provide further proof of principle demonstrating that available oral agents such as RG7388 (HDM2 inhibitor) and CPI-0610 (BET inhibitor), which are currently in clinical trials, can selectively inhibit CML cell growth in vivo. In future studies, it will be interesting to assess whether such dual inhibition has an effect on overall survival of treated mice and to test whether the combination treatment leads to reduction or elimination of leukemia-initiating cells in secondary transplantation assays.

Interestingly, the authors found that the p53/c-Myc network is also deregulated in TKI-non-responder (TKI-NR) patients and more advanced forms of CML. And indeed, combination treatment significantly induced apoptosis in CD34+ cells from TKI-NR patients.

In conclusion, Abraham et al. (2016) demonstrate the power of using an unbiased systems biology approach to identify novel regulatory pathways and therapeutic vulnerabilities in leukemic stem cells using primary patient samples. Their findings strongly support the further exploration and testing of dual p53 and c-Myc targeting, in addition to TKI therapy, for patients with CML. Furthermore, as transcriptional and epigenetic dysregulation is currently emerging as one of the hallmarks of the earliest origins of cellular transformation and cancer stem cells (Corces-Zimmerman et al., 2014; Will et al., 2015), the combinatorial targeting of key dysregulated transcription factors may be an approach with broader applicability including other types of cancer.

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Fate by Chance, not by Choice: Epidermal Stem Cells Go Live

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The skin epidermis is constantly renewed by epidermal stem cells. In a recent *Science* paper, Rompolas et al. utilize live imaging to track epidermal stem cells over their lifetimes. Their findings provide new insights into epidermal stem cell behaviors and unravel how newly generated cells are integrated into pre-existing tissues.

Homeostasis involves the replacement of old tissues with new tissues generated from somatic stem cells. Advances in lineage-tracing strategies have enabled the identification of stem cells and lineage relationships of their progeny. However, the vast majority of these studies rely on fixed samples taken at different time points, which do not inform how an individual stem cell behaves throughout the process. Using two-photon microscopy combined with live imaging, Rompolas et al. (2016) followed individual stem cells over their lifetimes, and in so doing, elucidated new principles of epidermal homeostasis.

The skin epidermis undergoes constant turnover. Proliferating cells are located at the basal layer, while differentiating cells move upward to first form the spinous layer, followed by the granular layer, and eventually the most outer stratum corneum, a dead cell layer that sheds. A classical hypothesis, inspired by the columnar stacks seen with the cornified layer, suggests that each stack of cornified cells is maintained by the basal cells underneath and each is an "epidermal proliferative unit" (the EPU hypothesis). Each EPU contains one slow-cycling stem cell that divides asymmetrically to renew itself and generate the fast-dividing transit-amplifying cells, which undergo limited rounds of divisions before differentiating upward (Potten, 1974) (Figure 1A).

Recent studies have suggested that the EPU hypothesis is not accurate in

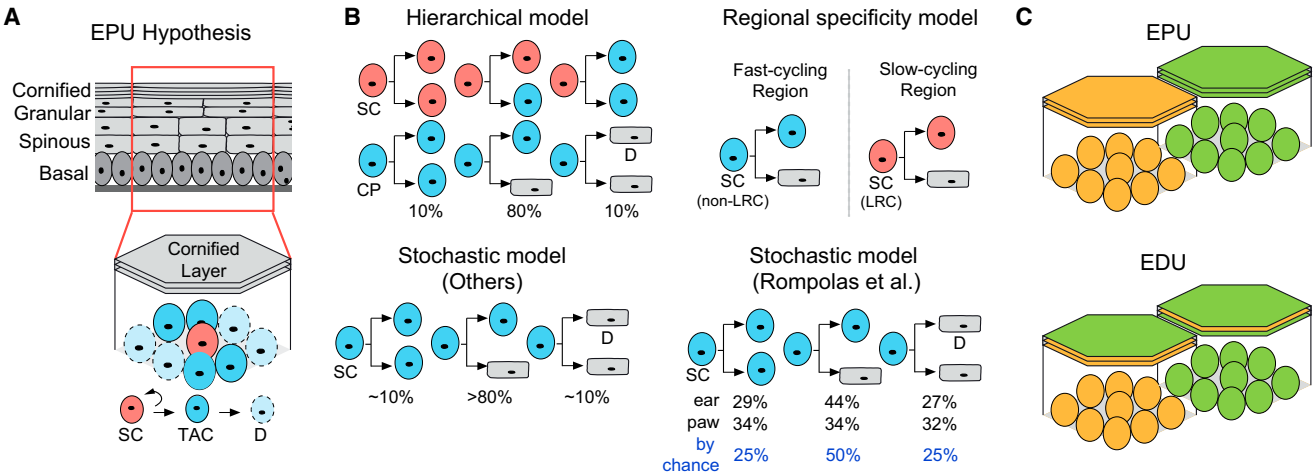

Figure 1. Models of Epidermal Homeostasis

(A) Schematic of the epidermal proliferative unit (EPU) hypothesis. Each EPU contains one stem cell (SC) that divides asymmetrically to generate one SC and one transit-amplifying cell (TAC) each time. TACs undergo rapid divisions before becoming differentiated cells (D) that migrate upward.

(B) Different models proposed for epidermal basal cells. Note that except for the model proposed by Rompolas et al., all models predict a large percentage of asymmetric cell division in that after division, sibling cells adopt distinct fates. SC, stem cell; CP, commited progenitor; D, differentiated cell; LRC, label-retaining cell.

(C) The EPU hypothesis predicts that each stack of cornified layer is derived from a single stem cell. Rompolas et al. observe that cells can switch to neighboring columns during differentiation, consistent with a model in which each stack of cornified layer comprises an epidermal differentiation unit (EDU) derived from multiple basal cells.

the strictest sense. Lineage-tracing using different Cre lines combined with label-retaining assays and mathematical modeling have led to the proposal of three alternative models (Figure 1B): (1) A hierarchical model that shares similarities with the EPU hypothesis, which proposes that the basal layer is composed of two populations of cells: slow-cycling long-term stem cells (SCs) that in turn give rise to fast-dividing committed progenitors (CPs) (Mascré et al., 2012). (2) A regional specificity model, in which slow-cycling and fast-cycling stem cells co-exist, but occupy different regions and each is capable of renewing its own domain (Gomez et al., 2013; Sada et al., 2016). (3) A stochastic model, in which all basal cells are equivalent in terms of their potential to divide or differentiate (Clayton et al., 2007; Doupé et al., 2010; Lim et al., 2013). Although conceptually different, a common theme among all these models is the assumption that most basal cell divisions are asymmetric—one of the sibling cells remains basal while the other ultimately differentiates.

With live-imaging to track the fate of marked cells combined with pulse-chase, Rompolas et al. examined the behavior of basal cells in the ear and paw epidermis. Their data are supportive of a stochastic model, but with some interesting twists.

They observed that individual basal cells either divide or differentiate directly by upward migration. However, no hierarchical relationships were observed among basal cells, and no slow-cycling cells were found. Perhaps most surprisingly, asymmetric division occurs at a much lower rate than anticipated: in the ear epidermis, the percentage of cells undergoing asymmetric division suggests that they likely do so by chance. In the paw epidermis, mechanisms might even exist that prevent asymmetric outcomes by coupling sibling cell fate (Figure 1B). Together, these data support a simple model proposed more than 50 years ago when Marques-Pereira and Leblond examined the rat esophagus (Marques-Pereira and Leblond, 1965): only a single population of basal cells exists, and they randomly choose between dividing or differentiating.

The ability to track individual cells over time also reveals dynamics during epidermal differentiation. First, individual cells can transition from the basal to the cornified layer independently of their neighbors. Newly differentiating cells arrive at the same space and adopt the same shape as their predecessors, so that the same architecture is maintained despite constant cell turnover. Second, progression from the basal layer to the cornified layer takes only a few days, which implies that cells go through very rapid cycles of breaking existing connections with neighboring cells in one layer, followed by forming new connections with the next layer, while synthesizing specialized proteins required at each layer. Lastly, Rompolas et al. observed that while most of the differentiating cells move along a vertical column that feeds into a stack of cornified layer directly on top, approximately 10% of the cells switch to a neighboring stack or generate a new stack. This result explains previous lineage-tracing data showing that each stack of cornified layer contains both labeled and non-labeled cells, and therefore is not likely the progeny of the same basal cell (Doupé et al., 2010). In this sense, the structure that inspired the EPU hypothesis is not a proliferation unit, but rather, functions as a highly organized epidermal differentiation unit (EDU) that collects progeny from different basal cells (Figure 1C).

Can a unified model for epidermal stem cells now emerge? Further studies are still required to reconcile some of the differences. However, it is likely that these different models explain co-existing behaviors, but apply to different parts of the body or cells occupying distinct domains. Of note, slow-cycling cells are specifically identified in the interscale region of the tail and backskin (Gomez et al., 2013; Sada

et al., 2016), which were not included in the analysis conducted by Rompolas et al. In addition, each approach has its unique advantages and limitations. While live-imaging is unparalleled for its ability to resolve individual cell fate, and the labeling approaches used by Rompolas et al. are perhaps the most unbiased among all, imaging the same skin area constantly might become a minor insult that could lead to a slightly higher turnover rate than other methods. In all, a consensus will likely require comprehensive approaches including applying tools developed by Rompolas et al. to other areas of the skin, lineage-tracing at saturation (Wuidart et al., 2016), and single-cell transcriptome analysis.

In conclusion, the study by Rompolas et al. shows that a stochastic model without regulated asymmetric divisions can sustain epidermal turnover. In this sense, self-renewal and differentiation are balanced at the tissue level but not controlled at the individual stem cell level. It is tempting to speculate that a seemingly random system like this might actually be more capable of adapting to changes than a hierarchical system, which in theory is more rigid. In the future, it will be particularly intriguing to reveal how different insults and conditions might shift this balance and uncover how dynamic differentiation processes described are controlled.

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