Differential Expression of Microtubule-Associated Protein 2 in Melanocytic Skin Lesions

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Abstract

Neoplastic melanocytes may exhibit certain differentiation characteristics of other neural-crest derivatives. We aimed to study the expression of microtubule-associated protein 2 (MAP-2) in different types of melanocytic skin lesions. Paraffin-embedded sections of 42 benign nevi (BN), 22 dysplastic nevi (DN), 45 superficial spreading melanomas (SSMs), and 15 subcutaneous melanoma metastases were immunohistologically assessed using the monoclonal mouse MAP-2ab antibody (Zytomed, Berlin, Germany). The percentage MAP-2 expression of DN and SSMs was significantly increased compared with BN. Moreover, subcutaneous melanoma metastases showed significantly decreased MAP-2 expression compared with DN and SSMs. In SSMs, MAP-2 expression significantly correlated with the Breslow vertical tumor thickness, Clark level, and stage of disease. We observed that MAP-2 is differentially expressed during the development and progression of benign and malignant melanocytic skin lesions. In contrast with the findings of previous studies, our data indicate that MAP-2 is a moderately positive predictor of the progression of SSMs.

Cutaneous malignant melanoma (MM) is a tumor that develops by transformation of melanocytes. Benign and atypical moles have been shown to exist in clinical and histologic contiguity with cutaneous MM, suggesting that these melanocytic nevi are also susceptible to malignant transformation.1-3

It is known that melanocytes arise from the neural crest that also gives rise to peripheral neurons, glial cells, and neuroendocrine cell types. Neoplastic melanocytes may exhibit certain differentiation characteristics of other neural-crest derivatives. Benign nevus cells that migrate into the dermis can morphologically resemble Schwann cells of the peripheral nervous system. Furthermore, desmoplastic (neurotropic) melanomas share many characteristics of peripheral nerve sheath tumors. Other studies have shown expression of neuron-associated markers (eg, neuropeptide substance P and neuron-specific enolase) in primary and metastatic melanomas. Hence, it is assumed that human cutaneous melanocytes maintain plasticity of differentiation, whereas neoplastic transformation likely allows them to exhibit characteristics of other neural-crest derivatives.4-6

Fang et al7 and Soltani et al8 investigated the expression of a neuron-selective marker, microtubule-associated protein 2 (MAP-2), in melanoma and its precursor lesions. MAPs are a family of proteins expressed predominantly in neuronal cells and are associated with the dendritic morphologic features of neurons. MAP-2, a neuron-specific MAP primarily localized to dendrites, stabilizes microtubule bundles and allows outgrowth of cellular processes.9,10 The data of Fang et al7 indicate that MAP-2 is expressed abundantly in a majority of melanocytic nevi and primary melanomas, but only weakly and heterogeneously in metastatic melanomas in vivo. Soltani et al8 demonstrated that MAP-2 induces mitotic defects, inhibits...
growth of melanoma cells, and predicts metastatic potential of MM. Because data on MAP-2 in melanocytic skin lesions are relatively sparse, we aimed to investigate the protein expression of MAP-2 in melanoma and its precursor lesions.

**Materials and Methods**

**Cases**

A computerized search was performed of our dermatopathology archive. We aimed to select paraffin-embedded sections of benign nevi (BN), dysplastic nevi (DN), and primary and metastatic MM from patients who underwent surgery between 2002 and 2008 in the Department of Dermatology, Ruhr-University Bochum, Bochum, Germany. Clinical data, including the Breslow vertical tumor thickness and stage of disease according to the American Joint Committee on Cancer melanoma staging system, were recorded from the original reports. Diagnosis and staging of nevi and MM had been performed on the basis of clinical, histopathologic, immunohistochemical, ultrasonic, and computed tomographic findings. This study adhered to the Declaration of Helsinki, and ethics approval for research was obtained from the local review board of the Ruhr-University Bochum.

**Immunohistochemical Studies**

Immunohistochemical staining was performed for MAP-2 as follows: 4-µm paraffin-embedded sections were used. Staining of formalin-fixed tissues required boiling tissue sections in 10 mmol/L citrate buffer, pH 6.0, for 10 minutes followed by cooling at 25°C. All sections were covered with monoclonal mouse MAP-2ab antibody (catalog No. 513-6104, Zytomed, Berlin, Germany) at a dilution of 1:100 for 30 minutes each. Specificity testing was performed by blocking of the primary antibody, and negative control staining was performed by omitting the primary antibody. All immunohistochemical slides were separately evaluated by the same observer (K.R.) for patterns of MAP-2 labeling of melanocytes. Microscopic evaluation (magnification ×100) involved an analysis in coincidental order of the tumor entities assessed. Three randomly chosen fields of view were assessed within the tumor tissue. The percentage of positively stained tumor cells per field of the total tumor cell count per field was determined. Quantitative results were expressed as the averaged percentages of positively stained tumor cells within the fields selected.

**Statistical Analysis**

Data analysis was performed using the statistical package MedCalc (MedCalc Software, Mariakerke, Belgium). Distribution of data was assessed by using the D’Agostino-Pearson test. The 1-way analysis of variance, including the Levene test for equality of variances and the Student-Newman-Keuls post hoc test for pairwise comparisons, was used for analysis of independent data. Categorical data were assessed by using the $\chi^2$ test. The Pearson coefficient of correlation ($r$) was also calculated. A $P$ value less than .05 was regarded as statistically significant.

**Results**

Clinical data are given in Table 1. All in all, we assessed the data for 80 men and 44 women. The mean ± SD age of the patients was 47.7 ± 14.1 years. A total of 124 individual paraffin-embedded specimens of melanocytic skin lesions were immunohistologically assessed, including 42 BN, 22 DN, 45 superficial spreading melanomas (SSMs) with a median Breslow vertical tumor thickness of 0.97 mm (range, 0.1-4.6 mm), and 15 subcutaneous melanoma metastases. Of the 45 primary SSMs, 11 (24%) had Clark level II, 14 (31%) had Clark level III, and 20 (44%) had Clark level IV. The disease stage in the 45 cases was as follows: IA, 21 (47%); IB, 7 (16%); IIA, 8 (18%); IIB, 1 (2%); IIIA, 4 (9%); IIIB, 1 (2%); and IV, 3 (7%).

As demonstrated in Image 1, MAP-2 showed relatively strong cytoplasmic staining in benign and malignant melanocytes. However, MAP-2 immunoreactivity tended to be stronger within the central components of the lesions. Quantified immunohistologic data were normally distributed. The Levene test result for equality of variances within the groups was not

### Table 1

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<th>Clinical Characteristics and Lesional Expression of MAP-2 in Patients With Benign and Malignant Melanocytic Skin Lesions</th>
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<td>Sex (M/F)</td>
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<td>Mean ± SD age (y)</td>
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<td>Mean ± SD MAP-2 expression (%)</td>
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MAP-2, microtubule-associated protein 2.

* Cytoplasmic protein expression in melanocytes.
significant \((P > .05)\). The 1-way analysis of variance including the Student-Newman-Keuls post hoc test for pairwise comparisons revealed that MAP-2 expression of DN \((26\% \pm 8.5\%)\) and SSMs \((26.9\% \pm 10.1\%)\) was significantly increased compared with BN \((18.6\% \pm 10.5\%; P < .05)\). Moreover, subcutaneous metastases of melanomas \((19.3\% \pm 7.1\%)\) showed significantly decreased MAP-2 expression compared with DN \((26\% \pm 8.5\%)\) and SSMs \((26.9\% \pm 10.1\%; P < .05)\)

**Figure 1.** MAP-2 expression observed in BN \((18.6\% \pm 10.5\%)\) and subcutaneous melanoma metastases \((19.3\% \pm 7.1\%)\) did not differ significantly \((P > .05)\). In SSMs, MAP-2 expression significantly correlated with the Breslow vertical tumor thickness \((r = 0.36; P = .016)\), Clark level \((r = 0.4; P = .0078)\), and stage of disease \((r = 0.31; P = .039)\).

**Discussion**

MAPs are a heterogeneous group of proteins associated with microtubules and are known to regulate the stability of microtubules, primarily in the axons and dendrites of neurons. Although MAPs are expressed most abundantly in neuronal cells, certain MAPs such as MAP-1 and MAP-4 are widely expressed in nonneuronal cells. Expression of MAP-2, one of the most extensively studied MAPs, seems to be restricted to neurons, and MAP-2 expression is used as a marker for neuronal differentiation.\(^{11-13}\) MAP-2 is localized primarily in the dendrites but not in axons. Multiple isoforms of MAP-2, which arise from alternative splicing of messenger RNA, have been characterized. It is interesting that this splicing event seems to be developmentally regulated. Whereas the juvenile MAP-2c form is found only in immature neurons, the mature MAP-2ab forms are found throughout the life of the neurons. In postmitotic terminally differentiated neurons, MAP-2 is localized to the dendrites.\(^{14,15}\) Altered MAP-2 expression has previously been reported in a variety of tumors with neuroendocrine differentiation, including carcinoid tumors, gliomas, and Merkel cell carcinomas.\(^{8,16-18}\)

Fang et al\(^7\) investigated 10 congenital and acquired melanocytic nevi, 9 primary MMs, and 42 metastases. However,
they did not include DN in their study.\textsuperscript{7} Whereas the majority of nevi (60\%) and many primary melanomas (44\%) were strongly MAP-2+, only a small percentage of metastatic melanomas (24\%) had foci of MAP-2–stained cells.\textsuperscript{7} Soltani et al\textsuperscript{8} recently showed that adenoviral-mediated expression of the mature form of MAP-2 leads to mitotic spindle defects, cell cycle arrest, and apoptosis of metastatic melanoma cells in vitro and that MAP-2 expression in vivo in primary tumors correlates with metastatic disease-free survival of patients with cutaneous melanoma.

In the present study, we investigated MAP-2 expression in a reasonable number of melanocytic skin lesions. We observed significantly increased expression of MAP-2 in DN and SSMs compared with BN and subcutaneous melanoma metastases. Our data differ from the results reported by Fang et al,\textsuperscript{7} who observed decreasing MAP-2 expression from nevi to melanoma metastases. Our results indicate that MAP-2 expression apparently increases with tumor progression up to a certain degree and finally decreases following metastasis, possibly due to dedifferentiation of the tumor. Moreover, we found a moderate correlation between MAP-2 expression of SSMs and Breslow tumor thickness, Clark level, and stage of disease. MAP-2 overexpression observed in SSMs was rather a negative prognostic factor in the present study. By contrast, Soltani et al\textsuperscript{8} found an inverse correlation between MAP-2 expression of primary melanomas and metastatic disease-free survival.

Hendrix et al\textsuperscript{19} suggested that melanoma exhibits plasticity of differentiation and is known to differentiate along multiple cellular pathways, including endothelial and neuronal. Indeed, the progression of MM from the locally invasive, but metastasis-incompetent radial growth phase to the rapidly proliferating, metastasis-competent vertical growth phase is accompanied by invasion of tumor cells into the dermis and subcutaneous tissue and a dynamic interaction between tumor cells and stroma.\textsuperscript{20} This reciprocal interaction results in differentiation of melanoma cells along different pathways, including neuronal pathways.\textsuperscript{4,7,19} In fact, the data of Soltani et al\textsuperscript{6} suggest that neuronal differentiation of primary melanoma cells in the dermis, as indicated by MAP-2 expression, can profoundly affect cell cycle progression, induce apoptosis, and influence clinical outcome.

It is known that neoplastic melanocytes, in particular dermal nevus cells, are able to differentiate along pathways of other neural crest–derived cell types. Hence, in melanocytic nevi, the terminal differentiation of type C nevus cells deep within the dermis results in Schwann cell–like morphologic features and activation of Schwann cell markers.\textsuperscript{21,22} Cellular and stromal interactions within the dermis are thought to provide the signals for such transdifferentiation of melanocytes. Only rarely do malignant melanocytes follow the Schwann cell pathway of differentiation. For example, desmoplastic neurotropic melanomas follow the Schwann cell pathway of differentiation, and these lesions are distinctive in that they tend to metastasize much later in their course than do conventional melanomas.\textsuperscript{23,24} In contrast, most MM seem to acquire characteristics more similar to neurons as shown by the expression of the intermediate filament protein peripherin and other neuronal markers. These observations have led to the hypothesis that during malignant progression, cutaneous melanocytes may follow divergent differentiation pathways.\textsuperscript{4-6} Our findings on the overexpression of MAP-2 in DN and SSMs seem to support the aforementioned hypothesis. Recently, Bhat et al\textsuperscript{25} suggested that Notch signaling, which is implicated in melanoma progression, and the transcription repressor HES1 have a role in MAP-2 gene regulation during melanoma progression.

Limitations of the present study include the absence of survival data and functional studies. Nevertheless, we observed that MAP-2 is differentially expressed during the development and progression of benign and malignant melanocytic skin lesions. Hence, neuronal differentiation of melanocytes seems to be altered depending on the type of melanocytic neoplasm considered. In contrast with the findings of previous studies, our data indicate that MAP-2 is a moderately positive predictor of SSM progression. More comprehensive studies are needed to fully establish the role of MAP-2 expression in melanocytic skin lesions.

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References