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Cells of origin and tumor-initiating cells for nonmelanoma skin cancers

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Abstract

The epidermis of the skin is a multilayered stratified epithelium whose primary function is to provide a barrier against our external environment. As a result, cells in the epidermis are subject to constant assault from environmental pathogens, many of which can cause deleterious mutations. However, most of these mutations do not lead to skin cancer. One explanation is that most genetic hits are sustained by mature or transit cells with limited proliferative capacity and only stem cells that acquire genetic alterations have the potential to propagate a frank tumor. In this mini-review we will discuss recent studies that provide some of the first genetic evidence to support a stem cell origin for a number of skin cancer types.

Keywords

epidermis; hair follicle; keratinocyte; stem cell; cancer stem cell

1. Introduction

The epidermis, the outer layer of mammalian skin, is a multilayered epithelial barrier tasked with protecting the body from environmental stresses, such as UV radiation, dehydration, extreme temperatures, and chemical and biological pathogens. The epidermis is comprised of numerous keratinocyte progenitor cell populations that are responsible for maintaining skin homeostasis and tissue repair following insult. However, the same keratinocyte progenitors that provide the epidermis with its self-regenerating properties are also susceptible targets for genetic damage induced by environmental pathogens and have been hypothesized to be usurped by cancers to lead to aberrant growth [1]. The development of skin cancer, based on the Knudson hypothesis [2], requires the accumulation of multiple genetics hits within a single, and this is relatively difficult to achieve within the epidermis due to the rapid cycling and shedding of keratinocytes through the process of terminal

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Conflicts of interest

None declared.

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differentiation [3]. Because stem cells reside in the epidermis for extended periods of time, they have the ability to accumulate the necessary genetic hits to initiate bona fide tumors [3].

Historically, carcinogenesis was initially portrayed by an egalitarian paradigm, which posits that tumors are comprised of differentiated cancer cells possessing similar proliferative potentials. During tumor progression, certain neoplastic cells will stochastically evolve abilities to invade and metastasize through mutations and unique influences of the tumor microenvironment. However, one shortcoming of this earlier model is that it does not account for the phenotypic diversity within many neoplastic tissues that has been well characterized over the last two decades. Recent findings support a hierarchical model indicating where tumor bulk is maintained by a rare population of multipotent tumor-initiating cells (TICs) with unlimited capacity for self-renewal and proliferation. In the TIC model, a select few neoplastic cells exhibit stem cell characteristics and have the ability to generate both differentiated highly proliferative tumor cells committed towards growth as well as additional self-renewing TICs that can regenerate all cell types comprising the tumor [1]. Like normal somatic stem cells, TICs likely overexpress multidrug resistance and anti-apoptotic genes and exhibit a slow-cycling nature that make them more resistant to radiation and chemotherapy compared to differentiated tumor cells [1]. Conventional tumor therapy, which indiscriminately targets rapidly proliferating cells, will preferentially target differentiated tumor cells and shrink tumors but are unable to eradicate TICs and offer a long-term cure. The identification and characterization of TICs should allow improved therapeutic targeting that offers a higher potential of cure as well as reduced toxicity. This paper will review the scientific advances made thus far including genetic pulse chase technology that has helped to uncover the cells of origin as well as TICs for a number of tumor types emerging in the epidermis of skin.

2. The landscape of epidermal progenitor niches in adult skin

The perpetual renewal of mammalian skin is known to be maintained by permanently residing stem cells that are able to sustain three principal differentiated lineages: the interfollicular epidermis (IFE), sebaceous gland (SG) and hair follicle (HF) [4]. In addition, recent studies identified Merkel cell mechanoreceptors residing in specialized epithelial structures termed touch domes in the hairy skin as a fourth lineage maintained by keratinocyte progenitors [5]. While it has long been accepted that skin homeostasis is dependent on the ability of stem cells to replenish the turnover of these mature epithelial lineages, it is the work over the last decade that has significantly enhanced our understanding the location and function of multiple stem or progenitor niches in the skin. These findings have dramatically changed our view of the cutaneous epithelial stem cell landscape rendering a highly compartmentalized epithelium maintained by multiple classes of phenotypically distinct regional niches [4].

In some cases progenitor niches have been labeled using mouse genetics approaches and characterized under normal conditions to be long-lived and able to sustain the cellular input to certain epithelial structures including the interfollicular epidermis [6,7], sebaceous gland [6,8] and hair follicle [9–12] (Table 1). In other cases, antibodies against cell surface proteins have been utilized to mark and isolate epithelial progenitors located in the IFE and HF (Table 1). These efforts have facilitated our understanding of the relative proliferative capacity of progenitor pools as well as their capacity to regenerate IFE, HF, SG or Merkel lineages in surrogate assays. Collectively, these studies have illustrated the role of epithelial progenitors during skin homeostasis as well as their ability to respond to skin insult. Importantly, the phenotypic diversity of these progenitor pools within the epidermis and the various differentiated lineages they maintain under normal conditions may provide important implications for the various classes of nonmelanoma skin cancers (NMSCs) that

emerge in the skin, including basal cell carcinoma (BCC), squamous cell carcinoma (SCC), trichofolliculoma, pilomatricoma, sebaceous adenoma and Merkel cell carcinoma (MCC).

Indeed, keratinocyte stem cells have long been implicated as target cells for NMSC. Morris et al. provided key initial findings to support this concept through their observation that murine papillomas and SCCs can be elicited even despite long time intervals between the initiation (genetic insult) and promotion (neoplastic proliferation) stages of skin carcinogenesis [13]. This temporal separation argues that the cell of origin for SCC must retain stem cell characteristics since these would be the only cells expected to persist for a long duration in the epidermis. Furthermore, studies were later published showing that keratinocytes demonstrating stem cell characteristics, including high label retaining and clonogenic capacities, were especially prone to uptake of the chemical carcinogen benzo[a]pyrene [14] and were slow to repair DNA adducts induced by chemical carcinogen treatment to murine skin [15]. Later Morris and colleagues demonstrated that killing of actively cycling cells but not quiescent epidermal stem cells by 5-fluorouracil treatment in carcinogen initiated mice does not influence the overall tumor burden in the skin [16] indicating that slowly cycling, i.e. stem cells, are likely the primary cellular targets for skin tumors induced by chemical carcinogens. However, the precise location for these originating cells and molecular defects necessary for transformation of such a cell remained unknown. More recently, the advent of conditional knock in mouse models has provided an elegant platform for genetic pulse chase labeling studies that enable one to mark discrete subsets of epidermal progenitors and follow their cellular progeny into developing cutaneous neoplasms. This technology has helped to uncover cells of origin for a number of cutaneous tumors, which are discussed in more detail below.

3. Squamous cell carcinoma

Cutaneous squamous cell carcinomas (SCC) represent 20% of non-melanoma skin cancer cases in humans and are associated with substantial risk of metastasis [17]. Human SCC are primarily induced by sunlight exposure and SCC can also be induced in mice through i) multistage carcinogenesis models employing either UV irradiation (primary etiological agent in sunlight) or chemical carcinogens such as the mutagen 7,12-Dimethylbenz(a)anthracene (DMBA) in combination with the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or ii) forced expression of oncogenes targeted to the proliferative or differentiated layers of the epidermis.

Brash and coworkers employed a whole mount labeling approach in areas of sun-exposed human skin to detect patches of early neoplastic keratinocytes that contained UV-induced TP53 mutations [18], a molecular signature of sunlight induced SCC. Using this approach Brash found that these large patches of mutant TP53 cells typically localized in the stem cell region of human epidermis and not in areas harboring transit-amplifying cells [18]. These studies were some of the first to provide *in vivo* evidence for an epidermal stem cell origin for UV-induced SCC in humans and have since been corroborated in mutant Trp53 mouse models exposed to UV radiation [19].

The Ras pathway has been implicated the development of murine and human SCCs. Activating mutations in Ha-ras are a molecular fingerprint of mouse skin tumors induced by polycyclic aromatic hydrocarbons such as benzo[a]pyrene and DMBA [20]. Although activating point mutations in RAS, are only found in 10–30% of human SCC cases [21], RAS overexpression, which phenocopies oncogenic RAS, is observed in most human cutaneous SCCs and SCC-precursors [21]. In addition, RAS mutations have been found to be enriched in SCCs developing in patients taking the melanoma BRAF inhibitor, vemurafenib, and these tumors developed rapidly within months of initiating anti-melanoma

therapy [22]. Brown et al. conducted a pivotal study showing that cutaneous SCC, when induced by overexpression of a mutant Ha-ras transgene, can arise from the hair follicle in mouse skin [23]. This experiment used a truncated keratin 5 promoter, which selectively targeted the proliferative keratinocyte layer including stem cells in the bulge, to drive mutant Ras expression within the hair follicle [23].

To pinpoint the cellular origin of Ras mediated SCC, two studies, by White et al. and Lapouge et al., utilized inducible Cre mouse models driven by bulge-selective keratin promoters (K15 or K19) to target this stem cell niche for mutant Kras induction [24,25]. Both groups also employed a Shh inducible mouse model for comparison to target the TA cell compartment. Kras induction in the bulge compartment via K15 and K19 promoters led to papilloma formation, whereas the same induction stimulus applied to the TA compartment via Shh promoter did not yield any macroscopic tumors [24,25]. Lapouge et al. additionally discovered that Kras induction in the postmitotic IFE layers via the Involucrin promoter was also able to induce papilloma formation [25]. The latter observation supports previous findings of papilloma formation in mouse models exhibiting constitutive v-Ha-ras [26] or c-Myc [27] oncogene expression in the suprabasal epidermal layers and collectively suggests that some degree of plasticity in otherwise mature epidermal keratinocytes may allow these cells to serve as a cell of origin for squamous neoplasia under certain conditions. Both groups then demonstrated that two genetic hits, first Kras induction then followed by p53 ablation, were necessary for progression from papilloma to SCCs [24,25]. Overall, both studies confirmed that HF bulge cells are susceptible to Ras overexpression and serve as a cell of origin for SCC, while HF TA cells are unable to initiate SCC under the same conditions [1,2]. These findings have been supported by a recent study by the Morris lab utilizing genetic pulse labeling to confirm that cells derived from the K15+ bulge compartment harbor DMBA-induced Ha-ras mutations and substantially contribute to the cellular input in murine squamous papillomas [28].

Using transplantation experiments, Malanchi et al. sought to identify an enriched subpopulation of murine SCC cells that could serve as TICs when orthotopically transplanted onto *Nude* mice. The authors showed that SCC cells enriched for CD34, a marker of normal bulge stem cells (Table 1), demonstrated 100-fold more *in vivo* potency in initiating secondary tumors after transplantation compared to the total pool of SCC cells [29]. In contrast, CD34-negative SCC cells never produced tumors after transplantation. Additionally, secondary tumors derived from CD34+ transplanted cells still maintained a small population of CD34+ cells while producing differentiated progeny, lending further credence to the possibility that CD34+ SCC cells act as TICs. The authors proceeded to further characterize the CD34+ SCC-initiating cells by proving that β -catenin signaling is essential for tumorigenesis and maintenance of SCC-initiating cells. They found that β -catenin gene ablation resulted in complete regression of DMBP/TPA-derived tumors, and notably, led to a depletion of CD34+ cells within the tumor [29]. The resultant CD34+ depleted and β -catenin deficient tumor loses tumorigenic capacity and is unable to initiate secondary tumors following transplantation. The overall findings of this study were supported by the observation made by Trempus and co-workers that CD34 null mice are resistant to DMBA-induced cutaneous SCCs [30]. However, the implications of these findings for TICs in human SCC remain unclear as neither CD34 expression nor Wnt signaling have been reported in human SCC. Schober and Fuchs established that TICs of murine SCCs were enriched for $\alpha 6 \beta 1$ integrin through transplantation studies showing that transplanted $\alpha 6 \beta 1^{hi}$ SCC cells can initiate secondary tumors in *Nude* mice whereas their $\alpha 6 \beta 1^{lo}$ counterparts cannot [31]. Importantly, this study further refined the paradigm of CD34+ as a requirement for TICs by demonstrating that the $\alpha 6 \beta 1^{hi}$ TICs possessed tumor-initiating abilities regardless of their CD34 enrichment status. Surprisingly, the CD34^{lo} integrin-enriched TICs reconstituted tumors more rapidly following transplantation

compared to CD34^{hi} counterparts. Moreover, CD34^{hi}α6β1^{hi} and CD34^{lo}α6β1^{hi} TICs were found to shift reversibly between CD34 high/low states *in vitro*, and both populations yield progeny tumors *in vivo* that individually contain CD34^{hi} and CD34^{lo} cells [31]. Schober and Fuchs proceeded further to demonstrate that tumorigenic potency of CD34^{hi} versus CD34^{lo} TICs was modulated by the balance of the TGF-β/TGF-β receptor II signaling pathway (which generally inhibits SCC formation) and the integrin/focal adhesion kinase (FAK) signaling pathway (which generally promotes SCC carcinogenesis). Specifically, ablation of TGF-β signaling relatively enhanced CD34^{hi} TIC proliferation rate (as measured by EdU incorporation) while ablation of integrin/FAK signaling further enhanced CD34^{lo} proliferation. However, the most tumorigenic TIC group among all those studied appeared to be the CD34^{hi} CSCs in the TGF-β knockout group, despite the inherent higher baseline proliferative potential of CD34^{lo} TICs, thereby stressing that cellular escape of TGF-β signaling was more important than level of CD34 expression [31].

In summary, Schober and Fuchs' study suggests that high integrin expression is a more important marker of TICs within murine SCC, and that CD34 status can distinguish between 2 interconvertible groups of integrin-enriched TICs whose tumorigenic properties are controlled by TGF-β and FAK signaling pathways. Altogether, these studies suggest that the genetic background of the lesion has as much of an effect on tumor-initiating properties of TICs as that of which specific stem cell population is being targeted.

3. Basal cell carcinoma

Basal cell carcinomas (BCC) represent 80% of non-melanoma skin cancer and are the most commonly diagnosed human cancer in the United States [32]. UV radiation appears to be principal mutagen in BCC pathogenesis, and dysregulation of the Shh/Ptch1/Smo pathway has been central to BCC development [33,34]. Overexpression of the Hedgehog pathway, either through deletion of Ptch1, mutational activation of Smo, or overexpression of Gli1 or Gli2 have been reported in sporadic human BCCs [33,35,36]. Inherited BCCs, most commonly secondary to Gorlin syndrome, is due to an autosomal dominantly inherited PTCH1 mutation. Despite the well-elucidated understanding of Hedgehog signaling to BCC, the cell of origin of BCC remains controversial. Further complicating this issue are the multiple subtypes of BCCs, including superficial and nodular variants, which raise the possibility that different subtypes may arise from different epidermal cellular compartments. Murine BCC pathogenesis is highly analogous to human BCC and thus offers an ideal model to tackle this question.

Early immunohistochemical studies of BCCs demonstrated that these tumors expressed a cytokeratin profile similar to follicular ORS and distinct from IFE, suggesting that BCCs arise from the hair follicle [37]. The observation that BCCs preferentially arise during anagen stage in mice lacking one allele of Ptch1 seems to further verify this notion. However, recently Gratchchouk et al. demonstrated that BCCs can arise from the bulge, sebaceous glands, and IFE after Gli2 induction, and interestingly, different subtypes of BCCs (superficial versus nodular) developed depending on which compartments were being targeted for induction [38]. Prior to this observation, studies that utilized inducible expression of overactive Smo targeted to murine IFE, hair matrix transit amplifying progenitors, and bulge stem cells found that BCCs were induced in tail and ear skin, although Smo expression is not typically observed in the skin these anatomical locations. Interestingly, the authors observed that BCC only arose from the IFE and the infundibulum but not from the bulge [39]. Soon afterwards, Wang et al. presented conflicting data using lineage tracing (K15CrePR transgenic mice) in adult Ptch1^{+/-} mice to demonstrate that X-ray induced BCC arise exclusively from the follicular bulge [40], and that deletion of p53 enhanced BCCs development from both the bulge and IFE in Ptch1^{+/-} mice. Taken together,

these studies suggest that BCC primarily originate from hair follicle bulge stem cells [40,41] but may be derived from other epidermal compartments under certain conditions [39,40]. Differences in the localization of BCCs to the bulge or IFE as revealed by the above experiments likely highlight the different impact of various HH signaling components on bulge versus IFE cells. For example, constitutive Smo activation may be unable to induce BCCs in the bulge secondary to unique HH suppressive pathways that exist in the bulge but not in IFE. These same pathways may only be activated in light of an activating abnormal allele and may be quiescent in the face of allelic deletion of an HH suppressor (i.e. Ptch1).

The observation that BCC can arise from multiple epidermal compartments is further supported by recent studies demonstrating the contributing role of wounding in BCC formation. Both Kasper et al. and Wong and Reiter used lineage tracing to observe that bulge stem cells, when activated with the HH pathway, can migrate to full-thickness wound IFE and initiate superficial BCC-like tumors in the IFE [42,43]. Wounding seemed to increase the tumorigenic potential of HH-activated bulge stem cells (but not IFE cells), leading to more BCC lesions and larger BCC lesions through unclear mechanisms [42]. However, both studies provide a plausible explanation for increased skin tumor incidence that is commonly associated with wounding.

4. Merkel cell carcinoma

Merkel cell carcinoma (MCC) is a rare cutaneous malignancy that consists of neuroendocrine Merkel cells and displays a very aggressive behavior typified by high rates of local recurrence and distant metastasis [44]. While MCCs are relatively rare compared to other types of nonmelanoma skin cancer, the incidence of MCC cases has tripled over the last decade with less than half of MCC patients surviving more than a few years following detection of lymph node involvement. Importantly, MCC incidence is at least 10-fold greater in AIDS and organ transplant patients as well as patients exposed to ultraviolet radiation [45–48] indicating that immune suppression may be a critical risk factor MCC. Human epidemiological evidence also correlates MCC incidence with higher geographical latitudes and lack of cutaneous melanin pigment [49] implicating ultraviolet radiation as an important etiological agent in MCC formation.

MCC research has been revolutionized in recent years following the discoveries that a hitherto unidentified Merkel cell polyomavirus (MCPyV) is clonally integrated in the majority of MCCs [49] and that viral expression is essential for tumor development in MCPyV+ MCC [50]. Polyomaviruses are known to infect progenitor cells preferentially [51]. This suggests that MCC may be derived from Merkel cell progenitors rather than from mature, differentiated Merkel cells, a theory that is supported by case reports of MCCs exhibiting multilineage (e.g. sarcomatous) differentiation [52]. Efforts to successfully identify cells of origin for MCC will likely benefit from more thorough characterization of the progenitor niche responsible for maintaining normal Merkel cells.

Merkel cells are putative mechanosensory receptor cells that exhibit neuroendocrine origin. Their developmental origins were recently elucidated, when Van Keymeulen et al. and Morrison et al. both independently demonstrated that Merkel cells arise from epidermal progenitors and not from the neural crest [53,54]. Woo et al recently advanced the identity of Merkel cell progenitors. The authors discovered a hitherto undescribed stem cell population residing in the touch domes (specialized epithelial structures on the IFE containing unusual columnar keratinocytes and Merkel cell-neurite complexes) of murine skin that can be identified by the co-expression of $\alpha 6$ integrin, Sca1, and CD200 surface proteins [5]. These so-called “touch dome progenitor cells” exhibit bipotent behavior and

can reconstitute both IFE and Merkel cell-containing touch domes following grafting onto Nude mice, whereas the remainder of IFE keratinocytes cannot produce Merkel cells [5].

Additional insight into MCC-initiating cells come from immunolabeling analyses of MCCs demonstrating positive staining for stem cell markers such as keratin 19, but interestingly, absent staining for keratin 15 [55]. Notably, the negative K15 expression suggests that bulge stem cells are unlikely to play a significant role in MCC pathogenesis. Overall, our understanding of MCC pathogenesis is still in its infancy. Fortunately, the recent discoveries of the MCPyV and Merkel cell progenitors should facilitate future efforts to characterize the putative cells of origin for MCC.

5. Role of the microenvironment in NMSC tumorigenesis

The paradigm of cancer holds that a tumor is an uncontrolled proliferation initiated by dividing cells harboring oncogenic genetic hits. Cutaneous stem cells are uniquely able to accumulate genetic lesions necessary for tumor formation due to their persistence in the epidermis, and thus are thought to play central roles in pathogenesis of skin cancers [3]. However, the transformation of otherwise normal adult stem cells is not sufficient to form a frank tumor in the skin and a number of studies have highlighted the importance of non-dividing, differentiated epidermal cells in promoting the clonal expansion, via regulation of a pro-tumorigenic epidermal microenvironment, of mutant stem cell niches.

Using an involucrin promoter to target differentiated, suprabasal keratinocytes, Hobbs et al. showed that constitutive activation of the ERK/MAPK pathway in post-mitotic suprabasal cells (targeted expression via the Involucrin promoter) stimulated proliferation of the basal epidermal layer leading to epidermal hyperproliferation and even papilloma formation in older mice [56]. This pro-tumorigenic effect of suprabasal keratinocytes on basal cells was driven by recruitment of immune cells to the epidermis and dermis and posits the possibility that diffusible mediators released by inflammatory cells are vital to early tumor proliferation. The role of inflammation was again reaffirmed when this same transgenic mouse system was later employed to establish that papillomas and keratoacanthomas could be induced through full-thickness wounding of the skin [57]. Notably, this study conclusively established that ERK activation in transgene-positive suprabasal keratinocytes recruited transgene-negative basal cells to contribute to tumor formation, by using chimeric mice to tag transgene-negative cells with GFP. GFP+ cells, which did not possess constitutive ERK activation, were fully integrated into the tumors (histologically indistinguishable from transgene-positive cells) and were responsible for the bulk of tumor growth. Thus, these studies established that non-dividing, differentiated epidermal cells can recruit undifferentiated stem cells to become integrated into a tumor become the primary source of growth for the tumor, perhaps through release of a paracrine inflammatory mediator [56,57]. IL-1 α was discovered to potentially play this role, as knockdown of IL-1 with an IL-1 receptor antagonist led to fewer and slower-growing tumors [57]. Aberrant expression of $\alpha 6\beta 4$ integrin in the suprabasal epidermal layers is a molecular signature of human and murine SCC pathogenesis. Further evidence for the role of differentiated epidermal cells regulating the tumor microenvironment has been demonstrated using an integrin transgenic mouse model, Inva $\alpha 6\beta 4$, where the $\alpha 6\beta 4$ integrin heterodimer is targeted to the suprabasal layers via the Involucrin promoter [58]. Forced expression of $\alpha 6\beta 4$ integrin in the suprabasal layers of murine epidermis stimulates DMBA-induced SCC formation; however, the reported increase in SCCs in $\alpha 6\beta 4$ integrin transgenic mice was not due proliferation in the differentiated keratinocyte layers [58]. Instead it was discovered that suprabasal $\alpha 6\beta 4$ expression actually blocked TGF- β growth inhibition in basal epidermal cells [58]. More recently it has been shown that suprabasal keratinocytes in Inva $\alpha 6\beta 4$ release increased levels of M-CSF in response to acute tumor promoter treatment compared to wild-

type mice [59]. The significance of increased M-CSF production was linked to an increased influx of inflammatory cells, including myeloid-derived suppressor cells (MDSCs), into the dermis, which could be blocked by systemic administration of M-CSF neutralizing antibodies [59]. Collectively, these studies highlight multiple roles for differentiated epidermal cells in nonmelanoma skin cancer by their ability to directly influencing the proliferation of transformed basal cells and by stimulating the recruitment of pro-tumorigenic inflammatory leukocytes.

Lastly, studies have established that differentiated cells can regain the ability to self-renew and dedifferentiate into less committed lineages. Mannik et al. showed that involucrin⁺ (terminally-differentiated) keratinocytes were able to reform multi-lineage, self-renewing skin epithelia following transplantation *in vivo* [60]. This raises the possibility that TICs may arise from differentiated progenitor cells that through dysregulation of signaling pathways gain stem cell characteristics enabling them to behave as TICs. Therefore, non-dividing differentiated keratinocytes should not be overlooked as a source of potential TICs in cutaneous tumors [25–27], as these cells can re-enter the cell cycle and acquire stem cell like properties when transplanted to a suitable environment.

6. Concluding remarks

TICs are already well described in hematologic and central nervous system malignancies [61], and convincing evidence exists suggesting that epidermal tumors are also propagated and maintained by TICs. Besides the evidence laid out in this review for SCC, BCC, and MCC, pathologists have observed for decades that epidermal tumors can display characteristics of multi-lineage differentiation (e.g. BCCs with squamous differentiation), thus hinting that skin malignancies may driven by cells that once had multipotent characteristics. Though, skin stem cells represent a logical target for carcinogenesis, their contribution to initiation and formation of NMSC remains incompletely understood. Given that non-dividing differentiated cells have been shown to initiate skin tumors, it remains unproven whether epidermal TICs evolve from somatic stem cells or can also arise from differentiated, non-stem cells. The emerging evidence that certain pro-oncogenic signals are equally important as determinants of TICs as epidermal lineage origin further complicates this situation. Understanding both the localization of TICs (and their associated unique markers), as well as the perturbed pathways that enable their tumorigenic capabilities, will be important to enable therapies that can specifically target malignant self-renewing cells without destroying normal stem cells responsible for skin homeostasis.

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References

1. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer*. 2003; 3:895–902. [PubMed: 14737120]
2. Knudson AG Jr. Genetics of human cancer. *Genetics*. 1975; 79(Suppl):305–316. [PubMed: 238902]
3. Owens DM, Watt FM. Contribution of stem cells and differentiated cells to epidermal tumours. *Nat Rev Cancer*. 2003; 3:444–451. [PubMed: 12778134]
4. Yan XY, Owens DM. The skin: a home to multiple classes of epithelial progenitor cells. *Stem Cell Rev*. 2008; 4:113–118. [PubMed: 18491239]
5. Woo SH, Stumpfova M, Jensen UB, Lumpkin EA, Owens DM. Identification of epidermal progenitors for the Merkel cell lineage. *Development*. 2010; 137:3965–3971. [PubMed: 21041368]

6. Ghazizadeh S, Taichman LB. Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. *EMBO J.* 2001; 20:1215–1222. [PubMed: 11250888]
7. Clayton E, Doupe DP, Klein AM, Winton DJ, Simons B, Jones PH. A single type of progenitor cell maintains normal epidermis. *Nature.* 2007; 446:185–189. [PubMed: 17330052]
8. Horsley V, O'Carroll D, Tooze R, Ohinata Y, Saitou M, Obukhanych T, Nussenzweig M, Tarakhovsky A. Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland. *Cell.* 2006; 126:597–609. [PubMed: 16901790]
9. Levy V, Lindon C, Harfe BD, Morgan BA. Distinct stem cell populations regenerate the follicle and interfollicular epidermis. *Dev Cell.* 2005; 9:855–861. [PubMed: 16326396]
10. Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, Cotsarelis G. Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med.* 2005; 11:1351–1354. [PubMed: 16288281]
11. Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, Clevers H, Toftgard R. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat Genet.* 2008; 40:1291–1299. [PubMed: 18849992]
12. Snippert HJ, Haegerbarth A, Kasper M, Jaks V, van Es JH, Barker N, van de Wetering M, van den Born M, Begthel H, Vries RG, Stange DE, Toftgard R, Clevers H. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. *Science.* 2010; 327:1385–1389. [PubMed: 20223988]
13. Morris RJ, Tacker KC, Fischer SM, Slaga TJ. Quantitation of primary in vitro clonogenic keratinocytes from normal adult murine epidermis, following initiation, and during promotion of epidermal tumors. *Cancer Res.* 1988; 48:6285–6290. [PubMed: 2460219]
14. Morris RJ, Fischer SM, Slaga TJ. Evidence that a slowly cycling subpopulation of adult murine epidermal cells retains carcinogen. *Cancer Res.* 1986; 46:3061–3066. [PubMed: 3698024]
15. Morris RJ, Fischer SM, Klein-Szanto AJ, Slaga TJ. Subpopulations of primary adult murine epidermal basal cells sedimented on density gradients. *Cell Tissue Kinet.* 1990; 23:587–602. [PubMed: 2177380]
16. Morris RJ, Coulter K, Tryson K, Steinberg SR. Evidence that cutaneous carcinogen-initiated epithelial cells from mice are quiescent rather than actively cycling. *Cancer Res.* 1997; 57:3436–3443. [PubMed: 9270010]
17. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. *N Engl J Med.* 2001; 344:975–983. [PubMed: 11274625]
18. Jonason AS, Kunala S, Price GJ, Restifo RJ, Spinelli HM, Persing JA, Leffell DJ, Tarone RE, Brash DE. Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc Natl Acad Sci USA.* 1996; 93:14025–14029. [PubMed: 8943054]
19. Zhang W, Remenyik E, Zeltermann D, Brash DE, Wikonkal NM. Escaping the stem cell compartment: sustained UVB exposure allows p53-mutant keratinocytes to colonize adjacent epidermal proliferating units without incurring additional mutations. *Proc Natl Acad Sci USA.* 2001; 98:13948–13953. [PubMed: 11707578]
20. Quintanilla M, Brown K, Ramsden M, Balmain A. Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis. *Nature.* 1986; 322:78–80. [PubMed: 3014349]
21. Uribe P, Gonzalez S. Epidermal growth factor receptor (EGFR) and squamous cell carcinoma of the skin: molecular bases for EGFR-targeted therapy. *Pathol Res Pract.* 2011; 207:337–342. [PubMed: 21531084]
22. Su F, Viros A, Milagre C, Trunzer K, Bollag G, Spleiss O, Reis-Filho JS, Kong X, Koya RC, Flaherty KT, Chapman PB, Kim MJ, Hayward R, Martin M, Yang H, Wang Q, Hilton H, Hang JS, Noe J, Lambros M, Geyer F, Dhomen N, Niculescu-Duvaz I, Zambon A, Niculescu-Duvaz D, Preece N, Robert L, Otte NJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Kockx M, Andries L, Lestini B, Nolop KB, Lee RJ, Joe AK, Troy JL, Gonzalez R, Hutson TE, Puzanov I, Chmielowski B, Springer CJ, McArthur GA, Sosman JA, Lo RS, Ribas A, Marais R. RAS mutations in cutaneous squamous-cell carcinomas in patients treated with BRAF inhibitors. *N Engl J Med.* 2012; 366:207–215. [PubMed: 22256804]
23. Brown K, Strathdee D, Bryson S, Lambie W, Balmain A. The malignant capacity of skin tumours induced by expression of a mutant H-ras transgene depends on the cell type targeted. *Curr Biol.* 1998; 8:516–524. [PubMed: 9560338]

24. White AC, Tran K, Khuu J, Dang C, Cui Y, Binder SW, Lowry WE. Defining the origins of Ras/p53-mediated squamous cell carcinoma. *Proc Natl Acad Sci USA*. 2011; 108:7425–7430. [PubMed: 21502519]
25. Lapouge G, Youssef KK, Vokaer B, Achouri Y, Michaux C, Sotiropoulou PA, Blanpain C. Identifying the cellular origin of squamous skin tumors. *Proc Natl Acad Sci USA*. 2011; 108:7431–7436. [PubMed: 21502497]
26. Greenhalgh DA, Rothnagel JA, Quintanilla MI, Orenge CC, Gagne TA, Bundman DS, Longley MA, Roop DR. Induction of epidermal hyperplasia, hyperkeratosis, and papillomas in transgenic mice by a targeted v-Ha-ras oncogene. *Mol Carcinog*. 1993; 2:99–110. [PubMed: 7681293]
27. Pelengaris S, Littlewood T, Khan M, Elia G, Evan G. Reversible activation of c-Myc in skin: induction of a complex neoplastic phenotype by a single oncogenic lesion. *Mol Cell*. 1999; 5:565–577. [PubMed: 10360173]
28. Li S, Park H, Trempus CS, Gordon D, Liu Y, Cotsarelis G, Morris RJ. A keratin 15 containing stem cell population from the hair follicle contributes to squamous papilloma development in the mouse. *Mol Carcinog*. 10.1002/mc.21896
29. Malanchi I, Peinado H, Kassen D, Hussenet T, Metzger D, Chambon P, Huber M, Hohl D, Cano A, Birchmeier W, Huelsken J. Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling. *Nature*. 2008; 452:650–653. [PubMed: 18385740]
30. Trempus CS, Morris RJ, Ehinger M, Elmore A, Bortner CD, Ito M, Cotsarelis G, Nijhof JG, Peckham J, Flagler N, Kissling G, Humble MM, King LC, Adams LD, Desai D, Amin S, Tennant RW. CD34 expression by hair follicle stem cells is required for skin tumor development in mice. *Cancer Res*. 2007; 67:4173–4181. [PubMed: 17483328]
31. Schober M, Fuchs E. Tumor-initiating stem cells of squamous cell carcinomas and their control by TGF-beta and integrin/focal adhesion kinase (FAK) signaling. *Proc Natl Acad Sci USA*. 2011; 108:10544–10549. [PubMed: 21670270]
32. Rogers HW, Weinstock MA, Harris AR, Hinckley MR, Feldman SR, Fleischer AB, Coldiron BM. Incidence estimate of nonmelanoma skin cancer in the United States, 2006. *Arch Dermatol*. 2010; 146:283–287. [PubMed: 20231499]
33. Athar M, Tang X, Lee JL, Kopelovich L, Kim AL. Hedgehog signalling in skin development and cancer. *Exp Dermatol*. 2006; 15:667–677. [PubMed: 16881963]
34. Aszterbaum M, Beech J, Epstein EH Jr. Ultraviolet radiation mutagenesis of hedgehog pathway genes in basal cell carcinomas. *J Invest Dermatol Symp Proc*. 1999; 4:41–45.
35. Tojo M, Kiyosawa H, Iwatsuki K, Nakamura K, Kaneko F. Expression of the GLI2 oncogene and its isoforms in human basal cell carcinoma. *Br J Dermatol*. 2003; 148:892–897. [PubMed: 12786818]
36. Kallassy M, Toftgard R, Ueda M, Nakazawa K, Vorechovsky I, Yamasaki H, Nakazawa H. Patched (ptch)-associated preferential expression of smoothened (smoh) in human basal cell carcinoma of the skin. *Cancer Res*. 1997; 57:4731–4735. [PubMed: 9354432]
37. Asada M, Schaart FM, de Almeida HL Jr, Korge B, Kurokawa I, Asada Y, Orfanos CE. Solid basal cell epithelioma (BCE) possibly originates from the outer root sheath of the hair follicle. *Acta Derm Venereol*. 1993; 73:286–292. [PubMed: 7506469]
38. Grachtchouk M, Pero J, Yang SH, Ermilov AN, Michael LE, Wang A, Wilbert D, Patel RM, Ferris J, Diener J, Allen M, Lim S, Syu LJ, Verhaegen M, Dlugosz AA. Basal cell carcinomas in mice arise from hair follicle stem cells and multiple epithelial progenitor populations. *J Clin Invest*. 2011; 121:1768–1781. [PubMed: 21519145]
39. Youssef KK, Van Keymeulen A, Lapouge G, Beck B, Michaux C, Achouri Y, Sotiropoulou PA, Blanpain C. Identification of the cell lineage at the origin of basal cell carcinoma. *Nat Cell Biol*. 2010; 12:299–305. [PubMed: 20154679]
40. Wang GY, Wang J, Mancianti ML, Epstein EH Jr. Basal cell carcinomas arise from hair follicle stem cells in Ptc1(+/-) mice. *Cancer Cell*. 2011; 19:114–124. [PubMed: 21215705]
41. Seykora JT, Cotsarelis G. Keratin 15-positive stem cells give rise to basal cell carcinomas in irradiated Ptc1(+/-) mice. *Cancer Cell*. 2011; 19:5–6. [PubMed: 21251609]

42. Kasper M, Jaks V, Are A, Bergstrom A, Schwager A, Barker N, Toftgard R. Wounding enhances epidermal tumorigenesis by recruiting hair follicle keratinocytes. *Proc Natl Acad Sci USA*. 2011; 108:4099–4104. [PubMed: 21321199]
43. Wong SY, Reiter JF. Wounding mobilizes hair follicle stem cells to form tumors. *Proc Natl Acad Sci USA*. 2011; 108:4093–4098. [PubMed: 21321207]
44. Schrama D, Ugurel S, Becker JC. Merkel cell carcinoma: recent insights and new treatment options. *Curr Opin Oncol*. 2012; 24:141–149. [PubMed: 22234254]
45. Engels EA, Frisch M, Goedert JJ, Biggar RJ, Miller RW. Merkel cell carcinoma and HIV infection. *Lancet*. 2002; 359:497–498. [PubMed: 11853800]
46. Penn I, First MR. Merkel's cell carcinoma in organ recipients: report of 41 cases. *Transplantation*. 1999; 68:1717–1721. [PubMed: 10609948]
47. Lunder EJ, Stern RS. Merkel-cell carcinomas in patients treated with methoxsalen and ultraviolet A radiation. *N Engl J Med*. 1998; 339:1247–1248. [PubMed: 9786759]
48. Miller RW, Rabkin CS. Merkel cell carcinoma and melanoma: etiological similarities and differences. *Cancer Epidemiol Biomarkers Prev*. 1999; 8:153–158. [PubMed: 10067813]
49. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science*. 2008; 319:1096–1100. [PubMed: 18202256]
50. Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, Moore PS, Becker JC. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. *J Virol*. 2010; 84:7064–7072. [PubMed: 20444890]
51. Hou J, Seth P, Major EO. JC virus can infect human immune and nervous system progenitor cells: implications for pathogenesis. *Adv Exp Med Biol*. 2006; 577:266–273. [PubMed: 16626042]
52. Hwang JH, Alanen K, Dabbs KD, Danyluk J, Silverman S. Merkel cell carcinoma with squamous and sarcomatous differentiation. *J Cutan Pathol*. 2008; 35:955–959. [PubMed: 18494821]
53. Morrison KM, Miesegaes GR, Lumpkin EA, Maricich SM. Mammalian Merkel cells are descended from the epidermal lineage. *Dev Biol*. 2009; 336:76–83. [PubMed: 19782676]
54. Van Keymeulen A, Mascré G, Youseff KK, Harel I, Michaux C, De Geest N, Szpalski C, Achouri Y, Bloch W, Hassan BA, Blanpain C. Epidermal progenitors give rise to Merkel cells during embryonic development and adult homeostasis. *J Cell Biol*. 2009; 187:91–100. [PubMed: 19786578]
55. Abbas O, Bhawan J. Expression of stem cell markers nestin and cytokeratin 15 and 19 in cutaneous malignancies. *J Eur Acad Dermatol Venereol*. 2011; 25:311–316. [PubMed: 20629844]
56. Hobbs RM, Silva-Vargas V, Groves R, Watt FM. Expression of activated MEK1 in differentiating epidermal cells is sufficient to generate hyperproliferative and inflammatory skin lesions. *J Invest Dermatol*. 2004; 123:503–515. [PubMed: 15304090]
57. Arwert EN, Lal R, Quist S, Rosewell I, van Rooijen N, Watt FM. Tumor formation initiated by nondividing epidermal cells via an inflammatory infiltrate. *Proc Natl Acad Sci USA*. 2010; 107:19903–19908. [PubMed: 21041641]
58. Owens DM, Romero RM, Gardner C, Watt FM. Suprabasal $\alpha 6 \beta 4$ integrin expression in epidermis results in enhanced tumorigenesis and disruption of TGF β signaling. *J Cell Sci*. 2003; 116:3783–3791. [PubMed: 12902406]
59. Maalouf SW, Theivakumar S, Owens DM. Epidermal $\alpha 6 \beta 4$ integrin stimulates the influx of immunosuppressive cells during skin tumor promotion. *J Dermatol Sci*. 2012; 66:108–118. [PubMed: 22464766]
60. Mannik J, Alzayady K, Ghazizadeh S. Regeneration of multilineage skin epithelia by differentiated keratinocytes. *J Invest Dermatol*. 2010; 130:388–397. [PubMed: 19675579]
61. Lobo NA, Shimono Y, Qian D, Clarke MF. The biology of cancer stem cells. *Annu Rev Cell Dev Biol*. 2007; 23:675–699. [PubMed: 17645413]
62. Yuspa SH, Kilkenny AD, Steinert PM, Roop DR. Expression of murine epidermal differentiation markers is tightly regulated by restricted extracellular calcium concentrations in-vitro. *J Cell Biol*. 1989; 109:1207–1217. [PubMed: 2475508]
63. Peters B, Kirfel J, Bussow H, Vidal M, Magin TM. Complete cytolysis and neonatal lethality in keratin 5 knockout mice reveal its fundamental role in skin integrity and in epidermolysis bullosa simplex. *Mol Biol Cell*. 2001; 12:1775–1789. [PubMed: 11408584]

64. Liu Y, Lyle S, Yang Z, Cotsarelis G. Keratin 15 promoter targets putative epithelial stem cells in the hair follicle bulge. *J Invest Dermatol.* 2003; 121:963–968. [PubMed: 14708593]
65. Vassar R, Rosenberg M, Ross S, Tyner A, Fuchs E. Tissue-specific and differentiation-specific expression of a human K14 keratin gene in transgenic mice. *Proc Natl Acad Sci USA.* 1989; 86:1563–1567. [PubMed: 2466292]
66. Michel M, Torok N, Godbout MJ, Lussier M, Gaudreau P, Royal A, Germain L. Keratin 19 as a biochemical marker of skin stem cells in vivo and in vitro: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donor age and culture stage. *J Cell Sci.* 1996; 109(Pt 5):1017–1028. [PubMed: 8743949]
67. Reichelt J, Magin TM. Hyperproliferation, induction of c-Myc and 14-3-3sigma, but no cell fragility in keratin-10-null mice. *J Cell Sci.* 2002; 115:2639–2650. [PubMed: 12077355]
68. Trempus CS, Morris RJ, Bortner CD, Cotsarelis G, Faircloth RS, Reece JM, Tennant RW. Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. *J Invest Dermatol.* 2003; 120:501–511. [PubMed: 12648211]
69. Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E. Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell.* 2004; 118:635–648. [PubMed: 15339667]
70. Kloepper JE, Tiede S, Brinckmann J, Reinhardt DP, Meyer W, Faessler R, Paus R. Immunophenotyping of the human bulge region: the quest to define useful in situ markers for human epithelial hair follicle stem cells and their niche. *Exp Dermatol.* 2008; 17:592–609. [PubMed: 18558994]
71. Jensen UB, Yan X, Triel C, Woo SH, Christensen R, Owens DM. A distinct population of clonogenic and multipotent murine follicular keratinocytes residing in the upper isthmus. *J Cell Sci.* 2008; 121:609–617. [PubMed: 18252795]
72. Vidal VP, Chaboissier MC, Lutzkendorf S, Cotsarelis G, Mill P, Hui CC, Ortonne N, Ortonne JP, Schedl A. Sox9 is essential for outer root sheath differentiation and the formation of the hair stem cell compartment. *Curr Biol.* 2005; 15:1340–1351. [PubMed: 16085486]
73. Nowak JA, Polak L, Pasolli HA, Fuchs E. Hair follicle stem cells are specified and function in early skin morphogenesis. *Cell Stem Cell.* 2008; 3:33–43. [PubMed: 18593557]
74. Rosenblum MD, Olasz EB, Yancey KB, Woodliff JE, Lazarova Z, Gerber KA, Truitt RL. Expression of CD200 on epithelial cells of the murine hair follicle: a role for tissue-specific immune tolerance? *J Invest Dermatol.* 2004; 123:880–887. [PubMed: 15482475]
75. Ohyama M, Terunuma A, Tock CL, Radonovich MF, Pise-Masison CA, Hopping SB, Brady JN, Udey MC, Vogel JC. Characterization and isolation of stem cell-enriched human hair follicle bulge cells. *J Clin Invest.* 2006; 116:249–260. [PubMed: 16395407]
76. Brownell I, Guevara E, Bai CB, Loomis CA, Joyner AL. Nerve-derived sonic hedgehog defines a niche for hair follicle stem cells capable of becoming epidermal stem cells. *Cell Stem Cell.* 2011; 8:552–565. [PubMed: 21549329]
77. Nijhof JG, Braun KM, Giangreco A, van Pelt C, Kawamoto H, Boyd RL, Willemze R, Mullenders LH, Watt FM, de Gruijl FR, van Ewijk W. The cell-surface marker MTS24 identifies a novel population of follicular keratinocytes with characteristics of progenitor cells. *Development.* 2006; 133:3027–3037. [PubMed: 16818453]
78. Tani H, Morris RJ, Kaur P. Enrichment for murine keratinocyte stem cells based on cell surface phenotype. *Proc Natl Acad Sci USA.* 2000; 97:10960–10965. [PubMed: 11005869]
79. Jensen KB, Collins CA, Nascimento E, Tan DW, Frye M, Itami S, Watt FM. Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. *Cell Stem Cell.* 2009; 4:427–439. [PubMed: 19427292]
80. Greco V, Chen T, Rendl M, Schober M, Pasolli HA, Stokes N, Dela Cruz-Racelis J, Fuchs E. A two-step mechanism for stem cell activation during hair regeneration. *Cell Stem Cell.* 2009; 4:144–169.

Table 1

Keratinocyte (stem and non-stem cells) markers

Marker(s)	Labeled regions	Comments	References
Krt5	Basal cells of IFE and ORS of HF	Maintains integrity of basal layer	[62,63]
Krt15	Bulge	Multipotent; can form HF, SG, and IFE; participates in epidermal repair	[10,64]
Krt14	Basal cells of HF, IFE, and sebaceous glands	Marks LRC in ORS	[62,65]
Krt19	Bulge	Not as specific for stem cells as Krt15. May label some TA cells.	[66]
Krt10	Suprabasal layer of IFE	Maintains epidermal integrity and inhibits cellular proliferation	[62,67]
Shh	Hair matrix transient amplifying cells	Controversial stem cell role. Cannot form BCCs even with constitutive Smo	[9,39]
$\alpha 6\beta 1$ integrin	ORS, bulge, IFE	Marks SCC TICs	[31]
CD34	Bulge	Specific marker of HF bulge in mice; Multipotent; Can form HF, SG, and IFE	[68,69]
Tenascin-C	Bulge	Also stains human bulge	[70]
Blimp1	Sebaceous gland	Unipotent, can only form SG	[8]
Lgr5	Lower bulge	Multipotent; can form HF, SG, and IFE	[11]
Lgr6	Isthmus	Multipotent; can form SG, IFE, and HF; participates in long-term wound repair	[12]
$\alpha 6^{\text{lo}}\text{CD}34^{\text{lo}}\text{Sca-1}^{\text{lo}}$	Isthmus	Multipotent; can form permanent SG, IFE, and HF lineages	[71]
Sox9	Bulge and hair follicle ORS	Required for development of HF stem cells	[72,73]
CD200	Bulge and touch dome progenitor cells	Found in human and mouse HF bulge	[5,74,75]
Gli1	Bulge and isthmus	Repairs IFE on wounding	[76]
MTS24 (Plet-1)	HF isthmus and infundibulum	Highly clonogenic <i>in vitro</i> and can reconstitute IFE, HF, and SG.	[77]
$\alpha 6^{\text{bright}}\text{CD}71^{\text{dim}}$	IFE	Enriched for IFE progenitors	[78]
Lrig1 ⁺	Junctional zone	Marks multipotent stem cells	[79]
P-cadherin	Hair germ	Progenitors provide cellular input into anagen HF	[80]