

## Topical Tazarotene Chemoprevention Reduces Basal Cell Carcinoma Number and Size in *Ptch1*+/- Mice Exposed to Ultraviolet or Ionizing Radiation

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### Abstract

Oral retinoids can reduce basal cell carcinoma (BCC) incidence in genetically susceptible patients, and one topical retinoid, tazarotene, has been reported to cure some sporadic BCCs. Therefore, we have tested whether this agent would affect BCCs in *Ptch1*+/- mice in a controlled chemoprevention trial. We found that topical tazarotene dramatically inhibits the formation of BCCs induced with either UV or ionizing radiation. The ability of tazarotene to inhibit BCC formation in this mouse model provides encouragement for the use of tazarotene in skin cancer chemoprevention trials in humans.

### Introduction

Retinoids have intriguing anticancer effects in many epithelia (1), including the skin, where epidermal differentiation is aberrant both in hyper- and hypo-vitaminosis A (2). At high doses, oral retinoids can reduce markedly the growth of new tumors in patients genetically susceptible to skin cancers: patients with the basal cell nevus syndrome or xeroderma pigmentosum (3). However, past trials of the effects of topical retinoids on murine photocarcinogenesis have been somewhat inconclusive; all-*trans*-retinoic acid has been shown in some trials to augment photocarcinogenesis and in others to have a mild protective effect (4–7). Tazarotene is an acetylenic retinoid with binding specificity for retinoic acid receptors (RARs)  $\beta$  and  $\gamma$  (8). It is approved in the United States for topical treatment of acne, psoriasis, and photoaging. Intriguingly, in one open-label study, 16 of 30 sporadic human basal cell carcinomas (BCCs) disappeared after 8 months of treatment with topical tazarotene (9). Therefore, we have tested in vehicle-controlled trials the chemopreventive efficacy of tazarotene in our *patched* heterozygote (*Ptch1*+/-) mouse against UV radiation- or ionizing radiation (IR)-induced BCCs. We find that tazarotene is impressively efficacious when given continually before and during UV radiation treatment and also before and after one dose of <sup>137</sup>Cs IR exposure. UV-exposed *Ptch1*+/- mice also develop large numbers of spindle cell tumors (10), and topical tazarotene treatment reduces the development of these tumors as well.

### Materials and Methods

**Mice.** Our *Ptch1*+/- mice are heterozygous for deletions in exons 1 and 2 of the *Ptch1* gene and are carried in our laboratory on an approximately 50:50 mixed C57BL/6 and DBA/2J background (*i.e.*, bred continuously to C57BL/6.DBA/2 F<sub>1</sub> mice; Ref. 10). Mice were housed four to a plastic cage with metal

lids and fed laboratory chow (5008; Purina, St. Louis, MO) and water *ad libitum*. The mice were housed at 70°F–74°F, 50% humidity with 12 h of fluorescent light from overhead 34-W bulbs. Hair was removed from the backs of the mice with electric clippers when needed for the application of topical agents or to allow exposure to the UV radiation.

**Tazarotene Treatment.** A 0.1% topical tazarotene cream (pharmaceutical grade) or vehicle cream was applied topically 5 consecutive days/week to the dorsal skin of mice at 2 mg/cm<sup>2</sup> until animals were 16 months of age.

**UV Exposure.** UV irradiation was administered using a UVB irradiation unit with UVB integrating dosimetry (Daavlin Corp., Bryan, OH) equipped with full spectrum fluorescent bulbs. UV light was filtered through Kodacel plastic sheeting (Eastman Kodak, Rochester, NY) to remove the  $\leq 280$  nm (UVC) light. Mice were exposed to 515 mJ/cm<sup>2</sup> UVB, 3 $\times$  the determined minimal erythema dose, 3 times/week.

**IR Exposure.** Five Gy of  $\gamma$ -radiation were given once, when animals were 3 months of age, using the Best Industries (Springfield, VA) <sup>137</sup>Cs radiation device (half-value layer, 0.60 cm Pb; dose, 0.94 Gy/min).

**Study Design.** In the first UV chemopreventive study, 26 female and 24 male *Ptch1*+/- mice were randomized by sex and litter and treated topically 5 times weekly, beginning at approximately 46 days of age, with either 0.1% tazarotene cream or vehicle control cream. When given on the same day, tazarotene or vehicle cream was applied after irradiation. UVB irradiation was started at 60 days of age. Skin biopsies were performed at 7 ( $n = 50$ ), 9 ( $n = 44$ ), and 11 ( $n = 30$ ) months of age. In a second UV chemopreventive study, 33 female and 10 male *Ptch1*+/- mice were treated as described for the first UV study (described above), and a single biopsy was taken at 9 months of age ( $n = 43$ ). For the IR chemopreventive arm of the study, 17 female and 14 male *Ptch1*+/- mice were randomized, as described above, and treated topically with 0.1% tazarotene or vehicle cream, 5 times weekly from 79 days of age. IR was administered at 91 days of age, and a single skin biopsy was performed at 10 months of age ( $n = 27$ ).

**Standardized Biopsies.** Mice were anesthetized using a 1:1 solution of 20 mg/ml xylazine and 100 mg/ml ketamine. For the first UV study, 1-cm<sup>2</sup> sections of skin were excised from standardized locations of the back. All biopsy samples were sliced vertically into three even, full-thickness slivers. For the IR and second UV study, 1.0  $\times$  1.5 cm of skin was excised from a standardized location of the back and cut vertically into five even, full-thickness slivers.

**$\beta$ -Galactosidase Staining.** LacZ-encoded bacterial  $\beta$ -galactosidase activity was detected by incubation of glutaraldehyde and formalin-fixed tissue with 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside and iron buffer solution (Boehringer Mannheim, Indianapolis, IN) for 48 h.

**Quantitation of Microscopic Tumors.** For the first UV study, single tissue sections of each of three slivers taken from the standard 1-cm<sup>2</sup> biopsy were analyzed for microscopic BCC number. Cross-sectional area was taken by measuring the greatest perpendicular diameters through the tumor. For the IR study and the second UV study, single tissue sections of each of the five slivers from the 1  $\times$  1.5-cm biopsy were analyzed for microscopic BCC number and size. A single observer, blinded with regard to treatment group, performed the histological evaluation of all skin sections for each of the UV and IR studies. Tumor numbers were standardized for an area of 1-cm skin surface length for comparison between the two UV studies and the single IR study. For purposes of this quantitation, tumors with the appearance of BCCs and of related tumor types (*e.g.*, trichoblastomas, and so forth; Ref. 10) were classified collectively as BCCs.

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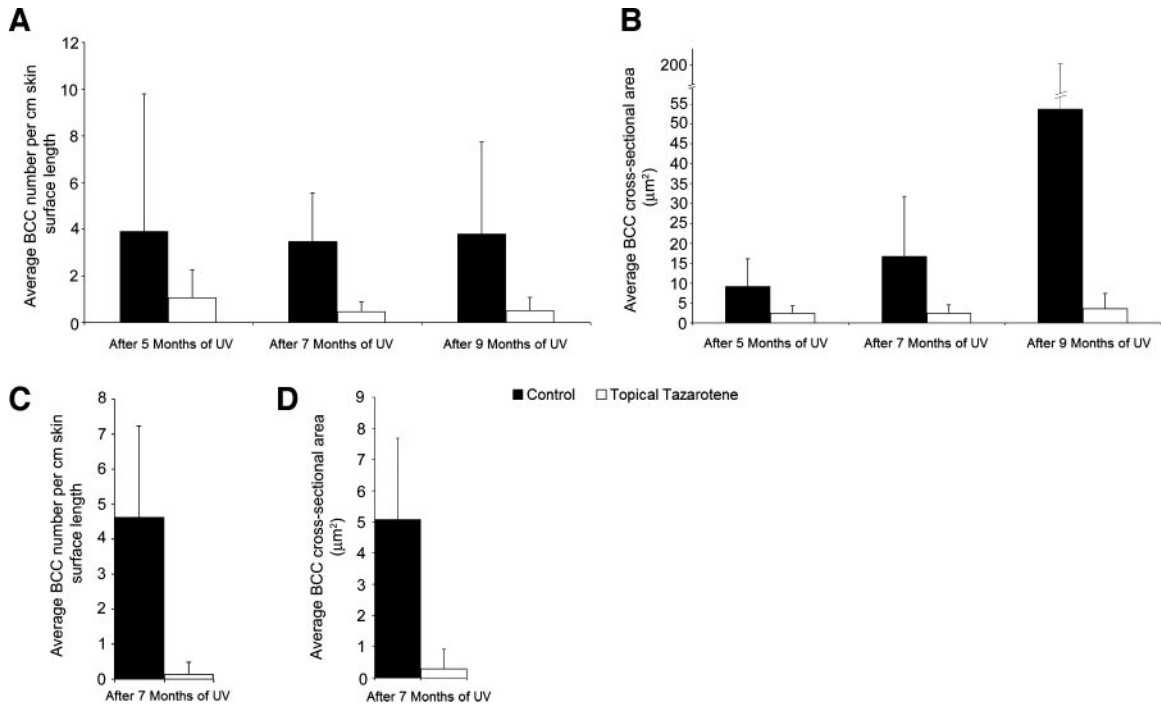


Fig. 1. Tazarotene treatment reduced the number and size of microscopic basal cell carcinomas (BCCs) in UV-treated *Ptch1*<sup>+/-</sup> mice. **A**, UV study 1, the average number of BCCs in mice after 5 ( $n = 50$ ), 7 ( $n = 44$ ), and 9 ( $n = 30$ ) months of UV exposure and treatment with topical tazarotene or control vehicle. Tazarotene-treated mice showed a significant decrease in the number of microscopic BCCs at all biopsy time points. **B**, UV study 1, the average cross-sectional area of BCCs in the same tazarotene- or control vehicle-treated mice after 5, 7, and 9 months of UV exposure. Mice treated with topical tazarotene showed a significant decrease in tumor size at all biopsy time points. **C**, UV study 2, the average number of BCCs in mice treated with topical tazarotene or control vehicle after 7 months of UV exposure ( $n = 43$ ). Similar to the first UV study, topical tazarotene treatment resulted in significantly lower numbers of microscopic BCCs, compared with control vehicle treatment. **D**, UV study 2, the average cross-sectional BCC area after 7 months of UV exposure for mice treated with topical tazarotene and control vehicle. Similar to the first UV study, topical tazarotene reduced the size of BCCs, compared with control vehicle.

**Macroscopic Tumor Analysis.** Visible tumors were initially examined by gross examination to determine the tumor type. Mice with tumors exceeding the animal welfare guidelines were euthanized, and tumors were sent for histological preparation to confirm tumor identities.

**Statistics.** Graphs and statistical analyses were plotted using Microsoft Excel. Kaplan-Meier graphs were plotted using the EcStat program linked to Microsoft Excel.

## Results

Consistent with our previous experience (10), all *Ptch1*<sup>+/-</sup> mice treated with vehicle control cream and exposed to UV or IR developed microscopic BCCs (Fig. 1). In the first UV study, *Ptch1*<sup>+/-</sup> mice treated topically with 0.1% tazarotene had fewer microscopic BCCs per centimeter of skin surface length than did those in the vehicle control group after 5 months of UV radiation at 7 months of age (1.05 versus 3.9;  $P < 0.03$ ); after 7 months of UV radiation at 9 months of age (0.46 versus 3.48;  $P < 0.0001$ ), and after 9 months of UV radiation at 11 months of age (0.51 versus 3.79;  $P < 0.011$ ; Fig. 1A). The average cross-sectional BCC size in the mice treated with tazarotene was also smaller than that in control animals after 5 months of UV radiation at 7 months of age (2.5 versus 9.1  $\mu\text{m}^2$ ;  $P < 0.0001$ ); after 7 months of UV radiation at 9 months of age (2.4 versus 17  $\mu\text{m}^2$ ;  $P < 0.0001$ ), and after 9 months of UV radiation at 11 months of age (3.5 versus 54  $\mu\text{m}^2$ ;  $P < 0.0023$ ; Fig. 1B). In the second controlled UV trial, tazarotene-treated mice after 7 months of UV radiation (age, 9 months) also had significantly fewer BCCs, compared with control vehicle-treated mice (0.13 versus 4.62;  $P < 0.0001$ ), as well as smaller BCCs (0.49 versus 8.5  $\mu\text{m}^2$ ;  $P < 0.0001$ ; Fig. 1). In the IR study, *Ptch1*<sup>+/-</sup> mice treated with 0.1% tazarotene developed fewer microscopic BCCs at 10 months of age (7 months after a single dose of IR at 3 months of age) compared with vehicle control-treated mice treated with IR (0.29 versus 6.18;  $P < 0.0001$ ; Fig. 2A). Furthermore,

the average cross-sectional BCC size in tazarotene-treated mice was considerably smaller compared with that in control vehicle-treated mice at 7 months after IR, although this difference was not statistically significant (3.8 versus 14  $\mu\text{m}^2$ ;  $P < 0.204$ ; Fig. 2B). In both UV and IR studies, BCCs expressed  $\beta$ -galactosidase, indicating activated Hedgehog (Hh) signaling in these tumors (Fig. 3; Ref. 10). Also, in many skin biopsies, topical tazarotene treatment resulted in a thicker

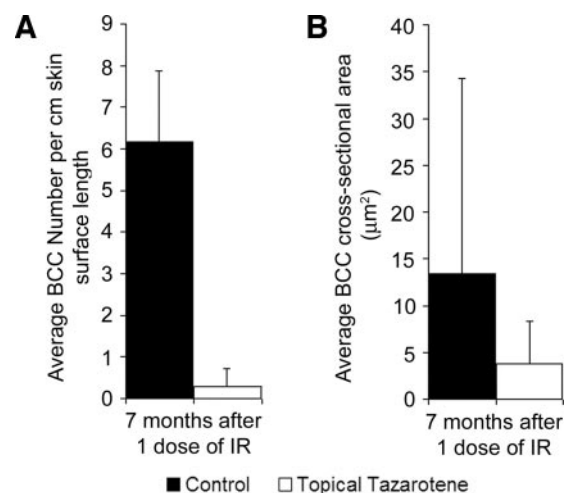


Fig. 2. Tazarotene treatment reduced the number and size of microscopic basal cell carcinomas (BCCs) in ionizing radiation-treated *Ptch1*<sup>+/-</sup> mice. **A**, average number of microscopic BCCs in mice treated with topical tazarotene or control vehicle 7 months after a single dose of ionizing radiation exposure ( $n = 27$ ). Tazarotene-treated mice had a significantly lower number of BCCs at 10 months of age than did control vehicle-treated mice. **B**, average cross-sectional area of BCCs 7 months after a single dose of ionizing radiation and treatment with topical tazarotene or control vehicle ( $n = 27$ ). Mice treated with topical tazarotene showed a reduction in BCC tumor size.

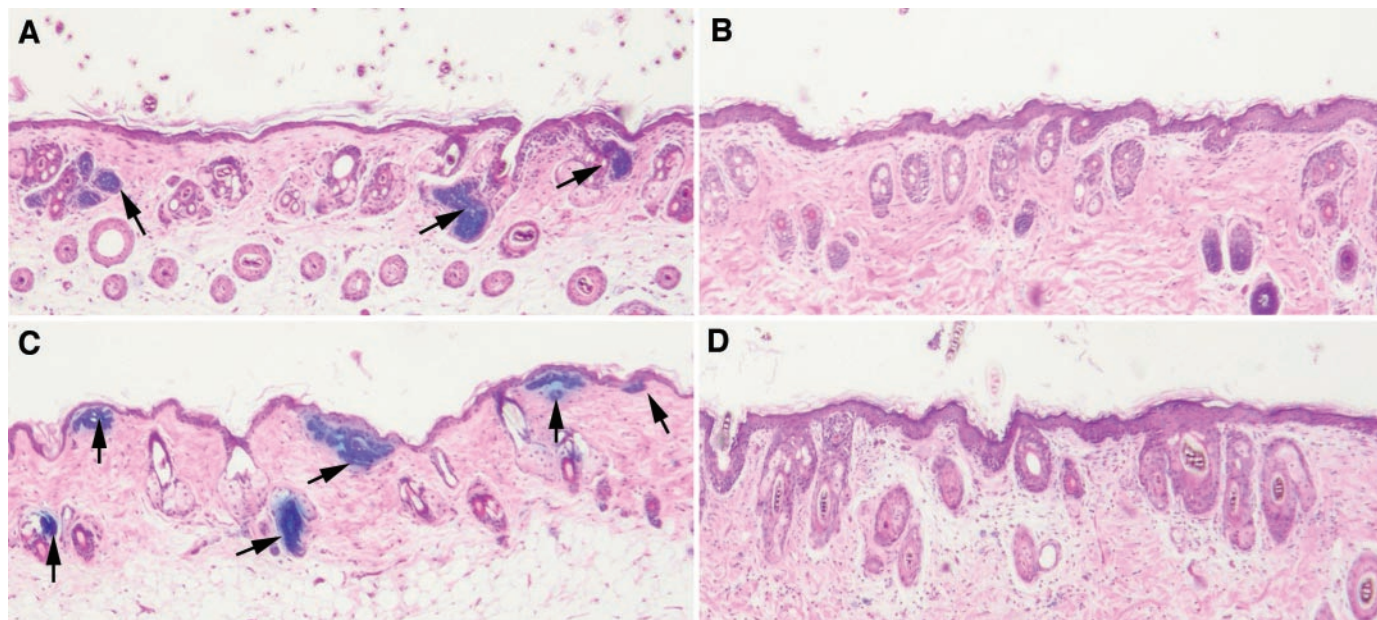


Fig. 3. Dorsal skin biopsies from control vehicle-treated *Ptch1*<sup>+/-</sup> mice exposed to UV radiation (A) or ionizing radiation (IR; C), compared with topical tazarotene-treated, mice exposed to UV (B) or IR (D). Biopsies were taken at 9 months of age, and microscopic basal cell carcinomas that developed stained blue for  $\beta$ -galactosidase activity (A and C, arrows). Almost all tazarotene-treated skin biopsies were microscopic basal cell carcinoma free (B and D). Also, tazarotene treatment resulted in some thickening of the epidermis in both UV- and IR-irradiated mice. Magnification,  $\times 40$ .

epidermis, as compared with control vehicle-treated mice (Fig. 3). Topical tazarotene treatment did not affect the normal weight gain of the UV- or IR-treated mice (data not shown).

Previously, we have shown that *Ptch1*<sup>+/-</sup> mice exposed to UV radiation (albeit using an alternative method of UV radiation delivery to the one described here) displayed macroscopic (visible) skin tumors comprising approximately 50% spindle cell tumors and 20% BCC or BCC-like tumors, whereas all skin tumors arising in IR-exposed mice were BCCs (10). The proportions of macroscopic BCC development in UV- and IR-irradiated *Ptch1*<sup>+/-</sup> mice treated with vehicle control in this study were in agreement with this (data not shown). Therefore, to investigate the effect of topical tazarotene on the development of macroscopic BCCs, we focused on mice from the IR study because all tumors that develop in these mice are BCCs. The results were plotted in a Kaplan-Meier graph to take into account those mice that did not reach the end point of observation due to death or other causes. Macroscopic tumors were first detectable in control vehicle-treated mice ( $n = 9$  at 10 months of age) at 11 months of age, and by 16 months of age, almost all of the remaining mice developed macroscopic BCCs (Fig. 4). In contrast, no tazarotene-treated mouse ( $n = 9$  at 10 months of age) had a macroscopic BCC by 16 months of age (Fig. 4;  $P < 0.003$ ). Both male and female control vehicle-treated, IR-exposed *Ptch1*<sup>+/-</sup> mice developed macroscopic BCCs; this is in contrast to a recent report using mice carrying a different *Ptch1* knockout allele showing that only IR-exposed *Ptch1*<sup>+/-</sup> males, but not females, developed macroscopic BCCs (11).

Also consistent with our previous experience (10), the majority of UV-induced macroscopic tumors were spindle cell tumors. We focused on the second UV study to analyze potential tazarotene efficacy against macroscopic spindle cell tumor development. The proportions of spindle cell tumor-free UV-treated control vehicle- or tazarotene-treated mice are shown in a Kaplan-Meier graph (Fig. 5). These tumors were first detectable in a small number of vehicle-treated mice at approximately 10 months for age (8 months after initial UV treatment;  $n = 26$ ), and the proportion remaining spindle cell tumor free decreased sharply in the following 4 months of observation. In

contrast, from 9 months ( $n = 28$ ) to 14 months of age, the majority of tazarotene-treated mice were macroscopic spindle cell tumor free (Fig. 5;  $P < 0.00023$ ).

## Discussion

Our results show clearly that tazarotene has significant anti-BCC efficacy in *Ptch1*<sup>+/-</sup> mice exposed to UV or IR, suggesting that the anti-BCC efficacy of tazarotene is fundamental to BCCs irrespective of the inciting environmental insult, and argue against the role of tazarotene as a potential sunscreen, a role ascribed to retinoids in the skin (12, 13). Human studies in which withdrawal of oral retinoids generally resulted in a rapid recurrence of tumors are consistent with an inhibitory effect on promotion and progression rather than on initiation (14). Tazarotene inhibited the formation of the majority of microscopic BCCs and reduced BCC size, suggesting that it is effi-

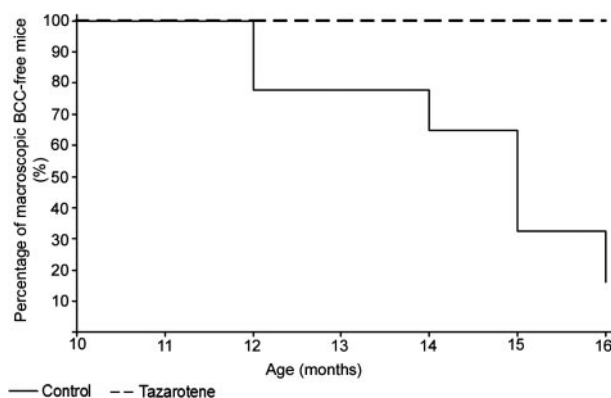


Fig. 4. Kaplan-Meier graph showing the percentage of ionizing radiation-exposed, control- or tazarotene-treated *Ptch1*<sup>+/-</sup> mice remaining macroscopic basal cell carcinoma free from 10 to 16 months of age. Control vehicle-treated mice ( $n = 9$ ) developed macroscopic basal cell carcinomas from 11 months of age, and by 16 months, nearly all of the remaining mice had developed at least one macroscopic tumor. Tazarotene-treated mice ( $n = 12$ ) did not develop any macroscopic tumors during the same observation period. Solid line, control; dashed line, tazarotene.

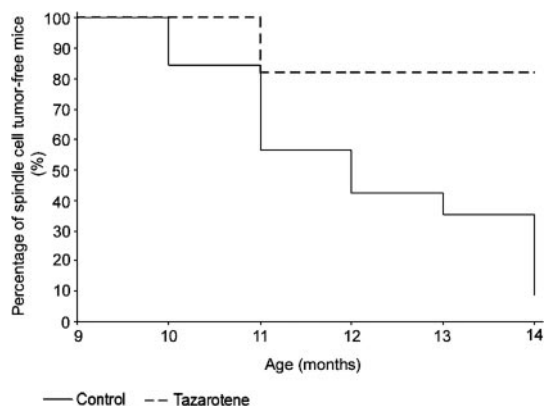


Fig. 5. Kaplan-Meier graph showing the percentage of UV-exposed, control- or tazarotene-treated *Ptch1*<sup>+/-</sup> mice remaining macroscopic spindle cell tumor free until 14 months of age. Control vehicle-treated mice ( $n = 26$ ) developed macroscopic spindle cell tumors from 10 months of age, and by 14 months, the majority of the remaining mice had developed at least one macroscopic spindle cell tumor. The majority of tazarotene-treated mice ( $n = 28$ ) did not develop any macroscopic tumors by 14 months of age. Solid line, control; dashed line, tazarotene.

caxious against tumor promotion and progression. For both BCC number and size, the SD values in our data reflected in part the background genetic variation in this mouse population (*i.e.*, mixed C57BL/6 and DBA/2J strain). Supporting this, our recent studies show that inbred *Ptch1*<sup>+/-</sup> mice exposed once to IR had significantly less variation of BCC number and size than did mice of more variable genetic background.<sup>7</sup> Tazarotene was also highly efficacious against spindle cell tumor development. Mouse dermal spindle cell tumors are commonly referred to as “fibrosarcomas.” The tumors that arise in our UV-irradiated *Ptch1*<sup>+/-</sup> mice indeed express vimentin;<sup>7</sup> however, whether they arise *de novo* from fibroblasts or derive from keratinocyte malignancies by epithelial to mesenchymal transition is unknown (15–17). Irrespective, tazarotene appears to have a significant inhibitory effect on their growth, as well as on that of BCCs.

Patched protein is a receptor for the hedgehog protein and represses the hedgehog signaling pathway (18, 19). *PATCHED1* has emerged as an important tumor suppressor gene, which is mutated in the germ line of basal cell nevus syndrome patients who have a pronounced predisposition to BCCs and also in many sporadic BCCs (20–22). *Ptch1* wild-type mice develop papillomas and cancers of the squamous but not basal cell lineage after treatment with UV radiation or chemical carcinogens (23); *Ptch1*<sup>+/-</sup> mice appear to be the first practical mouse model of BCC carcinogenesis (10). Current data do not explain the apparently far stronger inhibitory effect of tazarotene against BCCs in the *Ptch1*<sup>+/-</sup> mouse than of all-*trans*-retinoic acid on squamous cell carcinoma (SCC) carcinogenesis in *Ptch1* wild-type mice (4–7). It is possible that the differences are agent specific because tazarotene has relatively little RAR $\alpha$  binding activity, whereas all-*trans*-retinoic acid binds to all three RARs. Nonetheless, the predominant epidermal RAR is RAR $\gamma$ , and the binding of all-*trans*-retinoic acid to RAR $\beta$  and to RAR $\gamma$  is considerably stronger than is that of tazarotene (24). It is also possible that the differences are cancer-type specific because dysregulated hedgehog signaling is specific to BCCs and is not known to be important in SCCs (25). However, there is some evidence that aberrant signaling in both types of tumors converges on the mitogen-activated protein kinase pathway and on activator protein-1 activation (26, 27), inhibition of which is one postulated mechanism for retinoid anticancer efficacy (1). One possible site of retinoid action specific to BCCs is indicated by retinoid inhibition of transactivation by the downstream hedgehog

transcription factor GLI (28). Of note, mice with loss of epidermal retinoid X receptor  $\alpha$ , which is the predominant retinoid X receptor type in this tissue, not only have abnormal epidermal and hair differentiation but also may develop BCCs (1, 29). This formation of BCCs in keratinocytes lacking one of the major retinoid transactivators and the marked inhibition of their formation by a retinoid expected to enhance retinoid transactivation are parallel findings that, at least initially, are mutually reinforcing. Of note, human BCCs have been reported to express RAR $\alpha$  and RAR $\gamma$ , whereas expression of RAR $\alpha$  and RAR $\gamma$  is down-regulated in human SCCs (30, 31).

In human epidermal keratinocytes, tazarotene induces expression of the tazarotene-induced gene 3 (TIG3), which has significant homology to the tumor suppressor gene *H-rev*: increased TIG3 expression is correlated with decreased proliferation of human keratinocytes (32). TIG3 is highly expressed in normal human epidermis, and its expression is reduced in SCCs and BCCs (33), paralleling the reduction in retinoid receptor expression during skin carcinogenesis, at least in SCCs (30). Thus, TIG3 up-regulation by tazarotene may be required for the inhibition of BCC formation. In support of this, a recent study correlated the efficacy of topical tazarotene against human BCCs with induction of TIG3 expression (34). Also, tazarotene causes growth suppression in retinoid-responsive breast cancer cell lines by up-regulating TIG3 (32), whereas in prostate cancer, another tazarotene-induced gene, TIG1 (induced also in keratinocytes), is deleted, and transfection of TIG1 into prostate cancer cell lines reduces their invasiveness *in vitro* and their growth *in vivo* (35, 36).

It is uncertain whether the ability of tazarotene to inhibit BCC development resulted in inhibition of cell proliferation, cell differentiation, and/or apoptosis in our model. Retinoids are believed to cause tumor regression through induction of cell differentiation (as well as inhibition of cell growth, proliferation, and induction of apoptosis; Ref. 1); however, we did not note histological changes consistent with basal cell differentiation (*i.e.*, decreased nuclear to cytoplasmic ratio or evidence of keratinization) in short-term exposure of previously untreated mouse BCCs to topical tazarotene (data not shown). However, we cannot be sure that we examined tumors at the “snap-shot” of time appropriate to the capture of any such pro-differentiating effect. Whether tazarotene down-regulates Hh signaling in BCCs and spindle cell tumors in our *Ptch1*<sup>+/-</sup> mice is unknown. If this were the case, tazarotene could potentially be effective against other cancers with dysregulated Hh signaling such as some pancreatic (37), lung (38), and gastrointestinal (39) cancers.

The inhibitory effects of systemic retinoids on human BCCs are rapid in onset, and various experimental models argue that retinoid chemoprevention has a major effect against tumor promotion. Therefore, it seems likely that an effective topical retinoid might be particularly useful in patients who already have sustained significant sun damage and have experienced the discomfort and scarring of skin cancer treatment. The relevance of our findings in the *Ptch1*<sup>+/-</sup> mouse to possible benefits for *PTCHI*<sup>+/-</sup> humans (basal cell nevus syndrome patients), let alone for *PTCHI*<sup>+/+</sup> humans, has yet to be assessed. However, the development of the same tumors with the same dysregulated signaling patterns in the same tissue after the same environmental insults in man and mouse gives hope that the murine findings will indeed closely reflect those in humans. Furthermore, the *Ptch1*<sup>+/-</sup> mouse may well be an excellent model in which to elucidate further mechanisms of retinoid cancer chemoprevention. We conclude that tazarotene is a promising agent for skin cancer prevention in populations at risk for BCCs, such as patients with the basal cell nevus syndrome, and that this approach is deserving of evaluation in human clinical trials.

<sup>7</sup> E. H. Epstein, Jr., unpublished data.

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## References

- Altucci L, Gronemeyer H. The promise of retinoids to fight against cancer. *Nat Rev Cancer* 2001;1:181–93.
- Fisher GJ, Voorhees JJ. Molecular mechanisms of retinoid actions in skin. *FASEB J* 1996;10:1002–13.
- Peck GL, DiGiovanna JJ, Sarnoff DS, et al. Treatment and prevention of basal cell carcinoma with oral isotretinoin. *J Am Acad Dermatol* 1988;19:176–85.
- Epstein JH, Grekin DA. Inhibition of ultraviolet-induced carcinogenesis by all-trans retinoic acid. *J Investig Dermatol* 1981;76:178–80.
- Forbes PD, Urbach F, Davies RE. Enhancement of experimental photocarcinogenesis by topical retinoic acid. *Cancer Lett* 1979;7:85–90.
- Kligman LH, Kligman AM. Lack of enhancement of experimental photocarcinogenesis by topical retinoic acid. *Arch Dermatol Res* 1981;270:453–62.
- Kligman LH, Crosby MJ. Topical tretinoin enhances corticosteroid-induced inhibition of tumorigenesis in hairless mice previously exposed to solar simulating radiation. *Cancer Lett* 1996;107:217–22.
- Nagpal S, Chandraratna RA. Recent developments in receptor-selective retinoids. *Curr Pharm Des* 2000;6:919–31.
- Peris K, Fargnoli MC, Chimenti S. Preliminary observations on the use of topical tazarotene to treat basal-cell carcinoma. *N Engl J Med* 1999;341:1767–8.
- Aszterbaum M, Epstein J, Oro A, et al. Ultraviolet and ionizing radiation enhance the growth of BCCs and trichoblastomas in patched heterozygous knockout mice. *Nat Med* 1999;5:1285–91.
- Mancuso M, Pazzaglia S, Tanori M, et al. Basal cell carcinoma and its development: insights from radiation-induced tumors in Ptch1-deficient mice. *Cancer Res* 2004;64:934–41.
- Saurat JH. Skin, sun, and vitamin A: from aging to cancer. *J Dermatol* 2001;28:595–8.
- Antille C, Tran C, Sorg O, et al. Vitamin A exerts a photoprotective action in skin by absorbing ultraviolet B radiation. *J Investig Dermatol* 2003;121:1163–7.
- Peck GL. Long-term retinoid therapy is needed for maintenance of cancer chemopreventive effect. *Dermatologica* 1987;175(Suppl 1):138–44.
- Morison WL, Jerdan MS, Hoover TL, Farmer ER. UV radiation-induced tumors in haired mice: identification as squamous cell carcinomas. *J Natl Cancer Inst* (Bethesda) 1986;77:1155–62.
- Portella G, Cumming SA, Liddell J, et al. Transforming growth factor beta is essential for spindle cell conversion of mouse skin carcinoma in vivo: implications for tumor invasion. *Cell Growth Differ* 1998;9:393–404.
- Cui W, Fowles DJ, Bryson S, et al. TGFbeta1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. *Cell* 1996;86:531–42.
- Ingham PW. The patched gene in development and cancer. *Curr Opin Genet Dev* 1998;8:88–94.
- Murone M, Rosenthal A, de Sauvage FJ. Hedgehog signal transduction: from flies to vertebrates. *Exp Cell Res* 1999;253:25–33.
- Hahn H, Wicking C, Zaphiropoulos PG, et al. Mutations of the human homolog of *Drosophila* patched in the nevoid basal cell carcinoma syndrome. *Cell* 1996;85:841–51.
- Johnson RL, Rothman AL, Xie J, et al. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* (Wash DC) 1996;272:1668–71.
- Aszterbaum M, Rothman A, Johnson RL, et al. Identification of mutations in the human PATCHED gene in sporadic basal cell carcinomas and in patients with the basal cell nevus syndrome. *J Investig Dermatol* 1998;110:885–8.
- Bogovski P. Tumours of the skin. *IARC Sci Publ* 1994;111:1–45.
- Chandraratna RA. Tazarotene: first of a new generation of receptor-selective retinoids. *Br J Dermatol* 1996;135(Suppl 49):18–25.
- Eklund LK, Lindstrom E, Uden AB, et al. Mutation analysis of the human homologue of *Drosophila* patched and the xeroderma pigmentosum complementation group A genes in squamous cell carcinomas of the skin. *Mol Carcinog* 1998;21:87–92.
- Xie J, Aszterbaum M, Zhang X, et al. A role of PDGFRalpha in basal cell carcinoma proliferation. *Proc Natl Acad Sci USA* 2001;98:9255–9.
- Yuspa SH. The pathogenesis of squamous cell cancer: lessons learned from studies of skin carcinogenesis. *J Dermatol Sci* 1998;17:1–7.
- Goyette P, Allan D, Peschard P, et al. Regulation of gli activity by all-trans retinoic acid in mouse keratinocytes. *Cancer Res* 2000;60:5386–9.
- Li M, Indra AK, Warot X, et al. Skin abnormalities generated by temporally controlled RXRalpha mutations in mouse epidermis. *Nature* (Lond) 2000;407:633–6.
- Xu XC, Wong WY, Goldberg L, et al. Progressive decreases in nuclear retinoid receptors during skin squamous carcinogenesis. *Cancer Res* 2001;61:4306–10.
- Kamradt J, Reichrath J. Expression of retinoic acid receptor proteins in basal cell carcinomas: an immunohistochemical analysis. *J Histochem Cytochem* 1996;44:1415–20.
- DiSepio D, Ghosn C, Eckert RL, et al. Identification and characterization of a retinoid-induced class II tumor suppressor/growth regulatory gene. *Proc Natl Acad Sci USA* 1998;95:14811–5.
- Duvic M, Helekar B, Schulz C, et al. Expression of a retinoid-inducible tumor suppressor, tazarotene-inducible gene-3, is decreased in psoriasis and skin cancer. *Clin Cancer Res* 2000;6:3249–59.
- Duvic M, Ni X, Talpur R, et al. Tazarotene-induced gene 3 is suppressed in basal cell carcinomas and reversed in vivo by tazarotene application. *J Investig Dermatol* 2003;121:902–9.
- Jing C, El-Ghany MA, Beesley C, et al. Tazarotene-induced gene 1 (TIG1) expression in prostate carcinomas and its relationship to tumorigenicity. *J Natl Cancer Inst* (Bethesda) 2002;94:482–90.
- Lotan R. Is TIG1 a new tumor suppressor in prostate cancer? *J Natl Cancer Inst* (Bethesda) 2002;94:469–70.
- Thayer SP, di Magliano MP, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. *Nature* (Lond) 2003;425:851–6.
- Watkins DN, Berman DM, Burkholder SG, et al. Hedgehog signalling within airway epithelial progenitors and in small-cell lung cancer. *Nature* (Lond) 2003;422:313–7.
- Berman DM, Karhadkar SS, Maitra A, et al. Widespread requirement for Hedgehog ligand stimulation in growth of digestive tract tumours. *Nature* (Lond) 2003;425:846–51.