A PMLRARA Transgene Results in a Retinoid-deficient Phenotype Associated with Enhanced Susceptibility to Skin Tumorigenesis

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ABSTRACT

The construction of transgenic FVB/N mice targeting the PMLRARA fusion gene under the control of a human MRPS promoter recapitulated the phenotype of acute promyelocytic leukemia but had the unexpected result of multiple squamous papillomas of the skin (Brown et al., Proc. Natl. Acad. Sci. USA, 94:2551–2556, 1997). In addition, transgenic MRPS-PMLRARA mice exhibited a skin phenotype characteristic of vitamin A deficiency. The severity of the skin phenotype and spontaneous papilloma development correlated with the level of transgene expression. Papilloma formation was preceded by follicular hyperplasia and the expression of epidermal differentiation markers in the follicular epithelium. Mutations in the Ha or Ki alleles of ras were not detected in papillomas that developed on transgenic skin, and papilloma formation was accentuated in the Ha or Ki alleles of ras. The severity of the skin phenotype and spontaneous papilloma formation, and retinoid receptors are modified in both murine and human cutaneous neoplasms (20–24). Furthermore, genetic ablation of RARs and RXRs signaling (2) is typically expressed more highly than other retinoid receptors, whereas retaining an affinity for RXR that is similar to RARs, PML-RARα expression also results in sequestration of RARs from RXRs (2, 14). PML-RARα binds ATRA with similar affinity as RARα, although RARE-mediated transcription occurs only on pharmacological doses of ATRA (2, 15). Pharmacological doses of ATRA cause degradation of the fusion protein as well as up-regulation of RARα activity resulting in restoration of the normal function of both RARα and PML, and full maturation of the leukemic cells (reviewed in Ref. 2). In APL patients, disease remission in response to ATRA therapy is believed to be a consequence of ATRA-induced differentiation of leukemic cells (reviewed in Ref. 1). Thus, PML-RARα signaling appears to modulate cell differentiation and tumorigenesis, at least in part, by dysregulating retinoid signaling pathways.

PML-RARα also represses PML-modulated transcriptional regulation by forming heterodimers with PML and altering its subcellular localization. PML acts as a tumor suppressor by regulating cell growth and apoptosis. PML also interacts with viral oncoproteins (2). These functions for PML are apparent in APL cells as well as in the skin. PML nullizygous mice exhibit enhanced susceptibility to chemical carcinogen-induced skin carcinogenesis (16). In APL cell lines, disease of PML correlates with the latency of APL onset in PMLRARA transgenic mice (17).

A murine model for APL, developed in one of our laboratories, used the MRPS promoter to target the human fusion gene to bone marrow (9). MRPS was originally described as a member of the S100 family of proteins that is expressed during differentiation of myelomonocytic cells (18) but was subsequently found expressed in differentiating epithelial cells including skin keratinocytes (19). During the course of the analysis of the MRPS-PMLRARA mouse, a marked sensitivity to spontaneous skin tumor formation, often limiting the life span of these animals, was noted. Retinoids are well known to have a major influence on skin differentiation and tumor formation, and retinoid receptors are modified in both murine and human cutaneous neoplasms (20–24). Furthermore, genetic ablation of RXRα results in a marked alteration in skin development (24). We now report an additional characterization of the skin phenotype of the MRPS-PMLRARA mouse, and correlate that to transgene expression and retinoid response. The results implicate both PML function and retinoid metabolism as contributing to the sensitivity to skin tumor induction, thus illuminating a novel pathway leading to cutaneous neoplasia.

INTRODUCTION

Fusion of most of the RARA and PML genes via a reciprocal chromosomal translocation, thus creating the PMLRARA fusion gene, occurs in the majority of APL cells (1–3). Several laboratories have developed PMLRARA transgenic mice as a model for APL or to analyze the biological properties of the fusion gene (4–9). The PML-RARα protein exerts dominant-negative effects on both PML, a nuclear body protein that can limit cell growth and survival, and RARα signaling (2). Consequently, interrupted signaling prevents full maturation of promyelocytes resulting in the accumulation of immature leukemic cells. PML-RARα can homodimerize or form heterodimers with both PML and RXRs (reviewed in Refs. 1, 2). PML-RARα oligomers or PML–RARα RXR dimers cause transcriptional repression of RAR–RXR signaling by recruiting histone deacetylase together with the transcriptional corepressors SMRT and N-CoR to RAREs of target genes (10–13). Because PML–RARα is typically expressed more highly than other retinoid receptors, whereas retaining

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3 The abbreviations used are: APL, acute promyelocytic leukemia; RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, retinoic acid response element; MRPS, migration inhibitory factor-related protein; ATRA, retinoic acid.

MATERIALS AND METHODS

Animals. Transgenic mice of the FVB/N strain were created using a human MRPS8 promoter and a human PMLRARA cDNA with a chromosome 15 breakpoint in breakpoint cluster region 1 (9, 25). Tail DNA was prepared as described elsewhere (9) for genotyping using PCR. ATRA was administered to mice via s.c. implantation of a 21-day-release pellet containing 5 mg ATRA or placebo (Innovative Research of America, Sarasota, FL). For wounding experiments, a wound clip was attached to the back skin of 6–8-week-old mice. After 12 days, the wound clip was removed, and tumor development was monitored weekly for 6 weeks.
Cell Culture and Grafting. Eagle’s MEM and penicillin-streptomycin were obtained from Life Technologies, Inc. (Gaithersburg, MD), and FCS was from Gemini Bio-Products (Calabasas, CA). Primary keratinocytes were obtained from newborn transgenic PMLRARA and littermate controls. Keratinocytes were prepared as described previously (26) and cultured in calcium- and magnesium-free Eagle’s MEM with 8% chelated (Bio-Rad Laboratories, Hercules, CA) serum, 20 units/ml penicillin, 20 μg/ml streptomycin in Eagle’s MEM, and 0.05 mM calcium chloride. Cells were initially plated in medium adjusted to 0.25 mM calcium and changed to 0.05 mM calcium-containing medium ~18 h later. Viral infection with a v-rasH2 replication-defective retrovirus was performed using diluted supernatant from ψ-2 producer cells in the presence of 1 μg/ml Polybrene (27). Grafting of MR8-PMLRARA and nontransgenic keratinocytes with or without v-rasH2-expression together with primary BALB/c dermal fibroblasts onto athymic nude mice was performed as described previously (26). Tumor volume was measured weekly using digital calipers. Sequence analysis of tumor DNA for mutations in the rasH2 or rasK alleles was performed as described previously (28).

Immunohistochemistry and Immunofluorescence. Skin and skin tumors were removed from 4, 8, 12, and 16-week-old FVB/N and PMLRARA transgenic mice after euthanasia, fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned. For immunofluorescence experiments, sections were incubated with polyclonal rabbit antibodies recognizing mouse keratin 1 (K1), loricrin, filaggrin, keratin 6 (K6), and keratin 10 (K10; Covance, Berkeley, CA). After incubation with the primary antibodies, sections were incubated with an antirabbit secondary antibody conjugated to fluorescein (Vector Labs.) and then counterstained using 4',6-diamidino-2-phenylindole mounting medium (Vector Labs.). To detect transgene expression, sections were incubated with an antihuman PML antibody (PG-M3 from Santa Cruz Biotechnology), biotinylated anti-F(ab)2 fragment antiserum secondary antibody (Jackson Immunoresearch Labs), horseradish peroxidase-conjugated streptavidin (Jackson Immunoresearch Labs), diamino benzidine substrate, and counterstained with hematoxylin (Sigma).

Immunoblotting. Dorsal skin from transgenic and control mice was depilated with Nair, excised, and quick frozen. Minced frozen skin was extracted in 4°C buffer containing 500 mM Tris, 25 mM KCl, 5 mM MgCl2, 0.32 mM sucrose, 5 mM DTT, and 0.1 mM phenylmethylsulfonyl fluoride by polytron homogenization for 1 min. Fat and insoluble keratinous material was removed by polytron homogenization and then centrifuged at 1500 g for 15 min. The 1500-g pellet was re-extracted in Laemmli buffer and centrifuged at 75 K rpm for 15 min. The protein concentration of the supernatants was measured by the Bio-Rad protein assay. This assay is based on the principle that the absorbance at 595 nm is proportional to the amount of protein in the sample, up to a certain concentration. The method is sensitive and specific for most types of proteins, and it is widely used in laboratories worldwide.

RESULTS

MR8-PMLRARA Transgenic Mice Display a Hair Phenotype, Epidermal Thickening, and Spontaneous Papillomas at Sites of Wounding. A skin phenotype that varied in severity among founder lines was observed in 10 of 11 founder transgenics and their offspring. The abnormalities varied from slight thickening of the skin at the site of the ear tag (Fig. 1B) to regions of thickened, hairless, corruget skin skin tumors. Many distinct papillomas covered a large portion of the body of the animal (Fig. 1, A, C, E, and F). Regions of thickly furrowed hyperkeratotic skin resolved frequently into thin hairless patches. PMLRARA transgenic mice developed grossly visible papillomas as early as 3 weeks of age (data not shown). Although rare, progression to carcinoma was detected. In the line with the highest transgene expression, line 556, transgenic pups had a sparser haircoat (Fig. 1A), which clearly distinguished them from nontransgenic littermates. Transgenic mice also exhibited scaly skin, particularly around the snout, and peri orbital reddening (Fig. 1C), features characteristic of retinoid deficiency (35, 36).

Papilloma development was often associated with sites of bite wounds (Fig. 1E) or inflammation, such as the site of the ear tag (Fig. 1C). To verify that wounding could trigger tumor formation, wound clips were attached to the back skin of 6-week-old FVB/N control and PMLRARA transgenic mice as shown in Fig. 2A. The skin at the clip site was edematous in the FVB/N mice (Fig. 2B), whereas a tumor was already visible in the transgenic mice (Fig. 2D) when clips were removed 12 days later. Six weeks after placement of the clip, the site had returned to normal in the FVB/N mice (Fig. 2C), but the transgenic mice had sizeable tumors (Fig. 2E).

Histologically, regions of hyperplasia characterized the hair follicles and epidermis of the transgenic mice before the appearance of papillomas (Fig. 3). The interfollicular epidermis displayed patchy hyperplasia with occasional apoptotic or dysplastic cells (Fig. 3; data not shown). In 11-day-old pups, fewer hair follicles appeared to breach the surface of the epidermis, perhaps explaining the sparser hair coat, and the interfollicular epidermis was frequently thicker, although the overall pattern of differentiation, from basal cells to corneous cells, was intact (data not shown). In older mice, follicular hyperplasia was apparent, particularly in the upper, permanent portion of the hair follicle (Fig. 3), and excessive keratinization in the follicle ducts preceded papilloma formation (Fig. 3; data not shown).

Transgene Expression and Localization Correlates with the Skin Phenotype. Both the penetration and expressivity of this phenotype were in accord with the levels of transgene expression seen in the bone marrow. All of the mice from the high-expressing line 556 had visible skin lesions by 6 weeks of age, and their lesions were

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generally more severe than those seen in the low-expressing lines (Figs. 1 and 4). Mice bred to have increased transgene dosage generally had more severe phenotypes, as shown for lines 553 and 565 in Fig. 4.

Immunohistochemical analysis using an antihuman PML antibody that does not cross-react with mouse PML revealed PMLRARA transgene expression in the cutaneous epithelium, particularly in the follicular epithelium (Fig. 5). In hyperplastic epidermis, the transgene was expressed in the stratum granulosum as well. Expression was increased in papillomas with the strongest levels in the stratum granulosum (Fig. 5). Within the limits of immunohistochemical detection, transgene expression was consistently nuclear. No PMLRARA expression was detected in nontransgenic mouse skin using the antihuman antibody (data not shown). Taken together, these results strongly support the hypothesis that the skin lesions were associated with transgene expression.

Hair Follicles in PMLRARA Transgenic Mice Exhibit Epidermis-type Differentiation. Sebaceous and keratinizing differentiation were observed histologically in hyperplastic hair follicles from transgenic mice, in apparent association with the expression of the PMLRARA transgene in the hair follicles. Differentiation of the cutaneous epithelium was examined by immunohistochemistry using several well-characterized markers of epidermal differentiation. Expression of early markers of epidermal differentiation, keratins 1 and 10, was restricted to suprabasal stratum spinosum of the interfollicular epidermis in control FVB/N skin as shown in Fig. 6. In PMLRARA mice, in contrast, expression of K1 and K10 were localized not only to stratifying stratum spinosum, but aberrantly extended into the follicular epithelium (Fig. 6). K10 expression was detected in the upper, permanent portion whereas the entirety of many hair follicles was K1 positive (Fig. 6). Filaggrin, expressed in the stratum granulosum of the interfollicular epidermis as seen in skin from control FVB/N mice, was increased in the hyperplastic transgenic skin and abundant in transgenic follicles (Fig. 6). The pattern of differentiation markers in transgenic skin suggested that PMLRARA expression caused an epidermalization of hair follicles in association with the development of follicular papillomas.
Transgenic Papillomas Are Not the Consequence of Mutations in Proto-oncogenic ras Genes. Oncogenic ras\(^{Ki}\) and ras\(^{Ha}\) mutations are the most common reported initiating events in mouse skin papillomas (28). To determine whether activation of ras\(^{Ha}\) and ras\(^{Ki}\) proto-oncogenes contributed to papilloma formation in PMLRARA transgenic mice, ras\(^{Ha}\) and ras\(^{Ki}\) exons 1 and 2, containing the frequently mutated codons 12, 13, 59, and 61, were sequenced after nested PCR in spontaneously arising PMLRARA transgenic papillomas and nontumor-bearing skin as described previously (28). Sequencing of 7 tumors for the ras\(^{Ha}\) gene and 3 tumors for the ras\(^{Ki}\) gene revealed only wild-type sequence. Thus, in contrast to spontaneous and chemically induced papillomas in nontransgenic mice, papilloma development in PMLRARA transgenic mice does not frequently involve ras\(^{Ha}\) or ras\(^{Ki}\) mutations. This suggests that expression of PMLRARA in mouse skin may serve as an alternative initiating pathway for tumorigenesis.

C57/Bl6 Mice Are Readily Susceptible to PMLRARA-driven Papilloma Formation. Additional evidence that the mechanism of PMLRARA induced papillomagenesis was different from previously characterized mouse skin tumorigenesis models was discovered on crossing the PMLRARA transgene onto the skin tumor-resistant C57/BL6 strain. Mice from two PMLRARA transgenic lines (506 and 556) were crossed to inbred C57/BL6 mice to minimize the impact of the skin lesions on the analysis of the hematopoietic phenotype. The crosses were only followed for two generations, but the progeny
rapidly developed substantial skin lesions (Fig. 4; data not shown), which made them impractical to maintain. The histology of the lesions resembled those seen in the inbred FVB/N background (data not shown). This result suggested that tumor induction by the PMLRARA transgene occurred through a novel mechanism unrelated to the strain-dependent tumor susceptibility documented previously by other investigators (37–39).

Administration of ATRA Prevents Papilloma Development. The hyperkeratotic, scaly skin and periorbital edema and reddening of the PMLRARA transgenic mice were consistent with the appearance of retinoid deficiency (35, 36). To determine whether administration of ATRA could prevent the skin phenotype of the PMLRARA mice, transgenic mice were treated with placebo or 5-mg ATRA pellets implanted in each of 3 15-day-old pups of line 565, before the development of cutaneous papillomas. Three weeks after the pellets were implanted, the animals that received the placebo developed skin changes in the characteristic pattern, but the others had not (see eye reddening and scaly snout phenotype in Fig. 7). Therefore, RA was able to inhibit the development of the skin phenotype in the MRP8-PMLRARA transgenic mice. RA normalized the altered hematopoiesis of MRP8-PMLRARA transgenic mice as well (9). Implantation of ATRA pellets also abrogated skin tumor formation for both spontaneous and wound-induced tumors (including intentionally wounding by applying a surgical wound clip).

PMLRARA Transgenic Mice Exhibit Altered Metabolism of Retinoids. The similarity of the skin phenotype to vitamin A deficiency and the response to pharmacological ATRA prompted us to examine retinoid metabolism in the PMLRARA transgenic mice. Liver levels of retinyl palmitate in PMLRARA transgenic and control mice were analyzed by high performance liquid chromatography. Although there was a 25% reduction in the level of retinyl palmitate between transgenic and age-matched control livers, the difference was not statistically significant (Table 1). However, the ability to synthesize RA from retinal was reduced by 24% in liver extracts of transgenic mice (Table 1). An even greater decrease (37%) was found when the assay was conducted in the absence of exogenous retinal (Table 1). Both of these changes are statistically significant. These data suggest that a decrease in endogenous retinal as well as a decreased ability to convert retinal to RA may contribute to the retinoid-deficient phenotype of MRP8-PMLRARA transgenic mice. No measurable RA was detected in the absence of NAD (data not shown). These data suggest that decreased endogenous retinal may have been partly responsible for the difference in RA production between the two genotypes. Attempts to measure retinoid metabolism in the skin were not successful.

Skin from PMLRARA Mice Has Reduced Retinoid Receptors. Components of the retinoid signaling pathway were evaluated in extracts of nonlesional skin samples from 2 control and 2 transgenic mice. A clear reduction of >75% in both RARα and RXRα was detected in transgenic skin (Fig. 8). Using an antibody that recognizes
both human RARα and PML-RARα (Fig. 8, left top panel), the transgene is clearly detected in the skin lysates. Endogenous PML levels are not consistently altered in mice of either genotype.

**The PMLRARA Transgenic Skin Phenotype Is Independent of the Hematopoietic Phenotype.** To determine whether papilloma development was linked to the hematopoietic phenotype, we transplanted transgenic bone marrow cells to nontransgenic hosts and monitored the skin phenotype. Most recipients of bone marrow transplants developed the hematopoietic phenotype, acute myeloid leukemia, including anemia and thrombocytopenia in the absence of increased numbers of WBCs, pale bone marrow, hepatosplenomegaly, and lymphadenopathy. Morphologically and by fluorescence-activated cell sorter analysis (including low level expression of Gr-1 and Mac-1 antigens, characteristic of APL cells; Ref. 40) the leukemic cells were identical to leukemias that arose in nontransplanted MR8 PMLRARA transgenic bone marrow. However, cutaneous papillomas or other features of the skin phenotype did not develop (data not shown). Thus, the papillomas appeared to represent the result of expression of the PMLRARA transgene in the skin and other organs of the mice. To address this issue, keratinocytes from PMLRARA mice were grafted to athymic nude hosts, and the resulting skin grafts were monitored for phenotypic alterations or tumor formation. Although the grafting procedure creates a wound environment, grafted transgenic and control keratinocytes formed an identical integument, and papillomas did not occur (Fig. 9). When v-rasH1a was introduced into transgenic and control keratinocytes through retroviral

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**Table 1 RA synthesis is decreased in PMLRARA transgenic nonleukemic mouse liver**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Amount of RA produced in 3 h (pmol/ng protein)</th>
<th>Liver retinyl palmitate concentration (μg/g liver), n = 4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVB/N</td>
<td>3.3 ± 1.10 (37% decrease)</td>
<td>1206 ± 278 (25% decrease)</td>
</tr>
<tr>
<td>PMLRARA</td>
<td>1.98 ± 0.71</td>
<td>909 ± 197</td>
</tr>
</tbody>
</table>

*Values are reported as mean ± SD with the % of FVB/N control in parenthesis. Value is significantly different from the corresponding FVB/N control using a two-way ANOVA test, where P < 0.05.

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**Fig. 7.** ATRA pellets prevent the skin phenotype of PMLRARA transgenic mice. Six homozygous litters of line 565 received s.c. 21-day timed-release 5 mg ATRA or placebo pellets when they were 15 days old. Photographs show the mice at 6 weeks of age. Those that received ATRA pellets (+) did not develop the cutaneous manifestations characteristic of this line, whereas those that received placebo pellets (−) developed peri-orbital edema, erythema, and scaling.

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**Fig. 8.** Nonlesional skin extracts from 2 control FVB/N and 2 transgenic mice were independently assayed for RARα and RXRα protein levels by immunoblotting (left panel). The top left panel probe was antibody RPα F-115 (29), which recognizes human RARα and PMLRARα (30). The source of the other antibodies is described in “Materials and Methods.” The right panel quantitates relative expression of RARαs and RXRαs corrected for actin levels in the same sample.
transduction in culture and recipient cells grafted to nude mice, papillomas did develop in grafts, and the kinetics of tumor growth and tumor sizes in the two genotypes were similar (Fig. 9). Thus, the presence of the transgene did not interfere with ras-mediated tumor formation, supporting the notion that the absence of ras mutations in transgenic papillomas indicates an alternative pathway in skin carcinogenesis rather than exclusion of a ras pathway. These results also suggest that papilloma development in intact PMLRARA transgenic mice requires the combined effect of the PMLRARA transgene expression in the skin together with a systemic retinoid deficiency.

**DISCUSSION**

Skin is an important target for retinoid activity. Expression of a wide variety of genes that control keratinocyte proliferation and differentiation are regulated by retinoids (41). In rodent models, retinoid deficiency causes hyperkeratosis with prominent follicular keratinization forming horn plugs, a mottled coat, facial erythema and alopecia, and keratitis (35, 36), changes observed in the skin of MRP8-PMLRARA mice. Nevertheless, it appears that this local effect must be coupled with a systemic deficiency in vitamin A levels as indicated by the grafting studies.

RA, the active form of vitamin A, is produced from retinol through two oxidation steps that use retinol as the immediate precursor for RA synthesis (1). Retinyl esters represent the storage form of retinol and are hydrolyzed to maintain the homeostatic retinoid physiology. Retinyl esters are derived from dietary retinol and/or carotenoids, and are stored in the liver (reviewed in Ref. 46). The reduced retinyl ester levels and diminished metabolic production of RA in the liver could, in part, reflect an activity of the transgene expressed in the liver perhaps through an effect of circulating myeloid cells. For example, circulating granulocytes that express PML-RARA might influence hepatocytes as they pass through the liver. In addition, MRP8 is expressed focally in the mouse fetal liver (18) in the hematopoietic population, and an influence of transgene expression on the subsequent metabolic activity in adult hepatocytes is conceivable. Altogether, our results indicate that PML-RARα-induced skin tumorigenesis appears to proceed through a novel mechanism involving retinoid
insufficiency. In contrast to the skin phenotype, leukemia was independent of systemic retinoid deficiency as demonstrated by the development of leukemia in nontransgenic recipients of transgenic bone marrow.

The PMLRARα transgene appears to act as a conditional initiating factor in skin tumorigenesis, because papillomas form at the site of wounds and evolve rapidly. Similar rapid onset of hepatic tumors in a background of induced hyperplasia was detected when the PMLRARα transgene was expressed at high levels in the liver under the control of the metallothionein promoter, supporting a more general oncogenic activity associated with suppression of PML and RAR pathways (6). Our studies indicate that transgene expression is predominant in the more differentiated compartments of hyperplastic and neoplastic skin lesions. These results raise an interesting puzzle as to how an oncogenic factor expressed predominately in differentiated cells can induce tumors. This example is not unique in experimental skin carcinogenesis. Targeting of oncogenic ras to the suprabasal differentiating compartment of the epidermis with a keratin 1 or keratin 10 promoter was papillomagenic previously, although these tumors rarely, if ever, progressed to carcinoma (47, 48). In another study, cutaneous suprabasal expression of c-myc driven by the involucrin promoter activated the cell cycle in postmitotic suprabasal cells producing a severe hyperplasia and papillomatous lesions (49). Considering the role for PML in cell cycle control through the Rb pathway and apoptosis through the p53 or DAXX pathways (50), disruption by PML-RARα could recapitulate the biological action of c-myc overexpression. Such a possibility is supported by evidence that the PML-RARα protein can interfere with the transcriptional repression activity of the tumor suppressor Mad (51). Recent evidence for PML involvement in telomere maintenance and DNA methylation (52) provide other potential targets to contribute to cutaneous cancer induction. It should be noted that PML has been implicated as a modulator of experimental cutaneous carcinogenesis, because PML null mice are more susceptible to skin tumor induction than control mice when treated with 7,12-dimethylbenz(a)anthracene and 12-O-tetradecanoylphorbol-13-acetate (16).

Our findings may have important implications for understanding the development and treatment of human skin tumors. Epidemiological evidence suggests that systemic retinoid status can have a substantial influence on UV-induced human squamous cell cancers (45). This model could contribute to the identification of novel pathways that act in concert with retinoid deficiency in cutaneous oncogenesis. More broadly it has become clear that interactions between neoplastic epithelial cells and abnormalities of non-neoplastic stroma are important in tumor development, maintenance, and progression. The novel observation that retinoid deficiency combines with aberrant gene expression in skin to generate cutaneous papillomas raises the possibility that such systemic/local interactions may be similarly important in the initiation or progression of a variety of human cancers.

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