermost cornified cell layers are arranged in stacks of large hexagonal cells, but in human epidermis the cornified layer is less clearly organized[16,51].

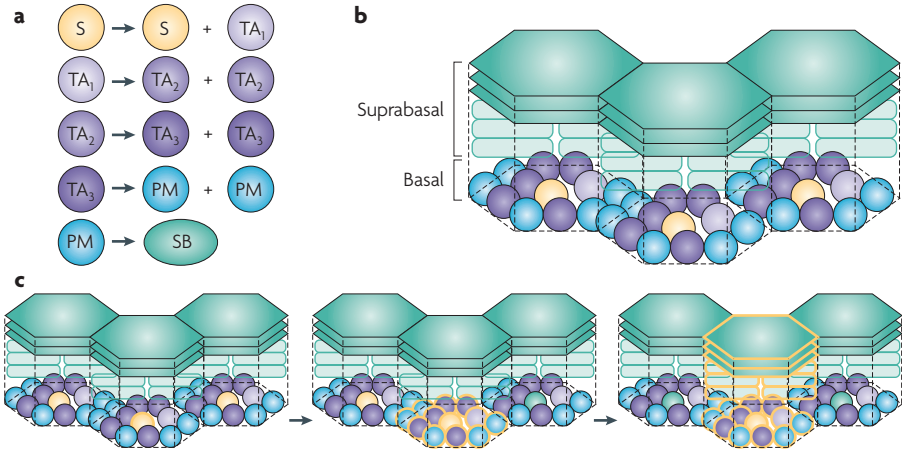


Figure 1 | **The EPU hypothesis. a** | The rules of cell fate as dictated by the epidermal proliferative unit (EPU) model[52]. Self-renewing stem cells (yellow) generate a transit-amplifying (TA) cell population (purple), which undergoes three rounds of symmetric division (TA_1 to TA_3) before terminal differentiation into post-mitotic (PM) cells (blue). These PM cells subsequently detach from the basal layer and become suprabasal (SB) cells (green). Note that in this model, the behaviour of TA cells is determined by their past history; for example, a TA_1 cell will differentiate after two further rounds of cell division. **b** | A schematic showing the spatial organization of cells in an EPU with cells coloured as in panel **a**. **c** | The prediction of the EPU model in a clonal labelling experiment in which a stem cell is labelled at induction, and cell fate follows the rules depicted in panel **a**. Cells expressing the label are shown with a yellow outline. Once all TA and differentiated cells are labelled, the size of the labelled clone is static.

number of basal layer cells in persisting clones (that is, the size of the 'footprint' of the labelled clone in the basal layer) increases linearly with time, as measured from induction. This is a manifestation of a striking and more general scaling behaviour that is displayed long term by the entire basal layer clone size distribution (BOX 2; FIG. 2e,f). A further significant observation is that the cell divisions of proliferating cells may adopt one of three fates: symmetric, resulting in two proliferating daughters; symmetric but resulting in two post-mitotic terminally differentiated daughters; or asymmetric, generating one proliferating and one post-mitotic cell.

Is the stem–TA hypothesis applicable?

The availability of a large body of clone-fate data enables predictions of models of epidermal homeostasis to be tested. Two features of the data challenge the stem–TA-cell hypothesis. First, the observed continuum of clone lifetimes is at odds with the prediction that the epidermis should contain discrete populations of short- and long-lived clones derived from the populations of TA and stem cells labelled at induction. Second, the observation that the clone size (as measured by its footprint in the basal layer) grows linearly with time in the long term contrasts with the prediction of the stem–TA-cell hypothesis: once the entire TA cell population supported by a labelled stem cell has been labelled, no further expansion of the clone can occur (compare this with the scenario in FIG. 1c). Moreover, the observed long-term scaling properties of the entire basal layer clone size distribution indicate that clonal evolution is controlled by a single rate-limiting process (BOX 2): any attempt to reconcile the data with the stem–TA-cell model by appealing to multiple processes such as cell proliferation plus stem-cell senescence and/or death and lateral migration of cells from adjacent EPUs, therefore, seems infeasible[27,28]. In conclusion, the observed data cannot be explained by a slow-cycling, long-lived stem-cell population supporting a short-lived TA cell population.

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A new model of epidermal homeostasis

Given the inadequacy of the stem–TA-cell model, is it possible to construct an alternative model from the data? Taken together, the entire range of clone-fate data, from the early time points to the long-term scaling behaviour, and the observation of symmetric and asymmetric cell division, is consistent with a remarkably simple model of epidermal homeostasis. The epidermis is maintained by a single population of progenitor cells with properties that conform neither to those of classical stem cells nor to TA cells. To discriminate between the properties of this cell population and those of the traditional TA cell compartment, we refer to these cells as committed progenitor (CP) cells. In homeostatic epidermis, this model (FIG. 3a) depends on just two parameters: the average cell division rate, λ, and the proportion of divisions that result in asymmetric fate, $1–2r$. To maintain the steady-state population of proliferating cells, the proportion of cells that generate daughters with each type of symmetric fate must be equal. Moreover, to maintain the population of post-mitotic cells in the basal layer, their rate of transfer to the suprabasal layer, Γ, is constrained by their rate of production leading to the relation $\Gamma = \rho\lambda/(1-\rho)$, where ρ denotes the fraction of basal layer cells that are cycling progenitors. By allowing the CP cell population to undergo an unlimited number of cell divisions before terminal differentiation, the epidermis can be maintained by a single progenitor-cell population.

In the stem–TA-cell model, the stem-cell population persists while TA cells are lost rapidly from the tissue. How can a single progenitor cell population generate both short- and long-lived clones? Although the division and migration processes imply a complex time-evolution of clone size, the principle characteristics can be inferred from the behaviour of the CP cell population alone. These cells conform to a simple 'birth–death' process in which the two symmetric channels of cell division either increase or decrease the CP cell population by one cell (with equal probability), whereas asymmetric division leaves the CP cell population unchanged. Models of this kind have a long history dating back to the works of Galton and Watson[40] and Bienaymé[41] on the 'extinction' of family surnames. In the case described here, the CP cells mimic the 'male' population, each capable of generating precisely two offspring with 'gender' chosen at random. Whereas the total number of CP cells (males) in a large population hardly fluctuates, the lineage of any given clone (family) may terminate through terminal differentiation (the female line). More formally, the probability of a CP cell labelled at induction (a patriarch) founding a clone (family) of size n at time t post-induction is given by equation 1:

$$p_n(t) = \frac{1}{(1+\tau/t)^{n+1}} \times \begin{cases} 1 & n = 0 \\ (\tau/t)^2 & n > 0 \end{cases} \qquad (1)$$

where $1/\tau = r\lambda$ is the rate of symmetric cell division. In particular, at times $t >> \tau$, the probability distribution of clones retaining at least one progenitor cell assumes the form

$p_n(t)/(1-p_0(t)) \approx (\tau/t)\exp[-n\tau/t]$, consistent with the observed scaling behaviour (BOX 2). The continual extinction of clones through terminal differentiation is compensated for by the steady growth of 'persisting' clones such that the total CP cell population is maintained.

How does CP cell behaviour compare with that of stem and TA cells in the stem–TA-cell model? CP cells are a self-maintaining population and, as such, might be thought of as stem cells. However, unlike stem cells, they are committed to terminal differentiation. In the clonal labelling experiment, most labelled CP cells are lost after 3 months, and even the few clones that persist for a year will ultimately be lost by differentiation. If CP cells are not classical stem cells, neither are they TA cells. The defining characteristic of a TA cell is that it undergoes terminal differentiation after a limited number of rounds of cell division; differentiation is thus linked to the past history of the cell. In contrast, CP cells have the same probability of undergoing terminal differentiation at each cell division, irrespective of the past history of the cell. There is no set limit to how many rounds of cell division the clonal progeny of a given CP cell may undergo.

It is important to stress that this new model has only been shown experimentally to describe homeostasis in mouse tail skin. Clones in back skin also expand progressively with time — a behaviour that conflicts with the predictions of the stem–TA-cell model and which is qualitatively consistent with the new model[39]. However, technical issues prevent the preparation of back skin to permit quantitative validation of the new model at this site. It remains to be seen whether the model can explain the clone size distribution at other body sites that can be whole-mounted, such as ear skin.

Implications of the new model

Interfollicular epidermal stem cells. The clone-labelling experiment we describe examined the behaviour of proliferating cells, so could not demonstrate or exclude the existence of a quiescent population of classical interfollicular stem cells. Although LRCs are found in murine interfollicular epidermis, it is unclear whether they represent stem cells[17,42]. Definitive proof of the existence of murine interfollicular stem cells is lacking. If cycling cells in human epidermis behave in a similar manner to the mouse cells, it would explain the quiescence of putative stem-cell clusters in human interfollicular epidermis[30,37].

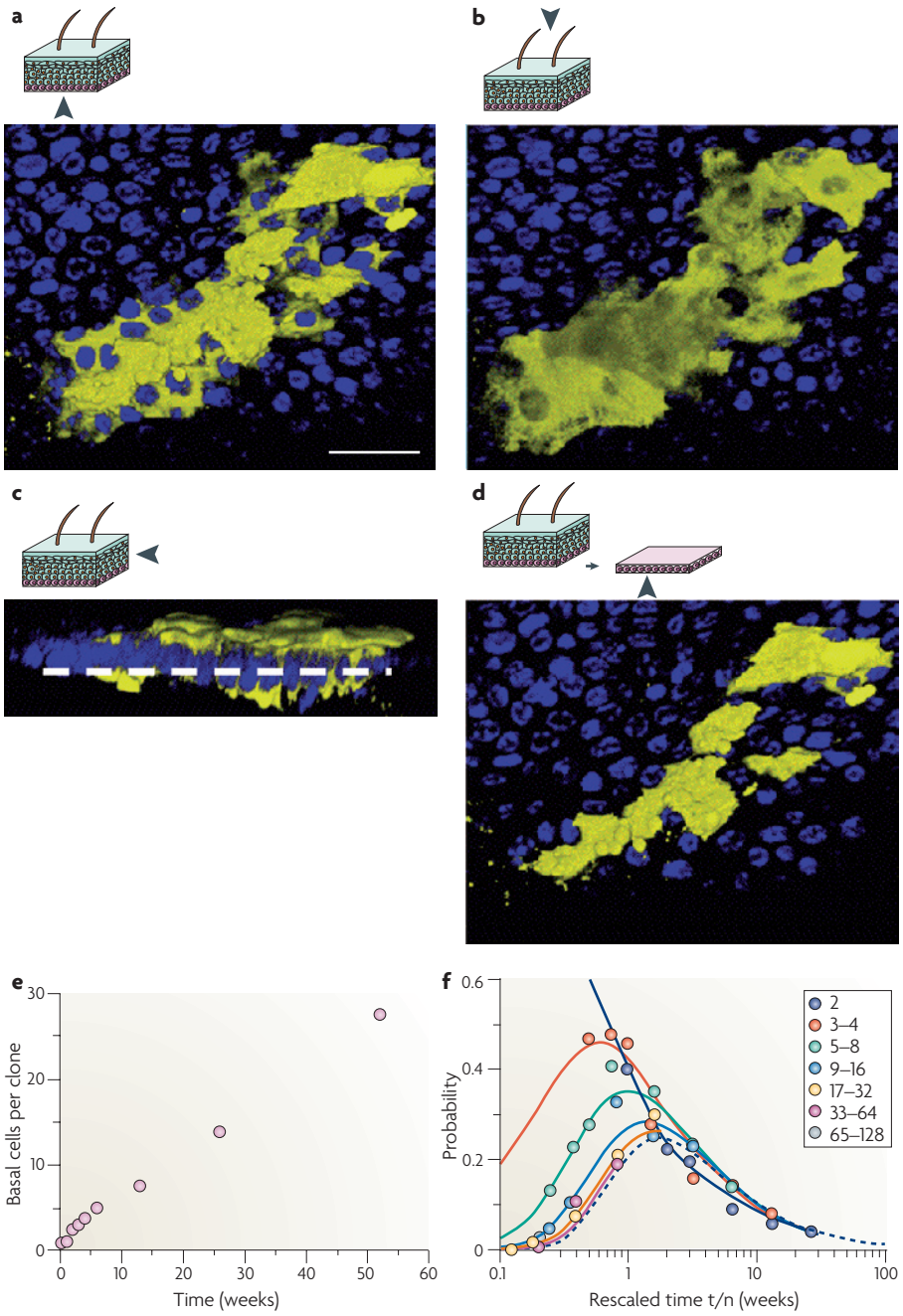

Figure 2 | **Clonal analysis in murine interfollicular epidermis.** Clone shapes are highly irregular and do not conform to the hypothesized epidermal proliferative unit (EPU) model. Confocal images show a typical mouse epidermal clone 6 months after labelling with enhanced yellow fluorescent protein (yellow)[39]. DNA is shown in blue. Arrows next to epidermis cross-sections indicate the viewing direction. Views from (**a**) the basal surface, (**b**) the external surface, (**c**) along the dotted line in side view and (**d**) of a section in the basal layer. Scale bar represents 50 μm. Note the irregular shape of the clone and that the apparent outlying cells in the basal layer are connected with the clone through the suprabasal layers (**b**,**c**). (**e**) The average number of basal cells in persisting clones as a function of time following induction of clonal genetic labelling of a representative sample of proliferating epidermal cells[39]. Note that average clone size grows linearly with time in the long term. (**f**) Basal layer clone size distribution (grouped in the range n+1 to 2n, n = 1, 2, …, 64) is plotted as a function of the rescaled time, t/n. The points show data and solid lines denote theoretical curves obtained from the committed progenitor (CP) cell model (FIG. 3a) with a cell-division rate of $\lambda = 1.1$ week^{-1}, $r = 0.08$ (that is, 84% of CP cell divisions result in asymmetric fate) and a progenitor cell fraction of $\rho = 0.22$ as measured by Ki67 immunostaining. Note that the long-term data collapse onto a single scaling curve (dotted), indicating that a single rate-limiting process determines the clone size distribution at longer time points. Panel **f** modified with permission from REF. 39 © (2007) Macmillan Publishers Ltd.

Box 2 | **Scaling**

A remarkable and revealing feature of the measured clone size distributions in murine epidermis is the collapse of the long-term data onto a single scaling curve (FIG. 2f). To expose this scaling behaviour, it is necessary to focus on the basal layer, defining $P_n(t)$ as the probability that a cell, labelled at induction, develops a clone with a total of $n = 0, 1, 2...$ basal layer cells after a time t. With this definition, $P_0(t)$ simply represents the 'extinction' probability of a clone, that is, the probability that the labelled cell and its progeny have all undergone terminal differentiation and have migrated out of the basal layer. To analyse the experimental data, it is helpful to exclude from consideration the population of extinct clones (which are difficult to monitor experimentally), leading to a distribution of persisting clones (see equation 2):

$$P_n^{pers.}(t) = P_n(t)/(1-P_0(t)) \quad (2)$$

Then, referring to FIG. 2f, one can see that after an initial transient behaviour, the clone size distribution acquires the simple scaling form shown in equation 3:

$$P_n^{pers.}(t) = \frac{1}{t} f\left(\frac{n}{t}\right) \quad (3)$$

That is, the probability of finding a clone with between, for example, $n/2$ and n basal layer cells at time t post-induction is the same as the probability of finding a clone with between n and $2n$ basal layer cells at time $2t$. This striking observation has important implications: first, the long-term evolution of the clone size distribution is governed by only one characteristic timescale. Second, the average (basal cell) size of those clones that do persist increases linearly with time (FIG. 2e). Because the labelled cell population is a representative sample of all basal layer cells[39], one is led to conclude that the continual loss of clones through differentiation is compensated for by the steady growth of persisting clones such that the total population remains constant.

CP cell behaviour. Although the behaviour of any individual CP cell is stochastic, even a small imbalance in the symmetric division rates would have drastic consequences for tissue homeostasis. An excess of divisions that generate two cycling daughters would lead to a rapid increase in the proportion of proliferating cells (one of the hallmarks of cancer), whereas an excess of divisions that generate two post-mitotic daughters would rapidly deplete the CP cell population. This raises several questions that merit further investigation. How is this balance achieved and regulated? Are the probabilities of CP cells undergoing a given fate fixed or variable? Is it possible that the relative proportions of cells that enter each of the cell-fate pathways depicted in FIG. 3 are 'hard-wired', as suggested by the observation that the proportion of asymmetric cell divisions is the same in adult and developing epidermis?[43]

Epidermal tissue injury. As a population, CP cells maintain a stem-cell-like capacity for self-renewal, although their individual characteristics suggest that they belong to a transient compartment. So, is there any requirement for a discrete population of epidermal stem cells at all? Although the CP cell population is capable of self-renewal, the model does not explain the rapid increase in the proportion of proliferating keratinocytes that is seen following injury[44]. One possibility is that there is a drastic change in the behaviour of the CP cell population requiring a temporary, but substantial, imbalance in the symmetric division rates to favour the production of cycling CP cells. An alternative explanation is that a discrete population(s) of quiescent stem cells is mobilized to generate additional CP cells. The observation that bulge-derived cells migrate into interfollicular epidermis following wounding indicates that stem cells are indeed mobilized following injury[9–12,45,46] (FIG. 3a). Further studies are required to resolve the extent to which either or both of these mechanisms operate.

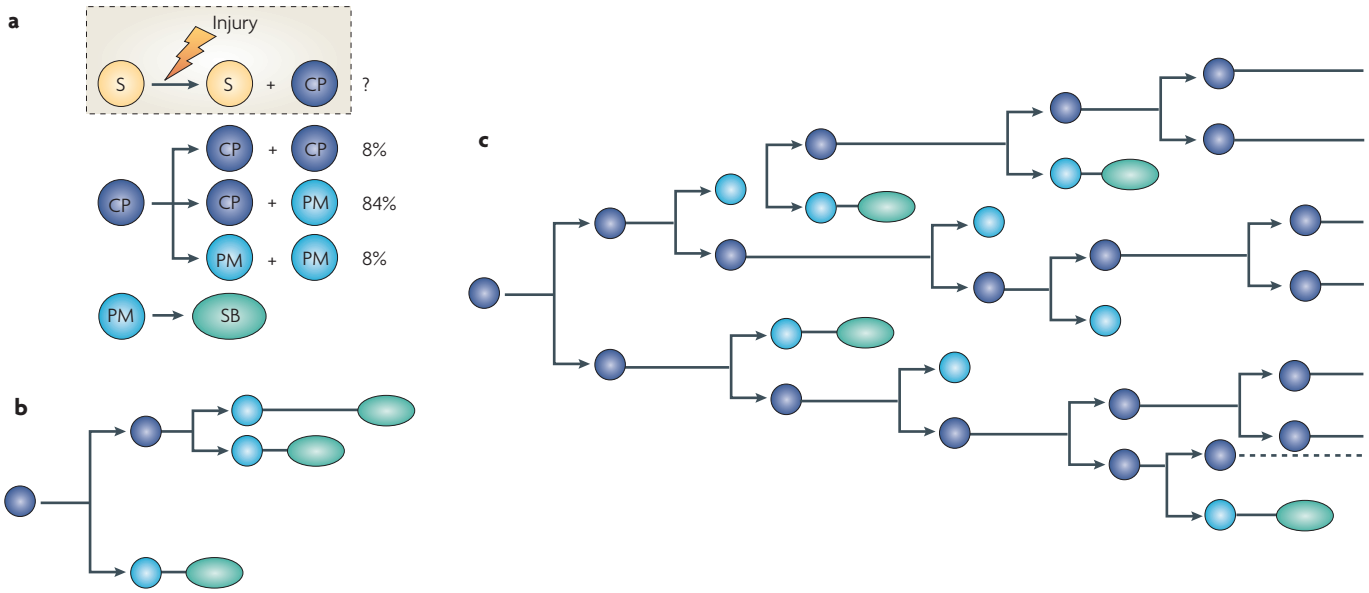

Figure 3 | **The stem–CP model of epidermal homeostasis. a** | The committed progenitor (CP) cell model. On division, daughter CP cells adopt one of three possible fates (the proportion of cells adopting each fate is shown by the percentages). Cells either remain as proliferative CP cells (dark blue) or become terminally differentiating, post-mitotic (PM) basal cells (blue), and subsequently leave the basal layer to become suprabasal cells (SB, green). The box indicates a stem-cell compartment that can generate CP cells, such as those in the hair follicle bulge or interfollicular stem cells. Stem cells (S; yellow) remain quiescent in normal homeostasis but can become activated following injury, dividing to produce CP cells and stem cells. **b** | The time evolution of a typical short-lived clone. **c** | The time evolution of a typical long-lived clone. Note that cell division and differentiation both occur in an asynchronous manner[53].

Carcinogenesis. The new model has significant implications for carcinogenesis. According to the stem–TA-cell hypothesis, TA cells are lost rapidly from the tissue so that cancer is predicted to arise from the accumulation of mutations in the cycling stem-cell population[47]. By contrast, the behaviour of CP cells protects stem cells from mutations that are acquired during DNA replication by allowing them to remain in a quiescent state. Moreover, the probability of a clone derived from a mutated CP cell persisting for long enough to accumulate further mutations is extremely low. Interestingly, studies of p53-mutant clones in UV-irradiated mice revealed clones that resemble those seen in normal mouse tail, which are then lost once UV irradiation ceases[48].

Conclusions and perspectives

The stem–TA-cell model of adult tissue homeostasis supposes that long-lived stem cells maintain a short-lived TA cell compartment[1]. Based on the observation of neat stacks of cornified layer cells and the assumption that there is no lateral cell migration through the suprabasal layers of the epidermis, it was further proposed that the epidermis was organized into EPUs[13]. Here, we have argued that the EPU hypothesis of epidermal homeostasis is not compatible with a wide range of experimental observations. Furthermore, the most recent evidence, which shows that clone size distributions conform to a simple scaling behaviour in the long term, is not only incompatible with the EPU hypothesis but also with the stem–TA-cell model itself[39]. The CP model, in which epidermis is maintained by a homogeneous population of cells committed to terminal differentiation, provides a quantitative account of the clone-fate data and is qualitatively consistent with the full range of published results. CP cells differ from TA cells in that they can undergo an unlimited number of cell divisions and, at each cell division, the probability of them taking any of the three possible cell fates remains the same.

Finally, could the concept of a self-renewing CP cell population be more widely applicable? The existence of a quiescent basal layer stem-cell population and the absence of patterning in the surrounding distribution of CP and post-mitotic cells suggest that the stem–CP-cell model may underpin homeostasis in human epidermis[30,37]. Intriguingly, looking beyond the epidermis, there is strong evidence to suggest that the pancreatic β-cell population is also capable of self-renewal without recourse to a stem-cell population[49]. Indeed, the advantages of releasing the stem-cell compartment from the routine maintenance of adult tissue suggest that many cell lineages may be supported by CP cells so that stem cells can sleep.

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DATABASES

UniProtKB: http://beta.uniprot.org/uniprot
β1 integrin | DSG3 | LRIG1

FURTHER INFORMATION

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