Wounding mobilizes hair follicle stem cells to form tumors

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A wide variety of human cancers are associated with injury. Although stem cells participate in tissue regeneration after wounding, it is unclear whether these cells also contribute to epithelial tumors. Human basal cell carcinomas (BCCs) are associated with misactivation of Hedgehog (Hh) signaling, commonly through acquisition of mutations in *Smoothened* (*Smo*). We have found that expression of an activated form of Smo by stem cells of the hair-follicle bulge and secondary hair germ does not induce robust Hh signaling or produce BCCs. However, wounding recruits these cells from the follicle to the wound site, where downstream Hh signal transduction is derepressed, giving rise to superficial BCC-like tumors. These findings demonstrate that BCC-like tumors can originate from follicular stem cells and provide an explanation for the association between wounding and tumorigenesis.

skin cancer | wound healing | carcinogenesis | reepithelialization

Wound repair is a complicated process involving inflammation, cell migration, proliferation, and tissue remodeling (1–3). Aberrant wound healing with chronic inflammation can promote malignant transformation, and a variety of human cancers, including those arising from the lung, liver, pancreas, bone, and skin, are associated with injury (2, 4). Epithelial stem cells are thought to play critical roles during wound repair, but, under homeostatic conditions, these cells are maintained in quiescent niches that preserve their ability to self-renew and give rise to differentiated progeny (5, 6). Whether stem cells are the cell of origin for most cancers is currently unclear.

Discrete stem-cell populations maintain the hair follicle and interfollicular epidermis (IFE) (7). Basal layer epidermal stem cells replenish the overlying stratified epidermis (6, 8), whereas stem cells located in the follicular bulge and secondary hair germ (SHG) regenerate the lower hair follicle and hair shaft during anagen (9–13). Upon cutaneous wounding, bulge-derived cells migrate into the epidermis, where they lose expression of follicle-specific genes and adopt an epidermal-like phenotype (14, 15). However, the majority of these cells contribute only transiently to the healed wound epithelium (14).

Human basal cell carcinomas (BCCs) are thought to arise from the hair follicle. Indeed, BCCs share certain properties of follicular stem cells, as these tumors are often composed of cells that are slow-growing, poorly differentiated, and, in some cases, capable of differentiating into adnexal structures (16). Recent studies, however, have suggested that cells in the bulge and SHG do not give rise to BCCs, and that these tumors originate instead from the IFE and follicular infundibulum (17). Given that follicular stem cells enter the IFE upon wounding, we examined whether oncogene-expressing bulge and SHG-derived cells also enter sites of injury to form BCCs.

Results

Misactivation of Hh signaling is the underlying cause of BCC (18–22), and mutations in *Smo*, a central mediator of the Hh pathway, are commonly found in these tumors (23). To assess whether activating the Hh pathway in follicular stem cells causes BCCs, we generated mice that express a conditional oncogenic allele of *Smo* previously isolated from human BCCs (*SmoM2*) (24) under the control of an RU486-inducible Cre recombinase driven by the *Keratin-15* promoter (*K15-Cre^{PR1}*, hereafter referred to as *K15:SmoM2*) (25). *Keratin-15* is expressed postnatally in the bulge and SHG, locations where follicular stem cells reside (25). We induced *SmoM2* expression in telogen skin at 7.5 wk of age and assessed tumor formation 11 wk later. Similar to previously reported results (17), we observed that *K15:SmoM2* skin developed few tumors (Fig. 1*A*). Most hair follicles remained in telogen and did not exhibit overt abnormalities, although occasional focal hyperplasias were detected in the bulge and SHG (Fig. 1*B*). Thus, follicular stem cells appear protected against tumorigenesis.

As expression of *SmoM2* in the bulge and SHG did not lead to BCCs, we examined whether cutaneous injury can collaborate with oncogene expression to promote tumor formation. We induced SmoM2 expression as described above and, 3 d later, generated 0.25-cm^2 full-thickness excisional wounds in the dorsal skin. In contrast to the paucity of tumors in unwounded *K15:SmoM2* skin, clusters of superficial BCC-like tumors formed at the wound site (Fig. 1 *C* and *D*). These tumors were composed of nests of Keratin-5-, Keratin-14-, and Keratin-17-positive cells that radiated downward from the healed epidermis and exhibited high nuclear-to-cytoplasmic ratios and histopathological features of BCC, such as nuclear palisading (Fig. S1). The small size of the wound did not induce follicular neogenesis within the healed epithelium, although intact follicles adjacent to the wound reentered anagen and were hyperplastic (Fig. 1*C* and Fig. S1). In the absence of SmoM2 expression, wounding did not induce tumorigenesis (Fig. 1*C*). These findings indicate that wounding stimulates the formation of BCC-like lesions at sites of injury in *K15:SmoM2* mice.

Unlike *K15:SmoM2* skin, expression of SmoM2 using a tamoxifen-inducible *Keratin-14* promoter-driven Cre recombinase (*K14-Cre^{ERT}*) induces widespread formation of BCC-like tumors (17, 24, 26). To understand how *K14-Cre^{ERT}*-driven expression of SmoM2 promotes tumorigenesis in intact skin, but *K15-Cre^{PR1}* fails to do so, we examined the specificity of *K14-Cre^{ERT}*, reported to be expressed in basal layer cells of the IFE and follicular outer root sheath (27). In tamoxifen-treated *K14-Cre^{ERT}* mice harboring a Cre-inducible *LacZ* reporter (*K14:LacZ*), we observed efficient recombination in the IFE but surprisingly little activity in the bulge and SHG, irrespective of oncogene expression (Fig. 1 *E* and *F* and Fig. S2).

Given that the *K15:SmoM2* wound-induced tumors formed outside of the bulge and SHG, we assessed whether *K15-Cre^{PR1}* induces recombination outside of these domains by using mice possessing the Cre-inducible *LacZ* reporter (*K15:LacZ*). In agreement with previous results (25), we detected β-galactosidase-

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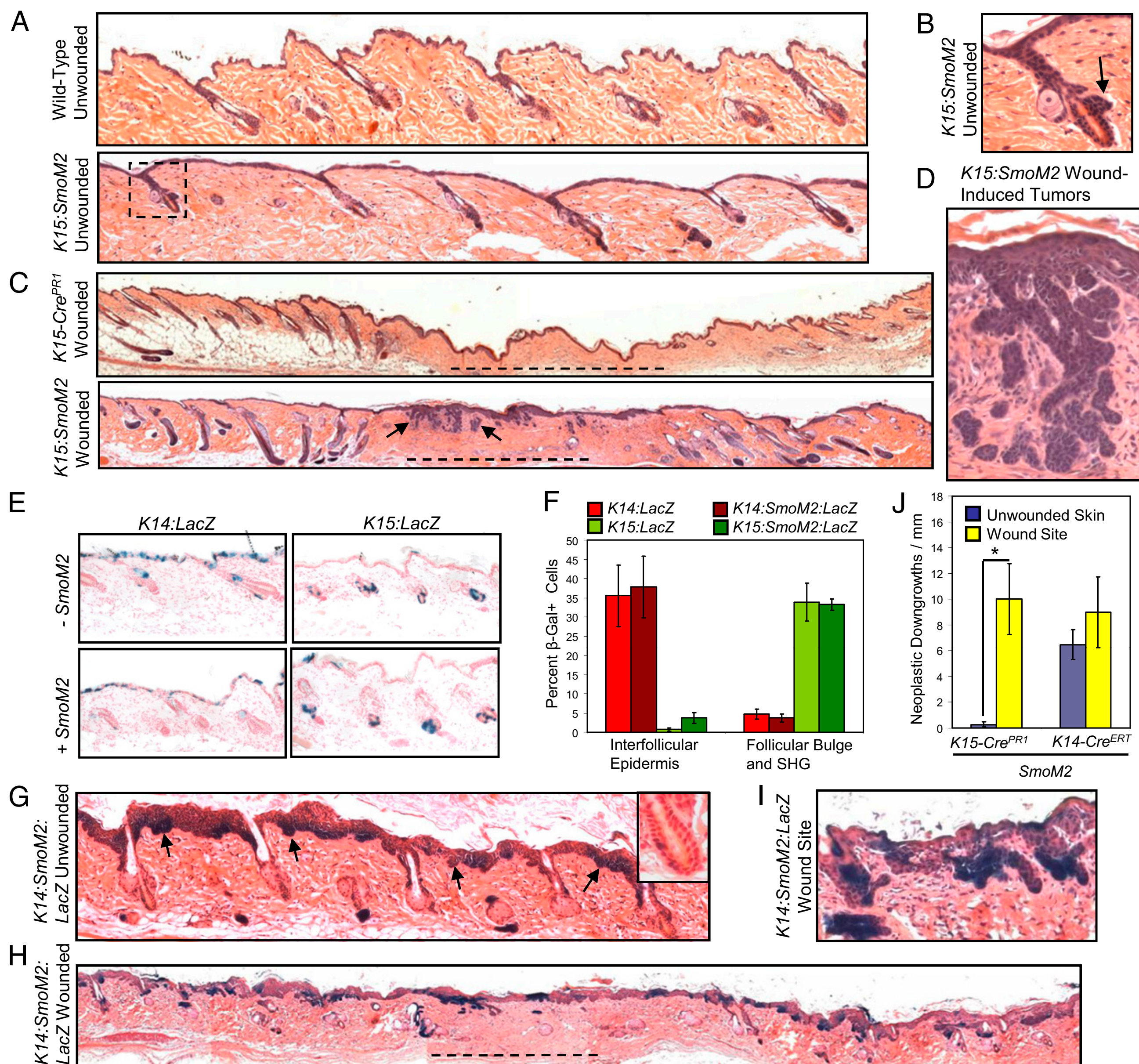

Fig. 1. SmoM2-expressing bulge or SHG-derived cells form superficial BCC-like tumors at sites of injury. (*A*) Intact *K15:SmoM2* dorsal skin, 11 wk after oncogene induction, displays largely normal IFE and telogen hair follicles, similar to wild-type skin. (*B*) Magnified view of the boxed area in *A*, showing a follicle with a focally hyperplastic bulge (arrow). (*C*) RU486-induced *K15:SmoM2* skin, 10 wk after wounding, possesses superficial BCC-like tumors (arrows) at the site of the healed wound (dashed line). Control skin does not form tumors after wounding. (*D*) Magnified view of BCC-like tumors in *C*. (*E*) β-Gal staining indicating that *K14-Cre*^{ERT} and *K15-Cre*^{PR1} exhibit largely reciprocal domains of activity, irrespective of SmoM2 expression, 3 d post induction. (*F*) Quantitation of β-Gal+ cell location 3 d after induction. (*G*) Intact *K14:SmoM2:LacZ* dorsal skin, 11 wk after oncogene induction, contains extensive IFE hyperplasia and BCC-like downgrowths (arrows), without obvious involvement of the bulge (*Inset*). (*H*) *K14:SmoM2:LacZ* skin, 10 wk after wounding, exhibits BCC-like downgrowths equally abundant at the healed wound site (dashed line) and in intact skin. (*I*) Magnified view of tumors from wounded *K14:SmoM2:LacZ* skin. (*J*) Quantitation of BCC-like neoplastic downgrowths, either at the healed wound site or in intact skin (**P* = 0.017). *G*–*I* were stained for LacZ expression. In non–*LacZ*-expressing control animals, no consistent β-Gal staining was detected (data not shown).

positive (β-Gal+) cells in RU486-induced *K15:LacZ* skin overwhelmingly in the bulge and SHG (Fig. 1 *E* and *F* and Fig. S2). In rare instances, we observed β-Gal+ cells in the IFE for both *K15:LacZ* and *K15:SmoM2:LacZ* skin (Fig. 1*F*). Therefore, *K15-Cre*^{PR1} and *K14-Cre*^{ERT} exhibit largely reciprocal domains of activity in the skin, inducing recombination primarily in cells of the bulge and SHG, or of the IFE, respectively.

Because *K15-Cre*^{PR1}-mediated recombination occurred in rare cells in the IFE, it is possible that these SmoM2-expressing IFE cells gave rise to the wound-induced BCC-like tumors in *K15:SmoM2* mice. To test the possibility that wounding promotes IFE cell formation of tumors, we took advantage of *K14:SmoM2:LacZ* mice that express SmoM2 primarily in IFE cells. In the absence of wounding, *K14:SmoM2:LacZ* skin developed widespread BCC-like tumors throughout the IFE, as expected (Fig. 1*G*). In nearly all cases, the follicular bulge appeared normal. After wounding, *K14:SmoM2:LacZ* skin developed superficial BCC-like tumors in the healed epithelium (Fig. 1 *H* and *I*). Unlike *K15:SmoM2* skin, however, wounding did not increase the frequency of tumorigenesis (Fig. 1*J*). Importantly, this result

indicates that wounding does not increase the tumorigenic capacity of SmoM2-expressing IFE cells. Thus, IFE cells are unlikely to account for the injury-induced enhancement in tumorigenesis displayed by *K15:SmoM2* mice. In addition, the absence of increased tumorigenesis in *K14:SmoM2:LacZ* mice suggests that wounding in SmoM2-expressing skin does not promote oncogenesis through a general mechanism, such as an injury-associated inflammatory response.

Because wounding induces the migration of follicular stem cells to sites of injury, we determined whether SmoM2-expressing bulge and SHG cells also migrate into the wound site. At 3 d after wounding, SmoM2-expressing follicular stem cells had migrated from the bulge or SHG into the epidermal leading edge of the wound margin (Fig. 2*A* and Fig. S3). After 10 wk, lineage analysis revealed that bulge or SHG-derived cells could still be detected at the healed wound site (Fig. 2*B*). SmoM2 expression did not affect the radial spoke cell-migration pattern previously reported (14). β-Gal staining revealed that bulge or SHG-derived cells at the wound site contributed normally to the simple stratified epithelium in *K15:LacZ* skin (Fig. 2*B*). In contrast, bulge or SHG-derived cells formed tumors at wound sites of *K15:SmoM2:LacZ* skin (Fig. 2*B*), and these tumors displayed increased proliferation over reepithelialized control skin (Fig. S4). However, we also observed that some tumors were negative for β-Gal, suggesting that β-Gal staining underestimates the number of tumor cells within the wound. Nonetheless, all wound-induced neoplastic downgrowths expressed SmoM2 and Keratin-5 (Fig. S4), indicating that these tumors were of epithelial origin. These data reveal that oncogene-expressing follicular stem cells migrate into sites of injury to seed BCC-like tumors.

We next examined whether SmoM2-expressing bulge or SHG-derived cells, similar to normal follicular stem cells, lose expression of follicular markers and adopt an epidermal-like phenotype upon wound-induced movement into the IFE (14). We assessed whether tumors in *K15:SmoM2* wounded skin express bulge-enriched proteins Keratin-15, Sox9, NFATc1, and CD34 (25, 28–31). These tumors strongly expressed Keratin-15 and Sox9, heterogeneously expressed NFATc1, and did not express CD34 (Fig. 2*C*). In addition, these lesions did not express P-cadherin, which is normally enriched in the SHG (13). Interestingly, BCC-like lesions originating from the IFE of *K14:SmoM2* mice expressed similar markers as the wound-induced tumors of *K15:SmoM2* animals (Fig. 2*C*). These results indicate that bulge or SHG-derived tumors lose some, but not all, markers of their follicular origin and are similar to tumors that arise from the IFE independent of wounding.

To determine how SmoM2 expression by follicular stem cells promotes the formation of BCC-like tumors only upon wounding, we compared the activation of signaling pathways between wounds from wild-type skin and tumorigenic wounds from *K15:SmoM2* mice. In normal skin, we found no evidence of up-regulated Hh or Wnt signaling subsequent to wounding, as assessed by in situ

Fig. 2. Wounding induces the migration of bulge and SHG-derived cells into sites of injury. (*A*) β-Gal+ bulge and SHG-derived cells from *K15:LacZ* and *K15:SmoM2:LacZ* skin migrate into the epithelial tongue (asterisk) at the leading edge of the contracting wound, 3 d after injury. (*Right*) Magnified views of the boxed areas. (*B*) β-Gal staining of healed dorsal skin, 10 wk after wounding, indicates that labeled cells infrequently persist in *K15-Cre^{PR1}* skin. (*Right*) Infrequently retained bulge or SHG-derived β-Gal+ cells contribute normally to the healed epithelium in the absence of oncogene expression but form tumors in the presence of SmoM2. (*C*) Immunofluorescence staining of normal telogen follicles (*Left*), IFE-derived tumors of *K14:SmoM2* skin (*Center*), and wound-induced tumors of *K15:SmoM2* skin (*Right*).

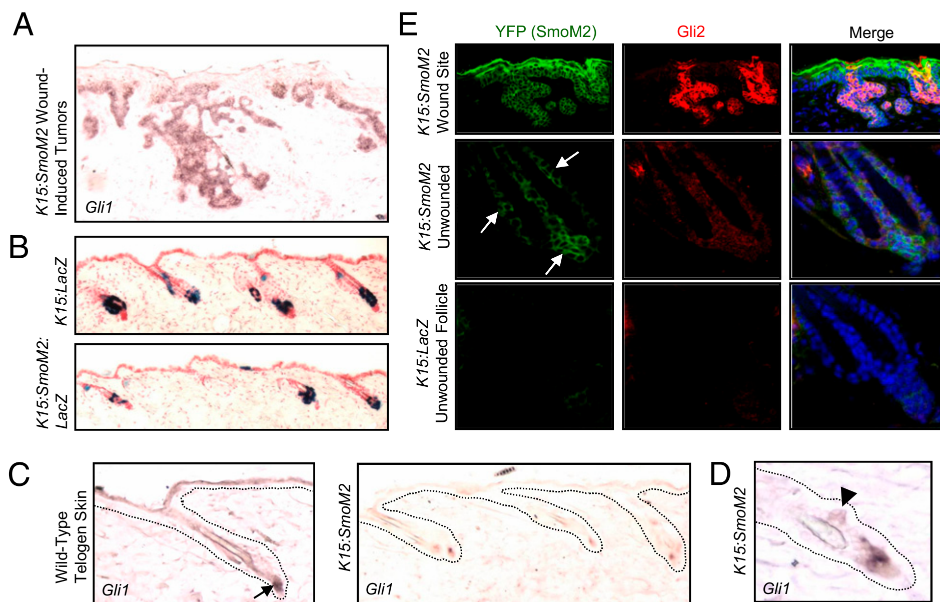

Fig. 3. SmoM2-expressing bulge and SHG cells fail to activate robust Hh signaling. (*A*) In situ staining for *Gli1* in wound-induced tumors from *K15:SmoM2* skin. (*B*) β-Gal staining reveals that cells that had undergone *K15-Cre*^{PR1}-mediated recombination are retained in the bulge and SHG of unwounded skin for at least 10 wk, irrespective of SmoM2 expression. (*C*) In situ staining for *Gli1* in wild-type telogen skin (*Left*) and unwounded *K15:SmoM2* skin (*Right*). Arrow indicates the site of normal *Gli1* expression near the SHG and dermal papilla. (*D*) *Gli1* is modestly up-regulated at a site of focal hyperplasia in the bulge of unwounded *K15:SmoM2* skin (arrowhead). (*E*) Immunofluorescence staining for the YFP tag of SmoM2 and Gli2 in *K15:SmoM2* wound-induced tumors (*Top*), in unwounded *K15:SmoM2* follicles (*Middle*), and in non-SmoM2-expressing control follicles (*Bottom*). Arrows indicate the SmoM2-expressing cells from unwounded *K15:SmoM2* follicles (blue, DAPI).

staining for the target genes, *Gli1* and *Axin2*, respectively (Fig. S5). Similarly, in mice expressing *LacZ* under the control of a Hh-responsive *Patched1* promoter (32), there was no evidence of pathway activation after wounding (Fig. S5). In wound-induced tumors, *Axin2* was modestly up-regulated, and most tumor cells did not display β-catenin nuclear localization (Fig. S6). In contrast, *Gli1* expression was strongly up-regulated in wound-induced tumors (Fig. 3*A*), indicating that misactivation of Hh signaling by bulge and SHG-derived cells specifically at the wound site may contribute to tumorigenesis.

Why does SmoM2 expression induce tumors in the IFE and upon wounding but not in the intact bulge or SHG? One possibility may be that follicular stem cells must leave their niche to activate tumorigenic signaling pathways. To begin testing this possibility, we assessed whether oncogene-expressing cells from *K15:SmoM2:LacZ* mice were retained in the bulge and SHG in intact skin but leave upon wounding. Indeed, staining for SmoM2 or β-Gal in unwounded skin at 11 wk after induction revealed that all follicles retained oncogene-expressing cells within the bulge and SHG (Fig. 3 *B* and *E*). Interestingly, neither *Axin2* nor *Gli1* was strongly up-regulated by SmoM2-expressing cells in the bulge and SHG (Fig. 3*C* and Fig. S6). In undamaged *K15:SmoM2* telogen skin, *Gli1* expression was almost exclusively localized near the SHG and dermal papilla, similar to control telogen skin (Fig. 3*C*). In contrast, wound-induced tumors expressed elevated levels of *Gli1*, even compared with sites of focal hyperplasia in the follicular bulges of unwounded *K15:SmoM2* skin (Fig. 3 *A* and *D*).

As with *Gli1*, we observed strong expression of Gli2, previously associated with increased Hh signaling in the skin (24, 26), in wound-induced lesions but not in control skin (Fig. 3*E*). Importantly, Gli2 up-regulation was not detected in SmoM2-expressing cells in the bulge and SHG (Fig. 3*E*). These differences in *Gli1* and Gli2 up-regulation are likely not attributable to regional differences in the abundance of primary cilia, which are necessary for Smo-mediated signaling, because Smo localization to cilia was frequently observed in SmoM2-induced bulge and SHG cells as well as in wound-induced lesions (Fig. S7). Together, our findings suggest that a block in downstream Hh pathway activation may contribute to protecting against SmoM2-mediated oncogenesis in the bulge and SHG, and that migration of SmoM2-expressing stem cells from their follicular niches is associated with derepressed Hh signaling and tumorigenesis.

During anagen, stem cells in the bulge and SHG proliferate and migrate from their niche to regenerate the lower follicular bulb and hair shaft (9, 11, 13, 15). To determine whether anagen-induced proliferation and migration of stem cells also promotes tumorigenesis, we induced SmoM2 expression in *K15:SmoM2* mice during telogen and, 3 d later, depilated the skin to induce anagen reentry. At 20 d after depilation, lineage analysis revealed that SmoM2-expressing cells contributed to the matrix, hair shaft, and lower anagen bulb (Fig. 4*A*). Similar to normal anagen follicles, these follicles activated the Wnt and Hh pathways in the lower follicle (Fig. S8). Although follicular hyperplasia was observed, severe aberrations in follicular morphology did not occur in skin that had exited anagen and subsequently reentered telogen, despite the fact that nearly all follicles retained SmoM2-expressing cells (Fig. 4*B*). These results indicate that, unlike wounding, anagen induction does not promote tumorigenesis in *K15:SmoM2* mice.

To examine whether the oncogene-expressing bulge and SHG cells that persist in the follicle remain competent long term to form tumors in response to wounding, we performed excisional wounding 5 wk, rather than 3 d, after SmoM2 induction. As before, we observed numerous tumor nests and aberrant downgrowths at the healed wound site (Fig. 4*C*). To assess whether less severe wounds can promote formation of superficial BCC-like tumors in *K15:SmoM2* mice, we induced expression of SmoM2 and subsequently performed incisional wounding. Indeed, we observed that bulge and SHG-derived cells were recruited to sites of injury and formed superficial tumors at the healed wound junction (Fig. 4*D* and Fig. S9). Together, these findings indicate that SmoM2-expressing follicular stem cells persist and remain competent for

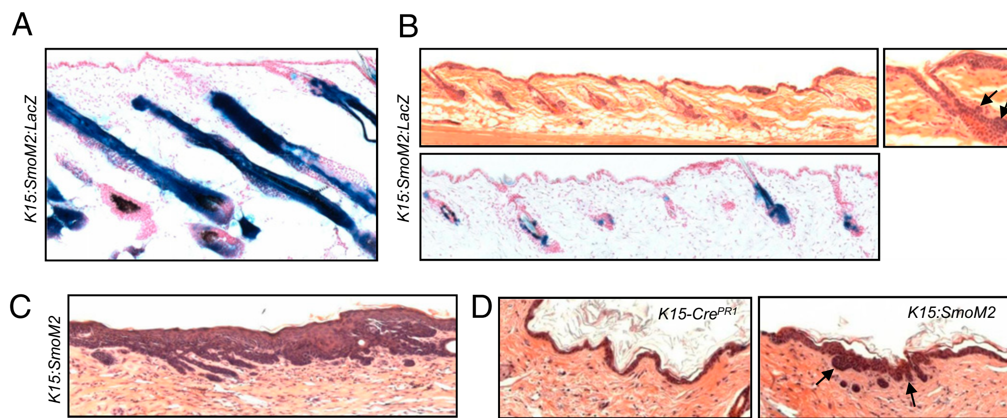

Fig. 4. Bulge or SHG-derived BCC-like tumors are induced by incisional or excisional wounding but not by depilation. (*A*) β-Gal staining indicates that bulge and SHG-derived cells that had undergone *K15-Cre*^{PR1}-mediated recombination contribute to cells of the lower anagen bulb in hyperplastic follicles from depilated *K15:SmoM2:LacZ* skin. (*B Upper*) Follicles of *K15:SmoM2* skin are grossly normal 10 wk after depilation. Note that anagen hair follicles had subsequently progressed to telogen without developing aberrations, aside from occasional focal hyperplasia (*Right*, arrows). (*Lower*) β-Gal staining demonstrates that bulge and SHG cells that had undergone *K15-Cre*^{PR1}-mediated recombination are retained in *K15:SmoM2* skin 10 wk after depilation. (*C*) BCC-like tumors arise from healed excisional wound sites of *K15:SmoM2* skin injured 5 wk after induction of SmoM2. (*D*) Tumors (arrows) arise at the healed incisional wound junction of *K15:SmoM2* mice 10 wk after injury but do not form in healed control *K15-Cre*^{PR1} skin.

tumorigenesis for at least several weeks, and that less severe incisional wounds also promote formation of BCC-like tumors.

Discussion

Rudolf Virchow postulated a relationship between injury and cancer in 1863, noting that both the frequency and extent of injury seemed to contribute to tumor formation (33). Indeed, human BCCs have been observed at sites of vaccination (34, 35), surgery (36, 37), burns (38), and other physical trauma (39, 40). Our findings suggest that, although BCCs likely originate from IFE cells in undamaged skin, as has been previously reported by Youssef et al. (17), oncogene-expressing follicular stem cells can give rise to tumors within sites of cutaneous injury. Ito et al. previously demonstrated that normal bulge-derived cells are recruited to sites of wounding (14), and our findings indicate that the wound-induced recruitment of SmoM2-expressing follicular stem cells releases these cells to form tumors. This paradigm may also apply to BCCs that display misactivation of Hh signal transduction due to loss-of-function mutations in *Patched1*, which normally suppresses Smo. However, it remains unclear whether the amplitude of downstream Hh activation is similar in tumors possessing loss-of-function mutations in *Patched1* and those with gain-of-function mutations in *Smo*. In addition, Patched1 may contribute to tumorigenesis independently of Hh signaling, as suggested by a study of skin squamous carcinoma (41).

We have also shown here that, in the absence of wounding, SmoM2-expressing follicular stem cells persist without forming tumors. However, these cells remain competent for wound-induced oncogenesis, even long after the onset of SmoM2 expression. Similarly, in a model of squamous cell carcinoma, oncogene-expressing cells can form tumors up to a year after an initial exposure to a chemical carcinogen (42, 43). These findings are concordant with step-wise models of cancer development, where long-lived progenitor cells residing in quiescent niches accumulate oncogenic insults before a final event exposes their tumorigenic phenotype. Our work suggests that this final event need not be an additional mutation but can be wound-induced mobilization of oncogenically initiated stem cells. Similarly, work by Kasper et al., has recently shown that wounding can recruit follicular keratinocytes to form tumors at sites of injury (44).

Importantly, our observation that wounding does not promote IFE cell-mediated tumorigenesis suggests that the rare IFE cells that had undergone K15-Cre^{PR1}-mediated recombination likely do not account for the enhanced tumorigenesis observed upon wounding in *K15:SmoM2* skin. Instead, migration into the wound may allow follicular stem cells or their progeny to acquire epidermal properties, as has been previously reported (14), and our findings suggest that these properties may include the ability to form tumors. In support of this hypothesis, wound-induced tumors derived from bulge or SHG cells display morphologies and marker expression similar to those of IFE-derived tumors.

Because SmoM2 expression in the IFE, but not in the bulge or SHG, is sufficient to induce tumors, we hypothesize that the bulge microenvironment inhibits SmoM2-mediated oncogenesis. Similar to our findings, recent studies have reported that expression of SmoM2 or truncated β-catenin in the bulge or SHG does not cause tumor formation (17, 45). Inefficient activation of downstream Hh signaling, as reflected by the absence of *Gli1* and Gli2 up-regulation, may explain the inability of SmoM2 to induce tumors in the bulge and SHG. This may be due to the activity of negative regulators of the Hh pathway, such as Gli3 or Sufu, whose expression was recently reported to be up-regulated in Lgr5-expressing bulge and SHG cells (10). In addition, proteolytic degradation of Gli transcription factors may attenuate Hh signaling in the bulge (46, 47). Because the bulge is thought to maintain a quiescent microenvironment through the action of BMPs (48, 49) and the suppression of Wnt signaling (15), these pathways may also interfere with transduction of downstream Hh signals and contribute to tumor suppression.

In addition to eliciting stem-cell escape from growth-restrictive niches, wounding also promotes transient epidermal hyperproliferation (2). In the wound microenvironment, fibroblasts and immune cells produce cytokines and extracellular matrix that promote cell division (4, 50). Indeed, inflammation accelerates tumorigenesis in a variety of cancer models, including those for skin (51), and is associated with increased formation of reactive oxygen species and genomic stress (4). Thus, wounding may promote oncogenic mutations that contribute to wound-induced tumorigenesis in some contexts. However, our finding that SmoM2-expressing IFE cells form numerous BCC-like tumors in *K14:SmoM2* skin in a manner unaffected by wounding suggests that injury-associated inflammation does not play a general role in SmoM2-mediated BCC formation. Nonetheless, we cannot rule out the possibility that inflammation may act specifically on bulge or SHG-derived cells to promote tumorigenesis by inducing mutations and increasing Hh signaling at the wound site.

Our study demonstrates that the cell of origin for BCC can depend on extrinsic factors such as wounding. Given the slow-growing nature of these tumors and the fact that even small incisional wounds can recruit oncogene-expressing stem cells from the follicle, it is possible that the formation of human BCCs may be temporally removed from their provocative injuries or that the associated injuries may go unnoticed. In future studies, it will be interesting to determine whether mobilization of oncogene-expressing stem cells underlies the development of other cancers whose pathogenesis has been associated with injury.

Materials and Methods

Mice. Mice expressing *K15-Cre*^{PR1}, *K14-Cre*^{ERT}, *SmoM2*, *ROSA26-LacZ* reporter, and *Patched1-LacZ* have been previously described (*SI Materials and Methods*) (24, 25, 27, 32, 51). Animals harbored single copies of the transgenes and were of a mixed background. At 7.5 wk of age, dorsal skin from *K15-Cre*^{PR1} mice was shaved and treated with 200 μL of 10 mg/mL mifepristone (Sigma-Aldrich) diluted in 50% DMSO/50% ethanol, applied topically for 5 d to induce Cre-mediated recombination. *K14-Cre*^{ERT} mice were induced with tamoxifen (Sigma-Aldrich) (26). At 3 d after the final treatment, a 0.5 × 0.5-cm full-thickness excisional wound was generated 3.5 cm from the base of the tail and 1 cm to the left of the spine. At 10 wk after wounding, biopsies spanning the healed wound site were removed, as were contralateral biopsies from the unwounded side (Fig. S10). For incisional wounds, a 0.5-cm incision was made 3.5 cm from the base of the tail and 1 cm to the left of the spine. For depilation, dorsal skin was treated with Nair (Church & Dwight) for 3 min. All mouse procedures were approved by the University of California San Francisco Institutional Animal Care and Use Committee.

Histology, Immunohistochemistry, and In Situ Hybridization. Skin biopsies were collected and stained by using standard protocols. Fixation conditions, staining procedures, and antibodies are described in *SI Materials and Methods*.

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