Expression of the *Ets-1* Proto-Oncogene in Melanocytic Lesions

Connie A. Keehn, M.D., Bruce R. Smoller, M.D., Michael B. Morgan, M.D.

Department of Pathology at the University of South Florida College of Medicine (CAK, MBM); Dermatopathology Division, University of Arkansas for Medical Sciences (BRS); and Department of Pathology, James A. Haley Veteran's Hospital (MBM)

Ets-1 oncoprotein is a transcription factor known to regulate the expression of numerous genes important in extracellular matrix remodeling and angiogenesis. Up-regulation of _Ets-1_ has been shown to be important in a variety of human malignancies and to correlate with prognosis. To our knowledge, this oncoprotein has not been examined in melanocytic lesions. A series of 10 cutaneous melanomas and 24 benign melanocytic lesions with patient records were independently examined for diagnosis confirmation and immunohistochemical expression by two dermatopathologists. The immunohistochemical expression for _Ets-1_ (Novocastra, Newcastle upon Tyne, UK) was scored by an average of the mean labeling intensity; no nuclear staining = 0, weak nuclear staining = 1, moderate = 2, and intense = 3. _Ets-1_ expression was statistically assessed by the one-way analysis of variance (ANOVA) comparing the mean labeling intensity of melanoma to benign melanocytic nevi. All of the benign melanocytic lesions exhibited negative to weak nuclear staining, with an average mean labeling intensity of 0.4. Melanoma _in situ_ exhibited moderate nuclear staining, for a mean labeling intensity of 2.0, whereas all conventional invasive melanomas exhibited moderate to strong nuclear staining, with a mean labeling intensity of 2.7. Metastatic melanoma exhibited very strong nuclear staining, with a mean labeling intensity of 3.0. Invasive desmoplastic melanoma, like melanoma _in situ_, showed moderate nuclear staining with a mean labeling intensity of 2.1. There was a trend toward more intense staining with melanoma progression. A statistically significant difference in the mean labeling intensity of _Ets-1_ was seen between invasive melanoma and benign melanocytic nevi ($P < .0001$). _Ets-1_ oncoprotein expression, however, does not distinguish among benign melanocytic lesions. Staining intensity and pattern might be a useful adjunct with histomorphology in distinguishing invasive melanoma from benign melanocytic nevi. Furthermore, _Ets-1_ expression may be an important pathogenic mechanism and predictor of aggressive biologic behavior of cutaneous melanoma, with a trend toward staining intensity increasing as Clark stage increases.

KEY WORDS: _Ets-1_, Immunohistochemistry, Melanocytic nevi, Melanoma.

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The *Ets-1* oncoprotein is a transcription factor known to regulate the expression of numerous genes important in cell proliferation and differentiation (1). It is implicated in cell migration and tumor invasion by inducing the expression of several genes encoding matrix-degrading proteases, including urokinase-type plasminogen activator, stromelysin, and collagenase (2–6). *Ets-1* also promotes angiogenesis by activating matrix metalloproteinases in endothelial cells (2, 5–7).

Up-regulation of *Ets-1* has been shown to be important in carcinogenesis and/or tumor progression in a variety of human malignancies, including carcinomas of the stomach (8, 9), pancreas (10), bile ducts (11), colon (12–14), liver (15, 16), esophagus (17), oral cavity (18–20), lung (21, 22), thyroid (23, 24), and ovary (25, 26), as well as in malignant meningiomas (27), malignant astroglial tumors (28), and angiosarcoma of the skin (29). To our knowledge, this oncoprotein has not been examined in melanocytic lesions.

Molecular markers important in the pathogenesis and prognosis of melanoma are an active area of current research. These include important downstream effectors of *Ets-1* regulation including urokinase-type plasminogen activator, stromelysin, and collagenase (30, 31). Known prognostic indica-

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Address reprint requests to: Connie A. Keehn, M.D., 15350 Amberly Drive #1714, Tampa, FL 33647; fax: 813-903-4813; e-mail: ckeehn@hsc.usf.edu.

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tors in cutaneous melanoma include the Breslow microstage, followed by the Clark level of invasion, growth phase, tumor mitotic rate, the presence of host tumor-infiltrating lymphocytes within the vertical growth phase, presence or absence of histologic regression, and ulceration. However, these indicators cannot precisely predict the clinical course of this disease for the individual patient. New molecular prognostic markers may be useful to provide for more accurate diagnosis and prognosis. In addition, such markers may help improve our understanding of the biology of melanoma and potentially offer new targets for novel treatment strategies. We studied a series of benign and malignant melanocytic lesions for the immunohistochemical expression of *Ets-1* in order to discern a possible difference in expression between benign and malignant lesions and to evaluate the relationship between intensity of staining and increasing tumor depth in melanoma.

MATERIALS AND METHODS

Cases and Tissues

We studied a series of 10 cutaneous melanomas and 24 benign melanocytic lesions. The cutaneous melanomas consisted of 2 melanomas *in situ*, 2 Clark Stage II melanomas, 1 Clark Stage III melanoma, 1 Clark Stage IV melanoma, 2 metastatic melanomas, and 2 desmoplastic melanomas. The benign melanocytic lesions consisted of 5 Spitz compound nevi, 6 dysplastic common nevi with moderate atypia, 6 common blue nevi, 4 intradermal nevi, and 3 congenital compound nevi. All cases along with patient records were independently examined for diagnosis confirmation by two dermatopathologists (MM, BS).

Immunohistochemistry

Formalin-fixed and paraffin-embedded tissues were cut into 3-μm sections and dried overnight at room temperature. Sections were deparaffinized in xylene and rehydrated through descending grades of ethanol to deionized water. High-temperature antigen unmasking was performed in a microwave using 10 mM citrate buffer, pH 6.0. Slides were cooled, washed in deionized water, and placed in TBS/Tween for 5 minutes. Deparaffinized sections were stained on the DAKO Autostainer using the DAKO LSAB+ Peroxidase detection kit (DAKO Corporation, Carpinteria, CA). All incubations were performed at room temperature (25° C). Endogenous peroxidase was blocked with 3% aqueous hydrogen peroxide, and endogenous biotin was blocked with the Vector Avidin/Biotin blocking kit (Vector Laboratories, Burlingame, CA). Sections were preincubated with DAKO Protein Block (surefire) to prevent nonspecific binding. A mouse monoclonal antibody against human *Ets-1* (prokaryotic recombinant protein corresponding to amino acids 226 to 454 of the human *Ets-1* oncoprotein; Novocastra Laboratories Ltd, Newcastle upon Tyne, UK via Vector Laboratories, Burlingame, CA) was used at a dilution of 1:50 for 60 minutes. The slides were sequentially incubated with biotinylated anti-mouse, -rabbit, -goat IgG, and streptavidin peroxidase and reaction products were visualized with the chromogenic substrate, NovaRed (Vector Laboratories, Burlingame, CA). Slides were removed from the Autostainer, counterstained with modified Mayer's hematoxylin, dehydrated, cleared, and mounted with resinous mounting medium. Mouse IgG was used as the negative control serum. Tonsil served as the positive control tissue. Lymphocytes served as the internal positive control. Analysis of the immunohistochemical staining was performed by two dermatopathologists (MM, BS). In each case, 100 consecutive melanocytic nuclei were evaluated for nuclear staining intensity at 400× magnification in nonoverlapping fields. An average score was reported for each slide. *Ets-1* expression was scored by an average of the mean labeling intensity; no nuclear staining of tumor cells = 0, weak nuclear staining = 1, moderate nuclear staining = 2, and intense nuclear staining = 3.

Statistical Analysis

Ets-1 expression was statistically assessed by the one-way analysis of variance (ANOVA), comparing the mean labeling intensity of melanoma to benign melanocytic nevi.

RESULTS

The positive control tissue (tonsil) showed intense nuclear staining of lymphocytes. Slides incubated with negative control serum exhibited no tonsillar cellular staining. The skin specimens showed weak to moderate nuclear staining of the basilar keratinocytes, with weak nuclear staining of the granular layer. Intraepidermal melanocytes showed no nuclear staining. Within the dermis, endothelial cells showed moderate nuclear staining. Dermal lymphocytes exhibited intense nuclear staining. Dermal fibrocytes and subcutaneous fat adipocytes were negative. Weak background cytoplasmic staining was seen within keratinocytes.

Immunopositivity of *Ets-1* in Melanoma and Benign Melanocytic Lesions

All of the benign melanocytic lesions studied (Spitz compound nevi, dysplastic common nevi,

common blue nevi, intradermal nevi, and congenital compound nevi) exhibited negative to weak nuclear staining, for an average mean labeling intensity of 0.4. Melanoma *in situ* exhibited moderate nuclear staining, for an average mean labeling intensity of 2.0. All conventional invasive melanomas exhibited moderate to strong nuclear staining, with an average mean labeling intensity of 2.7. Metastatic melanoma exhibited very strong nuclear staining, with an average mean labeling intensity of 3.0. Invasive desmoplastic melanoma, like melanoma *in situ*, showed moderate nuclear staining with an average mean labeling intensity of 2.1. All lesions exhibited diffuse staining patterns, with the intensity of staining similar throughout a given lesion. A statistically significant difference in the mean labeling intensity of *Ets-1* expression was seen between invasive melanoma and benign melanocytic nevi ($P < .0001$). *Ets-1* oncoprotein expression, however, did not distinguish among the different benign melanocytic lesions. (See Fig. 1)

Relation between *Ets-1* Expression and Clark Stage in Melanoma

There was a trend of increasing intensity of *Ets-1* expression with increasing Clark stage among conventional invasive melanomas. (See Figs. 2–6)

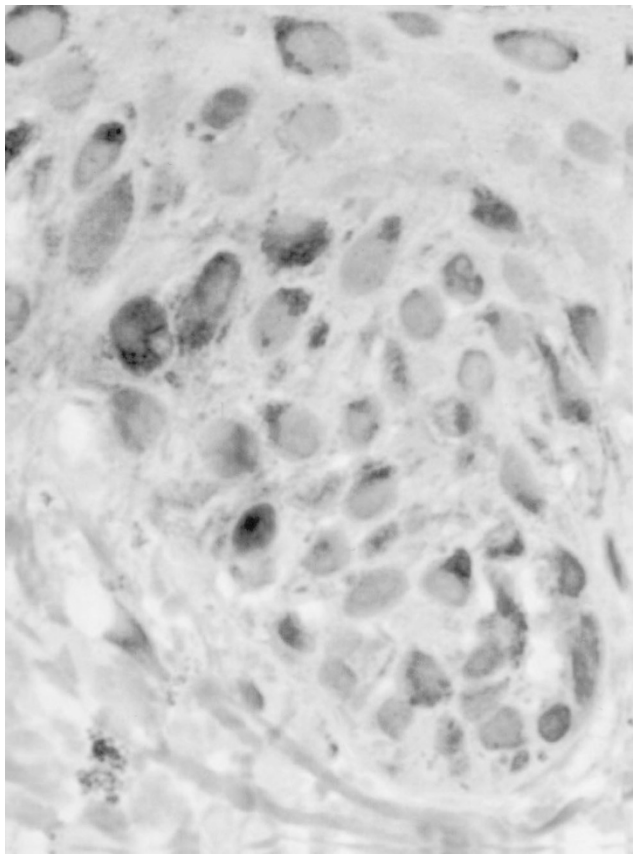

FIGURE 2. *Ets-1* expression for melanoma *in situ*.

DISCUSSION

ETS-1 is a proto-oncogene that is part of a family of proteins that play a central role in cellular differentiation, activation, and proliferation. *Ets-1* is specifically involved in lymphoid differentiation, as well as being expressed in adult lung, gut mesenchymal tissue, and bone marrow (32). It is also normally expressed within the skin and in proliferating endothelial cells. Chromosomal translocations involving the *Ets* gene are pathologically associated with some types of lymphoma and soft tissue neoplasms (33). Markedly elevated *Ets-1* expression has been demonstrated within endothelial cells in angiosarcomas but is not seen in benign endothelial proliferations such as hemangiomas and pyogenic granulomas (29). It has been suggested that *Ets-1* promotes angiogenesis by inducing expression of metalloproteinases and integrin B3 (7, 29). It has also been suggested that *Ets-1* may prevent apoptosis, but those same investigators found that endothelial cells demonstrated more apoptosis in the presence of *Ets-1* (7). It has also been shown that mutations in MMP-1 may result in additional *Ets-1* binding sites and in increased transcription of these proteins. In cell culture, melanoma cells demonstrated these mutations (34).

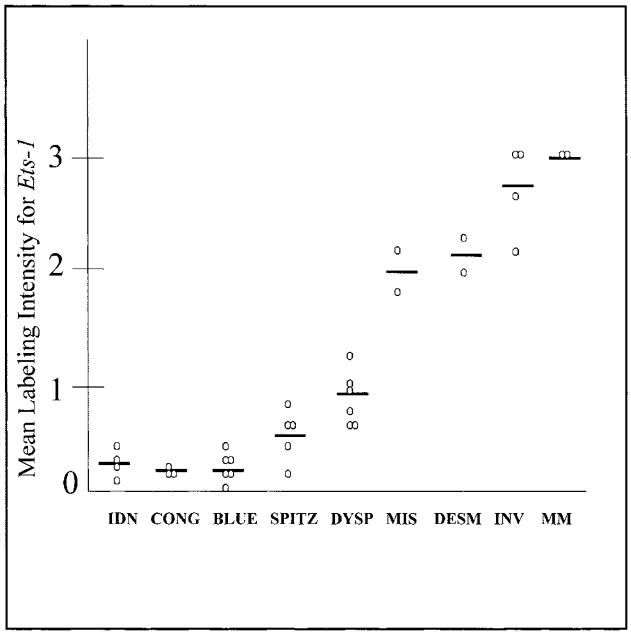

FIGURE 1. Mean labeling intensity for *Ets-1* expression in melanocytic lesions. IDN = intradermal nevi, CONG = congenital compound nevi, BLUE = common blue nevi, SPITZ = Spitz compound nevi, DYSP = dysplastic common nevi with moderate atypia, MIS = melanomas *in situ*, DESM = desmoplastic melanoma, INV = conventional invasive melanomas, MM = malignant melanomas.

In the present study, we demonstrated strong nuclear staining with *Ets-1* in malignant melanocytes but negative to weak nuclear staining in a variety of benign melanocytic lesions. Further, the intensity of expression of *Ets-1* increased with in-

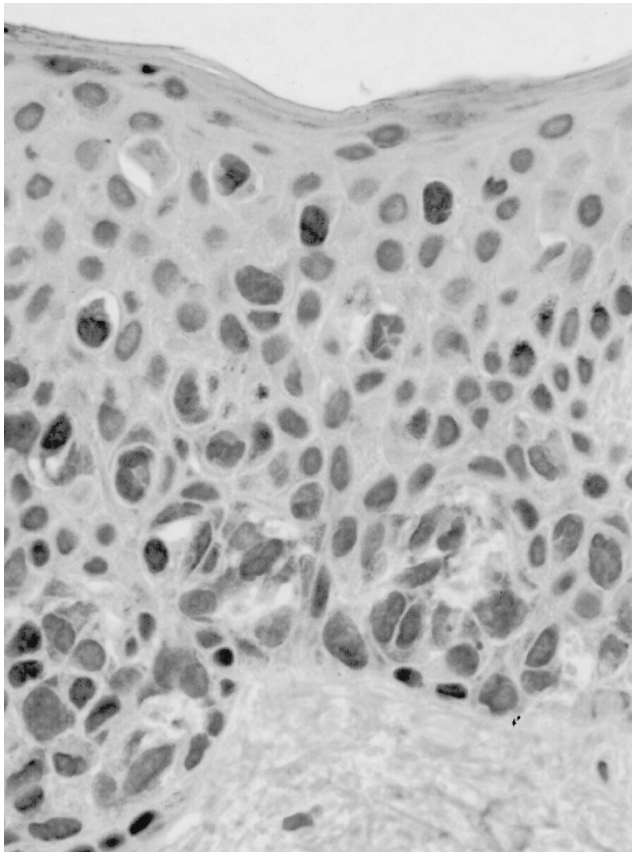

FIGURE 3. *Ets-1* expression for Clark Stage II melanoma.

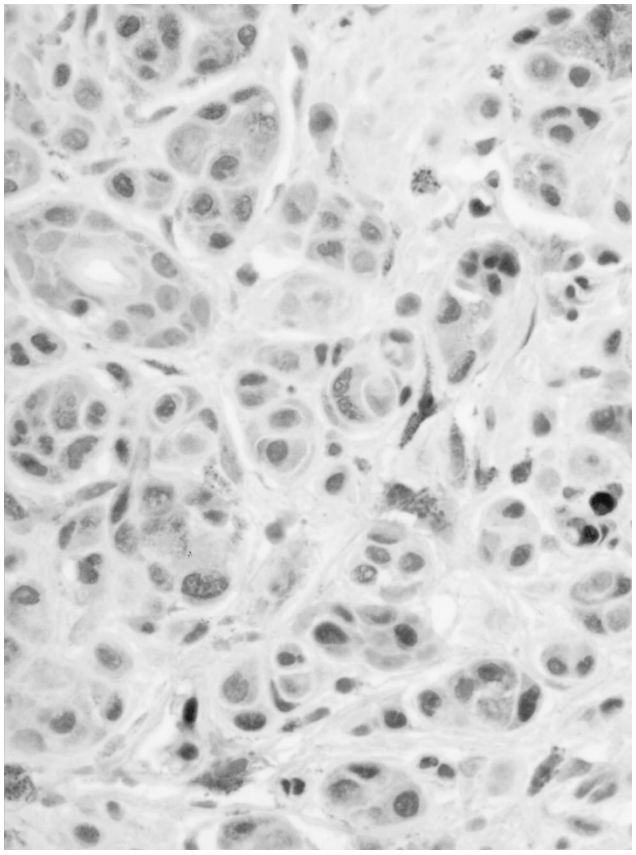

FIGURE 4. *Ets-1* expression for Clark Stage III melanoma.

creasing Clark stage in the cases studied. Although increased expression was seen within the intraepidermal melanocytes of melanoma *in situ*, the expression was more intense within deeply invasive tumors. Expression of MMP-1 has been shown to be up-regulated within dermal fibroblasts of deeply invasive melanomas (35). It can be speculated based on cell culture data (34) that the increase in MMP-1 expression is possibly related to *Ets-1* expression by the melanoma cells. It follows that increased metastatic potential and aggressive behavior in these lesions may be directly tied into the progressive capacity for melanoma cells to express *Ets-1*.

The ability of *Ets-1* to induce endothelial cell proliferation and activation, resulting in angiogenesis, provides another potential mechanism of action for this proto-oncogene. The correlation between angiogenesis and aggressive behavior and poor prognosis has been well documented for melanomas (36–40). It is possible that up-regulation of *Ets-1* by invasive melanoma cells documented in our study could facilitate access of melanoma to dermal vasculature and ultimate metastasis.

Ets-1 over-expression was not seen in a variety of benign melanocytic lesions. The differential expression of other proteins such as HMB-45, MART-1, and MIB-1, has been touted as a good discriminator of benign and malignant melanocytic proliferations (41, 42). However, in most of these situations, junctional melanocytes express these proteins in a similar manner regardless of their ultimate biologic behavior. Similarly, dermal melanocytes within Spitz's nevi, cellular blue nevi, dysplastic nevi, and other benign lesions have also been shown to overexpress these other proteins. These observations have relegated these markers to a limited role in discriminating among melanocytic nevi and melanoma. In the current study, we demonstrated negative to weak staining in all of our benign melanocytic proliferations, in contrast to the relatively strong nuclear staining in the vast majority of our melanomas. Although based upon a relatively small number of cases, our preliminary data suggest that overexpression of *Ets-1* may be a useful means for discriminating invasive melanoma from melanocytic nevi.

In summary, we have demonstrated that *Ets-1* is overexpressed within malignant melanoma cells, but similar overexpression is not seen within benign melanocytic proliferations. Our preliminary data suggest that *Ets-1* expression may be useful in helping to discriminate benign from malignant melanomas in difficult cases; however, this statement is made with caution, and additional studies are necessary to examine a more complete range of

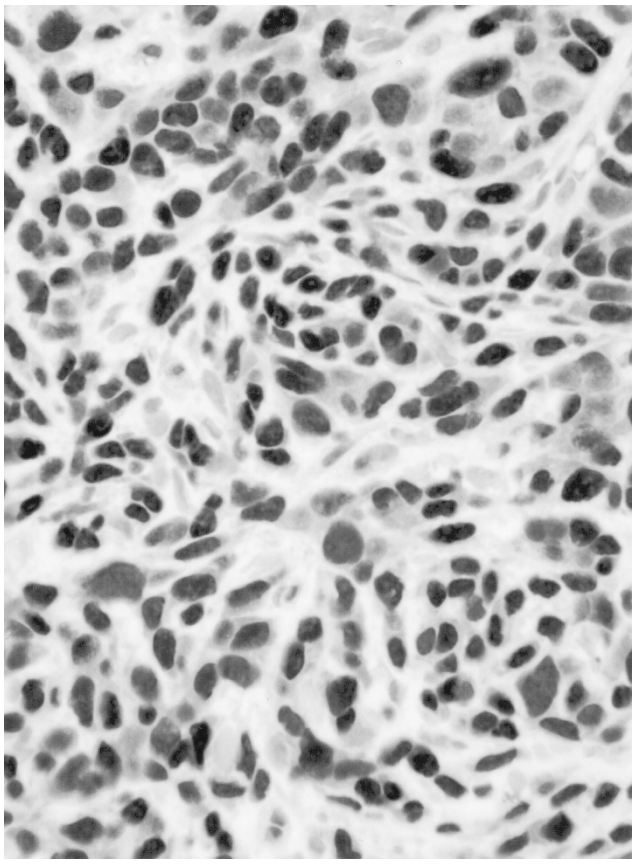

FIGURE 5. *Ets-1* expression for Clark Stage IV melanoma.

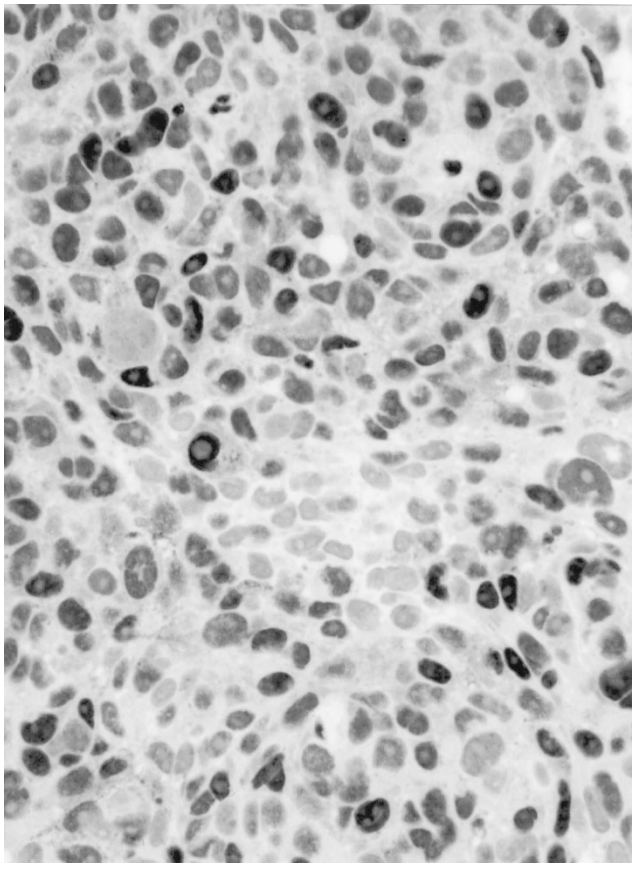

FIGURE 6. *Ets-1* expression for metastatic melanoma.

melanocytic proliferations and *Ets-1* expression. In addition, *Ets-1* expression by melanoma cells may explain the previously observed neovascularization underlying these lesions and the increased MMP-1 expression seen underlying highly aggressive tumors. Additional studies examining these relationships may result in potential therapeutic interventions and a better understanding of the pathogenesis of metastasis in primary cutaneous melanomas.

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