

Identifying the cellular origin of squamous skin tumors

Gaëlle Lapouge^{a,1}, Khalil Kass Youssef^{a,1}, Benoit Vokaer^b, Younes Achouri^c, Cindy Michaux^a, Panagiota A. Sotiropoulou^a, and Cédric Blanpain^{a,2}

^aInstitut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire, Université Libre de Bruxelles, B-1070 Brussels, Belgium; ^bInstitut d'Immunologie Médicale, Université Libre de Bruxelles, B-6041 Charleroi, Belgium; and ^cUniversité Catholique de Louvain de Duve Institute, B-1200 Brussels, Belgium

Edited by Brigid L. M. Hogan, Duke University Medical Center, Durham, NC, and approved February 25, 2011 (received for review September 9, 2010)

Squamous cell carcinoma (SCC) is the second most frequent skin cancer. The cellular origin of SCC remains controversial. Here, we used mouse genetics to determine the epidermal cell lineages at the origin of SCC. Using mice conditionally expressing a constitutively active KRas mutant (G12D) and an inducible CRE recombinase in different epidermal lineages, we activated Ras signaling in different cellular compartments of the skin epidermis and determined from which epidermal compartments Ras activation induces squamous tumor formation. Expression of mutant KRas in hair follicle bulge stem cells (SCs) and their immediate progeny (hair germ and outer root sheath), but not in their transient amplifying matrix cells, led to benign squamous skin tumor (papilloma). Expression of KRas^{G12D} in interfollicular epidermis also led to papilloma formation, demonstrating that squamous tumor initiation is not restricted to the hair follicle lineages. Whereas no malignant tumor was observed after KRas^{G12D} expression alone, expression of KRas^{G12D} combined with the loss of p53 induced invasive SCC. Our studies demonstrate that different epidermal lineages including bulge SC are competent to initiate papilloma formation and that multiple genetic hits in the context of oncogenic KRas are required for the development of invasive SCC.

cancer cell of origin | hair follicle stem cells

Squamous cell carcinoma (SCC) is one of two most frequent skin cancers and occurs most frequently in the sun-exposed regions of the skin and in immunocompromised patients. Approximately 250,000 patients per year develop skin SCC in the United States. Although skin SCC is often cured by surgical excision, ~8% of patients with skin SCC relapse, and 5% present metastasis within 5 y. In patients with metastatic SCC, the prognosis is very poor, with only a 10–20% survival rate over 10 years (1). A better understanding of the early steps of SCC initiation is thus warranted.

The most extensively used mouse cancer model for skin SCC is a multistage chemically induced carcinogenesis (2). In the first step, called initiation, mice are treated with a low dose of the mutagen 9,10-dimethyl-1,2-benzanthracene (DMBA). In the second step, called promotion, mice are treated continuously with a drug that stimulates epidermal proliferation, such as 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). During promotion, benign tumors (papillomas) arise, probably as a consequence of additional mutations, some of which will progress into invasive SCC. Interestingly, using this protocol, papillomas contain activating mutations in *HRas* gene, suggesting that this mutation confers a selective advantage to epithelial cells (3). However, not all papillomas contain mutations in the *HRas* gene, and some papillomas and SCC in both mouse and human present mutations in the *KRas* gene instead (4–6). Whereas *Ras* mutations seem to be an early step in skin cancer initiation, *p53* mutations are associated with malignant progression (7, 8), although a recent report suggests that gain of *p53* function increases tumor initiation and progression compared with *p53* loss of function (9).

Conflicting results have been reported regarding the determination of which epidermal lineages give rise to squamous cutaneous cancers. The skin epidermis contains different compartments, including the hair follicle (HF) and the sebaceous gland (SG), as well as the interfollicular epidermis (IFE), which

are maintained during homeostasis by different types of stem cells (SCs) (10). In the absence of injury, bulge SCs mediate HF regeneration (11–14), whereas the isthmus SCs mediate SG homeostasis (15–17). The maintenance of the IFE is ensured by the juxtaposition of many small epidermal proliferative units containing long-lived progenitors (18, 19). Upon wounding, bulge and isthmus SCs migrate upward and contribute to the repair of the IFE (11, 13, 17, 20). SCCs often present signs of squamous differentiation, as illustrated by the keratin pearls found in the center of the tumors, suggesting that SCCs may originate from the IFE (8). TPA administration stimulates papilloma formation even when TPA administration is started 1 y after the last DMBA treatment, suggesting that the initial mutation arises in long-lived SCs (21). There is some evidence that cells targeted by the benzo (*a*)pyrene or DMBA in the skin can be slow-cycling cells residing in the HF but also along the IFE (21–23). Dermabrasion techniques can selectively remove the IFE while leaving intact HFs, which then contribute to the repair of wounded skin. When TPA is applied after the repair of the epidermis, papilloma formation is reduced but the formation of SCC is not abolished, suggesting that initiating cells may reside in the IFE as well as in HFs (24). However, some studies suggested that oncogenic mutations could also occur in differentiated cells of the epidermis. For example, suprabasal expression of $\alpha 6 \beta 4$ integrins can increase the frequency of papillomas after DMBA-TPA treatment (25). In addition, transgenic mice expressing a mutated form of *HRas* under the promoter of *K10*, a marker of the committed suprabasal cells of the IFE, develop papillomas at sites of wounding (26). Transgenic mice expressing the same oncogene under the control of a truncated form of the *K5* promoter, which is expressed preferentially in the HFs of adult mice, develop papillomas and SCCs (27). In addition, these studies used transgenic mice expressing *Ras* mutant constitutively from embryonic development to adulthood and at supraphysiological level, leaving open the question of which cells of the skin epidermis are competent to give rise to SCCs upon mutated *Ras* expression at a physiological level in adult mice.

To determine which epidermal compartments are competent to initiate squamous tumors, we used mouse genetics to assess the incidence of skin cancer after the expression of oncogenic *KRas* at a physiological level in different epidermal compartments. To avoid the drawback of transgenic approaches that result in the random integration of several copies of the oncogene into differently accessible chromatin regions, we used a genetic mouse model to specifically activate a constitutively active mutant of *KRas* (G12D) knocked in into its own locus (*KRas*^{LSL-G12D}). Expression of this mutant *KRas* induced a broad variety of *Ras*-induced neoplasia (28), including oral (29) and skin (9) squamous tumors. The oncogene is only expressed when the *Lox-STOP-Lox*

Author contributions: G.L., K.K.Y., and C.B. designed research; G.L., K.K.Y., B.V., Y.A., C.M., and P.A.S. performed research; G.L., K.K.Y., and C.B. analyzed data; and G.L., K.K.Y., and C.B. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹G.L. and K.K.Y. contributed equally to this work.

²To whom correspondence should be addressed. E-mail: Cedric.blanpain@ulb.ac.be.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1012720108/-DCSupplemental.

(LSL) cassette is removed by a CRE recombinase. A short course of tamoxifen (TAM) administration in mice expressing CREER leads to a mosaic expression of $KRas^{G12D}$ in the epidermal compartments expressing the CREER, mimicking the natural occurrence of sporadic tumors.

Results

$KRas^{G12D}$ Expression in Bulge SCs and Their HF Progeny Induces Papilloma Formation. We first induced the expression of $KRas^{G12D}$ in bulge SCs and their progeny using mice expressing inducible CRE under the regulatory region of HF-specific promoter [K15-CREPR (12) and K19CREER (30)]. We did observe some leakiness in the K15CREPR/RosaYFP mice, visible by the presence of patches of YFP-expressing cells in the absence of RU486 (Fig. S1 A and B), whereas no leakiness was detected in the K19CREER/RosaYFP mice (Fig. 1 A and B) (31). Administration of RU486

in K15CREPR/Rosa-YFP mice induced the expression of YFP mostly in bulge SCs, hair germ (HG), and outer root sheath (ORS) cells but also to a lesser extent in the isthmus, infundibulum (the region that connects the bulge to the IFE) and the IFE (Fig. S1 C–G), as previously described (12). TAM administration in K19CREER/RosaYFP mice resulted in the labeling of bulge SCs and their HF progeny (Fig. 1 C–G). After TAM administration in K19CREER/ $KRas^{G12D}$ mice, recombination of $KRas^{G12D}$ allele was detected in bulge SCs (α 6HCD34H) but not in α 6HCD34[−] cells (Fig. S24), suggesting that the recombination of $KRas$ allele is restricted to the same cells labeled with the RosaYFP. In the K19CREER/ $KRas^{LSL-G12D}$, the expression of the $KRas^{G12D}$ oncogene in bulge SCs and their progeny led to a transient increase in bulge SC proliferation (Fig. S2B) and the enlargement of the majority of SGs 1 mo after TAM administration (Fig. S2C). These SGs became hyperproliferative, as shown by the increased number of Ki67-positive cells and the expression of K6 (Figs. S1–S3), although they were still able to undergo terminal differentiation, as shown by the presence of sebum (Figs. S1 and S3). The defect in SG homeostasis also led to the formation of cystic structures (Figs. S1 and S3). The SG hyperplasia observed here is reminiscent of the phenotype observed after c-Myc (32) and ΔN -Lef1 overexpression (33) or Blimp1 deletion (34) in the skin epidermis. Occasionally, the IFE was also abnormal after $KRas^{G12D}$ expression, as shown by the hyperthickening of the IFE located at the orifice of some abnormal HF with hypertrophic SGs (Figs. S1 and S3). In addition to sebaceous cysts, $KRas^{G12D}$ expression in bulge SCs and their progeny also led to the enlargement and abnormal differentiation of HF and to cystic structures negative for Oil-Red-O staining (Figs. S1 and S3). Altogether, these data show that $KRas^{G12D}$ expression in bulge SCs and their HF progeny led to frequent histological abnormalities, indicating that the majority of HFs expressing mutant $KRas$ developed pathologic features including SG hyperplasia and dysplasia, as well as hyperplasia and aberrant differentiation of the lower HF, some of which degenerated into cystic structures.

To investigate why $KRas$ expression in HF cells induces SG alterations, we performed lineage tracing together with $KRas$ expression (K19CREER/ $KRas^{LSL-G12D}$ /RosaYFP mice). Already at 2 wk after TAM administration, more YFP-positive cells were seen in the SG progenitors compared with wild-type mice (Fig. S44). At 4 wk after TAM administration, some YFP-positive cells were detected in hyperplastic SG and sometimes even in the IFE of $KRas$ /YFP mice, whereas no YFP-positive cells were observed in the SG or the IFE of control mice (K19CREER/RosaYFP mice) (Fig. S4B). These findings suggested that $KRas^{G12D}$ expression induces migration of bulge SCs toward the SG and the IFE. However, because there is not a perfect correlation between the hyperplastic phenotype and the YFP expression, our data cannot rule out that $KRas$ expression in HF cells promotes epidermal proliferation by a noncellular autonomous mechanism, and thereby promotes tumorigenesis as it occurs after *Notch1* deletion in the skin epidermis (35).

Tumors arose in the lips (57% of the animals treated), face (35%), and back skin (14%) of K15CREPR/ $KRas^{LSL-G12D}$ mice ($n = 14$) 2–4 mo after RU486 administration (Fig. S1). Similarly, tumors also arose with similar latency and frequency in the lips (78% of the mice treated) and back skin (33%) of K19CREER/ $KRas^{LSL-G12D}$ mice ($n = 9$) after TAM administration (Fig. 1 H and K). Consistent with the leakiness observed in K15CREPR/RosaYFP mice, all untreated K15CREPR/ $KRas^{LSL-G12D}$ mice eventually developed some papillomas, although the latency was increased in untreated mice (>6 mo) (Fig. S15). These tumors possessed all of the histological characteristics of benign papillomas, as demonstrated by the expression of K5 in the basal layers and K1 in the suprabasal layers (Fig. 1 I–M and Fig. S1 M–R), indicative of squamous differentiation. No sign of malignant transformation was observed in these mice up to 4 mo after $KRas^{G12D}$ expression. Altogether, these data indicate that bulge SCs and their HF progeny are competent to develop benign papillomas upon $KRas^{G12D}$ expression, but other genetic events are required to induce the development of invasive carcinomas.

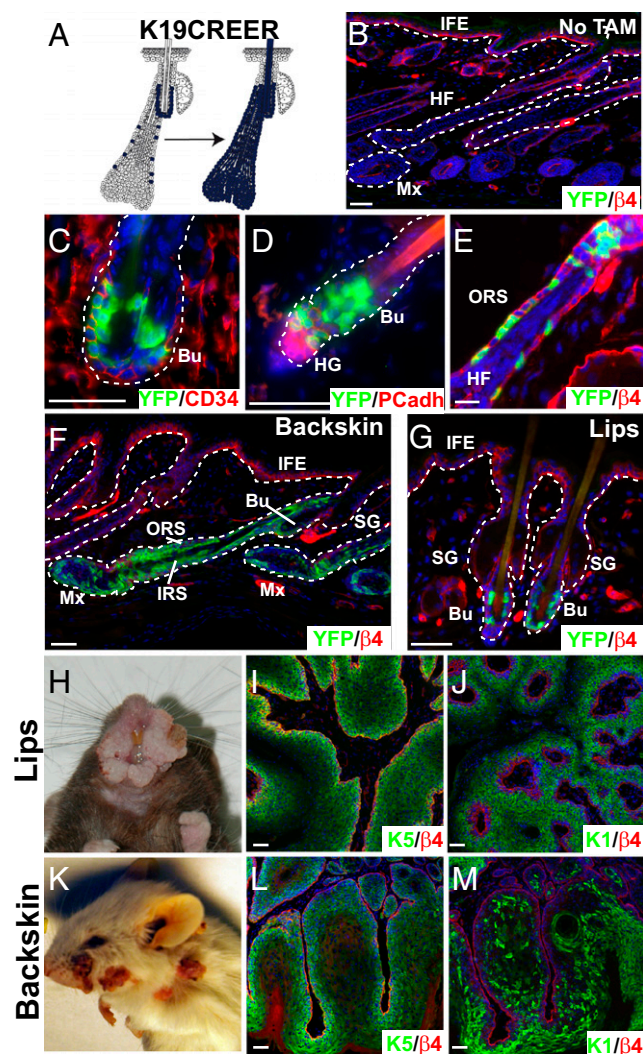


Fig. 1. Targeting $KRas^{G12D}$ expression in bulge SCs and their progeny induces papilloma formation. (A) Bulge SCs and their HF progeny targeted by K19 promoter are highlighted in dark blue. (B) YFP immunostaining in the absence of TAM administration. (C–G) Lineage tracings of K19CREER/RosaYFP mice are shown in the bulge (C), HG (D), and ORS (E and F) of the back skin and in the bulge of the lips (G) 1 wk after 10 mg TAM administration. (H–M) Papilloma formation in K19CREER/ $KRas^{LSL-G12D}$ mice. (H and K) Macroscopic pictures of tumors seen in the K19CREER/ $KRas^{LSL-G12D}$ lips (H), face, and back skin (K) 4 mo after TAM administration. Expression of K5 (I and L) and of K1 (J and M) in papilloma from lips and back skin arising from K19CREER/ $KRas^{LSL-G12D}$ mice. Bu, bulge; IRS, inner root sheath; Mx, matrix. (Scale bars, 50 μ m.)

HF Transit Amplifying Cells Are Not Competent to Initiate Papilloma Formation upon $KRas^{G12D}$ Expression. During HF regeneration, bulge SCs are activated, proliferate, and give rise to transit-amplifying (TA) cells, which differentiate into the hair shaft and its envelope (36). To determine whether $KRas^{G12D}$ expression in HF TA compartment is sufficient to transform these cells and induce squamous tumor formation, we administered TAM to ShhCREER/ $KRas^{LSL-G12D}$ mice at day 28 when HFs were in full anagen (Fig. 2A). No leakiness was detected in ShhCREER/RosaYFP mice (Fig. 2B). Administration of TAM to ShhCREER/RosaYFP mice preferentially labeled one side of the matrix. These labeled cells constitute a subset of matrix cells that differentiate into the different HF lineages (Fig. 2C–G), showing that ShhCREER (37) activity is indeed restricted to the HF TA cells, as we previously described in

the HF of the tail (31). Few marked YFP cells were still observed in the companion layer of the club hair 1 mo after TAM administration (Fig. S5A). RT-PCR analysis of FACS-isolated YFP cells from ShhCREER/RosaYFP demonstrated that $KRas$ is expressed at a similar level in Shh-derived cells, in bulge SC, and in cells of the IFE and the infundibulum (Fig. S5B). Despite the similar level of $KRas$ expression in matrix cells, no macroscopic tumors or microscopic HF or IFE defects (Fig. 2H–J and Fig. S5C and D) were observed in ShhCREER/ $KRas^{LSL-G12D}$ mice 4 mo after TAM administration ($n = 7$), suggesting that $KRas^{G12D}$ expression in HF TA cells is not sufficient to induce long-term renewal potential and initiate tumor formation.

$KRas^{G12D}$ Expression in the IFE Induces Papilloma Formation. Transgenic mice expressing a mutated form of HRas under the promoter of $K10$, a keratin expressed in the suprabasal cells of the IFE, induced hyperkeratosis and papilloma formation in response to me-

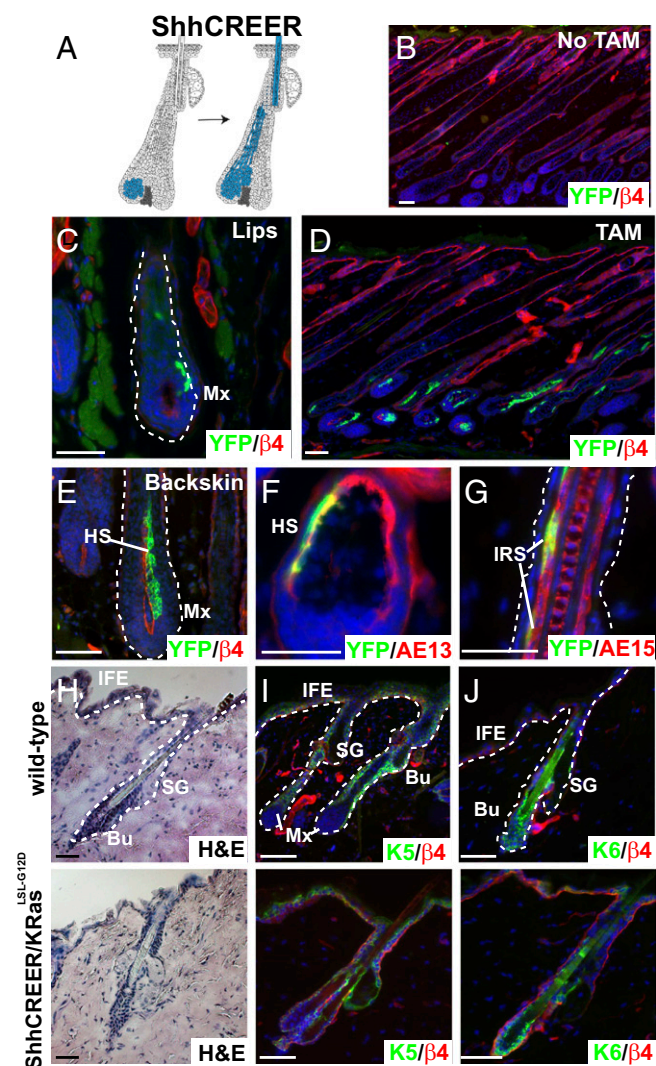


Fig. 2. Transit amplifying HF matrix cells do not give rise to papilloma upon $KRas^{G12D}$ expression. (A) Matrix cells and their progeny targeted by *Shh* promoter are highlighted in blue. (B) YFP immunostaining in the absence of TAM administration. (C–G) Lineage tracing in ShhCREER/RosaYFP mice showed the presence of YFP cells in the matrix cells of the lips (C), HF (D), matrix (Mx), and hair shaft (HS) (E and F); inner root sheath (IRS) of the back skin (G) shows that ShhCREER marked TA matrix cells and their HF progeny 1 wk after 2.5 mg TAM administration. (D–H) Comparison between wild-type and ShhCREER/ $KRas^{LSL-G12D}$ back skin 4 mo after 2.5 mg TAM administration. Hematoxylin-eosin staining (H) and expression of K5 (I) and K6 (J), (H) Oil-red-O staining in back skin, show no epidermal defect or tumor formation in mice expressing $KRas^{G12D}$ in matrix cells. Bu, bulge; Mx, matrix. (Scale bars, 50 μ m.)

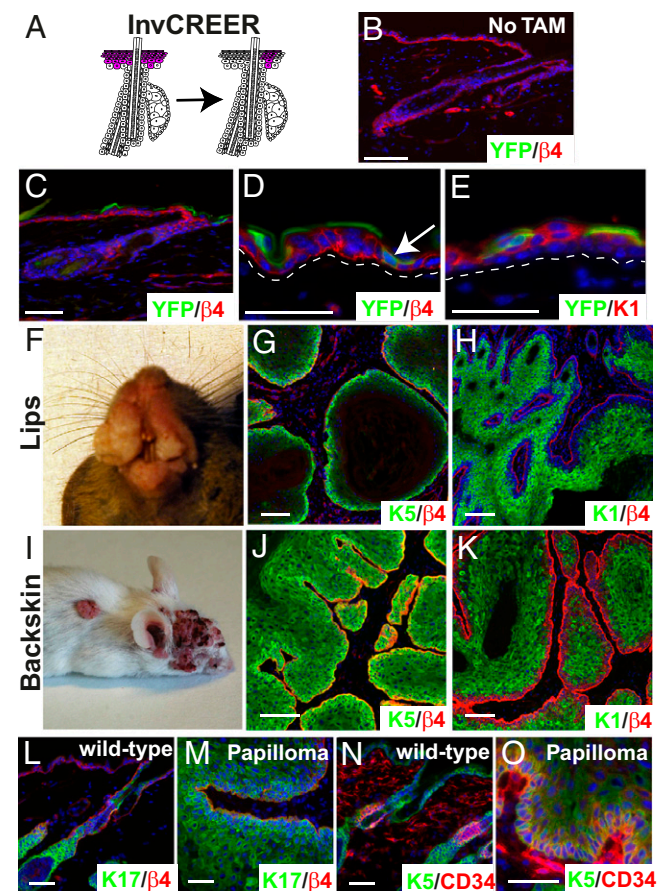


Fig. 3. $KRas^{G12D}$ expression in the IFE induces papilloma formation. (A) Interfollicular suprabasal cells and rare basal cells targeted by *involucrin* promoter are highlighted in dark pink. (B) YFP immunostaining in the absence of TAM administration. (C–E) Lineage tracing of InvCREER/RosaYFP back skin mice 1 wk after 5 mg TAM administration. Costaining of YFP and β 4, K5, and K1 shows that InvCREER mostly targets the suprabasal differentiated cells of the IFE, as well as some basal cells (D, arrow). (F–K) Papilloma formation in InvCREER/ $KRas^{LSL-G12D}$ mice. (F and J) Macroscopic picture of tumors in the lips (F) and back skin (J) of InvCREER/ $KRas^{LSL-G12D}$ mice 4 mo after TAM administration. Expression of K5 (G and I) and K1 (H and K) in papilloma from the back skin and lips of InvCREER/ $KRas^{LSL-G12D}$ mice. (L–O) Follicular marker expression in papilloma arising from InvCREER/ $KRas^{LSL-G12D}$ mice. Expression of K17 (L and M), a follicular marker, and CD34 (N and O), a bulge SC marker in wild-type epidermis (L and N) and papilloma (M and O) from InvCREER/ $KRas^{LSL-G12D}$ mice, indicating that tumor differentiation does not reflect their cellular origin. (Scale bars, 50 μ m.)

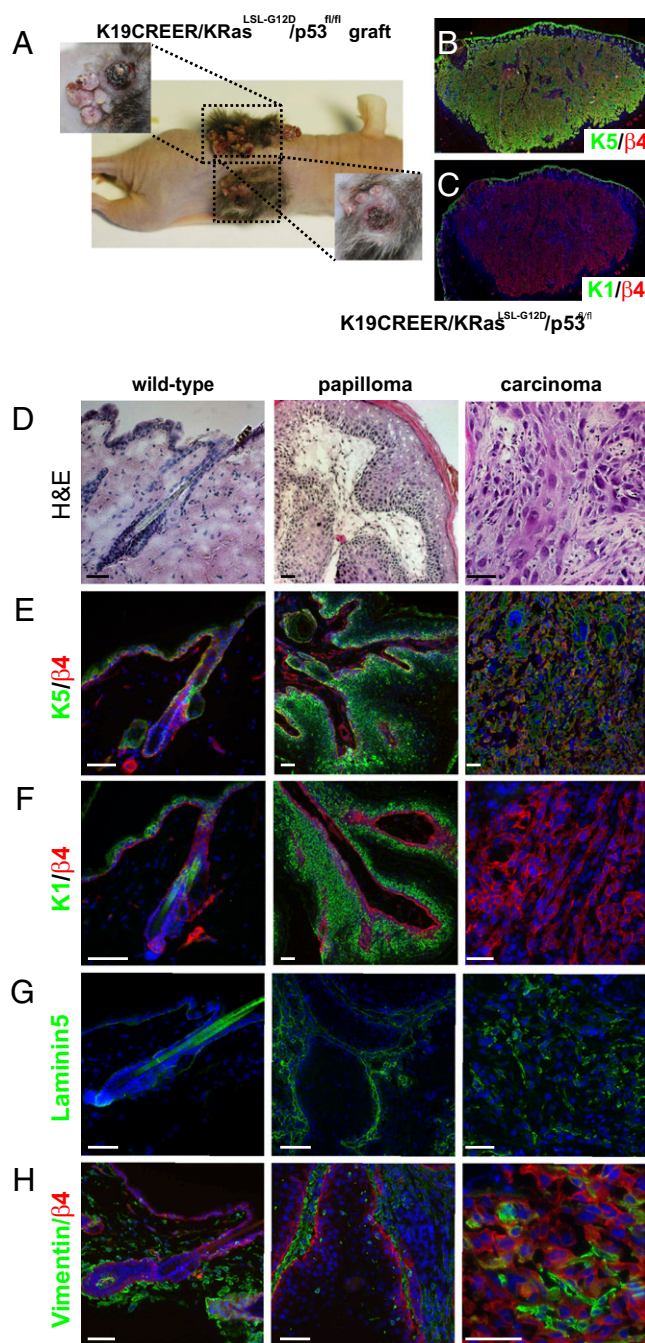


Fig. 5. $KRas^{G12D}$ expression and p53 deletion in bulge SCs and their HF progeny induce SCC. (A) Macroscopic picture of tumors arising in K19CREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ back skin grafted onto nude mouse. Immunostaining of K5 (B) and K1 (C) in K19CREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ carcinoma. (D–H) Comparison between wild-type back skin, papilloma, and carcinoma arising from grafted K19CREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ back skin. Hematoxylin-eosin staining (D) and expression of K5 (E), K1 (F), laminin5 (G), and vimentin (H) in wild-type back skin and in papilloma and SCC from K19CREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ mice. (Scale bars, 50 μm .)

demonstrate that, in the context of oncogenic $KRas$, at least two genetic hits (gain of Ras and loss of p53 function) are required for the development of invasive carcinoma.

We then determined more specifically whether the expression of $KRas^{G12D}$ combined with the p53 deletion in HF bulge SCs and their progeny also led to invasive SCC. To this end, we administered TAM to K19CREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ mice,

which specifically induce $KRas^{G12D}$ expression and p53 deletion in bulge SCs and their progeny (Fig. S7). However, these treated mice died within 2 mo after TAM administration from intestinal cancers before developing skin tumors ($n = 8$). To circumvent this issue, we grafted the back skin of K19CREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ mice onto the back skin of nude mice one month after TAM administration. All mice grafted with the skin of TAM-treated K19CREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ mice developed many skin tumors, including ulcerative lesions, within 2 mo after TAM administration (Fig. 5A) ($n = 10$ grafts). Microscopic examination revealed that the majority of these tumors were differentiated benign papillomas, as shown by K1 expression (Fig. 5C and F), but some of them presented all of the characteristics of invasive SCC (Fig. 5D–H), which were indistinguishable from the SCC observed in K14CREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ mice. To determine whether the expression of $KRas^{G12D}$ and the loss of p53 in matrix TA cells can induce skin tumor formation, we administered TAM to ShhCREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ mice. As in ShhCREER/ $KRas^{LSL-G12D}$ mice, no macroscopic or microscopic tumors (Fig. S8) were observed in ShhCREER/ $KRas^{LSL-G12D}/p53^{fl/fl}$ treated mice up to 4 mo after TAM administration ($n = 10$). These data clearly showed that the combined expression of $KRas^{G12D}$ and p53 deletion in bulge SCs and/or their HF progeny are required to induce full-blown invasive skin SCC, whereas TA matrix cells were not competent to initiate benign or malignant tumors.

Discussion

For most cancers, the precise identification of tumor-initiating cells remains elusive. We have recently demonstrated that upon oncogenic smoothened expression, basal cell carcinoma (BCC) arose most frequently from the IFE, whereas bulge SCs and their HF progeny were not competent to initiate BCC formation upon smoothened expression (31). In sharp contrast, $KRas^{G12D}$ oncogene expression in bulge SCs and their HF progeny gave rise to benign papillomas, and $KRas^{G12D}$ expression combined with p53 deletion in these cells led to invasive SCC. Because K15 and K19 inducible CRE lines also targeted cells located within the HG and the lower ORS, we cannot exclude the possibility that some short-lived HF progenitors could be as potent as bulge SCs in initiating squamous tumors. In addition to bulge SCs, cells of the IFE are also competent to give rise to benign squamous tumors upon physiological expression of $KRas^{G12D}$ oncogene without the need of exogenous inflammation or stimulation of tissue turnover by chemical abrasion or wounding. These data show that in contrast to the restricted epidermal lineages competent to initiate BCC upon smoothened expression (31), multiple epidermal compartments of the epidermis, but not matrix HF TA cells nor their hair shaft progeny, are competent to initiate squamous tumors upon $KRas^{G12D}$ expression. The absence of tumor formation upon $KRas^{G12D}$ expression in HF TA cells could be related to the transient period during which matrix cells actively proliferate. Indeed, it takes approximately 1 mo to detect the first microscopic lesions after $KRas^{G12D}$ expression in HF or IFE progenitors, and after 1 mo the lower part of the HF has degenerated during catagen stage, and only rare Shh-derived cells are still present within the HF, as terminally differentiated cells.

Papillomas arising from bulge SCs targeted by $KRas^{G12D}$ expressed K1, whereas bulge SCs and their HF progeny do not express K1 during physiological conditions. Similarly, papilloma arising from $KRas^{G12D}$ expression in the IFE expressed CD34, like papillomas from DMBA/TPA-treated mice (40), although in normal skin CD34 is specifically expressed by bulge SCs but not IFE cells (42, 43). These results indicated that the expression of differentiation markers by tumor cells does not necessarily reflect the cellular origin of squamous tumors. Similarly, oncogenic smoothened expression induced the expression of follicular markers in interfollicular progenitors during BCC initiation (31).

Whereas oncogenic smoothened expression drove the majority of targeted cells to invasive BCC (31), only a minor fraction of

KRas^{G12D} targeted cells formed squamous tumors, suggesting that papilloma initiation could require other additional genetic and/or epigenetic modifications in addition to oncogenic Ras expression. Interestingly, simultaneous deletion of p53 and KRas^{G12D} expression can initiate the formation of invasive SCC without first forming papilloma, suggesting that p53 loss might control SCC initiation, in addition to promoting tumor progression (41).

The mouse model of invasive SCC resulting from the expression of oncogenic KRas and p53 deletion in HF bulge SCs is likely to be relevant for human SCC because the same oncogenes (Ras and p53) are also implicated in the pathogenesis of human SCC (7, 8), and the histology of SCC found in our mouse model recapitulates the histology of human SCC. In addition, our findings, showing that squamous tumors arise from HF bulge SCs as well as from nonhairly epidermis, recapitulate the spectrum of locations of squamous tumors found in humans, such as hairy and nonhairly skin, oral cavity, head and neck, and esophagus.

Methods

Mice. K14CREER (44), ShhCREER (37), K15CREPR (12), K19CREER (30), Rosa-YFP (45), KRas^{LSL-G12D} (28), and p53^{fl/fl} mice (46) have been previously

described. Generation of InvCREER mice and induction of CRE-mediated recombination are described in *SI Methods*.

Histology, Immunostaining, and Imaging. These were performed as previously described (31) and are detailed in *SI Methods*.

Skin Graft Protocol. Mice were anesthetized with a mixture of 5% xylazine and 10% ketamine in PBS. Back skin of 2-mo-old TAM-treated mice were removed and grafted onto the back skin of nude mice. Skin grafts were monitored for carcinoma formation.

Isolation of Keratinocytes, DNA and RNA Extraction, Real-Time RT-PCR, and PCR Analysis of Cre-Mediated Recombination.

These are described in *SI Methods*.

ACKNOWLEDGMENTS. We thank our colleagues who provided us with reagents and whose gifts are cited in the text. P.A.S. and C.B. are Researchers of Fonds de la Recherche Scientifique (FRS)/Fonds National de la Recherche Scientifique (FNRS), K.K.Y. is a research fellow of the Formation à la Recherche dans l'Industrie et l'Agriculture (FRIA), and G.L. is post-doctoral fellow of the FRS/FNRS. This work was supported by a career development award from the Human Frontier Science Program Organization, the program CIBLES of the Wallonia Region, a grant from the Fondation Contre le Cancer and the fond Gaston Ithier, a starting grant from the European Research Council, and the European Molecular Biology Organization (EMBO) Young Investigator Program.

- Alam M, Ratner D (2001) Cutaneous squamous-cell carcinoma. *N Engl J Med* 344: 975–983.
- Kemp CJ (2005) Multistep skin cancer in mice as a model to study the evolution of cancer cells. *Semin Cancer Biol* 15:460–473.
- Quintanilla M, Brown K, Ramsden M, Balmain A (1986) Carcinogen-specific mutation and amplification of Ha-ras during mouse skin carcinogenesis. *Nature* 322:78–80.
- Spencer JM, Kahn SM, Jiang W, DeLeo VA, Weinstein IB (1995) Activated ras genes occur in human actinic keratoses, premalignant precursors to squamous cell carcinomas. *Arch Dermatol* 131:796–800.
- van der Schroeffer JG, Evers LM, Boot AJ, Bos JL (1990) Ras oncogene mutations in basal cell carcinomas and squamous cell carcinomas of human skin. *J Invest Dermatol* 94: 423–425.
- Sutter C, et al. (1993) ras gene activation and aberrant expression of keratin K13 in ultraviolet B radiation-induced epidermal neoplasias of mouse skin. *Mol Carcinog* 8:13–19.
- Perez-Losada J, Balmain A (2003) Stem-cell hierarchy in skin cancer. *Nat Rev Cancer* 3: 434–443.
- Owens DM, Watt FM (2003) Contribution of stem cells and differentiated cells to epidermal tumours. *Nat Rev Cancer* 3:444–451.
- Caulin C, et al. (2007) An inducible mouse model for skin cancer reveals distinct roles for gain- and loss-of-function p53 mutations. *J Clin Invest* 117:1893–1901.
- Blanpain C, Fuchs E (2009) Epidermal homeostasis: A balancing act of stem cells in the skin. *Nat Rev Mol Cell Biol* 10:207–217.
- Tumbar T, et al. (2004) Defining the epithelial stem cell niche in skin. *Science* 303: 359–363.
- Morris RJ, et al. (2004) Capturing and profiling adult hair follicle stem cells. *Nat Biotechnol* 22:411–417.
- Ito M, et al. (2005) Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med* 11:1351–1354.
- Levy V, Lindon C, Harfe BD, Morgan BA (2005) Distinct stem cell populations regenerate the follicle and interfollicular epidermis. *Dev Cell* 9:855–861.
- Jensen UB, et al. (2008) A distinct population of clonogenic and multipotent murine follicular keratinocytes residing in the upper isthmus. *J Cell Sci* 121:609–617.
- Jensen KB, et al. (2009) Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. *Cell Stem Cell* 4:427–439.
- Snippert HJ, et al. (2010) Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. *Science* 327:1385–1389.
- Ghazizadeh S, Taichman LB (2001) Multiple classes of stem cells in cutaneous epithelium: A lineage analysis of adult mouse skin. *EMBO J* 20:1215–1222.
- Clayton E, et al. (2007) A single type of progenitor cell maintains normal epidermis. *Nature* 446:185–189.
- Levy V, Lindon C, Zheng Y, Harfe BD, Morgan BA (2007) Epidermal stem cells arise from the hair follicle after wounding. *FASEB J* 21:1358–1366.
- Morris RJ (2000) Keratinocyte stem cells: Targets for cutaneous carcinogens. *J Clin Invest* 106:3–8.
- Morris RJ, Fischer SM, Slaga TJ (1986) Evidence that a slowly cycling subpopulation of adult murine epidermal cells retains carcinogen. *Cancer Res* 46:3061–3066.
- Morris RJ, Coulter K, Tryson K, Steinberg SR (1997) Evidence that cutaneous carcinogen-initiated epithelial cells from mice are quiescent rather than actively cycling. *Cancer Res* 57:3436–3443.
- Argyris TS, Slaga TJ (1981) Promotion of carcinomas by repeated abrasion in initiated skin of mice. *Cancer Res* 41:5193–5195.
- Owens DM, Romero MR, Gardner C, Watt FM (2003) Suprabasal alpha6beta4 integrin expression in epidermis results in enhanced tumorigenesis and disruption of TGFbeta signalling. *J Cell Sci* 116:3783–3791.
- Bailleul B, et al. (1990) Skin hyperkeratosis and papilloma formation in transgenic mice expressing a ras oncogene from a suprabasal keratin promoter. *Cell* 62:697–708.
- Brown K, Strathdee D, Bryson S, Lambie W, Balmain A (1998) The malignant capacity of skin tumours induced by expression of a mutant H-ras transgene depends on the cell type targeted. *Curr Biol* 8:516–524.
- Tuveson DA, et al. (2004) Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. *Cancer Cell* 5:375–387.
- Raimondi AR, Molinolo A, Gutkind JS (2009) Rapamycin prevents early onset of tumorigenesis in an oral-specific K-ras and p53 two-hit carcinogenesis model. *Cancer Res* 69:4159–4166.
- Means AL, Xu Y, Zhao A, Ray KC, Gu G (2008) A CK19(CreERT) knockin mouse line allows for conditional DNA recombination in epithelial cells in multiple endodermal organs. *Genesis* 46:318–323.
- Youssef KK, et al. (2010) Identification of the cell lineage at the origin of basal cell carcinoma. *Nat Cell Biol* 12:299–305.
- Arnold I, Watt FM (2001) c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. *Curr Biol* 11:558–568.
- Niemann C, Owens DM, Hülsken J, Birchmeier W, Watt FM (2002) Expression of DeltaNlcf1 in mouse epidermis results in differentiation of hair follicles into squamous epidermal cysts and formation of skin tumours. *Development* 129:95–109.
- Horsley V, et al. (2006) Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland. *Cell* 126:597–609.
- Demehri S, Turkoz A, Kopan R (2009) Epidermal Notch1 loss promotes skin tumorigenesis by impacting the stromal microenvironment. *Cancer Cell* 16:55–66.
- Blanpain C, Fuchs E (2006) Epidermal stem cells of the skin. *Annu Rev Cell Dev Biol* 22: 339–373.
- Harfe BD, et al. (2004) Evidence for an expansion-based temporal Shh gradient in specifying vertebrate digit identities. *Cell* 118:517–528.
- Carroll JM, Romero MR, Watt FM (1995) Suprabasal integrin expression in the epidermis of transgenic mice results in developmental defects and a phenotype resembling psoriasis. *Cell* 83:957–968.
- Schweizer J, Kinjo M, Fürstenberger G, Winter H (1984) Sequential expression of mRNA-encoded keratin sets in neonatal mouse epidermis: Basal cells with properties of terminally differentiating cells. *Cell* 37:159–170.
- Malanchi I, et al. (2008) Cutaneous cancer stem cell maintenance is dependent on beta-catenin signalling. *Nature* 452:650–653.
- Kemp CJ, Donehower LA, Bradley A, Balmain A (1993) Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. *Cell* 74:813–822.
- Trempe CS, et al. (2003) Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. *J Invest Dermatol* 120:501–511.
- Blanpain C, Lowry WE, Geoghegan A, Polak L, Fuchs E (2004) Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* 118:635–648.
- Vasioukhin V, Degenstein L, Wise B, Fuchs E (1999) The magical touch: Genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. *Proc Natl Acad Sci USA* 96:8551–8556.
- Srinivas S, et al. (2001) Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol* 1:4.
- Jonkers J, et al. (2001) Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat Genet* 29:418–425.