Nerve Growth and Expression of Receptors for Nerve Growth Factor in Tumors of Melanocyte Origin

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Nerve growth factor (NGF) stimulates growth and differentiation of sensory and sympathetic neurons. It is not known what role NGF plays in melanoma development, but nevus and malignant melanoma cells express NGF-receptor (NGF-R). We counted nerve fibers within melanocytic nevi, primary cutaneous melanomas, and cutaneous melanoma metastases using a monoclonal antibody (MoAb) as marker against a 200-kD glycoprotein that is expressed on human nerves. The expression of NGF-R was studied in serial cryostat sections using a MoAb against the NGF-R.

Compared to normal skin, increased numbers of nerve fibers were found in 72 melanocytic nevi. In congenital nevi their number significantly increased with age. In 47 primary cutaneous melanomas the number of nerve fibers decreased in proportion to tumor thickness. In 33 cutaneous melanoma metastases no accumulation of nerve fibers was found. NGF-R was not expressed in normal skin melanocytes and in the majority of nevus cells in melanocytic nevi. Considerable numbers of NGF-R-positive nevus cells were found only in some congenital nevi and few acquired nevi with dysplastic features. By contrast, in primary and metastatic melanomas higher expression of NGF-R was observed.

The increased number of nerve fibers in melanocytic nevi suggests that neurite-promoting factors are produced in situ. Production of such factors appears to be lost in malignant melanoma cells. The finding of an inverse correlation between an abundance of nerve fibers in NGF-R-poor nevi and a high expression of NGF-R in melanomas that show no evidence of nerve growth suggest a role for NGF and its receptor in malignant melanocytic tumors. J Invest Dermatol 96:662–665, 1991

Nerve growth factor (NGF) is known to be essential for the growth and differentiation of sensory and sympathetic neurons [1]. It is a neurotrophic factor, which generates changes in responsive neural cells [2] by binding to NGF receptors (NGF-R) on the cell surface [3]. Little is known about the synthesis and occurrence of NGF in human skin. The skin as the original tissue of melanocytic tumors was shown to produce NGF at least in the embryonic mouse [4]. Melanocytes are of neural crest origin. Whereas sensory and sympathetic neurons and chromaffine cells in vitro respond to NGF [5–7], normal and malignant melanocytes show little response to NGF [8,9]. NGF was shown to be able to increase the survival, but not the division, of melanoma cells from the human tumor line A875 [9]. NGF-R was detected in tissue sections of human neurofibromas, pheochromocytomas, and peripheral nerves [10], and in a variety of cultured cells of mesenchymal, epithelial, and hematopoietic derivation [11]. Human melanocytes, which normally lack NGF-R in situ [10], were shown to express NGF-R in vitro on stimulation with the tumor promotor TPA or UV irradiation [12]. NGF-R also was demonstrated on nevus cells and melanoma cells in vitro [13–15] and in situ [10]. Cultured melanocytes of normal skin and nevi showed moderate NGF-R expression in contrast to a strong NGF-R expression on cells cultured from primary melanomas and metastatic melanomas [14]. The NGF-R is heterogeneous with regard to its affinity [16]. In contrast to differentiated neural crest cells the majority of human melanoma cells express NGF-R with high affinity [3,16]. The effect of NGF on NGF-sensitive cells like pheochromocytomas and sensory and sympathetic neurons appears to be dependent on adhesion molecules for cell-matrix interactions [17–19]. The requirements for an effect of NGF on pigment cells are not yet known, so that the significance of NGF-R on melanoma cells is not clear. Different responses in the sense of growth and differentiation of cells to NGF were demonstrated in subclones of neuroblastoma [20].

Human skin contains sensory and sympathetic nerves, i.e., NGF-responsive structures. Also, in melanocytic nevus nerve fibers have been described [21]. With MoAb directed against a nerve-associated antigen and the NGF-R we studied a variety of benign and malignant melanocytic tumors of the skin in order to relate NGF-R expression and signs of nerve growth—promoting activity in situ.

MATERIALS AND METHODS

Tissues Representative portions of 72 melanocytic nevi (of 30 males and 42 females), 47 primary cutaneous melanomas (of 22 males and 25 females), and 33 cutaneous melanoma metastases were snap-frozen in liquid nitrogen and stored at −80°C. The melano-
Nerve fibers in Melanocytic Tumors

Table I. Nerve Fibers in Melanocytic Tumors

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of MoAb M-2-10-15+ Fibers per High-Power Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive/ Tested</td>
</tr>
<tr>
<td>Normal skin</td>
<td>50/58</td>
</tr>
<tr>
<td>Nevi</td>
<td>40/40</td>
</tr>
<tr>
<td>Congenital nevi</td>
<td>16/16</td>
</tr>
<tr>
<td>Common acquired nevi</td>
<td>16/16</td>
</tr>
<tr>
<td>Dysplastic nevi</td>
<td>72/72</td>
</tr>
<tr>
<td>Primary cutaneous melanomas</td>
<td></td>
</tr>
<tr>
<td>&lt;0.76 mm</td>
<td>6/8</td>
</tr>
<tr>
<td>0.76 - 1.5 mm</td>
<td>12/13</td>
</tr>
<tr>
<td>1.51 - 3.0 mm</td>
<td>3/13</td>
</tr>
<tr>
<td>&gt;3.0 mm</td>
<td>5/13</td>
</tr>
<tr>
<td>Cutaneous melanoma metastases</td>
<td>26/47</td>
</tr>
</tbody>
</table>

Cytic nevi comprised 40 congenital nevi, 16 common acquired nevi, and 16 acquired nevi with dysplastic features. The primary cutaneous melanomas comprised 30 superficial spreading melanomas, four lentigo maligna melanomas, three acral lentigious melanomas, five nodular melanomas, and three unclassified melanomas of different tumor thickness (see Tables I and II). Perilesional normal skin was available in 58 cases. Age of the patients and anatomic location of tumors were given by the patients' files.

Antibodies We studied the presence of intratumoral nerve fibers using the MoAb M-2-10-15. This antibody is directed against a 200-kD glycoprotein and was generated by immunization of a mouse with the human melanoma cell line McWo [22]. MoAb M-2-10-15 stains small nerve fibers in all tissues investigated and reacts occasionally with nevus and melanoma cells [23]. Expression of NGF-R was studied using the mouse MoAb 20.4 against a 75-kD protein of the NGF-R [10]. This antibody was generated by immunization with the human melanoma cell line WM245 [10].

The MoAb M-2-10-15 and 20.4 were used as primary antibodies in an indirect immunoperoxidase technique as described elsewhere [24]. The dilutions were 1:20 for MoAb M-2-10-15 and 1:3000 for MoAb 20.4.

Immunoperoxidase Assay Serial cryostat sections were studied with the MoAb M-2-10-15 [22] (all lesions) and the MoAb 20.4 [10] (all lesions except 32 congenital melanocytic nevi). Nerve fibers were determined by their reaction with MoAb M-2-10-15. Sectioned fibers or their tributaries were counted by two independent observers (EBB and HM) in three representative high-power fields (X 400). The average nerve fiber number was calculated in the tumors and in the surrounding normal skin. In Table I and Fig 2 the term "nerve fiber" means sectioned fibers or fiber tributaries. The percentage of NGF-R-positive tumor cells, indicated by the reaction with MoAb 20.4, was also assessed by two independent observers (EBB and HM).

The reaction with the nerves around sweat glands served as an internal positive control for the MoAb M-2-10-15. Many nerve fibers and, in addition, blood-vessel walls, reacted with the MoAb 20.4, and served as internal positive controls. As negative controls, sections were incubated with phosphate-buffered saline instead of the primary antibodies.

Statistical evaluation of data was performed with the Kruskal-Wallis assay [25]. The significance level was determined with z=0.05.

RESULTS

Nerve fibers in normal skin surrounding the tumors, stained with the MoAb M-2-10-15, were determined in 58 lesions. Compared to normal skin high numbers of nerve fibers were found within melanocytic nevi (Table I and Fig I). Only three of three junctional nevi showed no increased numbers of nerve fibers in contrast to all nevi reaching the dermis. As shown in Fig 1, single small fibers were arranged in a wave- and whorl-like pattern around the nevus cells. The morphologic feature of "neuroid transformation" of the nevus cells [26] was very rare in our material. The number of nerve fibers showed a correlation to neither the location of nevus nor to the sex of the patients. Acquired melanocytic nevi also did not show any relationship between the number of nerve fibers and the age of the patient. The number of nerve fibers in congenital melanocytic nevi was found to be positively correlated with the age of the nevus (= age of the patient). When divided into four age groups, congenital melanocytic nevi showed a statistical significance, in particular between the first two groups and the last two groups (z = 1.26 X 10^-4) (Fig 2).

In the total group of primary cutaneous melanomas the average number of nerve fibers did not differ from normal skin (Table I). The number of nerve fibers, however, was increased in thin melanomas and decreased in proportion to tumor thickness (Table I). The difference between melanomas with a thickness below 1.5 mm and

Table II. NGF-R Expression in Melanocytic Tumors

<table>
<thead>
<tr>
<th>Tissue</th>
<th>NGF-R+ Tumor Cells [%]</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Positive/ Tested</td>
</tr>
<tr>
<td>Normal epidermis</td>
<td>0/40</td>
</tr>
<tr>
<td>Nevi</td>
<td></td>
</tr>
<tr>
<td>Congenital nevi</td>
<td>6/8</td>
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<td>Common acquired nevi</td>
<td>10/16</td>
</tr>
<tr>
<td>Dysplastic nevi</td>
<td>12/16</td>
</tr>
<tr>
<td>Primary cutaneous melanomas</td>
<td>28/40</td>
</tr>
<tr>
<td>&lt;0.76 mm</td>
<td>8/8</td>
</tr>
<tr>
<td>0.76 - 1.5 mm</td>
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</tr>
<tr>
<td>1.51 - 3.0 mm</td>
<td>10/13</td>
</tr>
<tr>
<td>&gt;3.0 mm</td>
<td>9/13</td>
</tr>
<tr>
<td>Cutaneous melanoma metastases</td>
<td>39/47</td>
</tr>
</tbody>
</table>

![Figure 1](image-url) Nerve fibers in a congenital melanocytic nevus as visualized with the MoAb M-2-10-15. Small nerve fibers are M-2-10-15 positive (->). They are arranged in a wave- and whorl-like pattern around the nevus cells. Magnification X 200.
of 16 acquired nevi with dysplastic features contained 20% or more of NGF-R-positive tumor cells. The average percentage of NGF-R-positive nevus cells was higher in female (10.95 ± 7.46%) than in male subjects (5.95 ± 4.52%), but this difference did not reach statistical significance.

The mean percentage of NGF-R-positive tumor cells was significantly higher in primary cutaneous melanomas than in melanocytic nevi (z = 2.58 × 10^{-4}) (Table II). We found no relationship between Clark-level, tumor thickness, histologic type, location of tumors, sex and age of the patients, and the expression of NGF-R on tumor cells.

Cutaneous melanoma metastases showed the highest average percentage of NGF-R-positive tumor cells (Table II). We did not observe any sex relationship in cutaneous melanoma metastases. A considerable intralesional heterogeneity of NGF-R expression was observed in the tumors (Fig 4). The interlesional heterogeneity is the reason for the high standard deviation (Table II).

The difference in NGF-R expression between melanocytic nevi and primary cutaneous melanomas was statistically significant (z = 2.58 × 10^{-4}), whereas the differences between melanocytic nevi and cutaneous melanoma metastases and between primary cutaneous melanomas and cutaneous melanoma metastases did not reach statistical significance.

To sum up, NGF-R expression in malignant melanocytic tumors compared to benign melanocytic tumors is higher and shows a marked inter- and intralesional heterogeneity. In addition, we observed a decrease in intratumoral nerve fibers in proportion to tumor progression.

**DISCUSSION**

An inverse relationship between expression of the NGF-receptor on tumor cells and signs of nerve outgrowth was found in melanocytic tumors of the skin. This suggests a competition between malignant melanocytes and nerves for the binding of NGF. Is NGF produced in melanocytic tumors? We were unable to detect NGF immunoreactivity in the tumors studied (data not shown). The observation that in congenital melanocytic nevi a clear-cut increase of intratumoral nerve fibers occurs with the age of the patient (≈ age of the nevus) strongly suggests that a factor is produced in the tumors that induces the outgrowth of nerves.

NGF was shown to be produced independently from innervation in embryonic skin of rodents [27]. It remains to be determined whether NGF or other mediators, e.g., interleukin 6, cause nerve outgrowth in melanocytic tumors [28].

In primary melanomas, we found increased numbers of nerve fibers in areas adjacent to the tumor. NGF-R expression in malignant melanocytic tumors was not statistically different from that in cutaneous melanomas. However, primary cutaneous melanomas expressed NGF-R at a higher average percentage than cutaneous melanoma metastases (z = 2.58 × 10^{-4}).
fibers only within early lesions, i.e., <0.76 mm. In thicker primary melanomas and in cutaneous metastases, no outgrowth of nerve fibers was evident. This finding on the one hand may be explained by the much more rapid growth of advanced melanomas compared to nevi, which, as shown in congenital melanocytic nevi, need years to induce significant outgrowth of intratumoral nerves. On the other hand, NGF-R on melanoma cells might, dependent on density and affinity, compete with NGF-R on cutaneous nerves. The NGF-R was shown to be of higher affinity in melanoma cells than in differentiated neural crest cells [3,16]. This might explain the lack of outgrowth of nerve fibers in tumors with significant NGF-R expression.

In our material we found considerable expression of NGF-R not exclusively in melanomas but also in certain benign melanocytic tumors. These tumors belonged to potential melanoma precursor lesions, i.e., congenital melanocytic nevi and dysplastic nevi. We observed higher percentages of NGF-R–positive nevus cells in nevi of women than in nevi of males. This suggests a possible influence of sex hormones on the NGF-R expression, and is interesting insofar as, at least in Europe, more women than men develop melanoma [29]. All our patients derived from Northern Europe.

As reported previously [10,15], epidermal melanocytes of normal skin did not express NGF-R in situ. In vitro, human melanocytes were reported to require NGF-R on treatment with phorbol ester or UV light [12]. In our material, we could not find any relationship between NGF-R expression in nevus or melanoma cells and UV exposure as determined by the location of the lesions. A detailed evaluation of recent or previous UV exposure of the lesions, however, was not performed in this study.

The heterogeneity of NGF-R expression suggests either that NGF-R is only transiently expressed, or that the heterogeneous expression indicates different clones within a given lesion. Antigenic heterogeneity is well-known in melanomas [13–15,22,24] and also in congenital melanocytic nevi [30].

In conclusion, the inverse correlation of an abundance of nerves in tumors with low NGF-R expression (benign nevi) and a high expression of NGF-R in tumors without evidence of nerve growth (malignant melanoma) suggests a role for NGF and its receptor in autocrine growth regulation of melanocytic tumors.

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REFERENCES