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The HF Bulge Stem Cell Niche Resists Transformation by the Hedgehog Pathway

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Abstract

Similarities between basal cell carcinoma (BCC) tumor cells and hair follicle keratinocytes had previously suggested that BCC originates within the hair follicle bulge stem cell niche. However, in the current *Nature Cell Biology,* (Youssef et al.) show that BCC originates in the interfollicular epidermis.

Tumors have been commonly classified based on their similarity to the normal tissues from which they are derived, both at the level of architectural features and protein expression. Given the dynamic changes that occur during tumorigenesis, however, determining the specific cell of origin for any given tumor is not straightforward. Within normal somatic tissues, there is significant heterogeneity among the cells with regard to their proliferative capacity, differentiation, and susceptibility to malignant transformation. Identification of the specific cell subpopulations within individual tissues that give rise to particular tumors remains one of the current challenges in cancer biology. This is an important effort, as understanding mechanisms through which certain cell subpopulations resist malignant transformation, while adjacent subpopulations within the same tissue are vulnerable may lead to identification of new drug targets and therapeutic strategies for cancer. In the current issue of *Nature Cell Biology*, Youssef et al. (2010) characterize subsets of epidermal keratinocytes in mice that differ in their ability to give rise to basal cell carcinoma (BCC) driven by activation of the hedgehog signaling pathway.

BCC is the most common malignancy in the United States with nearly one million cases annually. These locally invasive skin tumors occur most commonly in sun-exposed skin and are driven by UV signature C-->T transitions at dipyrimidine sites in genes encoding proteins in the sonic hedgehog (Shh) pathway. The most common mutations result in inactivation of patched (the membrane receptor for Shh which, in the absence of Shh ligand, negatively regulates signaling through smoothened). Directly activating mutations also occur in other downstream pathway elements including Smoothened (Smo) and Gli transcription factors. UV signature mutations are also frequently present in the tumor suppressor p53, although p53 inactivation does not appear to be necessary for formation of BCC in either mouse or human models (Fan et al., 1997; Grachtchouk et al., 2000; Oro et al., 1997; Xie et al., 1998).

It is clear that the cell of origin in spontaneous human tumors, as well as murine model systems is the skin keratinocyte. However, within the skin there are multiple subpopulations of keratinocytes with divergent roles in tissue homeostasis. These groups include keratinocytes that reside within the epidermis between adjacent HFs (interfollicular epidermis or IFE), as well as keratinocytes localized to different regions within of the hair follicle (HF) itself. Which of these keratinocyte populations serve as the origin of the BCC

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tumors has not been clearly established, but it has long been theorized that they derive from the bulge region of the HF. There are several reasons for this. First, the hedgehog pathway is essential for HF development, and also for maintenance of the normal hair cycle in which HFs cycle between periods of active growth (anagen), regression (catagen) and quiescence (telogen) (Chiang et al., 1999; Wang et al., 2000). Progression from telogen to anagen requires hedgehog-mediated expansion of stem cells from within the specialized niche region called the bulge. These bulge stem cells have significant regenerative potential as they cycle for an entire lifetime, and it is this proliferative capacity that was thought to contribute to their potential to develop into cancer (Cotsarelis et al., 1990). It is important to note however, that epidermal stem cells also exist within the IFE. Second, the hedgehog pathway is the oncogenic driver for spontaneous human BCC. Finally, BCC tumors express keratins 15, 17, and 19 which are expressed preferentially in the outer root sheath of the HF. For all these reasons, it has long been thought that the cell of origin for BCC resides within the HF, with the bulge as the likeliest location harboring the cancer-competent sub-population.

Youssef et al. used inducible cre recombinase to activate the hedgehog pathway via expression of a constitutively active mutant human Smo (SmoM2) selectively within individual cellular compartments within postnatal murine epidermis. In the absence of cre recombinase, no SmoM2 was expressed and no BCCs developed spontaneously. Upon expression of SmoM2 throughout keratinocytes in both the IFE and HF using keratin 14-driven Cre, BCCs developed within 8 weeks, consistent with other murine BCC models in which active hedgehog pathway proteins were expressed from keratin 5 and keratin 14 promoters. Subsequent studies utilized a limiting amount of cre recombinase activity to activate SmoM2 in only a small percentage of K14 positive cells. This allowed a detailed histologic analysis of individual clones which revealed that 93% of BCCs arose within the IFE, with the remaining 7% derived from the upper part of the HF (infundibulum). Additionally, the fate of IFE cells expressing SmoM2 was followed over time and compared to control IFE cells expressing YFP. BCC tumors only seemed to arise in long-term progenitor cells, and did not induce renewing potential in K14 cells committed to differentiation. Surprisingly, no tumors were seen to arise from the stem cell rich bulge region.

Consistent with this lack of bulge cell derived BCCs, when keratin 15 and keratin 19 promoter constructs were used to selectively express SmoM2 only in the bulge region, no BCC tumors were observed. This was not the result of an ability of SmoM2 to activate the hedgehog pathway in bulge cells, as well known Shh target genes were induced. SmoM2 was also expressed in the lower portion of the HF (matrix) using an SHH promoter-driven Cre. Although the SmoM2 protein was observed in the matrix progenitor cells, no BCC tumors were seen.

Given that BCCs are UV-induced tumors, and that UV damage decreases with distance from the skin surface, it is not necessarily surprising that most BCCs are derived from the IFE. The fact that BCCs can occasionally occur on hairless areas of skin such as the palm of humans and footpad of mice is consistent with the studies here indicating that cells from the HF are not required for BCC formation. This study does suggest that long-lived progenitor cells resident within the IFE are the cell of origin for most basal cell carcinomas, and that the intrinsic renewal capacity of these stem cells may contribute to their susceptibility to malignant transformation.

While this elegant study clearly indicates that there are differences in keratinocyte susceptibility to malignant transformation by Shh activation, the mechanisms mediating the relative resistance of the bulge stem cell keratinocytes, which also possess the high

proliferative renewal capacity, remain to be explored. Are the bulge cells resistant because of factors intrinsic to the resident cells, or do differences in the environment including the supporting basement membrane and extracellular matrix of the HF offer protection from the oncogenic insult? Isolating SmoM2 expressing bulge cells and using them to regenerate IFE may be an experimental approach to address this question. It would also be useful to learn if the same susceptibilities observed here were recapitulated in older mice, as most human BCC occurs in older adults. Finally, it may be useful to learn whether the bulge stem cells are resistant to malignant transformation by other oncogenes or whether this observation is unique to the Shh pathway.

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