TO THE EDITOR

Seborrheic keratoses (SKs) are among the most common benign tumors in humans, occurring in 80–100% of people over 50 years of age (Yeatman et al., 1997; Kwon et al., 2003). Although not life-threatening, SKs may become irritated and itchy, are often unattractive and disfiguring, and may have a significantly negative psychological impact as daily reminders of aging. Several recent studies of the pathogenesis of SK have identified the importance of somatic activating mutations of fibroblast growth factor receptor 3 (FGFR3) (Logie et al., 2005; Hafner et al., 2006a, b, 2007a, b, c) and oncogenic mutations of the p110α subunit (PIK3CA) of phosphatidylinositol 3-kinase (PI3K) in an independent distribution (Hafner et al., 2007d, 2008).

Figure 1. PTEN (phosphatase and tensin homolog deleted on chromosome 10) hemizygosity is a predisposing factor for skin tumorigenesis following UVA irradiation. (a) Immunoblot analysis of PTEN protein levels in the epidermis from K14Cre;Pten+/+/ (+/+) and K14Cre;Ptenfl+/− (+/−) mice. (b) Percentage of tumor-free mice (n = 15). (c) Incidence of seborrheic keratosis (SK) and squamous cell carcinoma (SCC). (d) A mouse with an SK (indicated by a red arrow) developing in its skin. (e–h) Hematoxylin and eosin staining of the mouse specimens with SK and SCC. (e) SK at original magnification × 10. (f) SCC at original magnification × 10, with a smaller field of view. (e) Scale bar = 400 μm and (f–h) scale bar = 100 μm. The green arrow in (h) indicates a multinucleated cell.

Abbreviations: AKT, a serine–threonine kinase, downstream of PI3K, also called protein kinase B; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; SCC, squamous cell carcinoma; SK, seborrheic keratosis; +/+; Pten+/+, K14Cre, Pten+/+; +/−, Pten+/−, K14Cre, Pten+/−; +/−, Pten+/−, K14Cre, Ptenfl+/−
At the molecular level, PI3K signaling, upstream of AKT (a serine-threonine kinase downstream of PI3K, also called protein kinase B) activation, is negatively regulated by the tumor suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10) (Di Cristofano and Pandolfi, 2000; Suzuki et al., 2003; Backman et al., 2004). Our recent studies demonstrated that PTEN is downregulated by exposure to both UVB (Ming et al., 2010) and UVA (He et al., 2006).

Although SKs occur on both sun-exposed and non-sun-exposed skin, cumulative exposure to sunlight and aging have been proposed as independent risk factors for their development (Yeatman et al., 1997; Kwon et al., 2003). Here we report that in mice with a targeted PTEN downregulation in their epidermis, UVA causes the formation of tumors resembling human SKs as well as of squamous cell carcinomas (SCCs).

To determine the role of PTEN in UVA-induced skin tumorigenesis, we compared UVA-induced tumor formation between mice with normal PTEN (+/+) and mice with PTEN hemizygosity (+/−). All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Chicago. We deleted one allele of the Pten gene in the epidermal keratinocytes by crossbreeding mice expressing Cre recombinase under the control of the keratin 14 promoter (K14-Cre) with mice expressing a LoxP-flanked (floxed) Pten gene (Ptenfl/fl) to

**Figure 2. The PTEN (phosphatase and tensin homolog deleted on chromosome 10)/AKT (a serine-threonine kinase) pathway is involved in UVA-induced tumorigenesis in Pten+−/− mice.** (a-e) Immunohistochemical analysis of PTEN in nontumor and tumor samples using an anti-PTEN antibody. Scale bar = 50 μm. (a, b) Sham-irradiated normal epidermis from (a) Pten+/+ and (b) Pten+−/− mouse. (c) UVA-irradiated nontumor epidermis, (d) seborrheic keratosis (SK), and (e) squamous cell carcinoma (SCC) from Pten+−/− mouse. (f) Immunoblot analysis of PTEN, p-AKT (serine 473), AKT, and GAPDH (equal loading control) in +/+, +/−, SK, and SCC. (g) Quantification of PTEN protein levels in (f). (h) Quantification of the ratio of p-AKT/AKT in (f). N in (g, h), normal Pten+/+ skin. (i) Real-time RT-PCR analysis of the GLTSCR2 mRNA levels in +/+, +/−, and SK (n = 3). (j) Immunoblot analysis of p-AKT, AKT, p-ERK (extracellular signal-regulated kinase), ERK, PTEN, and β-actin (equal loading control) in individual +/+, 1/−, and +/− mouse epidermis (n = 3) at 6 and 24 h post-UVA (15 cm−2). (k) Cell growth analysis of neonatal primary Pten+/− keratinocytes infected with empty vector (EV) alone or wild-type Pten adenovirus (Ad-Pten) at a multiplicity of infection of 50 at different times post sham or UVA irradiation (10 cm−2) (n = 3). The right panel shows immunoblot analysis of PTEN and β-actin. Error bars show standard error (SE). (g, h) *P<0.05, significant difference between comparison groups. NS, not statistically significant, P>0.05. **P<0.05, significant difference between sham-irradiated +/−/EV and +/−/Ad-Pten groups. +/+, K14Cre/Pten+/+; +/−, K14Cre/Ptenfl/fl; EP, epidermis; D, dermis; HF, hair follicle; S, stroma; T, tumor.
To mimic the scenario of low-level UVA exposure that occurs in daily life for most people, we determined that the maximal dose of UVA that did not cause erythema was 15 J cm\(^{-2}\) and used that dose for all UVA experiments. The shaved mice were exposed to UVA or protected from UVA (sham group) for 25 weeks and then monitored for tumor development.

In the absence of UVA, Pten\(^{+/+}\) and Pten\(^{+-}\) did not grow tumors throughout the study period. UVA did not cause tumor formation in Pten\(^{+/+}\) mice. However, Pten\(^{+-}\) mice began to develop skin tumors at 37 weeks of UVA irradiation (Figure 1a). A log-rank test showed that PTEN hemizygosity significantly accelerated skin tumorigenesis following UVA irradiation (\(P<0.0001\)). Histopathological analysis of the tumors, using similar criteria for mice (Logie et al., 2005; Benavides et al., 2009), showed that in UVA-exposed Pten\(^{+-}\) mice 67% of the tumors were of the SK type, whereas 33% were SCCs (Figure 1c and d).

Thus, following UVA radiation, PTEN downregulation becomes a predisposing factor for skin tumorigenesis. Similar to the transgenic mouse model with an activating FGFR3 mutation (Logie et al., 2005), the skin lesions observed in our mouse model share several histological features with human SKs, including acanthosis, hyperkeratosis, papillomatosis, and keratin-filled structures (horn cysts and pseudo-horn cysts) (Figure 1e and f), while lacking significant cytological atypia, whereas the SCCs are characterized by the presence of atypical nuclei, mitotic figures, and multinucleated cells (Figure 1g and h).

To determine the molecular mechanisms by which UVA causes SK in Pten\(^{+-}\) mice, we examined the role of the glioma tumor-suppressor candidate region gene 2 (GLTSCR2), a nuclear protein that binds to and stabilizes PTEN (Okahara et al., 2004; Yim et al., 2007) and is reduced in SK (Kim et al., 2010). Real-time RT-PCR analysis showed that there were no significant differences in GLTSCR2 mRNA levels between +/+, +/−, and SK (Figure 2i), suggesting that GLTSCR2 is not involved in our SK mouse model. To determine the molecular impact of PTEN downregulation, we examined the phosphorylation of AKT and ERK (extracellular signal-regulated kinase) in parental and PTEN-downregulated epidermis. We found that phosphorylation of AKT and ERK in Pten\(^{+-}\) skin was increased at 6 and 24 h after 15 J cm\(^{-2}\) UVA irradiation (Figure 2j and Supplementary Figure S1 online). PTEN downregulation significantly increased AKT phosphorylation at both 6 and 24 h following 15 J cm\(^{-2}\) UVA irradiation (Figure 2j and Supplementary Figure S1A online). However, it significantly increased ERK phosphorylation only at 24 h following UVA irradiation (Figure 2j and Supplementary Figure S1B online). Furthermore, we found that UVA irradiation inhibited cell growth in vitro and in vivo (Figure 2k and Supplementary Figure S2 online). Constitutive PTEN reduction in the epidermis delayed cell exit from UVA-induced growth arrest (Supplementary Figure S2 online). While acute re-expression of PTEN in Pten\(^{+-}\) keratinocytes had little effect on cell growth post-UVA irradiation in vitro, it significantly inhibited cell growth in sham-irradiated cells (\(P<0.05\)) (Figure 2k). These data indicate that PTEN reduction exerts a critical role in UVA-induced development of SK as well as SCC, probably through the AKT and ERK pathways.

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
This work was supported by NIH grant ES016936 (YHY) and the NIH/NIEHS intramural program. Further support was provided by the UC Friends of Dermatology Fund. We thank Ramon Parsons (Departments of Pathology and Medicine, Columbia University, New York) for kindly providing us with the adenosaromal PTEN vector and Terri Li for PTEN immunohistochemistry. This paper is dedicated to the memory of Colin F. Chignell.

Ming M, Christopher R. Shea\(^{1}\), Li Feng\(^{2}\), Keyoumars Soltani\(^{1}\) and Yu-Ying He\(^{1}\)

\(^{1}\)Department of Dermatology, Department of Medicine, University of Chicago, Chicago, Illinois, USA

\(^{2}\)Laboratory of Pharmacology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina, USA

E-mail: yyhe@medicine.bsd.uchicago.edu

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES
Hafner C, Hartmann A, van Oers JM et al. (2007b) FGFR3 mutations in seborrheic keratoses are already present in flat lesions and associated with age and localization. Mod Pathol 20:895-903

www.jidonline.org 1585
Hafner C, Lopez-Knowles E, Luis NM et al. (2007d) Oncogenic PIK3CA mutations occur in epidermal nevi and seborrheic keratoses with a characteristic mutation pattern. Proc Natl Acad Sci USA 104:13450–4


Hafner C, Vogt T, Hartmann A (2006b) FGFR3 mutations in benign skin tumors. Cell Cycle (Georgetown, TX) 5:2723–8


