Melanocyte Expression of Survivin Promotes Development and Metastasis of UV-Induced Melanoma in HGF-Transgenic Mice

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Abstract

We previously found the apoptosis inhibitor Survivin to be expressed in melanocytic nevi and melanoma but not in normal melanocytes. To investigate the role of Survivin in melanoma development and progression, we examined the consequences of forced Survivin expression in melanocytes in vivo. Transgenic (Tg) mouse lines (Det-Survivin) were generated with melanocyte-specific expression of Survivin, and melanocytes grown from Det-Survivin mice expressed Survivin. Det-Survivin melanocytes exhibited decreased susceptibility to UV-induced apoptosis but no difference in proliferative capacity compared with melanocytes derived from non-Tg littermates. Induction of nevi in Det-Survivin and non-Tg mice by topical application of 7,12-dimethylbenz(a)anthracene did not reveal significant differences in lesion onset (median, 10 weeks) or density (4 lesions per mouse after 15 weeks). Det-Survivin mice were bred with melanoma-prone MH19/HGF-B6 Tg mice, and all progeny expressing either individual, neither, or both (Survivin/HGF) transgenes were UV-treated as neonates and then monitored for 43 weeks. Melanocytes in neonatal Survivin+/HGF+ mouse skin were less susceptible to UV-induced apoptosis than those from Survivin−/HGF− mice. Onset of melanocytic tumors was earlier (median, 18 versus 24 weeks; P = 0.01, log-rank test), and overall tumor density was greater (7.7 versus 5.2 tumors per mouse; P = 0.04) in Survivin+/HGF+ compared with Survivin−/HGF− mice. Strikingly, melanomas arising in Survivin+/HGF+ mice showed a greater tendency for lymph node (35% versus 0%; P = 0.04) and lung (53% versus 22%) metastasis and lower rates of spontaneous apoptosis than those in Survivin−/HGF− mice. These studies show a role for Survivin in promoting both early and late events of UV-induced melanoma development in vivo. [Cancer Res 2007;67(11):5172–8]

Introduction

Although the incidence of other cancers seems to be stabilizing, melanoma is increasingly more common with ~60,000 new cases expected in the United States in 2005 (1). Cutaneous melanoma arises in the skin from isolated melanocytes or from melanocytic neoplasms, termed nevi, and can be influenced by multiple genetic and environmental factors. Various genetic models based on melanocyte expression of oncogenes (i.e., Ras) or growth factors [i.e., hepatocyte growth factor (HGF)], or deficiency of tumor suppressors (i.e., Ink4a/Arf), show increased susceptibility to melanoma that may be enhanced by exposure to topical carcinogens or UV radiation (2). Patients with advanced melanoma respond poorly to conventional therapies, largely due to acquired apoptosis resistance in tumor cells (3). Although multiple mechanisms underlying apoptosis resistance in melanoma have been identified (3), including dysfunctional death receptor signaling (4), transcriptional repression of proapoptotic regulators (5), and up-regulation of apoptotic inhibitors (6, 7), the role of apoptosis inhibition in melanoma development is not well understood.

Survivin is an inhibitor of apoptosis (8) widely expressed in cancer and seems to be an attractive target for cancer therapy (9). Survivin has been implicated in mitotic control (10) and cytokinesis (11) and more recently shown to be an important component of a complex regulating chromosomes alignment during mitosis (12). However, transgenic expression of Survivin in skin keratinocytes was not associated with increased keratinocyte proliferation or epidermal hyperplasia (13), and overexpression of Survivin in HeLa cells did not affect cell cycle progression (14).

These experiments, and more recent work examining Survivin promoter activity in tumors (15), suggest that Survivin expression in tumors may be more important for inhibition of apoptosis than driving proliferation. Like other inhibitors of apoptosis, which function as caspase inhibitors (8), Survivin can protect cells against apoptosis induced by multiple stimuli (16). The mechanism(s) of apoptotic protection by Survivin, however, may vary with cell type and apoptotic stimulus. For example, its capacity to inhibit caspase-3 has not been observed consistently (17), and whereas Survivin interacts with caspase-9 and prevents its activation in HeLa cells (18), it seems to protect against caspase-independent apoptosis in other cell types (19, 20).

Our previous studies on Survivin suggested that it may be particularly important in melanoma development. First, unlike other apoptotic inhibitors, Survivin is not expressed in normal melanocytes (21). By contrast, Survivin was detected in >85% of localized melanomas and all metastatic melanomas examined (7). Its expression in nevi (7, 22), on the other hand, suggested that it may also be a marker of neoplastic melanocytes and play an important role in melanoma initiation. We and others have shown that blocking Survivin in melanoma cells is sufficient for induction of apoptosis (7, 23), sensitization to chemotherapy (23, 24), and inhibition of melanoma tumor growth in vivo (23, 24). Finally, in patients with metastatic melanoma, Survivin expression in tumor cells correlates with poor survival (25, 26).

To investigate the role of Survivin in nevus/melanoma formation and metastasis in vivo, we generated a transgenic mouse with melanocyte-specific expression of Survivin. We found
that constitutive Survivin expression in melanocytes was associated with increased formation and metastasis of UV-induced tumors, suggesting a role for Survivin in promoting both early and late events in melanoma development.

Materials and Methods

Dct-Survivin transgenic mice. The Survivin transgene was constructed using pTRP2-pA/StI (27) containing 1.7 kb of the Dct (dopachrome tautomerase, formerly known as TRP2) promoter and 0.8 kb of SV40 polyadenylation sequences. This expression vector was kindly provided by Dr. Paul Overbeek (Baylor College of Medicine) and has been used previously to confer melanocyte-specific expression in mice (27). A cDNA encoding the 140 amino acids and stop codon of mouse Survivin (28) was cloned into the XbaI and ClnI sites of pTRP2-pA/StI between the Dct and SV40 sequences, respectively, and the 3-kb Dct/Survivin/SV40 fusion product was released with KpnI and NotI. This fragment was sequentially purified by agarose gel electrophoresis, electroelution, and ion-exchange chromatography and dialyzed against injection buffer [10 mM L-Tris, 0.1 mM L EDTA (pH 7.4)] as described previously (13). Correct orientation and absence of mutations in the Survivin sequence were confirmed by routine DNA sequencing. Finally, the fragment was diluted in injection buffer (1 ng per μL) and microinjected into F1 (C57BL/6 × CBA) embryos, which were implanted into F1 (C57BL/6 × CBA) pseudopenetrant females.

Three mice (of 23 potential founders screened) were transgenic (Tg) by tail DNA genotyping, and all transmitted the transgene to offspring in Mendelian fashion. One founder (#15) did not express the transgene, whereas the two remaining founders (designated #5 and #21) showed comparable expression levels by in situ hybridization (see below). Separate lines were established from these two founders by mating with C3H/HeN mice (Charles River Laboratories). Animals from second and third backcrosses were further propagated by repeatedly mating Tg animals with non-Tg littermates.

Genotyping. Potential founders and littermates were screened for the transgene by PCR. Genomic DNA was prepared from tails using the DNeasy Tissue kit (Qiagen). PCR reactions were done using primers corresponding to Dct (5’-GTGCAAACGATCTCCTTAG-3’) and SV40 (5’-GCGAGATTGGA-TATCAAGGCA-3’) sequences, and the 0.5-kb product was visualized on ethidium bromide–stained agarose gels as described previously (13). For negative reactions, PCR was repeated using primers corresponding to diacylglycerol kinase-β (5’-CTGCAAGTGGACCACATTGGCC-3’) and 5’-CCACTGTGGAAGCCTAGGAACATC-3’) to confirm DNA integrity.

In situ hybridization. Given the paucity and predominantly follicular distribution of melanocytes in mouse skin (29), and the poor suitability of skin for the antigen retrieval procedure required for Survivin staining (7), we found that Survivin expression in Tg animals was best detected by in situ hybridization. Ten-micrometer sections were cut from paraffin tissue blocks and adhered to slides using Vectabond (Vector Laboratories). A full-length hybridization. Ten-micrometer sections were cut from paraffin tissue blocks and adhered to slides using Vectabond (Vector Laboratories). A full-length Survivin riboprobe was prepared by in vitro transcription from a T7-based plasmid using a biotin RNA-labeling kit (Roche Applied Science) as described elsewhere (30). We did not employ a sense riboprobe as a control given the possibility of reactivity with effector cell pro tease receptor-1, which is oriented in the opposite direction of the Survivin gene (31). Hybridization, washing, and detection were done with minor modifications as described previously (32). Briefly, the riboprobe was applied in 50% formamide, 2× SSC, 10% dextran sulfate, 0.01% herring sperm DNA, and 0.02% SDS at 35°C for 5 h followed by successive washing in 2× SSC overnight, 50% formamide/1× SSC at 55°C, and 1× SSC and TBS at room temperature. Sections were blocked in 10% normal sheep serum and 0.03% Triton X-100 and then reacted with alkaline phosphatase–conjugated anti-biotin antibody overnight at 4°C. The hybridized probe was visualized using 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazoli um substrate, and sections were counterstained with Nuclear Fast Red.

Melanocyte isolation and characterization in vitro. Skin from 1-day-old pups was excised and placed in 70% ethanol for 10 s followed by PBS, and residual s.c. tissue was dissected away. Following incubation in dispase II (25 mg/mL, Roche Applied Science) overnight at 4°C, the epidermis was lifted off and placed in 0.05% trypsin-EDTA (Invitrogen) for 10 min at 37°C with occasional vortexing. Residual tissue fragments were removed, and serum-containing medium was added; cells were pelleted and resuspended in Opti-MEM medium containing 7% FCS, 7% horse serum, 1% penicillin-streptomycin-glutamine, 0.05 μg/mL fungizone (all from Invitrogen), 50 mg/mL phosphor 12-myristate 13-acetate, 1 μmol/L sodium vanadate, and 0.2 μmol/L α-melanocyte stimulating hormone (all from Sigma). The medium was changed twice weekly, and fresh phosphor 12-myristate 13-acetate was added each week. Western analysis for Survivin was done as described previously (7). Proliferative responses were assessed by incorporation of 3H-thymidine. Cells (2.5 × 10⁶ per well) were incubated in a 96-well flat-bottomed plate with 1 μCi 3H-thymidine (Perkin-Elmer Life and Analytical Sciences) per well for 16 h and then lysed in water, and DNA was collected on A/E glass fiber filters (Gelman Sciences), and counts per minute were detected in Opti-fluor (Perkin-Elmer) in a scintillation counter. Apoptotic responses were determined by assessing nuclear morphology of cells stained with 6.5 μg per mL 4,6-diamidino-2-phenylindole, as described previously (7).

Chemical-induced nevi. Melanocytic nevi were induced using a modification of a previously described technique (33). Briefly, Dct-Survivin mice and non-Tg littermates ages 3 weeks were weaned and shaved to expose an −1.5 × 3 cm patch of dorsal skin. Four days later, 50 μg freshly prepared 7,12-dimethylbenz(a)anthracene (DMBA; Sigma) was applied topically (100 μL of 0.05% solution in acetone). An additional four applications of DMBA were applied at weekly intervals, and mice were monitored weekly for a total of 15 weeks for development and growth of pigmented lesions.

UV-induced tumors. Dct-Survivin mice were mated with HGF-Tg (BL6 MH19) mice (34), kindly provided by Glenn Merlino (National Cancer Institute). All offspring, representing four possible genotypes (Survivin+/HGF+, Survivin+/HGF−, Survivin−/HGF+, and Survivin−/HGF−), were irradiated unstrained 1 to 2 days after birth in open cages at a dose of ~4,000 J/m² (at a rate of 4 J/m²/s) using a bank of four fan-cooled unfiltered sun lamps (FS20T12-UVB, National Biological Corp.). These bulbs emit wavelengths between 250 and 420 nm (72.6% UVB, 27.4% UVA, 0.01% UVC), with peak emission at 313 nm, according to the manufacturer. Dosimetry was determined using a UVB-500C meter (National Biological Corp.). Following irradiation, mice were returned to their respective cages, weaned at 3 weeks of age, and monitored weekly thereafter for an additional 40 weeks until euthanized. Animals developing tumors ≥1 cm in diameter before 40 weeks were also euthanized.

Tissue analysis. All skin lesions ≥3 mm in diameter were excised from animals reaching experimental end points. Subcutaneous tumors were necropsied, and pieces of lung, liver, spleen, and regional (usually inguinal) lymph nodes were removed. Tissues were processed in 10% formalin and embedded in paraffin blocks. Sections were cut for routine H&E staining. Lymph node metastasis was interpreted as >50% effacement of node by pigmented cells, or definitive presence of malignant cells (resembling those of primary tumor) in the node. Lung metastasis was associated with the presence of either macroscopic dark dots on lung tissue or large collections of malignant cells seen microscopically. In some cases, sections were bleached to remove melanin before H&E staining to remove melanin, permit better visualization of cell morphology, and allow for special staining. For melanin bleaching, sections were deparaffinized, hydrated, and incubated in 0.25% potassium permanganate (Sigma) for 30 min. After washing in water, sections were treated with 5% oxalic acid (Sigma) for 5 min and then washed again in water. Some slides were stained with the Dct-specific rabbit antibody PEP8 (kindly provided by Vince Hearing, National Cancer Institute) at 1:30 dilution for 1 h at 37°C and developed with a rabbit Vectastain ABC kit (Vector Laboratories) and counterstained with hematoxylin. All slides were examined by a dermatopathologist (S.R.F.) who did not know the genotype. In some cases, fresh tissue was placed into Karnofsky’s fixative overnight and then subjected to standard processing, embedding, and sectioning for transmission electron microscopy.

Apoptosis in tissues. For measurement of apoptosis, tumor sections were stained by a terminal deoxynucleotidyl transferase–mediated nick-end labeling (TUNEL) technique incorporating fluorescein into fragmented DNA.
using the In Situ Cell Death Detection kit (Roche Applied Science) according to the manufacturer’s instructions. Slides were viewed on a fluorescent microscope, and positive cells were counted within four to five representative fields. Fields containing abundant inflammatory cells were excluded from examination. For examination of melanocyte apoptosis in vivo, deparaffinized sections were first TUNEL stained followed by staining with PEP8. After incubation with PEP8 antibody and washing with PBS, 1:200 dilution of Alexa Fluor–conjugated goat-anti-rabbit antibody (Invitrogen) in PBS was added to slides that were incubated for 1 h at 37°C. After washing with PBS, slides were viewed on a fluorescent microscope, and >300 PEP8-staining cells were scored for TUNEL positivity.

Statistics. Data derived from multiple animals or determinations were subjected to an unpaired t test using Welch’s correction (Prism, GraphPad software). The P values for some comparisons were obtained using contingency tables in Prism. P < 0.05 was considered statistically significant.

Results

Dct-Survivin mice have normal melanocyte distribution and pigmentation. To investigate Survivin function in melanocyte neoplasia and melanoma formation and progression in vivo, Tg mice were generated expressing wild-type mouse Survivin under the control of a Dct promoter. This cassette (Fig. 1A) has been used previously to direct expression of transgenes to melanocytes (27). Two independent Tg lines were generated, showing Survivin expression in hair follicles (Fig. 1B) where melanocytes in mouse skin are known to reside (29). Melanocytes were specifically identified by PEP8 staining and found in and around the hair follicles but were not increased in number (Fig. 1C) or present in the interfollicular epidermis (data not shown) of Dct-Survivin mice. Consistent with their similar skin histology, Dct-Survivin animals from each line were indistinguishable in appearance from non-Tg littermates (data not shown). Thus, constitutive Survivin expression

Figure 1. Construction and characterization of Dct-Survivin mice. A, the 0.5-kb mouse Survivin cDNA was cloned into the XbaI (X) and ClaI (C) sites of pTRP2/pA/StI between the Dct and SV40 sequences, respectively, and the 3-kb Dct/Survivin/SV40 fusion product was released with KpnI (K) and NotI (N) for microinjection. B, sections of skin from non-Tg and Dct-Survivin mice were incubated with a full-length biotinylated mouse Survivin riboprobe and then reacted with alkaline phosphatase–conjugated anti-biotin. Binding was visualized by addition of 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate (blue staining), and sections were counterstained with Nuclear Fast Red. Original magnification, ×400. C, sections of skin from adult non-Tg and Dct-Survivin mice were stained with PEP8 antibody (red) and counterstained with hematoxylin. Nonspecific staining of sebaceous glands are indicated by arrows. Original magnification, ×400.

Figure 2. Functional analysis of Dct-Survivin melanocytes. A, melanocytes were isolated from Dct-Survivin and non-Tg mice as indicated and subjected to SDS-PAGE followed by Western blotting for Survivin and actin, as assessed by incorporation of 3H-thymidine. Columns, mean of quadruplicate determinations; bars, SE. *, P = 0.57. C, melanocytes from Dct-Survivin (open column) and non-Tg mice (filled column) were treated with 480 J/m² UV and, 24 h later, assessed for apoptosis by nuclear morphology. Arrows indicate apoptotic cells.

D, UV-induced melanocyte apoptosis in vivo. Two-day-old Survivin+/HGF+ (filled column) and Survivin+/HGF mouse (open column) mice were UV irradiated (2400 J/m²) and 24 h later, dorsal skin was harvested and subjected to PEP8 and TUNEL staining. Percentages of melanocytes that were TUNEL positive are indicated. Columns, mean from four mice in each group; bars, SE. *, P = 0.02. Representative staining of melanocytes (PEP8, red) and apoptotic cells (TUNEL, green). Arrows indicate apoptotic melanocytes.
in melanocytes was not associated with aberrant melanocyte expansion or migration, or altered pigmentation in vivo.

Transgenic Survivin increases apoptosis resistance but not melanocyte proliferation. Mice isolated from Dct-Survivin mice maintained Survivin expression in culture (Fig. 2A) and were assessed for potential differences in proliferative capacity and susceptibility to apoptosis. There was not a significant difference ($P = 0.57$) in cellular proliferation between melanocytes derived from Dct-Survivin and non-Tg animals (Fig. 2B). By contrast, melanocytes from Dct-Survivin mice showed reduced susceptibility to UV-induced apoptosis compared with non-Tg melanocytes (Fig. 2C; $P = 0.02$). Thus, whereas transgenic expression of Survivin in melanocytes did not confer a proliferative advantage, it was associated with increased resistance to apoptosis.

Survivin expression does not increase susceptibility to chemical-induced nevi. To investigate whether Survivin expression predisposes melanocytes to neoplasia, Dct-Survivin mice and non-Tg littermates were treated topically with the chemical carcinogen DMBA and monitored for the appearance and growth of pigmented lesions over a 15-week period. Although standard skin carcinogenesis protocols employing the combination of topical DMBA plus phorbol 12-myristate 13-acetate yield keratinocytic neoplasms (35), application of DMBA without phorbol ester produces melanocytic neoplasms (nevus) in C57/HeN mice (33). Animals treated with DMBA developed darkly pigmented lesions, histologically characterized by intradermal collections of melanized cells (Fig. 3A). Approximately 90% of the animals developed nevi (Supplementary Table S1), with a mean time to lesion onset of ~10 weeks for both Dct-Survivin mice and non-Tg littermates (Fig. 4A; $P = 0.31$), and subset analyses of both transgenic lines of Dct-Survivin mice yielded similar results (data not shown). Untreated mice observed for up to 9 months did not spontaneously develop nevi. There were not significant differences in lesion density ($P = 0.89$), mean lesion size ($P = 0.41$), or development of large lesions ($P = 0.78$) between Dct-Survivin mice and non-Tg littermates (Supplementary Table S1). Thus, constitutive expression of Survivin in melanocytes did not increase susceptibility to chemical-induced nevi.

Reduced UV-induced melanocyte apoptosis and enhanced tumor formation in Dct-Survivin mice. To examine the effect of Survivin expression on melanocytic tumor formation, we placed the Dct-Survivin transgene on a melanoma-prone genetic background. Dct-Survivin animals were crossed with MH19/HGF-B6 Tg mice, which express an HGF transgene previously shown to predispose to melanoma development following a single neonatal UV exposure (36). Newborn litters from these crosses consisting of animal expressing either individual, neither, or both (Survivin/HGF) transgenes were UV irradiated and then monitored for 43 weeks for the development of melanocytic skin tumors. In a separate experiment, Survivin+/HGF+ and Survivin+/HGF− neognates were UV irradiated and the skin was examined for induction of melanocyte apoptosis in vivo by two-color fluorescence microscopy for PEP8 and TUNEL. As shown in Fig. 2D, melanocytes in neonatal Survivin+/HGF+ mouse skin were significantly ($P = 0.002$) less susceptible to UV-induced apoptosis than those from Survivin+/HGF− mice. Animals not carrying an HGF transgene (Survivin+/HGF−, Survivin+/HGF−) remained tumor-free for the duration of the observation period, whereas in singly Tg HGF (Survivin+/HGF+) mice, tumors developed with a mean onset of 24 weeks (Fig. 4B). In compound Tg Survivin+/HGF+ mice, tumor development was accelerated with a significantly earlier tumor onset (median, 18 versus 24 weeks; $P = 0.01$, log-rank test) compared with Survivin+/HGF+ mice (Fig. 4B). Subset analyses of mice derived from separate Dct-Survivin founder lines showed similar results (data not shown). Melanocytic tumors were clearly visible on the skin and histologically revealed infiltration of large melanized cells (Fig. 3B). Tumor density was significantly greater (7.7 versus 5.2 lesions per mouse; $P = 0.04$) in Survivin+/HGF+ compared with Survivin+/HGF− mice (Table 1). Thus, melanocytes in Survivin+/HGF+ mice showed increased resistance to UV-induced apoptosis and a predisposition towards UV-induced melanocytic tumor formation.

Melanoma metastasis in Dct-Survivin mice. A subset of animals with larger (>5 mm) tumors was necropsied, and lymph nodes, liver, spleen, and lung were examined both grossly and microscopically for metastasis. Metastasis to liver or spleen was
not seen in any of the animals examined. However, whereas none of the lymph nodes examined from Survivin+/HGF− mice (0 of 10) showed metastasis, lymph node metastasis was observed in 9 of 26 (35%; \( P = 0.04 \)) Survivin+/HGF+ mice (Fig. 3C and Fig. 5A). Melanocytic identity of these infiltrating cells was confirmed by PEP8 staining and visualization of melanosomal structures by electron microscopy (Fig. 3B, insets). Significantly higher rates of lung metastasis (53% versus 22%; \( P = 0.14 \)) were also seen in Survivin+/HGF+ mice compared with Survivin+/HGF− animals (Fig. 3D and Fig. 5A). The higher rates of lung metastasis compared with lymph node metastasis, seen in both Survivin+/HGF+ and non-Tg mice, may reflect hematogenous tumor spread in some cases. Indeed, prominent involvement of blood vessels (Supplementary Fig. S1A) as well as lymphatics (Supplementary Fig. S1B) by melanoma cells was frequently noted. A group of melanomas from Survivin+/HGF+ mice that metastasized was compared with a group (matched for depth: 5.4 mm ± 1.6 versus 4.3 mm ± 1.6; \( P = 0.64 \)) of melanomas from Survivin+/HGF− mice that did not metastasize for tumoral involvement of blood vessels, lymphatics, and muscle. Although tumoral involvement of these structures was seen in melanomas arising in Survivin+/HGF+ animals, it was a less common finding than in tumors from Survivin+/HGF+ mice (Fig. 5B). Finally, melanomas that metastasized in Survivin+/HGF+ mice contained a lower percentage of apoptotic (TUNEL staining) cells than matched non-metastasizing tumors from Survivin+/HGF− animals (Fig. 5C), consistent with the increased apoptosis resistance seen in melanocytes from Dct-Survivin mice (Fig. 2C and D). Thus, melanomas arising in Survivin+/HGF+ mice exhibited lower rates of spontaneous apoptosis that was associated with a greater tendency for tissue invasion and ultimately metastasis.

### Figure 4.
Survivin expression does not affect development of DMBA-induced nevi but accelerates UV-induced melanoma. A. Dct-Survivin (n = 31, □) and non-Tg (n = 37, ●) mice were treated with DMBA and monitored for 15 wks. Survival curves depicting percentage of animals without melanocytic lesions are charted. \( P \) for comparison of non-Tg and Dct-Survivin mice is 0.31. B. Survivin+/HGF− (n = 36, □), Survivin−/HGF+ (n = 29, ○), Survivin+/HGF+ (n = 34, □), and Survivin+/HGF− (n = 42, ○) mice were UV irradiated and monitored for 43 wks. Survival curves depicting percentage of animals without melanocytic tumors are charted. A total of 11 animals in the original groups died before 43 wks without tumors (Survivin+/HGF−, n = 1; Survivin−/HGF+, n = 5; Survivin+/HGF+, n = 5) and were not included in these analyses. Dashed lines indicate median time to first tumor formation. \( P \) for comparison of Survivin+/HGF− and Survivin+/HGF+ groups is 0.01.

### Discussion
Although previous studies have established Survivin as an important viability factor in melanoma cells (7, 23, 24) and correlated its expression in melanoma tumors with poor clinical outcome (25, 26), its role in melanocyte transformation has not been characterized in vivo. Thus, the importance of Survivin expression at particular stages of melanoma development and progression is unknown. In this study, we engineered a mouse with melanocyte-specific expression of Survivin that allowed us to investigate its potential role in normal melanocyte function (pigmentation), melanocyte neoplasia (nevus formation), and melanoma development and metastasis.

Although Survivin is potentially involved in both mitotic progression and apoptosis inhibition in malignant cells (37), our previous studies in K14-Survivin mice (with keratinocyte-specific expression of Survivin) revealed reduced susceptibility of keratinocytes to UV-induced apoptosis but no effect on keratinocyte proliferation in vivo (13). In other studies, we found that forced expression of Survivin in human melanocytes in vitro conferred protection against UV-induced apoptosis, reducing both caspase

### Table 1. Summary of UV-induced tumors

<table>
<thead>
<tr>
<th>Survival &amp; HGF</th>
<th>tumor* (%)</th>
<th>Total lesion number</th>
<th>Lesion density*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivin+/HGF+</td>
<td>31/34 (91%)</td>
<td>163</td>
<td>5.2 ± 0.83</td>
</tr>
<tr>
<td>Survivin+/HGF−</td>
<td>41/42 (98%)</td>
<td>315</td>
<td>7.7 ± 1.1</td>
</tr>
</tbody>
</table>

* Fraction of mice with at least one tumor at end point of 43 wks. Eleven animals that died before 43 wks without tumors (see Fig. 4B legend) are not included.

† Mean ± SE number of tumors per animal with tumors (\( P = 0.04 \)).
activation and mitochondrial AIF release (38). Here, constitutive Survivin expression in melanocytes did not cause melanocyte hyperplasia, or affect melanocyte localization or melanin production, as indicated by normal histology, PEP8 staining, and pigmentation of the skin and hair in Dct-Survivin mice. Thus, despite its pro-mitotic role in malignant cells (10) and expression in benign nevi (7, 22), Survivin expression in melanocytes was not sufficient to promote their spontaneous proliferation or neoplasia (nevus formation). A lack of histologic changes in association with transgenic expression of Survivin was similarly seen in the skin of untreated K14-Survivin mice (13) as well the bladder of untreated UPII-Survivin mice (39). Although both chemical- and UV-induced skin tumors in K14-Survivin mice showed an elevated rate of malignant conversion to squamous cell carcinoma (42, 43), overall tumor formation was paradoxically reduced due to the role of apoptosis in promoting clonal expansion of premalignant keratinocytes (43). Thus, the carcinogenic effects of Survivin expression may differ with cell type and tissue compartment, depending on the consequences of modulating apoptosis at various stages in tumor development.

The mechanisms for enhanced metastatic activity of melanomas arising in Survivin Tg mice are not entirely clear. Apoptotic inhibition may promote metastasis (44), and we did observe lower rates of spontaneous apoptosis in tumors that metastasized in Survivin Tg mice compared with size-matched tumors in non-Tg mice that did not metastasize. It is also possible that melanocyte expression of Survivin in this model may promote metastasis through additional mechanisms distinct from apoptosis. Salz et al. (39) found that Survivin expression in UPII-Survivin mice triggered dysregulated expression of multiple extracellular matrix and inflammatory genes. The potential role of these molecules in tumor metastasis has not been fully investigated but may represent indirect mediators of metastasis that are downstream of Survivin expression. The capacity of Survivin to promote both early and late events in melanoma development underscores its potential value as a therapeutic target in cancer. If Survivin-expressing melanocytes in nevi are susceptible to apoptosis induced by Survivin inhibition, then targeting Survivin might also be potentially useful as a preventive strategy in patients with numerous nevi and dysplastic nevi, who are at increased risk for melanoma (45).

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**References**


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