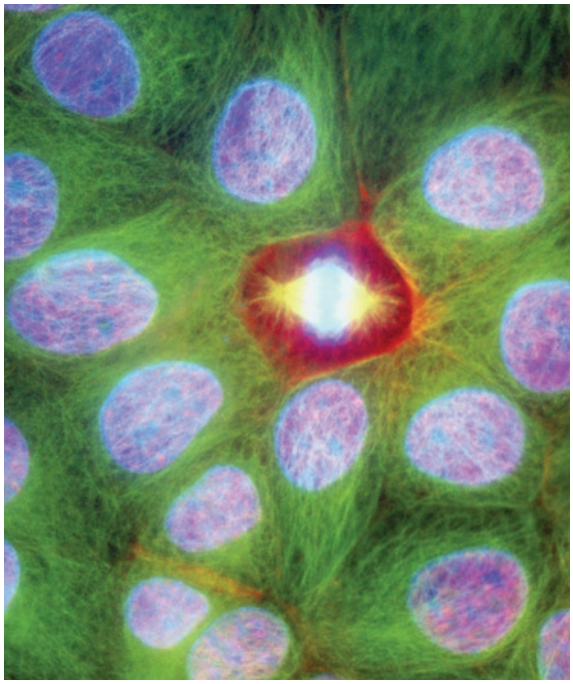
Tracing the roots of squamous cell carcinomas

There has been a recent surge of interest in identifying the 'cells of origin'—the cells that acquire the first cancer-promoting mutations in the tumorigenic process—in various types of cancer. Two new studies by Lapouge *et al.*[1] and White *et al.*[2] further fuel the ongoing debate over cells of origin by reporting the cellular origins of squamous cell carcinomas (SCCs), the second most common type of skin cancers. The two groups modeled SCC-initiating events in mice by driving oncogenic Ras expression and deleting the tumor suppressor p53 in different skin cell compartments. Intriguingly, tumors were initiated only from hair follicle stem cells and their immediate progenitors and not by more developmentally restricted transit-amplifying cells. We asked the experts to comment on how these studies might contribute to understanding SCC formation in humans.



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Slobodan Beronja and Elaine Fuchs

As we age, cells accumulate mutations that enable their escape from the proliferative checks and balances of normal tissue homeostasis. Identifying the origins of self-propagating cancerous cells is an important step in gaining insights into the molecular and cellular milieu that fostered their birth.

Two new studies[1,2] now identify the cellular roots of SCCs. In normal skin, epidermis constantly rejuvenates throughout life, as stem cell progenitors in the innermost layer commit to differentiate terminally, move outward and slough from the body surface. Stem cells also exist in hair follicles, where they periodically become activated to regrow hair. Many of the molecular mechanisms and distinct cell lineages underlying these tissue regenerative processes are now known[3].

The two groups used hair follicle and epidermal stem cell promoters to drive oncogenic *Kras*, yielding a high incidence of benign papillomas[1,2]. Upon concomitant removal of p53, the gatekeeper of a cell death switch, SCCs arose. In contrast, tumorigenesis did not occur when oncogenic Kras was targeted to the committed transit-amplifying progeny of hair follicle stem cells.

Why this difference? The simplest explanation, acknowledged by both groups[1,2], is that transit-amplifying cells are short lived and therefore less likely than stem cells to acquire additional genetic alterations that lead to cancer. If so, use of a larger sample size would be expected to 'up the odds' and uncover whether these cells have tumorigenic potential. Alternatively,

Allan Balmain

Like many interesting studies, these two papers[1,2] raise as many questions as they answer, some technical and some conceptual. Both groups used a keratin 15 (K15) promoter to drive oncogenic Ras expression in target skin cells, and both conclude that the K15-positive 'stem cell population' in the hair bulge, rather than the transit-amplifying compartment, harbors the target cells that form benign papillomas. However, the K15 promoter is active within a range of cell types in or near the bulge region, and so a subset of these cells might constitute the population responsible for tumor initiation. Tumor development is also initiated at the single-cell level rather than through the simultaneous activation of an oncogene in many cells, as modeled in these studies[1,2]. Thus these single initiated cells, which normally undergo clonal selection upon exposure to inflammatory or promoting agents, may be restricted to an even smaller compartment of epithelial cells within the skin.

> "One possibility is that virtually any cell can become a cancer cell when potent cancer genes such as those encoding Ras or p53 are mutated."

These papers[1,2] follow a recent flurry of studies that have identified

1. Lapouge, G. *et al.* Identifying the cellular origin of squamous skin tumors. *Proc. Natl. Acad. Sci. USA* **108**, 7431–7436 (2011).
2. White, A.C. *et al.* Defining the origins of Ras/p53-mediated squamous cell carcinoma. *Proc. Natl. Acad. Sci. USA* **108**, 7425–7430 (2011).
3. Blanpain, C. & Fuchs, E. Epidermal homeostasis: a balancing act of stem cells in the skin. *Nat. Rev. Mol. Cell Biol.* **10**, 207–217 (2009).
4. Greco, V. *et al.* A two-step mechanism for stem cell activation during hair regeneration. *Cell Stem Cell* **4**, 155–169 (2009).
5. Youssef, K.K. *et al.* Identification of the cell lineage at the origin of basal cell carcinoma. *Nat. Cell Biol.* **12**, 299–305 (2010).
6. Wang, G.Y., Wang, J., Mancianti, M.-L. & Epstein, E.H. Jr. Basal cell carcinomas arise from hair follicle stem cells in *Ptch*$^{+/-}$ mice. *Cancer Cell* **19**, 114–124 (2011).
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8. Epstein, E.H. Jr. Mommy—where do tumors come from? *J. Clin. Invest.* **121**, 1681–1683 (2011).
9. Chaffer, C.L. *et al.* Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc. Natl. Acad. Sci. USA* **108**, 7950–7955 (2011).

the molecular differences between tumor-compatible stem cells and committed transit-amplifying progeny may render transit-amplifying cells dependent upon specific mutations to progress to self-renewing cancer cells[4]. Meta-analyses of lineage-specific transcriptional profiles and cancer-associated mutations of these cells may be a good starting point for testing this possibility.

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Lastly, SCCs often show considerable diversity in their histological profiles, biological behaviors and clinical outcomes. It will be interesting to see whether the SCCs induced within distinct epidermal and hair follicle compartments will be useful in modeling the intertumoral heterogeneity observed in human cutaneous SCCs. If so, determining the molecular and cellular underpinning of distinct SCC subtypes might help to devise new and improved tools to treat cancer.

Slobodan Beronja is a postdoctoral fellow and Elaine Fuchs is an HHMI Investigator and Rebecca C. Lancefield Professor, Laboratory of Mammalian Cell Biology and Development, The Rockefeller University, New York, New York, USA.

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cells of origin for basal cell carcinomas (BCCs), many of which have reached contradictory conclusions. One possibility is that virtually any cell can become a cancer cell when potent cancer genes such as those encoding Ras or p53 are mutated, leading to reprogramming and the acquisition of more stem cell–like characteristics. The results obtained may therefore depend on the specific genetic strategy used. Youssef *et al.*[5] previously concluded that the K15-positive population is not susceptible to BCC formation by activation of the Sonic Hedgehog (SHH) pathway, which is known to be important in human BCC initiation. By contrast, Wang *et al.*[6] showed that radiation-induced BCCs in *Ptch1*$^{+/-}$ mice, in which SHH signaling is constitutively activated, can arise from K15-positive bulge cells. Wang *et al.*[6] used K15 as a neutral marker for the cell-of-origin population, rather than as a driver of the tumor-initiating event—a key difference between this and other studies.

An equally interesting issue is why Ras activation does not transform transit-amplifying cells expressing *Shh* but does lead to proliferation of K15-expressing cells[1,2]. This result is compatible with a model showing that suppression of SHH signaling by *Ptch1* overexpression promotes Ras-mediated transformation[7], suggesting some antagonism between the Ras and SHH pathways. It seems that the elusive cells of origin for many cancer types may remain incognito for some time to come.

Barbara Bass Bakar Distinguished Professor of Cancer Genetics Helen Diller Family Comprehensive Cancer Center, University of California–San Francisco, San Francisco, USA.

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Rune Toftgård

The cellular origin for SCC has been long debated, and evidence exists to support both hair follicles and interfollicular epidermis as its source. Two recent papers[1,2] provide important new pieces of the puzzle by showing that hair follicle stem cells but not transit-amplifying cells are cells of origin for SCC. This is consistent with observations in other rapidly renewing tissues, such as the intestine, where stem cells but not transit-amplifying cells give rise to persistent tumors upon inactivation of the APC tumor suppressor.

However, the hair follicle harbors a complex network of partly overlapping stem cell populations and, given the broad specificity of the genetic tools used in these studies, a remaining question is whether all or only some of these stem cells are potential SCC cells of origin. Conversely, experimental evidence showing a lack of tumor-initiating ability is only provided for a subset of fully committed transit-amplifying cells. Moreover, recent studies on BCC[8] have supported a cell-of-origin potential for similar populations of hair follicle stem cells upon activation of the Hedgehog pathway, illustrating that an oncogenic insult can dominantly influence phenotype and cell fate.

"One limitation is that we still know little about the identity of stem cells in human skin and hair follicles."

Earlier studies of chemically induced skin tumors have shown the presence of persistent latent tumor cells, hinting that these cells might have a stem cell origin. Alternatively, the oncogenic insult, such as a Ras mutation, may impart stem cell–like properties on non-stem cells. In support of this possibility, Lapouge *et al.*[1] show that SCC develops even if the *Kras* mutation and loss of p53 are preferentially induced in differentiated suprabasal epidermal cells. A similar remarkable cellular flexibility has been described by Chaffer *et al.*[9], who reported that both normal and tumor nonstem epithelial cells can spontaneously acquire stem cell properties. Taken together, the available evidence suggests the existence of a broad spectrum of cells with context-dependent potential to initiate tumorigenesis.

How relevant are these results for humans? One limitation is that we still know little about the identity of stem cells in human skin and hair follicles and how similar their characteristics and locations are to those in mice. Human skin has a different structure with many more cell layers, and a major inducer of mutations in humans, ultraviolet radiation, has a limited capacity to penetrate tissues. Hence, the identities of SCC cells of origin may differ. Further studies in model systems and human skin are needed before we know which cells are most at risk of becoming tumorigenic and how to apply this knowledge to prevent SCC.

Director of the Center for Biosciences, Karolinska Institutet, Stockholm, Sweden.

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