Proliferation, apoptosis, and survivin expression in a spectrum of melanocytic nevi

**Background:** Apoptosis is important for maintenance of tissue homeostasis and often dysregulated in cutaneous neoplasms. The apoptosis inhibitor survivin is expressed in melanoma and non-melanoma skin cancers and benign keratinocytic lesions. Its expression has not been studied in melanocytic nevi.

**Objective:** We determined the expression pattern of survivin in benign melanocytic nevi in comparison to markers of proliferation and apoptosis.

**Methods:** Six cases of each of the following melanocytic nevi were retrieved from a dermatopathology archive: compound dysplastic nevus, intradermal nevus, compound nevus, neurotized intradermal nevus, and Spitz nevus. Survivin expression was evaluated by in situ hybridization. Apoptotic and proliferation indices were calculated by counting immunoreactive cells in terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling and proliferating cell nuclear antigen immunostained sections, respectively.

**Results:** All nevi, regardless of histologic type, expressed survivin. Compound melanocytic lesions expressed survivin in both epidermal and dermal compartments. The apoptotic rate was low for dysplastic, compound, and Spitz nevi, and apoptotic cells were not identified in any neurotized nevus. The proliferative index was highest for Spitz nevus, while all other nevi demonstrated rare positive cells.

**Conclusions:** Survivin is consistently expressed in benign melanocytic lesions, while apoptotic cells are rarely identified, suggesting the dysregulation of apoptotic pathways with the accumulation of cells in these neoplasms.

neurotized areas in nevi, Bcl-2 expression is much weaker or absent. With respect to IAP proteins, most are expressed in melanoma cells but have not been examined in melanocytic nevi.

Apoptotic cells can be identified in tissue samples by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) immunostaining. Few apoptotic cells are found in malignant melanoma, with increased number in Spitz nevi and the deep component of ordinary nevi. Other than these reports, little additional information is known regarding apoptosis within melanocytic lesions.

Proliferation has been studied in malignant and benign melanocytic neoplasms. Commonly used markers of cell proliferation include topoisomerase II-α, proliferating cell nuclear antigen (PCNA), and MIB1/Ki-67. Proliferation in junctional, intradermal, and compound nevi, including dysplastic nevi, ranges from only rare positive cells (<1% of total nevus cell population) to about 3% in dysplastic nevi. Spitz nevi are considerably more mitotically active than other melanocytic nevi, with reported rates of proliferation ranging from 7% to about 40%. Malignant melanomas vary in proliferative activity from 20 to 40% of lesional cells.

Survivin is an IAP family member that has been implicated as an inhibitor of apoptosis and a regulator of cell division. Unlike other IAP proteins, survivin appears to function as a regulator of mitochondrial apoptosis rather than as a caspase inhibitor. Survivin associates with the mitotic spindle in the G2/M phase of the cell cycle, and disruption results in cell division defects and apoptosis. Survivin is not found in most normal tissues but is frequently expressed in human cancers. We have previously demonstrated that survivin is absent in normal skin but is present in melanoma and non-melanoma skin cancers, benign melanocytic nevi, and benign keratinocytic neoplasms.

The goal of the present study was to determine the expression pattern of survivin in various nevus types, including atypical (dysplastic), neurotized intradermal, and Spitz nevi and to correlate expression with apoptotic and proliferation markers.

Materials and methods

Case selection

A computerized search was performed of the dermatopathology archive at the Department of Dermatology (University of Utah) between 1990 and 1998 to obtain specimens of compound dysplastic nevus, intradermal nevus, compound nevus, neurotized intradermal nevus, and Spitz nevus. The first 10–12 specimens that could be obtained of each diagnosis were examined for adequate tissue remaining in the block, and a total of six were studied. The dysplastic nevi contained both epidermal and dermal components and exhibited architectural disorder as reflected by fibroplasia of the papillary dermis and bridging of adjacent rete ridges by nests of nevomelanocytes. In all these lesions, there was cytotologic atypia of individual melanocytes characterized by mild nuclear enlargement and finely granular pigmented cytoplasm. The architectural disorder and cytotologic atypia was not severe, nor was there a pagetoid epidermal distribution or significant mitotic activity characteristic of melanoma. Of the six specimens of Spitz nevi, one displayed a junctional pattern, two were dermal, and the remaining three were compound. Three were from children (ages 2, 6, and 13) and three were from young adults (ages 24–30). All diagnoses were confirmed by a dermatopathologist (S.R.F) by review of hematoxylin and eosin-stained slides.

Immunohistochemistry

Immunohistochemical studies were carried out on formalin-fixed, paraffin-embedded 4-μm thick sections of tissue. Peroxidase-based techniques for PCNA and TUNEL were performed as described previously. For PCNA staining, a 1:100 dilution of antibody (BD Biosciences, San Diego, CA, USA) was used. Strong nuclear staining of lesional cells was scored semiquantitatively as absent (−), weak or focal (+), or strong (++). Survival expression was revealed as dark blue diffuse granular cytoplasmic staining and was scored semiquantitatively as absent (−), weak or focal (+), or strong (++).

Results

Nevomelanocyte proliferation

Firstly, nevus cell proliferation was assessed by PCNA immunohistochemical staining. The PI was calculated for the junctional and/or intradermal components of each lesion. The mean PI for each lesion type is summarized in Table 1, and representative examples of staining are shown in Figures 1A and B. The PI was...
highest for Spitz nevi (16.6% epidermal component and 11.3% dermal component), followed by dysplastic nevi (1.0% epidermal component and 0.4% dermal component). We did not observe differences in mitotic activity between Spitz lesions derived from adults and children. Compound nevi, intradermal nevi, and neurotized nevi demonstrated only rare staining of cells (compound nevi demonstrated 0% epidermal component, 0.4% dermal component; intradermal nevi 0.7%, and neurotized intradermal nevi 0.6%). In the neurotized nevi, the rare positively staining cells were within the superficial component of the dermal populace; no staining was seen in neurotized nevomelanocytes.

Nevomelanocyte apoptosis

Next, apoptotic cells were identified by TUNEL immunostaining. Only rare positive cells were seen in the epidermal and dermal components of some dysplastic, compound, and Spitz nevi (Fig. 1C). Many nevi (particularly neurotized nevi) did not appear to contain any apoptotic cells (Fig. 1F), while two of the six compound nevi contained two TUNEL-positive cells per five ×400 fields in the dermal component.

Survivin expression

Survivin expression was identified by in situ hybridization, and the results are summarized in Table 2. All nevi, regardless of histologic type, expressed detectable levels of survivin. The dysplastic, compound, and Spitz nevi expressed survivin in both the epidermal and dermal compartments. We did not observe differences in survivin expression between Spitz lesions derived from adults and children. Dysplastic nevi showed strong cytoplasmic staining in nevomelanocytes of the epidermal and dermal compartments (four of six cases). While variable staining from focal to strong was observed in compound and intradermal nevi (Fig. 1D), survivin expression was generally weaker in Spitz nevi (Fig. 1E). Survivin expression was seen in both non-neurotized (six of six cases) and neurotized nevomelanocytes (four of six cases).

Discussion

In this study, we examined the expression of the apoptotic inhibitor survivin in a spectrum of melanocytic nevi, and attempted to correlate expression with apoptotic and proliferative rates. We found that all nevi, regardless of histologic type, expressed survivin at detectable levels. Despite its previously demonstrated functional activities as an inhibitor of apoptosis and a promoter of mitotic progression, we did not observe a correlation between level of survivin expression and apoptotic or proliferative indices for any of the lesion types. In addition, despite its association with malignant phenotypes, survivin expression did not correlate with the degree of differentiation or cytologic atypia in nevomelanocytes.

The low mitotic rates of compound nevi and high proliferative rate in Spitz nevi reported here are consistent with earlier results obtained by others. Our finding of decreased survivin expression in Spitz nevi relative to compound nevi, however, is somewhat puzzling, as we would have anticipated that the increased mitotic rate and cytologic atypia of Spitzoid nevomelanocytes might be associated with increased survivin expression. Our finding of different levels of survivin expression in Spitz and normal nevi are consistent with recent studies by Bastian et al. utilizing comparative genomic hybridization demonstrating molecular differences between Spitzoid nevomelanocytes and normal nevi. The lack of correlation between PI and survivin expression in these lesions is reminiscent of our findings in melanoma and keratinocytic neoplasms, and suggests that the survivin expression in nevi may be more important for control of apoptosis rather than proliferation.

Table 1. Summary of proliferating cell nuclear antigen (PCNA) immunostaining of melanocytic nevi

<table>
<thead>
<tr>
<th>Nevus type</th>
<th>Epidermal</th>
<th>Dermal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysplastic</td>
<td>1.0%*</td>
<td>0.4%</td>
</tr>
<tr>
<td>Compound</td>
<td>0.0%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Intradermal</td>
<td>no epidermal component</td>
<td>0.7%</td>
</tr>
<tr>
<td>Neurotized intradermal</td>
<td>no epidermal component</td>
<td>0.6%†</td>
</tr>
<tr>
<td>Spitz</td>
<td>16.6%</td>
<td>11.3%</td>
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*Percent PCNA-positive cells (proliferation index).
†Non-neurotized nevus cells (PCNA staining not observed in neurotized nevus cells).

Fig. 1. Representative proliferating cell nuclear antigen (PCNA), survivin, and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) staining in nevi. A) PCNA staining of compound nevus without significant proliferative activity. B) PCNA staining of intradermal Spitz nevus cells, demonstrating a high proliferative activity. C) TUNEL staining of same Spitz nevus, showing a single apoptotic cell (arrow). D) Survivin in situ hybridization of compound nevus depicted in A), showing ++ cytoplasmic reactivity. E) Survivin in situ hybridization of Spitz nevus depicted in B), showing little to no cytoplasmic staining. F) TUNEL staining of a compound nevus demonstrating absence of staining. Original magnification for all photomicrographs was ×400.
In that study, we examined a panel of both
(4)
(2), (1) – (1), (5), (1)*
and suggested to us that
and melanoma cells led
to spontaneous apoptosis and prevented tumor growth in vivo.20 These observations were consistent with its
expression in premalignant (or precursor) keratinocytic neoplasms (actinic keratoses)22 and suggested to us that
survivin expression may be an important and early step
in the transformation from melanocyte to melanoma. In
our previous study of keratinocytic lesions, we found
survivin expression to be consistently up-regulated in
13 of 15 invasive lesions and 15 of 15 metastatic lesions.23 Inhibition of survivin in melanoma cells led
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