# Keratin 15-Positive Stem Cells Give Rise to Basal Cell Carcinomas in Irradiated *Ptch1*$^{+/-}$ Mice

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The cell of origin for basal cell carcinoma (BCC) remains controversial. In this issue of *Cancer Cell*, Wang et al. provide strong evidence that BCC arise from hair follicle stem cells.

Over twenty years ago, we localized a population of presumptive stem cells to the hair follicle bulge (Cotsarelis et al., 1990). Both mouse and human bulge cells possess a quiescent phenotype that markedly contrasts with the much higher proliferative rate of the cells within the remainder of the cutaneous epithelium (Lyle et al., 1998). We proposed that bulge cells were the site of origin for skin cancers, primarily because their long-lived nature would allow for accumulation of multiple genetic hits and subsequent tumor formation. At that time, we asked, "What are the relative contributions of the follicle and IFE in giving rise to various human skin carcinomas?" With respect to cutaneous basal cell carcinoma, the most common type of cancer, this question awaited the development of molecular tools for targeting bulge cells as well as the development of mouse cancer models.

Genetic lineage analysis of bulge cells became possible with the discovery that the Keratin 15 (K15) promoter preferentially targets stem and progenitor cells in the bulge and adjacent secondary germ (Morris et al., 2004). The inducible K15-CrePR1;R26R mouse was used to demonstrate that bulge cells generated all epithelial lineages during hair follicle cycling when the lower follicle regenerates and that these cells contributed keratinocytes to healing wounds (Ito et al., 2005; Morris et al., 2004). The K15 promoter could then be used to target bulge cells in mouse models of skin cancer to determine if hair follicle stem cells contributed to cutaneous carcinogenesis.

Two recent studies took this approach and came to diametrically opposite conclusions. Blanpain and coworkers published that basal cell carcinomas in a transgenic overexpression model did not arise from hair follicle bulge cells (Youssef et al., 2010). In contrast, Epstein and colleagues report that irradiated *Ptch1*$^{+/-}$ mice develop BCCs almost exclusively from hair follicle bulge cells (Wang et al., 2011). Understanding this discrepancy is critical for the field and has implications on developing treatments for human BCCs.

BCCs in humans occur spontaneously or as part of hereditary syndromes such as basal cell nevus syndrome (BCNS). BCNS patients generally carry one mutated *PTCH1* gene, which encodes a receptor for hedgehog proteins, and develop BCCs through loss of heterozygosity of the remaining *PTCH1* allele. The large majority of spontaneous basal cell carcinomas also possess loss-of-function mutations in the *PTCH1* gene that lead to activation of the hedgehog pathway. Basal cell carcinomas without *PTCH1* mutations may possess activating mutations in genes encoding other components in the hedgehog pathway such as the signal transducer Smo (*SMO*). Thus, mutations leading to activation of the hedgehog pathway are a hallmark of human basal cell carcinomas. Since *PTCH1* is a hedgehog target gene, basal cell carcinomas overexpress *PTCH1* mRNA and this can be used as a marker for basal cell carcinoma.

The hedgehog pathway is critical for development of hair follicles. Interestingly, in the normal adult epidermis, little to no expression of Ptch or its ligand Shh is evident. These genes are expressed primarily in the hair follicle and only at specific times during hair follicle cycling in the adult. This raises the possibility that loss of Ptch in the interfollicular epidermis (IFE) may not immediately impact epidermal keratinocyte behavior. Consistent with this, transgenic mice that overexpress Shh in skin develop tumor-like changes primarily in their hair follicle epithelium rather than in their IFE (Oro et al., 1997).

Human BCCs generally possess two mutated alleles, often with UV signatures, of *PTCH1*. Loss of Ptch function results in overexpression of hedgehog target genes since Ptch normally represses these. Epstein and colleagues developed a *Ptch1*$^{+/-}$ mouse that forms BCCs following UV or ionizing radiation. This mouse phenocopies the BCNS patient in that both develop medulloblastomas and rhabdomyoscarcomas as well as BCCs. Thus, this mouse model seems ideal for studying the development of BCC and for answering the question of whether BCCs arise from hair follicle bulge cells or from other epithelial cells. Wang et al. crossed this mouse with the K15-CrePR;R26R-YFP mouse, induced labeling of the bulge cells, irradiated the mice, then evaluated the resulting tumors for expression of YFP that would indicate a bulge cell origin (Wang et al., 2011). Taking into account the efficiency of the inducible system, they showed that the great majority of BCCs arose from K15-positive bulge cells.

The cell of origin of human BCC has been debated for decades; however, dermatopathologists generally hold the view that "superficial (multicentric)" BCCs arise from IFE while some portion of nodular BCCs arise from the follicle. Previous studies on human BCCs showed that most of these tumors express keratins associated with follicular keratinocytes. Interestingly, when immunostained for K15 protein, the bulge cell marker, approximately one third of nodular BCCs stained positive, while none of the superficial BCCs stained, thus providing evidence that a substantial portion of

human nodular BCCs arise from the hair follicle bulge (Jih et al., 1999).

Other types of mouse models for basal cell carcinoma depend on overexpression of genes in the hedgehog pathway, such as Gli and Smo; for example, targeting of Gli expression to the follicle and IFE results in the formation of basal cell tumors that clinically resemble human basal cell carcinomas in that they have a translucent appearance and the presence of small vessels known as telangiectasias (Grachtchouk et al., 2000; Nilsson et al., 2000). These tumors are dependent on continuous Gli expression and regress if the transgene is turned off. Results of early clinical trials suggest that human BCCs are similarly "addicted" to hedgehog signaling and may be amenable to targeted therapy.

Dlugosz and colleagues previously published that constitutive overexpression of activated Smo in the epidermis resulted in basaloid hamartomas (Grachtchouk et al., 2003). These investigators were careful to distinguish between basaloid hamartomas and BCC because basaloid hamartomas, both in humans and in mice, have limited growth potential and rarely develop into BCC. In a more recent study, Blanpain and colleagues also overexpressed activated Smo in the epidermis, but with an inducible system, and described the formation of "basal cell carcinomas." (Youssef et al., 2010) One problem with both the Blanpain and the Wang paper rests on whether the tumors that developed are truly BCCs or whether they are basaloid hamartomas. Input from a dermatopathologist is essential for making the distinction, but marker studies would be ideal. Nonetheless, these findings do suggest that non-bulge cells have a lower threshold than bulge cells for tumor development in response to oncogenic Smo.

Wang et al. suggest that loss of p53 triggers Smo expression in epidermis of *Ptch1*$^{+/-}$ mice. Since Smo is an obligatory activator of Hh signaling, the resultant epidermal BCCs in irradiated p53-deficient *Ptch1*$^{+/-}$ mice suggests that loss of p53 may be a primary event in BCC formation, operating through the novel mechanism of Smo upregulation. This important concept deserves testing in both human epidermis with known p53 mutations and in mouse models. The findings could impact on future targeting of incipient BCC with chemotherapeutic agents.

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# Metastatic Colon Cancer Cells Negotiate the Intravasation Notch

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**In this issue of *Cancer Cell*, Sonoshita et al. report that Aes/Grg5 prevents metastasis of colorectal cancer cells by sequestering and inactivating Notch transcriptional effectors in distinct nuclear foci. Loss of Aes/Grg5 in invasive cancer cells where Notch is activated by stroma-expressed ligands promotes invasion, transendothelial migration, intravasation, and metastasis.**

Metastatic disease is the major cause of cancer-associated death. During the metastatic process, cancer cells need to overcome a number of hurdles, including invasion into neighboring tissue, intravasation into blood or lymphatic vessels, survival in the circulation, extravasation from vessels at distant organs, and colonization and outgrowth at the distant sites. Each of these events involves a number of signaling pathways. In this issue of *Cancer Cell*, Sonoshita and