Keratinocyte-specific Pten Deficiency Results in Epidermal Hyperplasia, Accelerated Hair Follicle Morphogenesis and Tumor Formation

Akira Suzuki, Satoshi Itami, Minako Ohishi, Koichi Hamada, Tae Inoue, Nobuyasu Komazawa, Haruki Senoo, Takehiko Sasaki, Junji Takeda, Motomo Manabe, Tak Wah Mak, and Toru Nakano

ABSTRACT

PTEN is a tumor suppressor gene mutated in many human cancers. We used the Cre-loxP system to generate a keratinocyte-specific null mutation of Pten in mice (k5Ptenfloxflox mice). k5Ptenfloxflox mice exhibit wrinkled skin because of epidermal hyperplasia and hyperkeratosis and ruffled, shaggy, and curly hair. Histological examination revealed that skin morphogenesis is accelerated in k5Ptenfloxflox mice. Within 3 weeks of birth, 90% of k5Ptenfloxflox mice die of malnutrition possibly caused by hyperkeratosis of the esophagus. All k5Ptenfloxflox mice develop spontaneous tumors within 8.5 months of birth, and chemical treatment accelerates the onset of tumors. k5Ptenfloxflox keratinocytes are hyperproliferative and resistant to apoptosis and show increased activation of the Pten downstream signaling mediators Akt/protein kinase B (PKB) and extracellular signal-regulated kinase. Pten is thus an important regulator of normal development and oncogenesis in the skin.

INTRODUCTION

PTEN (also called MMAC1 or TEP1) is a tumor suppressor gene mutated in both human sporadic cancers (1) and in hereditary cancer syndromes such as Cowden disease and Bannayan-Zonana syndrome (2, 3). Among the characteristic symptoms of Cowden disease are those involving the skin such as trichilemmomas in the face papules, papillomatosis in the mucosal and cutaneous tissues, and hyperkeratosis in the acral region of the skin (4). The association of cutaneous squamous cell carcinomas with Cowden disease has also been reported (5, 6).

PTEN is a dual protein and lipid phosphatase (7, 8). PTEN’s major substrate is PIP3, a second messenger molecule generated by PI3K activated in response to numerous growth factors such as EGF (9), hepatocyte growth factor (10), fibroblast growth factors (11), and IGF-I (12). PIP3 in turn activates the serine-threonine kinase Akt/PKB, which is involved in antiapoptosis, proliferation and oncogenesis. Thus, by dephosphorylating PIP3, PTEN negatively regulates cell survival.

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These individuals contributed equally as last authors.

To whom requests for reprints should be addressed, at Advanced Medical Discovery Institute, 620 University, Suite 708, Toronto, Ontario, M5G 2C1 Canada. Phone: (416) 204-2236; Fax: (416) 204-5300. E-mail: tmak@uhh雷斯.utoronto.ca

These individuals contributed equally as second authors.

The abbreviations used are: PIP3, phosphatidylinositol 3,4,5-trisphosphate; PI3K, phosphoinositide-3-kinase; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; IGF, insulin-like growth factor; MEF, mouse embryonic fibrosarcoma; SE, scanning electron microscopy; DBMA, 7,12-dimethylbenz(a)anthracene; TPA, 12-O-tetradecanoylphorbol-13-acetate; MAPK, mitogen-activated protein kinase; LOH, loss of heterozygosity; PKB, protein kinase B.

MATERIALS AND METHODS

Generation of k5Ptenfloxflox Mice. Ptenfloxflox mice (129 Ola x C57BL/6J F2) generated as previously described (29) were mated to Keratin5Cre transgenic mice (C57BL/6J background; Ref. 34) in which expression of Cre is controlled by the endogenous promoter of the keratinocyte-specific gene Keratin5. Offspring carrying Keratin5Cre and two copies of the floxed Pten
allele (k5CrePten<sup>flox/flox</sup>), Keratin5Cre plus one copy of the floxed Pten allele (k5CrePten<sup>lox/</sup>), and Keratin5Cre plus two copies of the wild-type Pten allele (k5CrePten<sup>+/+</sup>) were used in the analyses as homozygous mutant (k5Pten<sup>flox/flox</sup>), heterozygous mutant (k5Pten<sup>flox/+</sup>) and wild-type (k5Pten<sup>++</sup>) mice, respectively.

**PCR Analysis of Pten Genotypes.** Genomic DNA from mouse tails was isolated and amplified by PCR following a published protocol (29). Sense primer [5'-GTCAGCAGGTCTGTA-3'] and antisense primer [5'-GAACGGCTTACGAGT-3'] were used to detect the floxed Pten allele; sense primer [5'-GTCAGCAGGTCTGTA-3'] and antisense primer [5'-TGCTACAGCATGCAAC-3'] were used to detect the wild-type Pten allele; and sense primer [5'-ATGGCAGCTCTTCAGT-3'] and antisense primer [5'-TGCCTTTTTTACCTCAGA-3'] were used to detect the Keratin5Cre transgene. Amplified fragments of 512 bp, 413 bp and ~300 bp, respectively, were obtained.

**Preparation and in Vitro Culture of Keratinocytes.** Full-thickness skin taken from newborn mice was treated with 250 units/ml dispase (Godosbuseri, Tokyo, Japan) overnight at 4°C. The epidermis was peeled off from the dermis and trypsinized to prepare single cells that were suspended in Defined Keratinocyte-SFM medium (Life Technologies, Inc.) with supplements and 2% FCS. Cells were seeded at 1 × 10<sup>5</sup> cells/10 cm dish (for proliferation assays) or 5 × 10<sup>5</sup> cells/well of a 6-well plate (for apoptosis assays), which had been precoated with collagen type I (Iwaki Glass, Tokyo, Japan). Cells were cultured at 37°C in 5% CO<sub>2</sub> for 5 h until cells had attached and spread. Unattached cells were removed by washing with PBS, and the attached cells were additionally cultured in fresh medium without FCS for 24 h before proliferation and apoptosis assays (see below).

**Southern and Western Blots.** Genomic Southern blots of DNA obtained from keratinocytes were performed using a previously described probe and protocol (29). Genomic DNA from keratinocytes of new-born mice (k5Pten<sup>flox/flox</sup>), mice showing that Cre-mediated recombination of the Pten allele (Fig. 1A) were either left untreated or stimulated for 10 min or 16 h with 30 μg/ml EGF (Biomedical Technologies). Total cell lysates were prepared, and 15 μg of lysate aliquots were analyzed by Western blotting as described previously (30). Antibodies directed against the NH<sub>2</sub> terminus of Pten and antiactin were from Santa Cruz, whereas antiphospho-Akt/PKB (Ser<sup>473</sup>, antiaktal Akt/PKB, antiphospho-MAPK (p42/p44), and antitotal MAPK (p42/p44; Thr202/Tyr204) antibodies were from New England Biolabs.

**Histological Analysis and Immunohistochemistry.** For histological analysis, dorsal skin samples and tumors were fixed in formalin and embedded in paraffin before sectioning according to standard protocols. Sections of 5 μm were cut and stained with H&E. For immunohistochemical staining, freshly cultured at 37°C and trypsinized to prepare single cells that were suspended in Defined Keratinocyte-SFM medium without supplements and cultured in the presence of EGF (30 μg/ml) for 72 h. Cells were treated with 1 μCi [3H]thymidine (Amersham) for another 16 h before harvesting by trypsinization. Incorporated radioactivity was measured using a β-scintillation counter.

**Apoptosis Assay.** Keratinocytes cultured in 6-well plates coated with type I collagen were treated with either UV- or γ-irradiation at the doses indicated in Fig. 4. One day after treatment, cell viability was determined by staining with 7-aminocoumarin D (Sigma) as described previously (35).

**SE.** Hairs plucked from the anterior backs of mice were attached to aluminum mounts and coated with 350 angstrom gold using EIKO IB-5 sputter coater. Samples were observed under a JEOL JSM-T200 scanning electron microscope operated at 15-kV accelerating voltage. Photographs were taken using Fuji Neopan SS film.

**Tumor Induction.** Mice (6–7 weeks old) were shaved on their backs 2 days before tumor induction. To induce tumors, the shaved dorsal skin of mice was treated topically with 25 nmol of DMBA (Sigma) in acetone. After 1 week, each animal received subsequent topical treatments of 10 nmol of TPA (Sigma) in acetone twice weekly for 4 weeks. Control mice were treated with acetone only.

**RESULTS**

**Generation of Keratin5-CrePten<sup>flox/flox</sup> Mice.** Keratinocyte-specific Pten-deficient mice were generated using the strategy described in “Materials and Methods” and Fig. 1A. Briefly, Keratin5Cre transgenic (k5CreTyg) mice (34) were crossed to mice homozygous for the floxed Pten allele (Pten<sup>flox/flox</sup> mice) to generate Keratin5CrePten<sup>flox/flox</sup> mice (k5Pten<sup>flox/flox</sup>) mice. The Keratin5 promoter directs gene expression from day 13.5 postcoitum (d.p.c.) in the basal layer of epidermal and follicular keratinocytes so that expression of a floxed gene under the control of this promoter is disrupted throughout the epidermis and the outer root sheaths of hair follicles (36, 37). In mice bearing a floxed allele of the Pten gene, expression of Cre using the Keratin5 promoter induced the disruption of Pten not only in the vast majority of keratinocytes but also in the esophagus and stomach (34). These results are consistent with a previous report in which the Keratin5 gene was found to be expressed in esophagus and stomach as well as in epidermis (36, 38).

**k5Pten<sup>flox/flox</sup> mice** were born alive and initially appeared healthy. Genomic Southern blotting of DNA from keratinocytes of newborns showed that Cre-mediated recombination of loxp sites resulted in the deletion of much of the 6.0-kb Pten<sup>flox</sup> allele in almost all keratinocytes, leaving the 2.3-kb PtenΔ allele (Fig. 1B). The deletion of Pten was confirmed at the protein level by Western blotting using antibody recognizing the NH<sub>2</sub> terminus of Pten (Fig. 1C). Extracts of keratinocytes from k5Pten<sup>flox/flox</sup> mice showed a major reduction in

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Fig. 1. Generation of keratinocyte-specific Pten-deficient (k5Pten<sup>flox/flox</sup>) mice. A, targeting strategy. Exons of the murine Pten gene are represented by black arrowsheads. The probe used to analyze Southern blots is indicated and has been described previously (35). The floxed (Pten<sup>flox</sup>) and deleted (Pten<sup>Δ</sup>) alleles are shown. B, genomic Southern blot. DNA (20 μg) extracted from keratinocytes of the indicated genotypes was digested with HindIII. The vast majority of keratinocytes from k5CrePten<sup>flox/flox</sup> mice (k5Pten<sup>flox/flox</sup>) showed deletion of the Pten gene. C, Western blot analysis of Pten protein expression by keratinocytes of the indicated genotype. Actin, loading control.
Pten protein, whereas keratinocytes of k5Pten<sup>flox/+</sup> mice showed only a minor decrease.

**Lethality in the Lactation Period and Gross Skin Abnormalities in k5Pten<sup>flox/flox</sup> Mice.** We monitored the growth and health of k5Pten<sup>+/+</sup>, k5Pten<sup>flox/+</sup>, and k5Pten<sup>flox/flox</sup> mice for several months. All k5Pten<sup>flox/flox</sup> mice could be identified without genotyping because the skin of mutant animals 3 days of age or older was wrinkled because of hyperplasia (Fig. 2A). In addition, most k5Pten<sup>flox/flox</sup> mice were significantly smaller than their k5Pten<sup>+/+</sup> littersmates from 3–5 days of age on (Fig. 2B), and their hair coats were abnormally ruffled and shaggy (Fig. 2C). k5Pten<sup>flox/flox</sup> mice had hyperplastic noses and lips (Fig. 2, D and E) and slightly enlarged ears (data not shown), giving the mutants the appearance of a wrinkled bear (Fig. 2, D and E).

Surprisingly, >90% of k5Pten<sup>flox/flox</sup> mice died ~6 days after birth because of malnutrition during the lactation period (Fig. 2F). However, animals that survived this period and made it to the 2-month mark had normal life spans without severe malnutrition. Overall, these mutant mice looked similar to k5Pten<sup>+/−</sup> and k5Pten<sup>flox/+</sup> mice, although their skin and hair abnormalities were still obvious. Gross histological examination of k5Pten<sup>flox/flox</sup> pups revealed marked hyperkeratosis in the esophagus (Fig. 2, G and H). Milk was easily identified in the stomachs of wild-type mice but only faintly visible in the stomachs of k5Pten<sup>flox/flox</sup> pups. It is highly likely that esophageal dysfunction because of hyperkeratosis caused the dysphagia, perhaps accounting for the small body size of the mutant mice and their deaths because of malnutrition. Examination of the hair of k5Pten<sup>flox/flox</sup> mice showed that the cuticles, which normally cover the hair shaft, were almost completely detached from the hair shafts (Fig. 2, I and J). In contrast, k5Pten<sup>flox/+</sup> mice were indistinguishable from k5Pten<sup>+/+</sup> mice in both gross appearance and histological findings (data not shown).

**Precocious Hair Follicle Morphogenesis in Pten-deficient Epidermis.** To determine the cause of the gross abnormalities of k5Pten<sup>flox/flox</sup> skin, we analyzed the microscopic architecture of skin in tissue specimens taken at various time points after birth. Histological comparison with wild-type mice revealed that hyperkeratosis, hypergranulosis, and epidermal hyperplasia were present in Pten-deficient newborns (Fig. 3; day 0, top and middle panels). Moreover, the high density of the hair follicles in the mutant caused a reduction of interfollicular epidermis (Fig. 3; day 0, top panel and arrow in bottom panel; day 7, top panel), and sebaceous glands showed advanced development in k5Pten<sup>flox/flox</sup> skin (Fig. 3; day 7, arrows in top and bottom panels). At ~10 days after birth, the epithelia of k5Pten<sup>flox/flox</sup> mice became papillomatous. Immunostaining using antibodies specific for Keratin5 and Keratin10 showed no obvious difference between k5Pten<sup>+/+</sup> and k5Pten<sup>flox/+</sup> mice, except that ectopic staining by anti-Keratin10 was observed in the infundibular epidermis of the mutants (Fig. 3; day 10, middle panel).

In wild-type mice, skin morphogenesis is completed by the end of the first hair follicle cycle. Hair remodeling then begins its lifelong cycle of regression, resting, and spontaneous regrowth (39). In this study, the first anagen phase of hair development in wild-type mice continued until day 10, the day on which the thickest skin was present (Fig. 3; day 10, top left panel). In contrast, the skin of k5Pten<sup>flox/flox</sup> mice was much thicker than that of wild-type animals on day 7 but significantly thinner on day 10, suggesting that the mutants had already regressed into the catagen phase (Fig. 3; day 7, top panel and day 10, top panel). In wild-type mice, relative quiescence (telogen) was observed at day 19 (Fig. 3; day 19, top and middle left panels). However, on day 19 in k5Pten<sup>flox/flox</sup> mice, the epithelia were papillomatous and thicker than in the wild-type, and well-developed sebaceous glands were present (Fig. 3; day 19, top and middle right panels). Immunostaining using anti-Ki-67 antibody indicated that on day 19, basal cells in the mutant were proliferating more than those of wild-type littersmates (Fig. 3, day 19, bottom panels). Moreover, the proliferation of basal cells in the mutant on day 19 was greater than that of mutant basal cells on day 15 (data not shown), consistent with accelerated entrance into the next anagen phase. Although follicular remodeling was not impaired in the absence of Pten (data not shown), most epithelia in the mutant mice continued to show hyperkeratosis, hyperplasticy (especially the granular layer), and well-developed sebaceous glands even at 3 months of age (Fig. 3, 3 months, right panels). The hair was consistently abnormal (data not shown). The presence of hyperplastic epithelia and the acceleration of skin mor-

![Fig. 2. Gross abnormalities of k5Pten<sup>flox/flox</sup> mice. A–C, reduced size. k5CrePten<sup>+/+</sup> (left) and k5CrePten<sup>flox/flox</sup> (right) pups at (A) 4 days, (B) 6 days, and (C) 12 days after birth. The skin of k5CrePten<sup>flox/flox</sup> mice exhibits hyperproliferation and the hair is ruffled and shaggy. D and E, wrinkled bear appearance. Lower face regions of k5CrePten<sup>+/+</sup>/H11001 and k5CrePten<sup>flox/flox</sup>/H11001 mice (E) at 1 month of age. F, lethality of the k5CrePten<sup>flox/flox</sup> mutation. Numbers of dead mice (■) on the indicated day after birth and the total number of the surviving mice (□) are plotted. G and H, severe hyperkeratosis (arrow) in the lower esophagus. Comparative histology of lower esophagus of k5CrePten<sup>+/+</sup> (G) and k5CrePten<sup>flox/flox</sup> (H) mice. I and J, cuticle detachment. SE analysis of cuticles (arrow) of k5CrePten<sup>+/+</sup> (I) and k5CrePten<sup>flox/flox</sup> (J) mice. The cuticle of the k5CrePten<sup>flox/flox</sup> hair has almost completely separated from the hair shaft.
phogenesis in k5Pten^flox/flox^ mice indicate that Pten is indispensable for the normal development of skin.

**Skin Tumor Formation in k5Pten^flox/+ and k5Pten^flox/flox Mice.**

To further characterize the effects of Pten gene disruption on mouse epidermis, 32 wild-type, 31 heterozygous mutant, and 11 homozygous mutant mice that survived over 2 months after birth were monitored for spontaneous tumorigenesis. As shown in Fig. 4A, no skin tumors were detected on k5Pten^+/+ mice during the 8.5 month observation period. In contrast, spontaneous tumors developed in 23% of k5Pten^flox/^ mice and, most surprisingly, in...
100% of k5Pten<sup>flox/flox</sup> mice. Most of these spontaneous tumors were squamous papillomas that occurred on the face and palms of the front paws (Fig. 4, B and F). However, many of these papillomas went on to develop into squamous cell carcinomas with nuclear atypia and increased mitosis. These latter tumors were capable of invading the dermis (Fig. 4, C and G). In addition to papillomas and squamous cell carcinomas, we observed sebaceous carcinomas characterized by an obvious sebaceous gland-like structure (in 11% of tumor-bearing mice; Fig. 4, D and H) and adenocarcinomas of the sweat gland (in 11% of tumor-bearing mice; Fig. 4, E and I). To confirm that the tumors arising in k5Pten<sup>flox/flox</sup> mice arose because of a LOH, PCR assays on tumor DNA were carried out. The loss of the wild-type Pten allele was observed in 3 of 3 squamous carcinomas obtained from k5Pten<sup>flox/flox</sup> mice (Fig. 4J).

To examine induced carcinogenesis in Pten-deficient skin, k5Pten<sup>+/+</sup>, k5Pten<sup>flox/+</sup>, and k5Pten<sup>flox/flox</sup> mice of 6–7 weeks of age (n = 8/group) were treated with either DMBA plus TPA or TPA alone. DMBA initiates skin tumorogenesis, and TPA promotes growth of an established skin tumor. Surprisingly, 100% of k5Pten<sup>flox/flox</sup> mice treated with DMBA followed by TPA developed 5–15 skin papillomas in the treated area (only) within 5 weeks of the initial DMBA treatment (Fig. 4, K–M). In contrast, no tumors were present on the skin of either k5Pten<sup>flox/+</sup> or wild-type mice treated in the same fashion for the same duration. TPA treatment alone failed to induce tumorigenesis in any of the three groups during the 6-week observation period (data not shown). This result was not unexpected because spontaneous tumors did not appear in k5Pten<sup>flox/flox</sup> mice until 3.5 months of age. These observations indicate that Pten functions as a tumor suppressor for both spontaneous and induced skin tumors in mice.

Hyperproliferation and Resistance to Apoptosis in k5Pten<sup>flox/flox</sup> Keratinocytes. We next investigated whether the accelerated skin morphogenesis and oncogenesis in k5Pten<sup>flox/flox</sup> mice were associated with a defect in keratinocyte proliferation or apoptosis. Keratinocytes were stimulated in vitro with EGF to induce proliferation or subjected to UV- or γ-irradiation to induce apoptosis. As shown in Fig. 5A, Pten-deficient keratinocytes showed enhanced proliferation in response to EGF. In addition, the mutant keratinocytes were more...
resistant than wild-type keratinocytes to apoptosis induced by either high dose UV- or γ-irradiation (Fig. 5B).

Activation of Akt/PKB and MAPK in Pten-deficient Keratinocytes. We have previously reported that regulation of Akt/PKB activation by Pten is critical for normal proliferation of MEFs and for proliferation/apoptosis in T cells (30, 35). MAPK, a major signaling molecule downstream of Ras, is also activated downstream of PI3K (40). Our previous demonstration that both MAPK and Akt/PKB are activated in Pten-deficient T cells (35) prompted us to analyze the phosphorylation of Akt/PKB and MAPK in keratinocytes from k5Ptenfloxflox mice compared with k5Pten−/− mice and k5Ptenfloxflox newborn mice. After stimulation with EGF, the phosphorylation of Akt/PKB (Fig. 6A) and MAPK (Fig. 6B) was significantly elevated in k5Ptenfloxflox keratinocytes compared with k5Pten−/− or k5Ptenfloxflox keratinocytes. Thus, in keratinocytes, as in T cells, Akt/PKB and MAPK activation is subject to negative regulation by Pten.

DISCUSSION

In previous studies, we showed in MEFs, T cells, and neuronal cells that Pten deficiency causes accumulation of PI3K and constitutive activation of Akt/PKB. These events result in cellular hyperproliferation and resistance to apoptosis that can lead to abnormal development and malignancy (29, 30, 41). We now report similar findings in murine skin in vivo and in keratinocytes in vitro from studies of mice in which the Cre-loxP system was used to selectively disrupt the Pten gene in the epidermis.

Hereditary heterozygous mutation of PTEN in humans is associated with Cowden’s disease (2), a disorder characterized by the onset of multiple hamartoma in various tissues. These hamartoma frequently develop into malignancies such as breast and thyroid cancers (5). The characteristic clinical features of Cowden’s disease include skin abnormalities such as trichilemmomas in the face papules, papillomatosis in the mucosal and cutaneous tissues, and hyperkeratosis in the acral region of the skin (4). At the molecular level, skin lesions in Cowden’s disease patients have shown evidence of LOH for PTEN (42). In this study, we observed significant similarities to Cowden’s disease symptoms in k5Ptenfloxflox mice. The mutant animals exhibited hyperkeratosis and spontaneous tumors such as papillomas and cutaneous squamous cell carcinomas. Although trichilemmomas, a type of tumor of the epidermal appendages, did not occur in k5Ptenfloxflox mice, the mutants frequently displayed other types of epidermal appendage tumors. We therefore believe our k5Ptenfloxflox mice represent not only a reasonable model of the skin lesions characteristic of Cowden’s disease but also a suitable model of skin carcinogenesis in general.

The phenotypes observed in k5Ptenfloxflox mice are reminiscent of those of Ha-ras (26), transforming growth factor α (22), sons of sevenless (SOS) (24), and IGF-1 (25, 27) transgenic mice. The similarities between k5Ptenfloxflox and transgenic mice expressing IGF-1 under the control of the Keratin5 promoter (k5IGF-1 Tg) are particularly striking. k5IGF-1 Tg mice are small in size as neonates and have wrinkled, thick skin because of hyperkeratosis and epithelial hyperplasia. The hair of these mutants is ruffled and shaggy, and spontaneous tumors such as papillomas and squamous cell carcinomas develop with age. Cells of these mutants show evidence of apoptotic resistance and activation of PI3K and its downstream mediators Akt/PKB and ERK (25). These observations, taken together with the results of our study, suggest that signal transduction downstream of many molecules affecting cell growth/death involves common Akt/PKB- and ERK-mediated pathways. Disruption of these pathways may account for the phenotypes of k5Ptenfloxflox mice.

One of the most striking phenotypes in k5Ptenfloxflox mice is the precocious morphogenesis of skin. The wnt/β-catenin/Lef-1 pathway has been reported to be very important for the acceleration of developmental morphogenesis in the skin (43). In the embryonic skin of 14.5 d.p.c mice, pilosebaceous units develop from epidermal downgrowths under the influence of specific mesenchymal cell condensations. These condensations supply permissive and instructive signals that govern the position and type of hairs and other appendages developed (reviewed by Refs. 38, 44). The expression of patterning genes such as those in the wnt/β-catenin/Lef-1 signaling pathway are thought to regulate these signals (43). Perhaps significantly, Pten has been shown to negatively regulate the β-catenin/Lef-1 pathway by
inhibiting the nuclear accumulation of β-catenin and activation of Lef-1 in a prostatic cell line (45). However, in our hands, no difference in the subcellular distribution of β-catenin in k5Ptenfl/fl cells was observed (data not shown). Both k5Ptenfl/fl mice and k5Gf-1 Tg mice showed accelerated hair growth at day 5 (27), indicating that common molecules downstream of PKB/Akt in addition to β-catenin, or molecules downstream of ERK, may account for the accelerated skin morphogenesis in these mice.

Several lines of evidence suggest that Akt/PKB may be a key molecule regulating the onset of skin carcinogenesis in mice. The transplantation of keratinocytes overexpressing Akt/PKB results in highly aggressive skin tumors characterized by increased invasiveness and altered differentiation (33). In addition, Akt/PKB activation is one of the first events in the chemical induction of skin tumors (33). Finally, the onset of skin tumor formation in mice requires EGFR and Akt/PKB signaling in addition to SOS/Ras/ERK signaling (24). Indeed, MAPK/ERK has been reported to act in synergy with the PI3 K pathway to stimulate CycD1 transcription in NIH3T3 cells (46). Thus, the same mechanism underlies the phenotypes in k5Ptenfl/fl mice. Prolonged observation of k5Ptenfl/fl mice subjected to treatment with either DMBA or TPA alone may help to define the role of Pten in chemically induced carcinogenesis.

In this study, both k5Ptenfl/fl and k5Ptenfl/+ mice developed spontaneous skin tumors at high frequency. Fully 100% of k5Ptenfl/fl mice and 23% of k5Ptenfl/+ mice acquired papillomas and/or squamous cell carcinomas during the 8.5-month observation period. The result for the k5Ptenfl/+ mice is particularly surprising in light of the fact that skin tumors are observed in Pten−/− mice at a frequency of <5%. We speculate that the tissue-specificity of the Pten mutation examined in this study may in some way account for this discrepancy. For example, an immunosuppression mechanism able to block skin tumor formation may be triggered in response to global (only) inactivation of Pten in heterozygotes. Such a mechanism would be in line with our previous observations of mice with a T-cell-specific Pten deficiency. These mutants experience an accumulation of T cells that produce increased levels of Th1 and Th2 cytokines (35).

Our study is the first reported in vivo analysis of Pten function in mouse skin. We clearly demonstrate that Pten is an essential regulator of normal homeostasis and oncogenesis in the organ. Our results suggest that inhibition of the PIP3-Akt/PKB pathway may be an attractive therapeutic target for the treatment of skin malignancies. Studies to examine Pten expression in human keratinocyte malignancies are ongoing.

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ROLE OF PTEN IN SKIN MORPHOGENESIS AND ONCOGENESIS


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