Skin-specific Expression of a Truncated Ela Oncoprotein Binding to p105-Rb Leads to Abnormal Hair Follicle Maturation Without Increased Epidermal Proliferation

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Abstract. In cultured cells, mutants of the Adenovirus Ela oncoprotein which bind to a reduced set of cellular proteins, including p105-Rb, p107, and p60-cyclin A, are transformation defective but can still interfere with exogenous growth inhibitory and differentiating signals, such as those triggered by TGF-β. We have tested the ability of one such mutant, NTdI646, to interfere with keratinocyte growth and differentiation in vivo, in the skin of transgenic mice. Keratinocyte-specific expression of the transgene was achieved by using a keratin 5 promoter. Two independent lines of transgenic mice were obtained which expressed Ela specifically in their skin and exhibited an aberrant hair coat phenotype with striking regional variations. Affected hair shafts were short and crooked and hair follicles exhibited a dystrophic or absent inner root sheath. Interfollicular epidermis was normal, but its hyperplastic response to acute treatment with TPA (12-O-tetradecanoylphorbol-13-acetate) was significantly reduced. Primary keratinocytes derived from these animals were partially resistant to the effects of TPA and TGF-β. The rate of spontaneous or chemically induced skin tumors in the transgenic mice was not increased.

Thus, expression of a transgene which interferes with known negative growth regulatory proteins causes profound disturbances of keratinocyte maturation into a highly organized structure such as the hair follicle but does not lead to increased and/or neoplastic proliferation.

SELF-renewing epithelia are characterized by a fine balance between growth and differentiation. An important problem is how these processes are controlled. In the epidermis, proliferation is normally confined to keratinocytes of the basal layer while cells of the upper layers terminally differentiate (Fuchs, 1990). A more complex program of keratinocyte growth and differentiation is involved in formation of skin appendages such as hair follicles (Stenn et al., 1991). The mature hair follicle consists of several concentric epithelial layers, of which the innermost ones make up the hair shaft. Each follicle undergoes repeated cycles of growth (anagen), regression (catagen), and quiescence (telogen). In the growth phase, the relatively undifferentiated and actively dividing cells of the lower portion, or “bulb,” move upwards and differentiate into the several concentric epithelial layers of the upper region. In turn, the proliferating bulb cells appear to arise from stem cells situated in the “bulge,” in the upper region of the hair follicle (Cotsarelis et al., 1990). The importance of this region for keratinocyte self-renewal is indicated by both hair follicle (Oliver, 1966; Inaba et al., 1979) and epidermal (Lenoir et al., 1988; Limat et al., 1991) regeneration experiments. This upper portion of the follicle, rather than the epidermis, is also thought to contain target cells for cutaneous carcinogenesis (Berenblum et al., 1959; Argyris, 1980. Keratinocytes in both epidermis and hair follicles are dependent on intimate interactions with the underlying mesenchymal tissues (for example, see Billingham and Silvers, 1967; Saag et al., 1985; Oliver and Jahoda, 1988; Coulomb et al., 1989; Hirai et al., 1992).

Previous studies have shown that the Adenovirus Ela oncogene can be used to probe into complex regulatory functions, involved in keratinocyte growth and differentiation (Barrandon et al., 1989; Pietenpol et al., 1990; Missero et al., 1991a,b). The immortalizing and transforming activity of this oncogene depends on its binding to a specific set of cellular proteins, including p105-Rb, p107, and p60-cyclin A (for review see Nevins, 1992). It has been shown that Ela can interfere with the function of these latter proteins by causing their dissociation from specific transcriptional complexes (Bagchi et al., 1990; Chellapan et al., 1991; Mudryj et al., 1991; Cao et al., 1992; Ewen et al., 1992). Besides transformation, binding of Ela to these proteins interferes with transmission of exogenous growth inhibitory and differentiating signals of the kind triggered by TGF-β (Pietenpol et al.,…
transfection. Tumorogenicity of keratinocytes transformed by a single ras oncogene can be suppressed by dermal fibroblasts, through the production of a diffusible TGF-β factor. Resistance to dermal fibroblast-tumor inhibition was observed in keratinocytes concomitantly transformed by ras and E/ta or a truncated E/ta mutant which binds to p105, p107, and p60.

Complex growth inhibitory and/or differentiating signals may be operative in the intact skin, which cannot be taken into account in culture and/or in simple tumorigenicity assays. Our previous studies suggest that heterotypic cell–cell interactions, such as those mediated by TGF-β production, can play an important role in skin tumor control and, more generally, in normal skin homeostasis (Missero et al., 1991b). To further test this model, we have generated transgenic mice which specifically express in their epidermis a transformation defective E/ta mutant (NtdI646) which retain the ability to bind to the p105, p107, and p60 proteins (Whyte et al., 1989). Transgenic mice expressing the entire Adenovirus E1 region from the MMTV promoter have been previously described (Koike et al., 1989). The complex phenotype that was found in that case, multifocal minors at the epidermis of transgenics carrying the truncated E/ta oncogene was run as controls.

Histology and Immunohistochemistry

Formalin-fixed tissues were sectioned and stained with haematoxylin and eosin. For EM, specimens were fixed in glutaraldehyde as previously described (Filvaroff et al., 1990). For immunohistochemistry, tissues were fixed in 3% paraformaldehyde for 1 h at 4°C. Paraffin sections were subjected to PCR analysis with E/ta-specific primers corresponding to nucleotides +877 to +897 and +1073 to +1093 of the E/ta sequence. DNA from the E/ta-positive mice was cleaved with EcoRI and BglII and analyzed after agarose gel electrophoresis. PCR reactions with primers for the endogenous mouse aatn as controls.

Ribonuclease Protection Assays

Tissues were pulverized after quick freezing in liquid nitrogen. Total RNA was extracted by the method of Chomczynski and Sacchi (1987). Ribonuclease protection assays were performed as previously described (Missero et al., 1991b) using 50 μg of tissue RNA and an RNA probe corresponding to nucleotides +646 to +1007 of the E/ta sequence. An RNA probe for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Tso et al., 1985) was also used to control for mRNA levels in the various samples. Probes were prepared after plasmid linearization, by in vitro transcription with T7 polymerase (Stratagene) in the presence of α32P-UTP (10 μCi/ml, 400 Ci/mmol, Amerham Corp., Arlington Heights, IL).

Immunoblotting

Cells or pulverized tissues were dissolved in sample buffer (Laemmli, 1970), boiled for 5' and analyzed by SDS-PAGE and immunoblotting with anti-E/ta M73 mAbs (1:10 dil.; Oncogene Science, Inc., Manhasset, NY) overnight at 4°C. Antibody binding was detected by incubation with 125I-protein A (100 μCi/ml; ICN Biochemicals, Irvine, CA). The various samples were normalized for protein content by gel electrophoresis and staining with Coomassie blue.

**Materials and Methods**

**Plasmid Construction and Characterization**

A 900-bp portion of the keratin 5 promoter was obtained from a mouse genomic DNA library by PCR amplification, from position +807 to position +90 of the published sequence (Lersch et al., 1989) and cloned into DMBA, 7,12-dimethylbenz[a]anthracene; IRS, inner root sheath; ORS, outer root sheath; TPA, 12-O-tetradecanoylphorbol 13-acetate.

1. Abbreviations used in this paper: DMBA, 7,12-dimethylbenz[a]anthracene; IRS, inner root sheath; ORS, outer root sheath; TPA, 12-O-tetradecanoylphorbol 13-acetate.
(Lenexa, KA) and TGF-β 1 from R&D Systems (Minneapolis, MN). Cells were labeled by a 1-h pulse with [Methyl-3H]thymidine (3.3 μCi/ml; 40-60 Ci/mmol; ICN Biomedicals, Costa Mesa, CA). Epidermal transglutaminase activity was measured by [3H]putrescine incorporation as previously described (Filvaroff et al., 1990).

**Tumor Induction Experiments**

Ela-positive and -negative mice in the resting phase of the hair cycle (6-7 wk old) were shaved and treated with DMBA (20 μg in 200 μl acetone) 48 h after shaving. Mice were subsequently treated twice a week with TPA (10^{-4} M in acetone) for 7 mo. These experiments were performed with transgenics in a Senecar background (F4 and F5 generations) and the Ela-negative mice used as controls were derived from the same litters as the transgenics. DMBA was dissolved in acetone just before use. TPA solution was stored at -70°C. Papillomas were excised for histological examination at different stages of their growth.

For experiments involving DMBA, Ela-positive and -negative mice from the same litters were exposed to a single dose of this agent 1-3 d after birth via intraperitoneal injection. Relative to topical treatment of adult mice, this way of administration is equally effective for skin tumor induction (Hennings et al., 1981) and has the potential advantage of hitting target keratinocyte populations in mice of younger age, at a time when the effects of Ela expression appear to be more evident. For these experiments DMBA was first dissolved in DMSO, diluted in SSV buffer (Hennings et al., 1981) and injected at 20 μg per mouse in a 25 μl volume. Mice were kept for examination until 8 mo of age.

**Results**

**Construction of a Keratinocyte-specific Ela Expression Vector and Generation of Transgenic Mice**

A previous study has indicated that the first 900 nucleotides in front of the human keratin 5 gene are sufficient to drive specific expression of this gene in cultured keratinocytes (Lersch et al., 1989). For the present work, this 900-bp promoter sequence was used to drive specific expression of the Ela NTd1646 gene in keratinocytes of transgenic mice. This gene lacks the sequences for the first 29 amino acids of Ela. This deletion prevents binding of Ela to a p300 cellular protein without affecting association with p105, p107, and p60. The NTd1646 gene lacks the immortalizing and transforming activity of the full Ela gene (Whyte et al., 1989), but retains the ability to interfere with the growth inhibitory and differentiating effects of TGF-β, TPA, cAMP, and corticosteroids (Missero et al., 1991a; Florin-Christensen et al., 1992; our own unpublished observations).

A construct carrying the Ela NTd1646 gene behind the K5 promoter (KST/Ela646; Fig. 1 A) was transfected into established Pam212 keratinocytes (Yuspa et al., 1980). As previously described for the Ela NTd1646 gene driven by its own adenoviral promoter (Missero et al., 1991a), KST/Ela646 induced ~40% resistance to TGF-β growth inhibition (data not shown).

KST/Ela646 was used to generate transgenic mice, by injection of purified insert DNA (BamHI cassette, Fig. 1 A) into fertilized eggs from (C57BL/6 × SJL) F1 mice. 5 out of 18 newborn mice were found to be positive for the transgene, and each carried ~4 copies of unarranged DNA, as judged by Southern blotting and dot blotting of chromosomal DNA alongside serial dilutions of vector DNA standards. Transgenic lines for further analysis were generated by breeding all positive founders with Senecar mice. This strain of mice was chosen because of its intrinsic sensitivity to skin carcinogenesis (Hennings et al., 1981), which might enhance whatever growth-related effect the truncated Ela transgene might have. One of the Ela-positive founders failed to transmit the gene to the progeny. Of the other four, two, 901T and 906T, showed similar hair coat abnormalities (see below), which were transmitted to the offspring. The 901T founder was albino and gave rise only to albino mice. 906T was agouti and, when crossed with the Senecar albinos, gave rise to Ela-positive offspring with all possible coat color combinations, indicating that the inserted transgene was unlinked to either the agouti or albino loci.

**Macroscopic Abnormalities in the Ela NTd1646 Transgenics**

Positive mice were clearly distinguishable a few days after birth because of a striped hair coat, resulting from areas of shorter and wrinkled hair shafts alternating with bands of...
Aberrant hair coat phenotype in the Ela NTd1646 transgenies. (a) An Ela-positive 901T mouse at 7 d of age along with an Ela-negative littermate; (b) two Ela-positive 906T mice along with an Ela-negative sibling at 10 d of age; and (c) an Ela-positive 906T mouse with particularly strong phenotype at 1 mo of age. The well demarcated bald area occurred spontaneously.

The striped pattern occurred independently of hair color, sex, and sex of affected parent. Some 906T mice, especially in the F2 and F3 generations, exhibited a stronger phenotype, which included larger and irregular areas of very poor hair growth and/or baldness (Fig. 2 c). As with the stripes, these areas of baldness did not cross the midline but could sharply terminate there (Fig. 2 c). In these same areas, thin pigmented lines could be detected. With time (as early as 3-4 wk of age) some of the bald areas evolved into ulcers (data not shown). Since such destructive lesions could be found at the base of the neck, and in mice that were caged individually, it is unlikely that they were the result of trauma, such as biting or scratching. In the most severely affected animals, the hair abnormality was present universally on all skin surfaces with some hair gradually growing later in life. The weak and strong phenotypes were equally observed in inbred litters of Ela-positive transgenics and outbred litters of Ela-positive males with normal Sencar females.

Besides hair coat abnormalities, the only other macroscopic phenotypic alteration present in some newborn animals was a persistent crust in the eye, without effects on the opening of the lid. The crust was eventually eliminated by 2-3 wk of age. Ela-positive mice were often smaller than their Ela-negative littermates at birth, but this difference tended to disappear by 3-4 wk of age. Autopsy revealed no macroscopic or histologic abnormalities of the various internal organs.

**Histological Abnormalities**

Use of a dissecting microscope revealed that, relative to neighboring regions, hair shafts in the affected stripes were shorter and crooked (Fig. 3, a-c). These hair abnormalities were particularly evident in agouti mice, where the alternating transversal bands of yellow and black pigment—intrinsically to the agouti phenotype—produced an impression of abnormal pigmentation. Very short and irregular shafts were present also in the bald areas. Overhairs—including vibrissae—and underhairs were similarly affected. The shafts appeared thinner and wavy but the tips were tapered as in normal hairs and fractured forms were not seen (Fig. 3, c-e). Hair shafts from individual mice collected and analysed for amino acid content. Variations in individual amino acid were <1% except for citrulline and ornithine—an enzymatic conversion product of trichohyalin (Rogers et al., 1991): a 30% decrease of these particular amino acids was consistently observed in each of five Ela-positive mice versus five Ela-negative siblings.

Hair follicles were directly visualized after removal of dermal collagen by acid extraction (Schweizer and Marks, 1977). This technique revealed that hair follicle density was only slightly reduced in the Ela-positive mice, even in their striped and bald areas, but follicles were generally smaller and erratic in shape compared with the controls. Severely affected follicles were often located next to relatively normal ones. By high power light microscopy, no abnormalities were notable in the dermal papilla, or the lower bulb. Cells in the upper area of the bulb were slightly reduced in number and size and had an abnormal shape and organization. The most obvious changes were apparent in the inner root sheath (IRS) with disruption of the Huxley's and Henle's layers and dilated cells with a reduced content of trichohyalin granules (Fig. 4, c and d). The hair shaft was often prematurely separated from the IRS and showed substantial irregularities in width and a reduction of trichohyalin granules in the medulla. In contrast to IRS and hair shaft, the outer root sheath was usually normal. Focal disruption of the outer root sheath (ORS) could however be found at sites of ulceration (see below). These findings were confirmed by EM, with cells of the IRS lacking the typical differentiation features of both Huxley's and Henle's layers (Fig. 5).
Figure 3. Hair coat abnormalities as observed microscopically. (a-c) Striped hair coat of a 906T mouse as seen under a dissecting microscope at increasing magnification. (d and e) Hair shafts from an Ela-negative and -positive littermate, respectively, as seen by light microscopy. Bars: (a) 300 μm; (b) 150 μm; (c) 60 μm; and (d and e) 75 μm.

The epidermis of the Ela-positive mice was normal (Fig. 4, a and b), except for mild thickness and scaling in some markedly affected newborns. The healing process after full thickness wounding was normal. Areas of spontaneous ulceration were characterized by a marked epidermal hyperplasia surrounding the ulcer and an inflammatory and reparative response of the dermis which contained very few residual hair follicles. No signs of infections were detectable at these locations. To determine the histogenesis of these lesions, we examined a set of eight mice at a rather young age (3 wk), with a very strong phenotype and with already small ulcers. Limited foci of epidermal hyperplasia were found in the bald but intact areas. Much more pronounced hyperplasia was found along the margin of the small ulcers. The wall of the hair follicles in these areas was irregularly thinned and showed focal infiltration of acute inflammatory cells. At least in some cases, it was possible to demonstrate that the ORS was focally disrupted. This histological picture is similar to human perforating folliculitis and is consistent with a deep folliculitis being responsible for the observed ulceration and scarring.

No signs of spontaneous tumorigenic conversion was ever detected over a period of 12 mo either at these locations or anywhere else in the skin of the affected animals.

Correlation of Phenotype with Preferential KST/Ela646 Expression in the Skin

Total RNA was prepared from the skins of F1 mice and analyzed by ribonuclease protection assays with an Ela-specific probe. Ela expression was found in the two families with positive phenotype, 90IT and 906T, while the other two families showed no detectable expression (data not shown). Ela expression in 90IT mice was found to be consistently lower (two- to-threefold in three independent measurements) than in the 906T. For most of the biochemical and functional assays reported hereafter we have focused on the 906T mice.

Tissue specific expression of the transgene was assessed by ribonuclease protection assays with total RNA derived from various tissues. As shown in Fig. 1 B, Ela was preferentially expressed in the skin, with much weaker levels of expression detectable in the stomach and intestine. In all other organs...
Figure 4. Histology of the skin of Ela NTdl646 transgenics. (A and B) Low power magnification of the skin from an Ela-negative (A) and Ela-positive (B) littermates. Note the abnormal and crooked hair follicles with a normal, nonhyperplastic epidermis in the affected animal. Early and irregular separation of the hair shaft from the sheath could be typically observed. (C and D) High power magnification of a hair follicle from the Ela-negative and Ela-positive mice. Note the normal layering of the control follicle (C) with clearly distinguishable ORS, Henle's and Huxley's layers, cuticle, cortex, and medulla. Notice that the medulla has a regular width and the medullary spaces are filled with melanin and trichohyalin granules. The affected follicle (D) contains an irregularly shaped shaft with open medullary spaces, with normal melanin but substantially reduced trichohyalin granules. The wall lacks the typical layering of the normal follicle. In particular, the region corresponding to the putative IRS shows a markedly dystrophic structure and lacks the typical features of both Huxley's and Henle's layers. Haematoxylin and eosin. Bars: (A and B) 200 μm; (C and D) 15 μm.
EM of hair follicles from control (A) and transgenic (B) littermates. Note the normal structure of the control follicle with the ORS, Henle’s (Hen), and Huxley’s layers (Hux), cuticle (cut), cortex (cor), and medulla (med). The wall of the affected follicle is distinctly disorganized with swollen cells without trichohyalin granules in the area of the Huxley’s layer (Hux) and an irregular to absent Henle’s layer (Hen). The layering of the shaft is relatively normal, but the medullary spaces are wider and have a reduced number of granules. The differences in thickness of the cortex and keratinization levels between A and B are not significant as they are due to the slightly different level of the follicle from which these two sections were taken. Bar, 5 μm.

Ela expression remained undetectable, including the esophagus. This organ contains a stratified epithelium where the K5 keratin is usually expressed (Fuchs and Green, 1980; Moll et al., 1982). The lack of expression of the Ela transgene in this location may be due to the relatively small K5 promoter region used for these studies.

The Ela gene products migrate as a set of bands as a result of differential splicing and phosphorylation (Boulanger and Blair, 1991). Expression of Ela NTd1646 proteins was verified by immunoblot analysis of whole skin extracts of the transgenic mice with anti-Ela M73 mAbs (Fig. 1 C). Primary keratinocytes derived from the Ela transgenics were also found to express these proteins (Fig. 1 C), while dermal fibroblasts did not (data not shown). This analysis was complemented by an immunohistochemical study with the same antibodies to determine expression of the Ela protein in the skin in situ. Specific nuclear labeling was found in keratinocytes of both hair follicles (primarily in the ORS) and, to a lesser extent, epidermis (Fig. 6). Interestingly, staining was not homogeneous, but in patches, suggesting local variations in levels of Ela expression between neighboring hair follicles and epidermal keratinocytes.

Response of Transgenic Keratinocytes to TGF-β Growth Inhibition

Primary keratinocytes derived from the Ela transgenics were tested for TGF-β sensitivity. Newborn litters obtained from crosses of F2 906T males with Sencar females were separated into Ela-positive and -negative groups by PCR analysis of tail DNA. Primary keratinocytes were prepared from the two groups of mice and Ela expression was confirmed by immunoblotting with Ela-specific antibodies (Fig. 1 C). Mitogenic response of these cells was determined after 20 h incubation with TGF-β at various concentrations (Fig. 7 A). In five independent experiments, DNA synthesis of the Ela-positive cells was found to be consistently less inhibited than that of their Ela-negative counterparts. However, this difference was never greater than 20% and was not observed at high TGF-β concentrations (>1 ng/ml).

Thus, expression of a truncated Ela NTd1646 gene in the transgenic keratinocytes interferes only partially with TGF-β growth inhibition.

Resistance of Transgenic Keratinocytes to TPA Effects both In Vitro and In Vivo

Phorbol esters such as TPA (12-O-tetradecanoylphorbol-13-acetate) act as potent modulators of keratinocyte growth and differentiation, under both in vitro and in vivo conditions (Steinert and Yuspa, 1978; Balmain, 1978; Bhisey et al., 1982; Yuspa et al., 1982; Reiners and Slaga, 1983). Mitogenic assays similar to the ones described above were used as a first test of the sensitivity of Ela-positive and -negative...
primary keratinocytes to the growth inhibitory effects of TPA. DNA synthesis of Ela-positive cells was found to be inhibited by this agent but to a significantly lesser extent than that of their negative counterparts, and this difference was maintained even at high TPA concentrations (Fig. 7 B). In parallel with these results, epidermal transglutaminase, an enzymatic marker of keratinocyte differentiation, was induced by TPA to substantially lower levels of Ela-positive keratinocytes than in the negative controls (Fig. 7 C).

To test whether there was a decreased responsiveness to TPA in vivo, groups of Ela-positive and -negative mice were treated with this agent or acetone and sacrificed 72 h later (Fig. 8). Serial sections of the skin of each of these animals revealed a strong and homogeneous hyperplastic response in the Ela-negative mice (Fig. 8 C). In contrast, the epidermis

**Figure 6.** Expression of the ElaNTd1646 gene in the skin of transgenic mice as determined by immunohistochemistry. (A and B) Epidermis from a 906T Ela-positive mouse (2 wk old) stained with anti-Ela mAbs (A) or nonimmune antibodies (B); (C and D) a hair follicle of the same mouse stained with anti-Ela mAbs (C) or nonimmune antibodies (D). White arrows point to the specific nuclear labeling with the anti-Ela mAbs. Note in A the heterogeneity of Ela staining between adjacent epidermal keratinocytes. Bar, 90 μm.

**Figure 7.** In vitro response of transgenic primary keratinocytes to TGF-β and TPA. Primary keratinocytes derived from 906T Ela-positive mice (●) and Ela-negative littermates (□) were tested for their sensitivity to growth inhibition by TGF-β and TPA (A and B, respectively) and epidermal transglutaminase induction by TPA (C). Cells were tested in triplicate wells and standard deviation was as indicated. Cells were incubated with fresh medium plus/minus TGF-β 1 or TPA for 20 h. DNA synthesis was measured by a 1-h pulse with [Methyl-3H]thymidine and expressed as percentage values relative to untreated controls. Epidermal transglutaminase activity was measured by [3H]-putrescine incorporation and expressed as fold induction of treated versus untreated controls.
of the Ela-positive animals showed only regions of mild hyperplasia, which alternated with others where the hyperplastic response was totally absent (Fig. 8 B).

Thus the Ela-expression keratinocytes are substantially less responsive to the biological effects of TPA under both in vitro and in vivo conditions.

**Tumor Induction in Ela-positive and -negative Mice**

Spontaneous skin tumor development has not been detected so far in our Ela transgenics (at 15 mo from the beginning of the experiments). Similarly, initiation of mice with DMBA (7,12-Dimethylbenz[a]anthracene) without subsequent tumor promotion elicited no skin tumor development in either Ela-positive or -negative mice (Table I).

A complete initiation-promotion protocol with DMBA and TPA resulted in efficient papilloma formation in both positive and negative mice (Table I). There were no significant differences between the two groups in either time of appearance, size, and rate of tumor growth. The numbers of papilloma-bearing animals and of papillomas per mouse were somewhat lower with the Ela-positive mice than with the negative controls. The histological characteristics of the papillomas obtained in the two cases were very similar (data not shown). Only one carcinoma developed so far from one Ela-negative mouse (at 7 mo from the beginning of the experiment). We conclude from these studies that expression of the truncated Ela NTd1646 gene is unlikely to favor keratinocyte tumor development (at least as tested in the Sencar genetic background), nor does it influence its histological type.

### Table I. Chemically Induced Tumors in Ela-positive and Ela-negative Mice

|                  | DMBA* | DMBA + TPA† |
|------------------|-------|-------------|
|                  | Mice with tumors | Mice with papillomas | Papillomas per mouse |
| Ela-positive     | 0/15 | 28/38 [33/37] | 5.6 [9.7] |
| Ela-negative     | 0/12 | 13/13 [13/13] | 10.8 [16.1] |

* Mice were exposed to a single dose of DMBA either 1–3 d after birth via intraperitoneal injection (20 μg/injection, as described in Materials and Methods) or at 7 wk of age by topical application (4 μg/200 μl acetone). Duration of the experiment: 9 mo.
† Mice were exposed to a single dose of DMBA (4 μg/200 μl acetone) at 7 wk of age followed by biweekly treatments with TPA (10–4 M, 200 μl, in acetone). Values are given after 2 and 4 (brackets) mo. Tumor size varied significantly between and within individual animals, with no consistent differences observed between the Ela-positive and -negative groups.
Discussion

An increasing number of negative growth regulatory genes are being identified which are deleted and/or functionally suppressed in tumor cells (Stanbridge, 1990). The specific consequences of loss of these genes for normal tissue homeostasis and neoplastic development are currently being investigated (Donehower et al., 1992; Lee et al., 1992; Jacks et al., 1992). We have attempted to address this question in mouse skin, by using a transformation-deficient Ela mutant (NTdl646) which interferes with a specific set of cell negative regulatory proteins of which p105-Rb, p107, and p60-cyclin A are probably the best characterized ones (as reviewed by Boulanger and Blair, 1991).

Expression of the Ela NTdl646 transgene did not lead to detectable alterations of the epidermis while hair follicles were substantially abnormal. The main defect appeared to be an absent or markedly dystrophic IRS, with an impaired function as a guide and mold for the nascent hair shaft. A fine structure must exist between growth of keratinocytes in the bulbar part of the hair follicle and their differentiation and organization into the upper structure. In vivo [3H]thymidine incorporation experiments with Ela-positive and -negative siblings, revealed no differences in the number of dividing cells below the Auber's line of the bulb and along the bulbar part of the hair follicle and their differentiation consequences of loss of these genes for normal tissue are being identified which are deleted and/or functionally suppressed in tumor cells (Stanbridge, 1990). The specific aspects of the keratinocyte response to TPA are compromised. The function of the TPA-responsive transcription factor AP-1 will be worth investigating, as the c-fos protein, an integral AP-1 component (Chiu et al., 1988; Sassone-Corsi et al., 1988), is highly expressed in the hair follicle (Fisher et al., 1991).

Expression of an Ela gene able to interfere with the Rb tumor suppressor protein, did not lead to epidermal hyperplasia, nor increased skin tumor formation, or, if anything, seemed to reduce it. The histological type of chemically induced tumors was similar in the Ela-positive and -negative mice, suggesting that the target cell population and its growth-differentiation behavior is not influenced by Ela expression. Greenhalgh and Yuspa (1988) reported that carcinogenic conversion of ras-transformed papilloma cells is not increased by an intact Ela oncogene. Even in our transgenics, assuming that the carcinogenic effects of DMBA are due to activation of the Ha-ras gene (Balmain et al., 1984), expression of the truncated Ela oncogene does not appear to exert a ras-cooperating activity. However, it remains to be tested whether tumor-facilitating effects of this transgene can be caused by Ela expression, possibly through the induction of other growth regulatory genes.
be manifested in a genetic background less prone to skin carcinogenesis, such as that of the Balb/c strain (Hennings et al., 1981). In any case, it has to be stressed that many negative regulatory loops may act in vivo to maintain skin homeostasis, some of which are totally unaffected by Ela NTd3646 activity. For instance, gap junctional intercellular communication, which is an important mechanism to coordinate control of keratinocyte growth and differentiation (Yamazaki, 1990), is not changed in keratinocytes expressing this truncated oncogene (our own unpublished results).

In conclusion, expression of an Ela gene which interferes with known negative regulatory growths, such as p105-Rb and p60-cyclin A, does not cause gross abnormalities in keratinocyte growth and differentiation, nor facilitates neoplastic outgrowth. Rather, expression of this gene interferes with the regulatory mechanisms involved in keratinocyte hair follicle maturation. Besides p105/p107/p60, expression of this Ela gene may affect other unrelated regulatory proteins, for instance through its intact transactivation domain (domain 3; Moran and Mathews, 1987). An elucidation of the specific interactions involved should prove invaluable to understand how a complex epithelial structure such as the hair follicle is organized and maintained.

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The Journal of Cell Biology, Volume 121, 1993 1120