Induction of Primary Cutaneous Melanocytic Neoplasms in Urokinase-Type Plasminogen Activator (uPA)-deficient and Wild-Type Mice: Cellular Blue Nevi Invade but Do Not Progress to Malignant Melanoma in uPA-deficient Animals

Richard L. Shapiro, Julie G. Duquette, Daniel F. Roses, Irene Nunes, Matthew N. Harris, Hideko Kamino, E. Lynette Wilson, and Daniel B Rifkin


ABSTRACT

Evidence suggests that the plasminogen activators (PAs), in particular urokinase-type PA (uPA), play a pivotal role in tumor invasion and metastasis. We studied the contribution of the PAs to the malignant phenotype through the chemical induction of melanocytic neoplasms in uPA-deficient mice. Primary tumors were induced and promoted concomitantly in 35 uPA−/− deficient and 35 uPA+/+ wild-type mice using a single application of 7,12-dimethylbenz(a)anthracene followed by repetitive applications of croton oil. Animals were sacrificed at 60-day intervals for 1 year. At necropsy, the four largest pigmented lesions in each animal were excised, characterized histologically, and evaluated microscopically for evidence of metastasis. The regional lymph nodes, lungs, and solid abdominal visceral organs were sectioned and examined microscopically for evidence of metastatic disease. The regional lymph nodes, lungs, and solid abdominal visceral organs were sectioned and examined microscopically for evidence of metastatic disease. Enhanced expression of uPA has been reported in a variety of human cancers, including carcinomas of the breast (12), colon (13), lung (14), stomach (15), and prostate (16), glioma (17), and malignant melanoma (18). The demonstration of increased PA activity has been correlated with tumor invasiveness and may be a prognostic indicator of local recurrence, distant metastasis, and overall survival (19).

A variety of laboratory models have been developed to study the role of uPA in cellular invasion and metastasis. Studies of malignant cell lines in culture have demonstrated a correlation between uPA expression and the ability to penetrate synthetic protein gels and extracellular matrices (20, 21). Experiments performed in vivo have provided further evidence for the association of cell surface uPA activity and the invasive and metastatic potential of transplanted tumor cells in laboratory animals (22, 23). One strategy that has been used to assess the fundamental importance of uPA activity in tumor progression is to block its action. Both pharmacological agents and antibodies have been used to inhibit plasminogen activation (24–25). Ossowski and Reich (26) and Ossowski et al. (27) demonstrated that antialcatalytic antibodies to uPA diminish the metastatic potential of human HEp3 carcinoma cells in chick embryos and diminish local invasion in nude mice. Antisense oligonucleotides also block the invasive and metastatic potential of tumor cells transplanted into in vivo model systems (28). However, much of the data supporting a causal relationship between uPA production and cellular invasion and metastasis derive from experiments that oversimplify the sequence of events critical for the development of malignant cells in vivo. In addition, a number of studies have yielded conflicting results (29). These experiments typically entail the s.c. or i.v. transplantation of large numbers of tumor cells, derived from established cell lines, into immunodeficient laboratory animals (30, 31). The invasive and metastatic potential of these transplanted cells is assessed by recording the size and number of tumor emboli present in the lung or visceral organs at a predetermined time after implantation or injection. The validity of this model for the study of metastasis and the clinical value of data obtained, however, have been challenged on methodological grounds (32, 33). For example, the i.v. administration of tumor cells into an animal, although able to reproduce specific patterns of metastasis to various organs such as the lung (34), bypasses many of the early steps in the metastatic cascade. Moreover, the use of immunologically compromised recipients may diminish the importance of the local microenvironment (29, 35). In addition, the phenomena of these cell lines may have occurred after in vitro and in vivo passaging (36). The orthotopic implantation of histologically intact human tumor tissue into immu-
nosuppressed animals may more closely resemble human tumorigenesis (33). Although this model produces metastatic patterns and frequencies that resemble the biological behavior of the original human tumor, this approach requires that the recipient animals be immunosuppressed to avoid rejection of human tissue (37).

The recent development of PA-null strains of mice (38) provides an experimental system to evaluate the biological relevance of the PA system. The simultaneous deletion of both uPA and iPA genes results in a number of physiologically significant consequences. Unfortunately, the shortened life spans of these animals preclude their use for the study of tumorigenesis. Disruption of the uPA gene alone, however, results in no serious phenotypic defects and a normal life span. The invasive properties of nonpathological cell populations derived from uPA-deficient animals, such as thioglycollate-elicited peritoneal macrophages or trophoblasts, appear to remain unchanged. The effect of isolated uPA deficiency on the ability of transformed cells to invade local tissues or to metastasize to distant anatomical sites, however, has not been explored.

To evaluate the significance of uPA in cancer in the primary host, we have induced cutaneous melanocytic neoplasms in uPA-deficient and wild-type mice. Primary cutaneous malignant melanomas was selected as a model because of the similarities between the histological development and clinical progression of this tumor in laboratory animals and humans. In experimental animals and humans, malignant melanoma is a biologically aggressive neoplasm with a propensity for local invasion and rapid dissemination. A vertical growth phase and subsequent penetration of underlying tissues and neighboring structures correlate with the development of a malignant phenotype in murine models (39). In addition, up to 75% of laboratory animals in which cutaneous malignant melanomas are induced develop distant metastases before succumbing to their disease, again resembling the clinical course of human melanoma (28, 31).

Melanoma can be induced reliably and reproducibly in laboratory animals. Using a single dorsal application of DMBA as an initiator, followed by repetitive applications of croton oil as a promoter, Berkelhammer et al. (40) and Berkelhammer and Oxenhandler (41) induced primary cutaneous malignant melanomas in 15.7% of 70 and 10.0% of 20 C57BL/6 mice treated, respectively. The mean latent periods from initiation of treatment to the histological confirmation of melanoma were 11 and 6.5 months, respectively.

The sequence of cellular events associated with the histological development of DMBA-induced malignant melanoma has been described (39–42). Briefly, a spectrum of cutaneous lesions becomes clinically apparent approximately 2 weeks after the initiation of treatment. Initially, small pigmented macules arise from and around an area of dorsal hyperpigmentation. These lesions progress to larger, raised nevi histologically identical to human blue nevi and consist of heavily pigmented bipolar melanocytes and lightly pigmented to amelanotic spindle cells. These lesions progress to become CBN containing denser populations of spindle and epithelioid cells interspersed with melanin. Malignant melanomas ultimately appear as dermal spindle cell neoplasms frequently associated with areas of necrosis and ulceration. Squamous cell carcinomas and spindle cell sarcomas are also induced through similar methods.

In this study, melanocytic neoplasms were induced in uPA-deficient and wild-type uPA+/+ mice using the protocol of Berkelhammer et al. (40). A spectrum of melanocytic neoplasms was induced over a period of 1 year. Primary cutaneous melanocytic lesions were characterized histologically according to cell type and progression through the dermis. A microscopic examination of the regional lymph nodes, lungs, and solid visceral organs was also performed on all animals. A statistical comparison of the progression and growth characteristics of melanocytic lesions induced in uPA−/− and uPA+/+ wild-type animals was performed to assess the clinical significance of increased uPA activity and the ability of transformed cells to disseminate in the primary host.

MATERIALS AND METHODS

Mice. Urokinase-deficient (uPA−/−) and wild-type (uPA+/+) mice (C57BL: Ola 129) used for the induction of tumors were bred from stock developed and generously donated by Dr. P. Carmeliet (Center for Molecular and Vascular Biology, Leuven, Belgium). All animals used in this experiment were maintained under veterinary supervision at the New York University Medical Center Animal Facility in accordance with the guidelines established by the NIH for the care of laboratory animals.

Induction of Tumors. Thirty-five uPA-deficient and 35 wild-type mice were treated using the protocol described by Berkelhammer et al. (40) for the induction of primary cutaneous melanocytic neoplasms. Briefly, a single application of 50 µl 0.4% DMBA (Sigma Chemical Co., St. Louis, MO) in acetone was applied to the denuded dorsal regions of 4-day-old mice. This was followed 10 days later by twice weekly applications of 25 µl 2.5% croton oil (Sigma) in DMSO until a predetermined time point or until the animals became moribund or debilitated. All animals were examined on a daily basis, and the development of pigmented lesions and other neoplasms was recorded.

After a minimum of 3.5 months of chemical promotion, 7 of a total of 35 mice of each strain (uPA−/− and uPA+/+) were selected randomly and sacrificed by cervical dislocation at 60-day intervals for a period up to 1 year. Each animal was randomly assigned an identification number, allowing for an unbiased postmortem examination. Prior to necropsy, animals were placed in a buffered 10% formalin solution (Richard Allen Co., Richland, MI).

Histological Evaluation of Pigmented Lesions and Metastatic Survey. A detailed necropsy of each animal began with a complete cutaneous examination. An 8-cm2 area of the dorsal region of each animal was defined by a rectangular template, 4 cm long x 2 cm wide, carefully centered along the craniocaudal axis of the spine. The number of pigmented lesions induced in this 8-cm2 area was recorded, as was the presence of any other significant amelanotic lesions. The four largest cutaneous pigmented lesions in each animal were excised down through the underlying s.c. tissues and placed in separate tissue cassettes for further analysis. In addition, all other significant soft-tissue masses were excised for histological examination. The axillary and inguinal regions were dissected, and at least four regional lymph nodes were removed from each animal. The thoracic and abdominal cavities were explored, and the lungs, liver, spleen, kidney, adrenal glands, and any other grossly abnormal visceral organ or soft-tissue masses were dissected and placed in separate tissue cassettes. After paraffin embedding, 7-µm-thick sections were obtained serially from each fixed tissue specimen, mounted on glass slides, and stained with H&E. Each pigmented lesion was histologically characterized according to the description by Clark et al. (39) of induced melanocytic neoplasms in rodents. The radial and vertical progressions, in mm, of the four largest cutaneous melanocytic lesions induced in each animal were measured using an ocular micrometer. Dermal extension or invasion of the underlying adipose or s.c. tissues was noted.

Representative 7-µm sections of regional lymph nodes, lung, and solid visceral organs were similarly examined, and the presence of additional primary and metastatic lesions was recorded. The melanocytic nature of primary cutaneous lesions as well as the presence of metastases to the regional lymph nodes and solid visceral organs were confirmed by the immunohistochemical identification of the S-100 protein using commercially prepared polyclonal antibodies to the S-100 protein antigen (DAKO Corp., Carpinteria, CA). Heavily melanized tissue sections were counterstained with azure in an attempt to ascertain the true morphology of cells in ambiguous cases. On completion of the postmortem examination, the genotype (uPA−/− or uPA+/+) of each mouse was revealed, and a comparative analysis was performed between the two groups.

PA Assays. PA activity in primary malignant melanomas chemically induced in the wild-type group was measured in the fibrin plate assay as described (43). Briefly, samples of freshly harvested malignant melanomas were rinsed with PBS, finely minced, snap frozen in liquid nitrogen, and pulverized using a glass mortar and pestle. Cells were extracted with 0.5% Triton X-100 and clarified by microcentrifugation, and the supernatants were collected. Extracts were stored at −80°C until they were analyzed for enzymatic activity. The protein content of each sample was determined by the...
RESULTS

CBN Induced in uPA-deficient (uPA−/−) Mice Invade s.c. Tissues Despite a Reduction in Progression through the Dermis

Compared with CBN Induced in Wild-Type (uPA+/+) Mice. CBN were induced in 100% of uPA−/− and uPA+/+ animals except for the 9-month time point (Fig. 2a). The diminished vertical progression of CBN between the uPA−/− and uPA+/+ animals was statistically significant (P < 0.05) at all time intervals measured, with the exception of the 9-month time point (Fig. 2a). Quantitatively, the difference in the radial progression of CBN between the wild-type and uPA-deficient mice compared with the wild-type treatment group (Fig. 2, a and b) was statistically significant (P < 0.05, Paired t test), with the exception of the 9-month time point (Fig. 2a). The diminished vertical progression of CBN induced in the uPA−/− mice was also statistically significant (P < 0.05) at all time intervals measured, with the exception of the 5-month time point (Fig. 2b). However, despite differences in the progression of these lesions through the dermis, microscopic examination of H&E-stained fixed tissue specimens revealed that more than 98% of the CBN in both uPA−/− and uPA+/+ mice ultimately penetrates the underlying s.c. tissues (Fig. 1c).

Induction of Benign and Malignant Tumors. A variety of additional benign and malignant tumors was identified (Table 1). Bronchial adenomas resembling carcinoid tumors were noted in 11.2% (4 of 35) of wild-type treatment group (Fig. 1a). With continued chemical promotion, these nevi became more cellular (Fig. 1b) and ultimately progressed through the dermis and invaded the underlying s.c. tissues (Fig. 1c).

No significant difference was noted between the two groups in terms of the number of CBN present in a standardized 8-cm² area of promoted dorsal skin (data not shown).

Table 1 Histologic characterization of DMBA-induced lesions

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Mouse strain</th>
<th>uPA−/−</th>
<th>uPA+/+</th>
</tr>
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<tbody>
<tr>
<td>Melanocyte-derived neoplasms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBN</td>
<td>35/35 (100)</td>
<td>35/35</td>
<td></td>
</tr>
<tr>
<td>Amelanotic spindle cell tumor</td>
<td>2/35 (5.6)</td>
<td>4/35 (11.2)</td>
<td></td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>0/35 (0)</td>
<td>5/35 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Malignant nonmelanocytic tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>4/35 (11.2)</td>
<td>6/35 (17.1)</td>
<td></td>
</tr>
<tr>
<td>Malignant germ cell tumor (testis)</td>
<td>1/35 (2.8)</td>
<td>1/35 (2.8)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma (mediastinum, spleen)</td>
<td>1/35 (2.8)</td>
<td>0/35</td>
<td></td>
</tr>
<tr>
<td>Benign nonmelanocytic tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial adenoma (carcinoid)</td>
<td>4/35 (11.2)</td>
<td>5/35 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Sebaceous gland adenoma</td>
<td>3/35 (8.6)</td>
<td>1/35 (2.8)</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.

Squamous cell carcinomas were identified in 11.2% (4 of 35) uPA−/− and 17.1% (6 of 35) uPA+/+ animals. In the uPA−/− animals, none (0 of 4) of these tumors violated the underlying basement membrane, and all were classified as carcinoma in situ (Table 2). In contrast, 67% (4 of 6) of these neoplasms arising in the wild-type mice were locally destructive and invaded the s.c. tissues (P < 0.001).

A malignant germ cell tumor of the testis was discovered in one animal (2.8%) in each treatment group. Multiple pulmonary metastases were observed in the single wild-type mouse harboring the germ cell tumor. No distant metastases were noted in the uPA-deficient animal developing a similarly sized (9-mm-diameter) germ cell tumor. A poorly differentiated and locally aggressive lymphoma, widely infiltrating the mediastinum, periaortic lymph nodes, and spleen, was noted in a single uPA-deficient animal.

Malignant Melanomas Arise in Wild-Type uPA+/+ Mice but not in uPA−/− Mice. Malignant melanomas were induced in 14.3% (5 of 35) of the wild-type mice. No malignant melanomas were induced in the group of 35 identically treated uPA-deficient mice. All melanomas appeared in wild-type mice after 5–7 months of chemical promotion as rapidly growing soft-tissue tumors on the dorsal region or flank of each animal. These neoplasms frequently ulcerated and invaded deeply into the underlying tissues, penetrating the s.c. fat and musculature of the back. Two of these tumors penetrated the vertebral column. Microscopically, these tumors comprised sheets of highly pleomorphic spindle-shaped melanocytes (Fig. 3a). Mitotic figures were frequently noted (Fig. 3b). Although they appear to have originated from heavily pigmented CBN, these melanomas were predominantly amelanotic. All five tumors stained positive for the S-100 protein, a marker for cells of neuroectodermal origin, confirming histologically the diagnosis of malignant melanoma (45).

Two of 35 (5.6%) animals in the uPA-deficient group and 3 of 35 (8.6%) of wild-type mice developed amelanotic spindle cell tumors, described elsewhere as "melanoma without intraslesional transformation" (39, 41). These lesions comprised a uniform population of large, amelanotic spindle cells. No evidence of nuclear atypia, cellular pleomorphism, or mitotic figures was noted. As such, these lesions are not classified as malignant tumors by traditional histological criteria but are thought to represent an intermediate phase in the transformation of melanocytes into melanoma cells (39). With continued chemical promotion, it is conceivable that some of these lesions would ultimately give rise to malignant melanomas.

Tissue-Type PA Is the Predominant PA in Melanomas Induced in Wild-Type (uPA+/+) Mice. Because these tumors were extremely aggressive locally, and PAs have been correlated with melanoma invasiveness, the PA activity of the melanomas induced in the wild-type (uPA+/+) mice was analyzed in the fibrin plate assay. Plasminogen-dependent enzymatic activity (100–200 mPU/mg protein) was detected in extracts of minced tissue derived from melanomas induced in three wild-type mice (Fig. 4). The addition of amiloride to duplicate wells failed to inhibit significantly the plasminogen-dependent enzymatic activity, indicating that tPA is the predominant PA in these tumors. In the absence of plasminogen, significant enzymatic activity was not detected (data not shown).

Malignant Melanomas and CBN Do Not Metastasize to the Regional Lymph Nodes or Distant Anatomical Sites. Large pigmented cells were noted in more than 95% of the more than 280 regional lymph nodes dissected from both uPA-deficient and wild-type mice. These cells were heavily laden with melanin and most frequently observed in the subcapsular region of the regional node (Fig. 3c). To determine whether these cells represent metastatic melanoma or CBN, immunohistochemical staining for the S-100 neoplasms, such as hemangiomas, sebaceous gland adenomas, and squamous papillomas, were also detected in both groups of animals.
protein was performed on fixed tissue specimens of regional lymph nodes containing populations of these large, pigmented cells. None of the regional lymph nodes tested stained positive for the S-100 protein. The absence of the S-100 epitope indicates that these cells are not neuroectodermally derived (45) and, therefore, do not represent metastases from melanocytic neoplasms such as CBN or malignant melanoma, which stain positive for S-100 (data not shown). These cells are most probably melanin-laden macrophages. Further evidence of the nonmelanocytic, nonmetastatic nature of these melanin-containing cells is the fact that they were
Tumors were initiated in 35 uPA-deficient (●) and 35 wild-type (○) mice with DMBA and exception of the first time interval (5). b, vertical progression of induced CBN over time.

The four largest CBN induced in each animal were harvested and embedded in paraffin. Groups and sacrificed at 60-day intervals for a period of up to 1 year. After formalin fixation, CBN were mounted on glass slides and stained with H&E. Vertical progression was assessed microscopically by measuring the Breslow thickness of each lesion. Each datapoint represents a minimum of 3 months of chemical promotion, 7 animals were selected randomly from each group and sacrificed at 60-day intervals for a period of up to 1 year. After formalin fixation, the four largest CBN induced in each animal were harvested and embedded in paraffin. Representative sections (7 μm) of each CBN were mounted on glass slides and stained with H&E. The radial progression of each lesion was assessed microscopically by measuring the radial diameter in mm. Each data point represents the mean diameter in millimeters of 28 CBN (4 CBN from 7 mice of each strain per time point). Bars, SD. Time is measured in months of chemical promotion. The difference in the radial progression of CBN between uPA—/— and uPA+/+ mice is statistically significant (P < 0.05, paired t test) at all points, with the exception of the second time interval (*).

frequently present in lymph nodes harvested from untreated, tumor-free mice of both strains.

Gross and microscopic examination of the solid visceral organs failed to reveal metastatic melanocyte-derived cells in any animal from either treatment group, including the five uPA+/+ animals that developed large (10–15-mm) and locally invasive malignant melanomas.

**DISCUSSION**

In this investigation, we attempted to study the contribution of the PAs to the malignant phenotype through the chemical induction of tumors in a newly developed uPA-deficient strain of mouse (38). Primary lesions were induced concurrently in uPA—/— and wild-type uPA+/+ mice and permitted to progress and disseminate over time. By allowing these tumors an opportunity to proceed through the entire sequence of events required for tumorogenesis in vivo, we sought to assess the biological relevance of enhanced uPA activity in tumor invasion and metastasis.

We found that CBN arise in the majority of animals exposed to DMBA and repetitive applications of croton oil. CBN, whether they are induced experimentally in laboratory animals or arise spontaneously in man, do not demonstrate evidence of cellular atypia, nuclear pleomorphism, or mitotic figures and, therefore, are not classified as malignant neoplasms. However, these histologically benign lesions have been reported to progress through the dermis, invade underlying s.c. tissues, and metastasize to the regional lymph nodes (39–41). In addition, malignant melanoma has been noted to arise from preexisting CBN (46).

Important differences exist between experimentally induced melanocytic lesions in laboratory animals and spontaneously arising melanocytic neoplasia in humans. Although some human cutaneous malignant melanomas originate from a benign precursor lesion within a preexisting collection of intradermal melanocytes, the majority of these tumors arise de novo in normally pigmented skin at the dermoepidermal junction (47). Experimentally induced malignant melanoma in rodents, however, arises from a proliferation of melanocytes situated in the lower papillary dermis.

Despite a reduction in the progression of CBN induced in the uPA—/— mice when compared with the uPA+/+ animals, more than 95% of all CBN examined in both groups of mice invaded the s.c. tissues. The observation that the majority of CBN induced in uPA—/— mice progress through the dermis and infiltrate the s.c. tissues indicates that uPA activity is not essential for the invasive capability of these lesions. To determine whether invasiveness is associated with enhanced PA activity, CBN and randomly selected samples of normal skin devoid of nevi from uPA—/— mice were analyzed for PA activity in the fibrin plate assay. Plasminogen-dependent enzymatic activity (100–500 μM/mg protein) was detected in all extracts of uPA—/— CBN and normal nonpigmented skin analyzed (data not shown).

**Fig. 2. Progression of CBN induced in uPA-deficient and wild-type mice over time. a, radial progression of induced CBN over time. Tumors were initiated in 35 uPA-deficient (●) and 35 wild-type (○) mice with DMBA and promoted with croton oil twice a week. After a minimum of 3 months of chemical promotion, 7 animals were selected randomly from each group and sacrificed at 60-day intervals for a period of up to 1 year. After formalin fixation, the four largest CBN induced in each animal were harvested and embedded in paraffin. Representative sections (7 μm) of each CBN were mounted on glass slides and stained with H&E. The radial progression of each lesion was assessed microscopically by measuring the radial diameter in mm. Each data point represents the mean diameter in millimeters of 28 CBN (4 CBN from 7 mice of each strain per time point). Bars, SD. Time is measured in months of chemical promotion. The difference in the radial progression of CBN between uPA—/— and uPA+/+ mice is statistically significant (P < 0.05, paired t test) at all points, with the exception of the second time interval (*).

**b,** vertical progression of induced CBN over time. Tumors were initiated in 35 uPA-deficient (●) and 35 wild-type (○) mice with DMBA and then promoted with croton oil twice a week. After a minimum of 3 months of chemical promotion, 7 animals were selected randomly from each group and sacrificed at 60-day intervals for a period of up to 1 year. After formalin fixation, the four largest CBN induced in each animal were harvested and embedded in paraffin. Representative sections (7 μm) of each CBN were mounted on glass slides and stained with H&E. Vertical progression was assessed microscopically by measuring the Breslow thickness of each lesion. Each data point represents the mean thickness in mm of 28 CBN (4 CBN from 7 mice of each strain per time point). Bars, SD. Time is measured in months of chemical promotion. The difference in the vertical progression of CBN between uPA—/— and uPA+/+ mice is statistically significant (P < 0.05, paired t test) at all points, with the exception of the second time interval (*).

**Table 2** Frequency of local invasion of induced malignant neoplasms

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>uPA+/+</th>
<th>uPA—/—</th>
</tr>
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<tbody>
<tr>
<td>Malignant melanoma</td>
<td>n = 5/35</td>
<td>n = 0/35</td>
</tr>
<tr>
<td>Local invasion</td>
<td>5/5 (100%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>n = 6/35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>n = 4/35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Local invasion</td>
<td>6/6 (100%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td>Malignant germ cell tumor</td>
<td>n = 1/35</td>
<td>n = 1/35</td>
</tr>
<tr>
<td>Local invasion</td>
<td>1/1 (100%)</td>
<td>0/1 (0%)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Numbers in parentheses are percentages.

<sup>b</sup> P < 0.001.
Fig. 3. Malignant melanoma evolving in a cellular blue nevus. a, amelanotic spindle cell melanoma (mm) almost completely replacing the dermis and underlying s.c. tissues. Residual CBN (arrows) still evident at center of field. H&E stain, ×100. Bar, 110 μm. b, high-power view of chemically induced malignant melanoma. Note mitotic figure (arrow). H&E stain, ×500. Bar, 11 μm. c, axillary lymph node showing collections of pigment-laden macrophages (melanophages) within the lymph node parenchyma (arrow). H&E stain, ×250. Bar, 55 μm.

However, we could not demonstrate that CBN elaborate more PA than normal skin. The application of more sensitive methods, such as immunohistochemistry or in situ hybridization, may be required to resolve the question of whether CBN possess enhanced levels of tPA.

We were unable to confirm histologically the presence of regional lymph node metastases arising from CBN in our evaluation of more than 280 axillary and inguinal lymph nodes resected from the 70 mice (four or more lymph nodes per mouse) in either treatment group. This may be because in our investigation, representative fixed tissue specimens of lymph nodes demonstrating evidence of possible metastases...
The major contribution of uPA to the malignant phenotype is presumed to be an enhancement of the invasive capability of transformed cells. Plasmin, however, can act on a broad spectrum of substrates. Consequently, uPA-mediated plasmin generation, or its absence, may result in a variety of biologically significant effects. A recent study of tumorogenesis in a melanoma-susceptible transgenic mouse model suggests that enhanced uPA production during melanoma progression is associated with angiogenesis rather than tumor invasiveness (54). The malignant melanomas induced in the wild-type uPA+/+ mice are extremely vascular tumors (Fig. 3a). However, because melanoma did not arise in the uPA-deficient animals, we cannot compare the angiogenesis associated with melanoma induction in the two groups (uPA−/− and uPA+/+) of mice. We have attempted to measure the angiogenesis associated with the development of CBN in both groups of mice by quantifying the number of capillaries within the dermis adjacent to each induced lesion. An analysis was performed on many CBN by an observer blinded to the strain of origin (uPA−/− and uPA+/+) of each lesion but was inconclusive (data not shown).

We hypothesize that the deletion of the uPA gene may result in alterations in the cutaneous microenvironment. The biological activities of at least three growth factors, bFGF (55), transforming growth factor β (56), and hepatocyte growth factor (57), may be mediated by plasmin. The survival and proliferation of human melanocytes in vitro is dependent on the elaboration of bFGF by keratinocytes (58). In vivo, proliferating keratinocytes may produce bFGF, which supports the growth of neighboring melanocytes (55). However, bFGF is tightly bound to the ECM. Increased production of plasmin may release soluble complexes of heparin sulfate-bFGF that may represent the active form of bFGF (59). In the absence of uPA, sufficient amounts of bFGF may be unavailable to support the proliferation of dermal melanocytes and nevus cells undergoing PMA promotion, decreasing the probability of complete cellular transformation to malignant melanoma. In addition, bFGF is an angiogenic factor (60). By decreasing the availability of bFGF, uPA deficiency may interfere with neovascularization and result in a further disruption of tumorigenesis. Unfortunately, differences in bFGF localization within the dermis between the uPA-deficient and wild-type mice that may be biologically significant are likely to be undetected using immunohistochemical methods. To investigate our hypothesis that the mechanism underlying the failure of melanoma induction in the uPA-deficient animals may be a reduction in bFGF mobilization, we will be inducing melanocytic neoplasms in wild-type, bFGF−/−, and uPA−/−-bFGF−/− mice.

In this investigation, we sought to assess the contribution of uPA to the malignant phenotype. Although the ability of melanocytic lesions within the dermis to progress vertically and infiltrate underlying tissues was independent of uPA activity, melanoma induction was blocked. This suggests that uPA activity may significantly influence the cutaneous microenvironment. We hypothesize that a biologically significant effect of enhanced uPA-mediated plasmin generation may be the liberation of...
essential cytokine growth factors. In the absence of uPA, the local environment becomes unfavorable to tumorigenesis and/or wound healing.

REFERENCES


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