Influence of β1 Integrins on Epidermal Squamous Cell Carcinoma Formation in a Transgenic Mouse Model: α3β1, but not α2β1, Suppresses Malignant Conversion

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ABSTRACT

Although aberrant integrin expression has been documented in many epithelial tumors, little is known about how integrins influence neoplastic progression. To examine this issue, transgenic mice in which the α2β1 or α3β1 integrin was expressed in the suprabasal epidermal layers via the involucrin promoter were subjected to skin carcinogenesis. Equal numbers of benign squamous papillomas were observed in transgenic and wild-type animals. However, the frequency of conversion of papillomas to malignant squamous cell carcinomas was much lower in α3β1 transgenic than in α2β1 transgenic and wild-type mice. No differences were observed in apoptosis or in the expression of endogenous integrins in transgenic and wild-type papillomas. However, α3β1 transgenic papillomas displayed a diminished proliferative capacity and were more highly differentiated as judged by BrdUrd incorporation and keratin 10 expression, respectively, than α2β1 transgenic and wild-type papillomas. Two proteins that associate with α3β1 and not α2β1 are extracellular matrix metalloproteinase inducer and CD81. Extracellular matrix metalloproteinase inducer expression correlated inversely with the degree of differentiation in normal epidermis and in transgenic and wild-type papillomas. Up-regulation of CD81 was observed in 100% of wild-type and 88% of α2β1 transgenic papillomas but in only 25% of α3β1 transgenic papillomas. CD81 was undetectable in untreated epidermis and strongly expressed in all transgenic and wild-type squamous cell carcinomas. Our results demonstrate that the α3β1 integrin can suppress malignant conversion, and that the mechanism may involve CD81.

INTRODUCTION

Integrins are cell surface receptors that mediate cell-extracellular matrix and cell-cell adhesion events and are capable of transducing signals that are implicated in a wide variety of cellular responses (1, 2). In the epidermis, the major keratinocyte integrins are α2β1, α3β1, and α6β4 (3). In addition to mediating keratinocyte adhesion to extracellular matrix proteins, β1 integrins regulate epidermal proliferation and terminal differentiation (4–8).

Integrin expression is normally restricted to keratinocytes in the basal layer of the epidermis. However, integrin expression is altered when the epidermis is damaged and in benign and neoplastic diseases. Suprabasal integrin expression has been observed during wound healing and in psoriatic epidermis (9, 10). SCCs5 exhibit variable patterns of integrin expression, including normal expression, loss of expression, and overexpression, and variation is observed both within and between individual tumors. In squamous papillomas and SCC, suprabasal expression of α6β4, as well as increased basal α6β4 expression, is associated with tumor progression and invasion (11–13). Highly undifferentiated spindle cell carcinomas exhibit up-regulated expression of the α5β1 integrin (14). Increased expression of α2β1 and α3β1 integrin has been observed in basal cell carcinomas (3, 15). Complete or focal loss of expression of α2β1, α3β1, and α6β4 has also been observed in SCC (16, 17). Hence, both loss of expression and overexpression of keratinocyte integrins have been observed in SCC.

Various in vitro models have been established to examine the significance of alterations in keratinocyte integrin expression in SCC (13, 18, 19). The behavior of SCC lines has been analyzed before and after introduction of specific integrin subunits. Although such studies clearly demonstrate that abnormal integrin expression contributes to the failure of SCC cells to differentiate normally, they suffer from the limitation that it is not possible to study the role of integrins in tumor development. To overcome this difficulty, we have made use of a transgenic mouse model that targets the expression of human integrin subunits to the suprabasal layers of the epidermis via the involucrin promoter (20, 21). Mice expressing suprabasal α2β1 or α3β1 exhibit sporadic epidermal hyperplasia and skin inflammation, which are indicative of the benign hyperproliferative disease psoriasis (20, 21). However, no spontaneous epidermal tumors have been observed in these lines. To determine whether suprabasal integrin expression can alter the sensitivity of keratinocytes to malignant conversion, we have subjected transgenic mouse skin expressing α2β1 or α3β1 to a chemical carcinogenesis protocol that allows evaluation of the development of both benign (papillomas) and malignant (SCC) tumors (22, 23).

MATERIALS AND METHODS

Reagents. DMBA was purchased from Argos Chemicals. TPA was purchased from LC Laboratories. Acetone was purchased from BDH Chemicals. BrdUrd was purchased from Sigma Chemical Co. Cito Plus antigen retrieval solution was purchased from BioGenex (San Ramon, CA). Automation buffer was purchased from Biomedia. Fluorescein apoptosis detection system was purchased from Promega.

Animals. All transgenic and wt mice were generated and maintained in the Imperial Cancer Research Fund Animal Containment unit. Animals were kept on a 12 h light/dark cycle and fed R20 rodent chow (Special Dietary Services, Essex, United Kingdom) and water ad libitum. α2 (founder line 1070; 49 copies/cell), α3 (founder line 1120C; 20 copies/cell), and β1 (founder line 0840; 42 copies/cell) transgenic mouse lines previously on an F1 hybrid (CBA × C57BL/6) genetic background (20, 21) were backcrossed six generations onto a homogeneous C57BL/6 background. α2β1 and α3β1 double transgenic mice were then generated by crossing α2 and α3 single transgenics to β1 single transgenics.

Tumor Studies. Female α2β1 and α3β1 integrin transgenic and wt littermate mice 7 weeks of age (25 animals/group) were shaved once on the dorsal surface with electric clippers. One week later all animals that did not show signs of hair regrowth received one topical application of 100 nmol (25 μg) DMBA in 200 μl of acetone or 200 μl of acetone alone. One week later, mice received thrice-weekly applications of 6 nmol (3.7 μg) TPA in 200 μl acetone or 200 μl acetone alone. C57Bl/6 mice have been shown previously to be resistant to twice-weekly applications of TPA but do develop tumors with a thrice-weekly protocol (22, 23). Benign and malignant skin tumors were recorded once a week for a period up to 52 weeks after the start of promotion.

Student’s t test was conducted to statistically compare papilloma and malignancy formation between α2β1 and α3β1 transgenic mice and wt mice. The
experiment was repeated with a second group of animals, and the results obtained in the first and second experiments were the same.

**Short-Term TPA Treatment.** Female a2B1 and a3B1 transgenic and wt littermate mice 7 weeks of age (five animals/group) were shaved once on the dorsal surface with electric clippers and 1 week later received either 1, 4, 8, or 12 applications of 6 nmol TPA, in 200 μl acetone or 200 μl acetone alone at a frequency of three applications per week. All skin sections were harvested 24 h after the final TPA treatment.

**Tissue Harvesting.** One h before sacrifice, all mice received a single i.p. injection of 100 mg/kg BrdUrd. Skin tumors and dorsal skin sections were harvested, and portions of each tumor or skin section were fixed in 10% neutral-buffered formalin or frozen in liquid nitrogen-cooled isopentane for embedding in OCT medium. All formalin-fixed tumor sections were stained with H&E and graded as either a squamous papilloma or a SCC (24).

**BrdUrd Labeling.** To examine BrdUrd incorporation, formalin-fixed skin and tumor sections were processed as described previously (25). Briefly, tissue sections were deparaffinized and incubated in 2 M HCl for 30 min at 37°C, dipped in borate buffer for 3 min, and then digested in 0.01% trypsin for 3 min at 37°C. Sections were blocked in 10% normal goat serum for 20 min and then probed with antibodies for BrdUrd (Becton Dickinson) for 1 h. Sections were then incubated with a species-specific Alexa 488-conjugated (Molecular Probes) secondary antibody, after which sections were counterstained with 1 μg/ml propidium iodide and examined under a Zeiss Axiophot fluorescent microscope.

**Immunofluorescence.** For mouse keratin 10 staining, formalin-fixed tumor sections were deparaffinized and microwaved in Citra Plus antigen retrieval solution for 2 min 30 s and allowed to cool in the antigen retrieval solution for an additional 15 min. Sections were blocked in 10% normal goat serum for 20 min and then probed with antibodies for mouse keratin 10 (Babco) for 1 h. Sections were then incubated with a species-specific Alexa 488-conjugated (Molecular Probes) secondary antibody, after which sections were counterstained with 1 μg/ml propidium iodide. Fluorescence was observed using a Zeiss Axiophot fluorescent microscope.

Frozen skin and tumor sections were fixed in acetone at −20°C for 10 min prior to a 10% normal goat serum block and then probed with antibodies for mouse α6 integrin (CD49f; Serotec), mouse β1 integrin (MB1.2; courtesy of Bosco M. Chan, University of Western Ontario, London, Ontario, Canada; Ref. 26), rat EMMPRIN (CE9; courtesy of James Bartles, Northwestern University Medical School, Chicago, IL; Ref. 27), mouse CD81 (Eat2; courtesy of Shoshana Levy, Stanford University Medical Center, Stanford, CA; Ref. 28), mouse involucrin (29), human α3 integrin (VM-2; Ref. 21), or human α2 integrin (HAS4; Ref. 20). Tissue sections were then incubated with a species-specific Alexa 488-conjugated (Molecular Probes) secondary antibody for α6 integrin, EMMPRIN, and β1 integrin, or species-specific Alexa 594-conjugated (Molecular Probes) secondary antibodies for α2 and α3 integrin and involucrin, or FITC-conjugated antihuman (PharMingen) for CD81. After a 20 min secondary antibody incubation, sections were washed and mounted in Gelvatol. Immunofluorescence was visualized using a Zeiss Axiophot fluorescent microscope.

**TUNEL Assay.** Mouse papilloma and skin sections were processed for detection of apoptotic cells according to the manufacturer’s protocol. Deparaffinized formalin-fixed papilloma and skin sections were incubated in 0.85% NaCl followed by PBS and then fixed in 4% paraformaldehyde, permeabilized with 20 μg/ml Proteinase K for 10 min, and fixed again in 4% paraformaldehyde. Sections were incubated with terminal deoxynucleotidyl transferase in the presence of fluorescein-12-dUTP at 37°C for 1 h. Sections were counterstained with 1 mg/ml propidium iodide and examined under a Zeiss Axiophot fluorescent microscope.

**RESULTS**

**Suprabasal Keratinocyte Proliferation in a2B1 and a3B1 Transgenic Mouse Skin.** The previously reported sporadic hyperproliferative and inflammatory phenotype in a2B1 and a3B1 transgenic mouse skin is not observed in young animals either in the original F1 background (CBA × C57Bl/6) or after backcrossing onto a homogeneous C57Bl/6 background (20, 21). As shown in Fig. 1 panels A, C, and E, acetone vehicle-treated a2B1 and a3B1 transgenic mouse skin displayed a similar labeling index to wt mouse skin, with all BrdUrd-positive S-phase cells residing in the basal layer. However, the transgenics showed a markedly different response to TPA from wt mice. a2B1 and a3B1 transgenic and wt mouse skin was treated with 1, 4, 8, or 12 applications of 6 nmol TPA, and skin sections were analyzed for BrdUrd incorporation. After only a single application of TPA, before the onset of significant hyperplasia, both a2B1 and a3B1 transgenics exhibited BrdUrd incorporation in the suprabasal layers of the epidermis (Fig. 1, D and F), whereas no BrdUrd-positive keratinocytes were observed in the suprabasal layers of wt epidermis (Fig. 1B). The number of BrdUrd-positive keratinocytes in the basal layer did not differ between a2B1 and a3B1 transgenic and wt mouse skin after any of the TPA treatment regimens. The suprabasal BrdUrd labeling in transgenic epidermis was also present after 4, 8, and 12 applications of TPA, whereas only basal BrdUrd incorporation was observed in wt mice (data not shown).

**a2B1 and a3B1 Transgenic Mice and wt Mice Are Equally Sensitive to Papilloma Formation.** To determine whether the acute phenotypic response to short-term TPA treatment observed in transgenic mouse skin (Fig. 1) could be extended to tumor formation, we compared the responses of transgenic and wt mouse skin to chemical carcinogenesis. a2B1 and a3B1 transgenic and wt littermate female mice were initiated with a single dose of 100 nmol DMBA. One week later, mice were treated with 6 nmol TPA three times/week for 25 weeks. As shown in Fig. 2A, papillomas first emerged in all groups 8 weeks after the start of TPA promotion, and maximum papilloma responses reached a plateau by 20 weeks. No significant differences in papilloma frequency were observed between a2B1 (1.9 papillomas/mouse) or a3B1 transgenic mice (2.3 papillomas/mouse) and wt mice (1.6 papillomas/mouse; Fig. 2A; P = 0.637 for a2B1 versus wt; P = 0.352 for a3B1 versus wt). In addition, there was no difference in the incidence of papillomas (percentage of mice with papillomas) in a2B1 and a3B1 transgenic mice and wt mice (Fig. 2B). No papillomas were observed in transgenic or wt mice that were initiated with DMBA and promoted with acetone vehicle or that were initiated with acetone vehicle and promoted with TPA (data not shown). In a repeat experiment, no significant differences in papilloma incidence or frequency were observed between a2B1 (1.88 papillomas/mouse) or a3B1 transgenic mice (2.13 papillomas/mouse) and wt mice (3.12 papillomas/mouse; P = 0.128 for a2B1 versus wt; P = 0.164 for a3B1 versus wt).

**a3B1 Transgenic Mice Are Resistant to SCC Formation.** To determine whether aberrant integrin expression could influence malignant conversion, a2B1 and a3B1 transgenic and wt mice were maintained under weekly observation for SCC formation for a period of 52 weeks after the start of TPA promotion. As shown in Fig. 3A, malignancies first emerged in wt mice 20 weeks after the start of promotion, whereas the first malignancies did not arise in a2B1 and a3B1 transgenic mice until 26 and 27 weeks, respectively. No difference in the average number malignancies/mouse was observed between a2B1 transgenic and wt mice (P = 0.583; Fig. 3A). However, a3B1 transgenic mice developed 5–6 fold fewer malignancies than wt mice (P = 0.0005), and by 52 weeks, just three malignancies were observed in the entire group (Fig. 3A). As shown in Fig. 3B, a3B1 transgenic mice also displayed a profound decrease in SCC incidence compared with a2B1 transgenic and wt mice. H&E-stained histological sections from all malignancies were examined and graded to be SCCs in all groups (24). Similar decreases in SCC formation in a3B1 transgenic (0.30 malignancies/mouse; P = 0.020) but not in a2B1 (0.61 malignancies/mouse; P = 0.231) transgenic mice compared with wt (0.96 malignancies/mouse) mice were obtained in a repeat experiment (data not shown).
Phenotypic Characterization of α2β1 and α3β1 Transgenic and wt Skin Tumors. Mouse skin papillomas and SCCs from every tumor-bearing animal were subjected to histopathological examination (Fig. 4). Pedunculated and sessile papillomas were observed in all groups of mice, with pedunculated papillomas predominating. The extent of dysplasia and cellular atypia was similar in wt and transgenic papillomas (Fig. 4), and data not shown). All of the SCCs were well to moderately differentiated (Fig. 4, A, C, and E). Inserts in B, D, and F show higher magnification views (×6.5) of parts of each section. Scale bar, 100 μm.

Fig. 1. BrdUrd labeling. Immunofluorescent detection of BrdUrd-positive S-phase cells in wt (A and B), α2β1 (C and D), and α3β1 (E and F) transgenic epidermis after a single treatment of acetone (A, C, and E) or TPA (B, D, and F). Formalin-fixed skin sections were stained with a BrdUrd antibody (green) and then by a propidium iodide counterstain (red). Insert in B, D, and F show higher magnification views (×6.5) of parts of each section. Scale bar, 100 μm.
profile of α6β4 existed between the groups as a whole (data not shown).

The decrease in SCC formation observed in α3β1 transgenic mice might be attributable to an increased tendency for cells in the papillomas to undergo apoptosis. To examine this possibility, α2β1 and α3β1 transgenic and wt papillomas were subjected to TUNEL labeling to visualize apoptotic cells. Heterogeneity existed in the numbers of apoptotic cells between papillomas in the same group; however no overall differences were observed in the numbers or in the location (basal or suprabasal) of apoptotic cells between any of the papilloma groups (data not shown).

Collectively these results suggest that the reason why α3β1 transgenic mice show a reduced frequency of malignant conversion is that they develop a lower frequency of high-risk papillomas than α2β1 transgenic and wt mice.

α3β1 Transgenic Papillomas Exhibit Altered Expression of CD81 but not EMPRIN. A large number of proteins form complexes with integrins and several of these associate with α3β1 but not with α2β1 (31). To see whether differences in the expression or distribution of such proteins might account for the differences in the malignant conversion of α2β1 and α3β1 transgenic papillomas, we stained sections with antibodies to the TM4SF protein CD81 (32) and to the immunoglobulin superfamily protein EMPRIN (extracellular MMP inducer; also known as CD147, basigin; Ref. 33). EMPRIN regulates the expression of MMPs in stromal fibroblasts (34) and is thought to play a role in the progression of human oral SCC (35). Ligation of CD81 and other α3β1 associated TM4SF proteins stimulates tumor cell invasion by modulating the actin cytoskeleton and stimulating production of MMP-2 (36).

As illustrated in Fig. 5C, basal keratinocytes in phenotypically normal epidermis of wt and transgenic mice exhibited intense membrane-localized staining for EMPRIN, whereas suprabasal cells had a more diffuse and cytosolic staining. In some papillomas, intense membrane-localized staining was observed in the suprabasal layers and this expression pattern correlated inversely with the degree of differentiation. As shown in Fig. 5, F and G, the up-regulation of EMPRIN in the suprabasal layers correlated with a loss of involucrin expression. In the α3β1 transgenic papilloma shown in Fig. 5, D and E, the basal, transgene-negative layer stained intensely for EMPRIN, whereas the suprabasal, transgene-positive layers had weaker, diffuse staining. In all papillomas and SCCs examined, increased EMPRIN staining was associated with a loss of differentiation, and, as such, EMPRIN may serve as a marker of progression in mouse skin, as it does in human oral SCC (35). However, there were no differences in EMPRIN expression between α3β1 transgenic papillomas and α2β1 transgenic and wt papillomas beyond those reflected in the overall degree of differentiation.

CD81 expression was examined in normal and TPA-treated α2β1 and α3β1 transgenic and wt mouse skin and in skin papillomas and SCCs (Fig. 6 and Table 2). CD81 expression could not be detected by immunofluorescence in frozen sections of normal wt or transgenic epidermis (data not shown). However, CD81 was induced in the suprabasal layers of TPA-treated α2β1 and α3β1 transgenic and wt mouse skin (Fig. 6A and data not shown). As illustrated in Fig. 6 and in Table 2, CD81 was constitutively up-regulated in the majority of wt

![Fig. 2. Papilloma formation. Mouse skin papilloma frequency (A) and incidence (B) in wt, α2β1, and α3β1 transgenic mice are shown. Female mice received a single topical dose of DMBA (initiation). One week later, mice were promoted with topical applications of 6 nmol TPA three times/week for 25 weeks.](image1)

![Fig. 3. SCC formation. Frequency (A) and incidence (B) of malignancies in wt, α2β1, and α3β1 transgenic mouse skin are shown. Female mice received a single topical dose of 100 nmol DMBA (initiation). One week later, mice received topical applications of 6 nmol TPA three times/week for 25 weeks (promotion). Mice were observed for 52 weeks after the start of promotion.](image2)
SUPRABASAL α3β1 EXPRESSION INHIBITS SCC FORMATION

Fig. 4. Histopathological analysis of wt, α2β1, and α3β1 transgenic papillomas and SCCs. Formalin-fixed tumor sections were stained with H&E. wt papilloma (A) and SCC (moderately to well differentiated); B), α2β1 papilloma (C) and SCC (moderately differentiated; D), and α3β1 papilloma (E) and SCC (well differentiated; F). Scale bar, 100 μm.

and α2β1 transgenic papillomas (Fig. 6, B and C), but most α3β1 transgenic papillomas were CD81-negative (Fig. 6D and Table 2). In contrast, CD81 up-regulation was detected in 100% of α2β1 and α3β1 transgenic and wt SCCs (Table 2). These results indicate that constitutive up-regulation of CD81 is associated with tumor progression and suggest that the absence of CD81 observed exclusively in α3β1 transgenic papillomas may play a role in the suppression of SCC formation in α3β1 transgenic mouse skin.

DISCUSSION

In this study we examined the sensitivity of transgenic mice expressing suprabasal α2β1 and α3β1 integrins to chemically induced skin carcinogenesis. α2β1 and α3β1 transgenic and wt mice were equally sensitive to DMBA/TPA-induced papilloma formation. Wt and α2β1 transgenic mice exhibited similar frequencies of malignant conversion of papillomas to SCCs. However, α3β1 integrin-transgenic papillomas were resistant to SCC development.

Previously, it has been unclear why the hyperproliferative and inflammatory phenotype of α2β1 and α3β1 transgenics is sporadic, although the transgenes are constitutively expressed in the suprabasal epidermal layers (20, 21). We found that suprabasal proliferation, as measured by BrdUrd incorporation, was strongly induced by a single dose of TPA in the transgenics, whereas it was not induced in wt mice. This suggests that the transgenomes may act by increasing the sensitivity of keratinocytes to external proliferative or inflammatory stimuli. Although the presence of suprabasal S-phase cells in papillomas is indicative of a high risk of malignant conversion (12, 25), the suprabasal proliferation induced in normal epidermis by one dose of TPA did not correlate with an increased frequency of papilloma formation. Thus, suprabasal S-phase cells are more indicative of tumor progression than promotion.

One potential explanation for the low frequency of SCCs in α3β1 transgenics would be that α3β1 papillomas had a tendency to regress as a result of apoptosis. Induction of apoptosis through the loss of attachment to the extracellular matrix (anoikis) is mediated by integrins (37, 38), and resistance to anoikis is seen as a hallmark of malignant conversion in epithelial cell types (37). However, normal keratinocytes do not undergo anoikis (39), and suprabasal integrin expression does not lead to increased apoptosis in the epidermis of the integrin transgenic mice (20). Furthermore, there were no differences in the total number or distribution (basal or suprabasal) of TUNEL-positive keratinocytes in α3β1 transgenic papillomas compared with α2β1 transgenic and wt papillomas. Thus apoptosis does not play a role in the failure of α3β1 transgenic papillomas to undergo malignant conversion.

The progression of papillomas to SCC has been characterized phenotypically by inappropriate expression of certain growth factors, cyclins/cyclin-dependent kinases, and cell surface receptors for growth factors and adhesion molecules (40, 41). It is interesting that many of these gene products are known to interact with or to be regulated by integrins. Examples include gap junction proteins such as connexin 43 (42), cyclin D1 (43), transforming growth factor β (44), and components of the Ras signaling pathway (45). However, with the exception of the link between α3β1 and connexin 43 (42), there is no evidence for a differential interaction between any of these molecules and α3β1 or α2β1.

The low frequency of α3β1 transgenic SCCs was correlated with a tendency of α3β1 papillomas to be well differentiated (keratin 10-positive) with a low incidence of suprabasal BrdUrd incorporation. The explanation for the difference in malignant conversion of α2β1 and α3β1 papillomas may lie with the integrins themselves or with proteins that specifically associate with them. In addition to differences in the ligand specificities of α2β1 (collagen) and α3β1 (laminin), α3β1 has the distinct properties of being a transdominant inhibitor of other integrins and a suppressor of stress fiber formation (46). However, the suprabasal integrins in the transgenic mice do not have

Table 1 Phenotypic characterization of wt, α2β1, and α3β1 transgenic papillomas

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<th>BrdUrd&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Keratin 10&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>Basal</td>
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<td>wt papillomas (n = 17)</td>
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<td>23</td>
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<tr>
<td>α2β1 papillomas (n = 10)</td>
<td>10</td>
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<td>α3β1 papillomas (n = 23)</td>
<td>65</td>
<td>22</td>
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<sup>a</sup> The percentage of papillomas in each category is shown. Suprabasal BrdUrd labeling and keratin 10 expression is as follows: +, 0–20% papilloma; ++, 25–50% papilloma; ++++, >50% papilloma.
associated extracellular matrix ligands (20), and in the present experiments the presence of the transgenes did not affect expression of the endogenous mouse integrins. Another possibility is that suprabasal integrins influence tumorigenesis through a paracrine effect on the underlying transgene-negative basal cells (21), and that the nature of these signals differs between cells expressing \( \alpha_2 \beta_1 \) and \( \alpha_3 \beta_1 \).

Of the two \( \alpha_3 \beta_1 \)-associated proteins we examined, EMMPRIN was down-regulated in differentiating cells of normal epidermis, papillomas, and SCCs; but there was no evidence that EMMPRIN distribution differed between \( \alpha_2 \beta_1 \) and \( \alpha_3 \beta_1 \) transgenics. Potentially more significant was the observation that, whereas CD81 was absent in normal epidermis and in the majority of \( \alpha_3 \beta_1 \) papillomas, it was strongly up-regulated in \( \alpha_2 \beta_1 \) and wt papillomas and in all transgenic and wt SCCs. Inasmuch as \( \alpha_3 \beta_1 \) integrin-tetraspan complex activation has been shown to induce MMP secretion and tumor cell migration (36), and MMPs are invariably up-regulated in epithelial cancers (47), the absence of CD81 in the \( \alpha_3 \beta_1 \) papillomas could explain why they only convert to SCCs at low frequency. What is unclear at present is whether the \( \alpha_3 \beta_1 \) transgene contributes directly to the regulation of CD81 expression and, if so, by what mechanism.

Altered integrin expression has been extensively documented in human SCC (11, 15–17), and some changes are useful prognostic indicators (11). However, because tumor progression requires multiple genetic and epigenetic changes, the sequential order or timing of

Fig. 5. Immunofluorescent detection of BrdUrd incorporation and EMMPRIN. BrdUrd labeling of an \( \alpha_3 \beta_1 \) transgenic (A) and wt (B) papillomas. BL, basal layer (arrows); SL, suprabasal layers. Untreated wt mouse skin (C), an \( \alpha_3 \beta_1 \) papilloma (D and E), and wt papilloma (F and G) stained with antibodies to EMMPRIN (C, D, and F), human \( \alpha_3 \beta_1 \) (E) or involucrin (G). D and E, the same section has been double-labeled. F and G, serial sections. In F, --- demarcates a region of intense EMMPRIN staining, which corresponds to the involucrin-negative area in G. Scale bar, 50 \( \mu \)m.

Fig. 6. Immunofluorescent detection of CD81. A, \( \alpha_2 \beta_1 \) transgenic epidermis after 8 applications of 6 nmol TPA. B–D: papillomas from wt (C), \( \alpha_2 \beta_1 \) (B) and \( \alpha_3 \beta_1 \) (D) transgenic mice. BL, basal layer (arrows); and SL, suprabasal layer. --- in D, boundary between SL and BL. Scale bar: 50 \( \mu \)m.
alterations in integrin expression may be critical, and early changes in integrin expression may have more impact on the course of the disease than the changes that characterize a mature tumor. The value of our transgenic mouse model is that it can be used to discover how early changes in integrin expression affect later stages in the disease.

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REFERENCES


Table 2. Incidence of CD81 expression in papillomas and SCCs

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<tr>
<th>Tumor Type</th>
<th>CD81 Expression (%)</th>
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<tr>
<td>Papilloma</td>
<td>100 (7/7)</td>
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<tr>
<td>SCC</td>
<td>88 (5/6)</td>
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% CD81 positive tumors

Papilloma: 100 (7/7)

SCC: 88 (5/6)

The table shows the incidence of CD81 expression in papillomas and squamous cell carcinomas (SCCs) in a mouse model. The data indicate a significant difference in the expression levels of CD81 between papillomas and SCCs, with papillomas showing almost complete expression (100%) and SCCs expressing CD81 at a lower level (88%). This finding suggests that CD81 expression may be involved in the progression of squamous cell carcinomas.
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