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Diagnostic Assessment of Two Novel Proliferation-Specific Antigens in Benign and Malignant Melanocytic Lesions

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The aim of this study was to gain a thorough insight into the proliferative activity of benign and malignant melanocytic tumors. A total of 314 cases were examined by immunohistochemistry on paraffin-embedded material. The growth fraction was assessed by means of two monoclonal antibodies, Ki-S1 and Ki-S5, which react with two different proliferation-specific nuclear antigens. Additionally, HMB-45 was used as a marker of melanocytic activation. Statistically significant differences ($P < 0.01$) in the proliferation rates were found between common acquired nevi, Spitz's/Reed's nevi, primary cutaneous melanomas, and metastatic melanomas, whereas dysplastic nevi were hardly distinct from other nevi of the compound type. In melanoma, the growth fraction correlated well with the tumor stage but poorly with HMB-45 expression and mitotic count. Along with tumor progression, an increasing heterogeneity of proliferation indices was observed. Our results provide no evidence for a progression from dysplastic nevi into melanoma. They indicate that the assessment of the proliferative activity may be of considerable diagnostic help in cases of uncertain histology and that it might contribute to an alternative concept for the classification of melanocytic tumors. (Am J Pathol 1995, 147:1615–1625)

Melanocytic proliferations account for the vast majority of all skin tumors, but their histogenesis and possible progression into malignancy is yet poorly understood. Four categories of melanocytic neoplasia may be distinguished: congenital nevi, acquired common nevi, frankly malignant lesions such as invasive melanoma, and a borderline group of either unknown or unpredictable biological behavior, comprising so-called dysplastic nevi and melanoma *in situ*.

Since the first description of the B-K mole by Clark et al,[1] the concept of dysplastic nevus has given rise to considerable controversy.[2–4] In familial dysplastic nevus syndrome[1,5] associated with a particular genotype possibly inherited in an autosomal dominant fashion,[6] this kind of lesion appears to be a marker of increased risk for malignant melanoma.[7,8] On the other hand, the biological significance of so-called sporadic dysplastic nevi, ie, solitary lesions with corresponding gross and microscopic features occurring in normal individuals, remains unclear. Although some authors regard them as premalignant lesions in analogy with epithelial dysplasia,[3,5,9] eg, in solar keratosis, or envisage at least an equivalence with the moles of dysplastic nevus syndrome, their existence as a nosological entity is vigorously denied by others.[10] Moreover, the atypical nevus is clinically a clear-cut diagnosis,[11] whereas no complete agreement as to the correlated histopathological criteria could be achieved so far.[5,12–14] Similarly, melanoma *in situ* has been the subject of extensive debates[15–17] leading to a surfeit of suggestive designations[18,19] that occasionally blend with those applied to certain types of nevi. Although it is taken for granted that melanoma *in situ* is cured by complete excision and does not evolve into metastatic disease,[20] it remains uncertain whether, in a certain percentage of cases, it is the latest step of a progressive transformation in nevi,[5,21,22] even though it is generally accepted that up to 80% of malignant melanomas arise *de novo* and independently of previous dysplastic change.[3]

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Table 1. *Survey of the Different Diagnostic Entities of Melanocytic Tumors Investigated*

Diagnosis	Patients			Age (mean ± SD)
	Male	Female	Total	
Junctional nevus/jentigo	13	12	25	34.8 ± 23.1
Compound nevus/compendigo	25	18	43	32.4 ± 22.3
Dermal nevus	13	21	34	36.4 ± 24.8
Blue nevus	19	13	32	32.7 ± 21.2
Spitz's nevus	8	15	23	12.5 ± 7.0
Reed's nevus	3	1	4	23.4 ± 11.5
Dysplastic nevus	32	18	50	27.4 ± 12.6
Melanoma *in situ*/lentigo maligna	14	6	20	58.9 ± 12.8
Superficial spreading melanoma				
<0.8 mm thickness	8	15	23	46.6 ± 14.4
>0.8 mm thickness	5	12	17	55.6 ± 14.1
Nodular melanoma	18	7	25	60.2 ± 13.6
Metastatic melanoma	9	9	18	55.3 ± 18.8
Total	167	147	314	

The model of progression for melanocytic tumors as proposed by Clark et al,[21] emphasizing the transition from dysplastic nevus into malignant melanoma, is met with growing acceptance. For its corroboration, a panel of methods, including the search for remnants of dysplastic nevi,[23] immunohistochemistry,[24–27] morphometry,[28] Ag-NOR counting,[29] DNA determination,[30,31] and molecular genetics,[32–34] have been applied with divergent results. Only recently, a few studies were devoted to the assessment of proliferation in nevi and melanomas,[35–37] but they also failed to establish a unifying concept.

The present study is concerned with the proliferative activity in different kinds of melanocyte-derived neoplasia and its possible correlation with HMB-45 antibody-monitored cellular activation.[38] A precise assessment of the proliferating cell fraction in morphologically unaltered sections was facilitated by an immunohistochemical approach with the monoclonal antibodies Ki-S5[39] and Ki-S1[40]. Ki-S5 binds to a formalin-resistant epitope of the Ki-67 protein[41,42]; the antigen recognized by Ki-S1 is human topoisomerase IIα.[43] Both antigens, being exclusively expressed in actively cycling cells, allow a reliable evaluation of the proliferative activity. Although we took into consideration a wide spectrum of melanocytic tumors, our interest was centered on sporadic dysplastic nevi, Spitz's nevi, and early melanomas, as these are the groups in which difficulties in differential diagnosis may arise.[44–48] Moreover, we regard our paper as a contribution to the abiding discussion on premalignant lesions of melanocytic origin.

Materials and Methods

A total of 314 melanocytic tumors, viewed by at least two independent pathologists, were selected from the bioptic material of our institutions. The subclassifications and the number of corresponding specimens are listed in Table 1.

No congenital nevi were included in this study. Common nevi of either junctional, compound, or dermal type were diagnosed as such only when the lesions were symmetrical and neither nuclear atypia of melanocytes nor a host response was present. For the diagnosis of sporadic dysplastic nevus, the following conditions were considered as essential: (1) clinical diagnosis of atypical nevus and diameter of the lesion greater than 5 mm; (2) no history of familial dysplastic nevus syndrome; (3) concurrence of all histopathological criteria as defined by Elder,[49] ie, architectural disorder, lentiginous and/or epithelioid-cell hyperplasia, cytological atypia and a host response including a lymphoid infiltrate and either concentric or lamellar fibroplasia. No distinction was made between major and minor criteria,[12] and no grading was applied, as all of the selected lesions would score as moderate to severe dysplasia by definition.[11,50]

Only benign blue nevi of high nevocellular density were included in this study, most of them containing both dendritic and epithelioid cells. Spitz's/Reed's nevi as well as melanomas were diagnosed by the commonly applied criteria,[48] and for the classification of malignant melanoma, both Clark's level and tumor thickness were taken into account.

Immunohistochemistry was performed with minor modifications as described before.[39,51] Briefly, paraffin sections, 2 to 4 μm thick, from routinely processed formalin-fixed material, were mounted on 3-aminopropyl-triethoxy-silane-coated slides, dried overnight at room temperature, and subsequently deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase was blocked by 3% (vol/vol) hydrogen peroxide in methanol for 5 minutes, followed by rinsing

with phosphate-buffered saline. Primary antibody Ki-S1 was applied at a dilution of 1:60,000 to 1:100,000 for 30 minutes, preceded by a treatment with 0.1% trypsin (Sigma Chemical Co., Steinheim, Germany) for 45 minutes. Retrieval[52] of the KI-S5 antigen was carried out by heating the slides submerged in 0.01 mol/L citric acid at pH 6.0 in a microwave oven (Toshiba) for 15 minutes at the highest power setting before incubation with the antibody (undiluted culture supernatant) for 30 minutes. Nuclear immunostaining was enhanced either by the biotin-streptavidin complex with peroxidase and rabbit anti-mouse antibody or by means of the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique (if not otherwise specified, all reagents were purchased from Dako, Hamburg, Germany). For the cytoplasmic staining with HMB-45 (Ortho, Neckargemünd, Germany), the antibody was diluted 1:16,000 and incubated for 30 minutes, and the reaction was enhanced by the APAAP technique. After rinsing with Tris-buffered saline, the slides were briefly counterstained with Mayer's hematoxylin.

The growth fraction was assessed by counting a minimum of 1000 tumor cells. In larger lesions, multiple fields of viable tumor tissue were examined to minimize erroneous ratings caused by a focal or regional distribution of the proliferating cells. In small lesions, the total tumor surface was evaluated. Only nuclei with unequivocal reactivity were scored as positive, and the count was expressed as a percentage of the tumor cells. Whenever possible, the epidermal and the dermal component of the tumors were evaluated separately. All comparisons were based on mean values and standard deviations of the Ki-S1- and Ki-S5-positive cell fractions. A normal distribution of the values having been verified, Student's *t*-test was elaborated on a probability level of at least $P < 0.01$ for significant differences. In parallell, mitotic figures, if present, were counted by the usual procedure[53]; as a rule, no less than 20 high power fields were examined, but the results are expressed in mitoses per 10 high power fields.

The immunoreaction with HMB-45 was graded into four categories: strong positivity of more than 90% of cells (+++), somewhat pale cytoplasmic staining of the majority of tumor cells or strongly positive reaction in approximately 50% (++), weak staining of approximately one-half of the tumor cells or strong immunoreaction in just a few scattered units (+), and no detectable reactivity (−).

Results

For both antibodies, evaluation of the immunoreaction by two independent observers yielded an overall concordance of approximately 95%. Whenever the variability exceeded 10%, the cases were discussed and the immunoreactive scores were appropriately reconsidered.

A small fraction of proliferating cells could be detected in the majority of the benign lesions examined. However, in 20% of the common nevi and in 1 of 12 blue nevi, no reaction of the melanocytes with either of the two proliferation-associated antibodies was observed. False negative results could be excluded by the regular staining of suprabasilar keratinocytes, hair germs, or cutaneous adnexal glands usually present within the specimens. Normal melanocytes in the basal cell layer of the epidermis were always negative.

The percentage of Ki-S5-labeled cells generally were greater than that of Ki-S1-positive cells, but there was a linear correlation ($r = 0.85$) between the results obtained with both antibodies. In the following, we refer first to the percentage of Ki-S5-reactive melanocytes and in parentheses to Ki-S1.

In nevi of the compound type, the growth fraction was constantly higher in the epidermal/junctional compartment than in the dermal aggregations of nevus cells. The ranges, mean values, and standard deviations of the proliferation rates in the eight separate groups of melanocytic tumors are summarized in Table 2 with reference to the two antibodies, Ki-S1 and Ki-S5.

The percentage of positive melanocytes in the epidermis never exceeded 1.2% (1.1%) in common nevi (Figure 1b) and 1.6% (1.1%) in dysplastic nevi (Figure 1, c and e), with an exceptional peak to 2.5% (2.0%) in one single case. The scores were substantially higher in Spitz's/Reed's nevi, with a maximum of 5.3% (4.3%), amounting to a mean value of 1.76% (1.67%).

Intradermal nevus cells displayed a low proliferative activity, with a maximum of 0.6% in common nevi of the compound type (Figure 1b) and 0.8% (0.7%) in dysplastic nevi and corresponding mean values of 0.18% (0.16%) and 0.16% (0.15%). The growth fraction was minimal in Unna's nevi (<1% with both antibodies; Figure 1a), but in cellular blue nevi, it was comparable to that of the epidermal component of compound nevi, with a range from 0 to 0.8% and a mean value of 0.31% (0.24%). Again, in Spitz's nevi, the growth fraction reached higher levels (Figure 1d) that occasionally attained 4.3% but remained well below the proliferation indices in melanoma with similar histological features.

The proliferation index was significantly higher ($P < 0.01$) for melanoma *in situ* (Figure 2, a and b) and showed a marked increase with advancing tumor

Table 2. *Growth Fraction of Benign and Malignant Melanocytic Tumors as Determined by Immunohistochemistry with Two Novel Antibodies, Ki-S1 and Ki-S5, Directed Against Different Proliferation-Specific Nuclear Antigens*

Diagnosis	n	Antigen	Epidermal compartment, mean ± SD (range)	Dermal compartment, mean ± SD (range)
Common nevus	102	Ki-S1	0.30a ± 0.24 (0–1)	0.16a ± 0.18 (0–1)
		Ki-S5	0.31 ± 0.33 (0–1)	0.18 ± 0.21 (0–1)
Dysplastic nevus	50	S1	0.60a ± 0.42 (0.5–2.5)	0.15a ± 0.23 (0–1)
		S5	0.67 ± 0.49 (0.3–3)	0.16 ± 0.19 (0–1)
Blue nevus	32	S1		0.24a ± 0.28 (0–1)
		S5		0.31 ± 0.33 (0–1)
Spitz's/Reed's nevus	28	S1	1.67b ± 0.30 (0.5–4.3)	1.25b ± 0.31 (1–3.3)
		S5	1.76 ± 0.44 (0.3–5.3)	1.52 ± 0.50 (1–4.3)
Melanoma *in situ*	20	S1	5.29c ± 3.60 (3–11)	
		S5	6.40 ± 3.90 (4–13)	
Invasive melanoma <0.8 mm	22	S1	8.60c ± 7.10 (3–19)	8.40c ± 7.70 (3–28)
		S5	10.25 ± 6.10 (4–23)	10.3 ± 9.30 (4–29)
Invasive melanoma >0.8 mm	42	S1	9.90c ± 5.50 (3–19)	19.7c ± 8.3 (4–35)
		S5	13.25 ± 7.80 (4–26)	23.2 ± 8.7 (5–43)
Metastatic melanoma	18	S1		29.6d ± 19.4 (9–48)
		S5		37.8 ± 17.0 (8–66)

Items represent numbers of cases (n) and the percentage of positive tumor cells expressed as mean values ± standard deviations and ranges in parentheses (a < b, $P < 0.05$; b < c < d, $P < 0.01$).

stage. Strikingly, melanoma cells in the basal and suprabasal cell layers displayed a brisk proliferative activity that virtually disappeared with the ascendence of neoplastic cells into the granular or cornified layer (Figures 1f and 2a). Figure 3a shows a graphic representation of the results. Interestingly, the main fraction of proliferating cells was located in the epidermal and junctional compartment during early vertical growth phase (Figure 1f), whereas a reversal occurred approximately at Clark's level III, resulting in a massive overgrowth in the dermal part of the tumors. However, invasive melanomas presented as a highly heterogeneous population. Some level IV tumors exhibited a low proliferative activity, whereas flat invasive melanomas of Clark's level II occasionally peaked to more than 20% proliferating cells. Moreover, proliferation was often focal within one tumor sample, suggesting the coexistence of biologically dissimilar clones (Figure 2c). The average proliferation in relation to Clark's level is represented in Figure 3b.

Typically, there were foci of increased proliferation in all invasive tumors, mostly located at the periphery of dermal infiltrates. On the other hand, cytological atypia did not necessarily coincide with proliferation; in cases of nodular melanoma, large anaplastic cells were found to be negative for Ki-S5, whereas the surrounding small, moderately atypical tumor cells showed strong nuclear labeling (Figure 2d).

Frequently, the epidermal and adnexal keratinocytes displayed an increased proliferative activity in the vicinity of melanoma and of larger compounds of nevus cells compared with the normal proliferation index at the section borders.

Mitotic figures were occasionally seen in both common and dysplastic nevi. They were generally located in the epidermal or junctional compartment and frequently became apparent only in step sections. Their number was slightly increased in Spitz's/Reed's nevi but never exceeded four in the totality of sections from one tumor. It seems remarkable that in all but one cases of melanoma *in situ*, no mitotic figure was detected even in multiple sections. In invasive melanomas, mitotic figures were often numerous (up to 25 per high power field), but in some tumors, only a few or none at all were observed despite the expression of both proliferation-associated antigens in more than 20% of the tumor cells. Thus, even though there was a partial concordance of the proliferation index and the mitotic count, no linear correlation could be established between these parameters.

HMB-45 expression was present with variable intensity in all lesions and usually more accentuated in the epidermal/junctional compartment. Only invasive melanomas displayed a slightly increased positivity in the dermal tumor component, and the majority of blue nevi reacted strongly with the antibody. The pattern of distribution was not distinctive, and there was no correlation with the proliferative activity. The results are summarized in Table 3.

Discussion

Purely morphological criteria, such as growth pattern, nuclear atypia, and mitotic count, are increasingly being complemented by the employment of

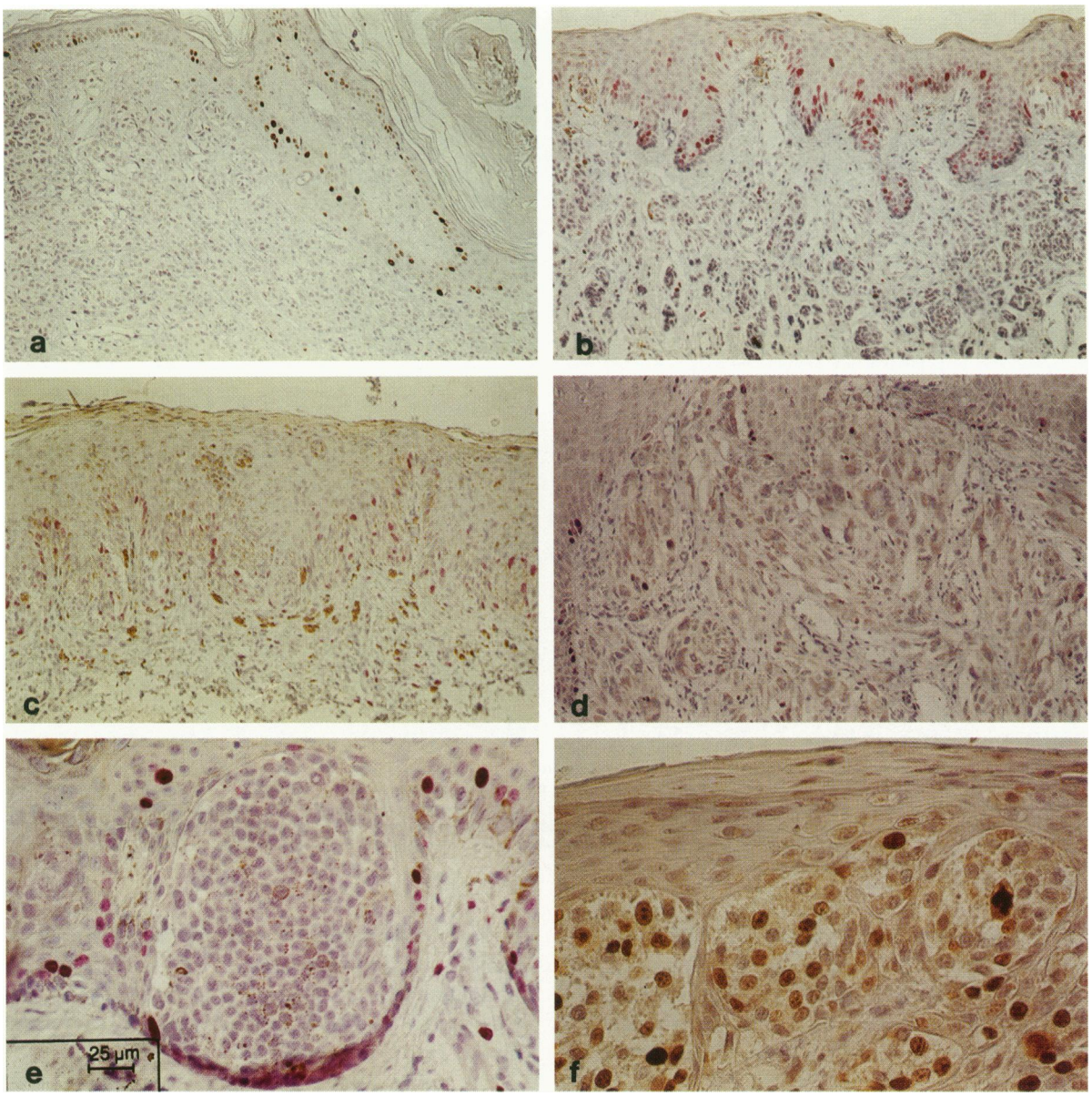

Figure 1. *Ki-S5 immunoreactivity in nevi (APAAP technique, red label; streptavidin-biotin-peroxidase technique, brown label). No proliferative activity is seen in the cells of a dermal nevus, whereas multiple basal keratinocytes are labeled* (a, ×*140*); *only a few scattered cells of a common acquired compound nevus show immunoreactivity* (b, ×*140*); *the proliferative activity is slightly increased in the junctional nests of a dysplastic nevus* (c, ×*140*); *in Spitz's nevus, despite a striking nuclear atypia, not more than 2% of the nevus cells are labeled* (d, ×*350*). *Note the virtual absence of proliferating cells in a dysplastic nevus* (e, ×*200*) *compared with the brisk proliferative activity in the nest-shaped compounds of tumor cells of a flat melanoma* (f, ×*350*).

additional variables of possible prognostic significance. Among these, the evaluation of the growth fraction has been proven to be the most reliable and independent indicator of prognosis. In a large number of studies, the proliferative activity of tumors could be significantly correlated to survival rates and disease-free interval for several types of carcinoma,[54–56] lymphomas,[57] and soft tissue tumors[58] as well as malignant melanoma.[59]

The qualities and limitations of the presently available techniques pertaining to cellular proliferation have been thoroughly discussed elsewhere.[40,60,61] The advantage of the present investigation consists of the combination of a comparatively easy quantitation of cellular growth with a well preserved morphology.

We used two novel monoclonal antibodies directed against different nuclear antigens associated with cellular proliferation. Ki-S5[39] binds to a formalin-resistant epitope of the Ki-67 antigen,[41,42] yields identical results in fresh material and fixed tissues,[39] and, unlike Ki-67, does not cross-react with cytoplas-

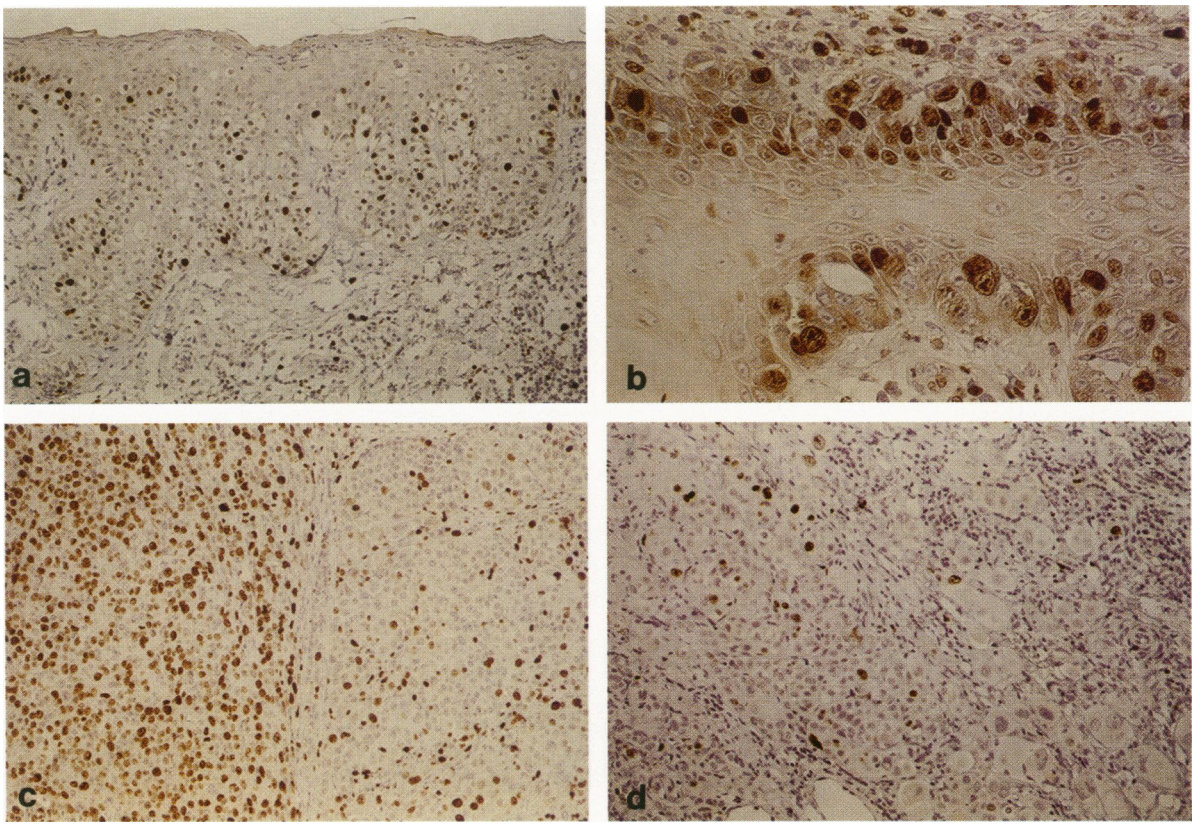

Figure 2. *Ki-S5 immunoreactivity in malignant melanoma (streptavidin-biotin-peroxidase technique). Numerous proliferating cells are seen in melanoma* in situ (**a**, *×140); note the decrease of tumor cell proliferation in the upper epidermal layers. More than 90% of the atypical melanocytes in a melanoma* in situ *of lentigo maligna type are actively proliferating* (**b**, *×350). Intra-tumor heterogeneity was frequently observed, as in this case of nodular melanoma* (**c**, *×140) exhibiting neatly separated foci of high* (**left**) *and low* (**right**) *proliferative activity. Occasionally, the growth fraction was restricted to small, moderately atypical melanoma cells* (**left**), *whereas large polymorphous nuclei* (**right**) *were unlabeled, indicating that proliferation is not necessarily correlated to tumor cell morphology* (**d**, *×140).*

mic antigens of epithelial cells. Ki-S1 reacts with a 170-kd protein expressed in late G_1, S, and G_2/M phase of the cell cycle, which we recently identified as human topoisomerase IIα[43] (also, R Parwaresch, HJ Heidebrecht, U Kellner, F Buck, M Domanowski, H-H Wacker, M Tiemann, J Felgher, P Rudolph, submitted for publication). The epitope recognized by Ki-S1 is characterized by a very low sensitivity to fixation and aggressive enzymatic tissue digestion, and its expression was found to be related to the histological and nuclear grade as well as to the clinical outcome in breast cancer.[51,62]

Our results show a linear correlation with a coefficient of $r = 0.85$ between the expression of the two

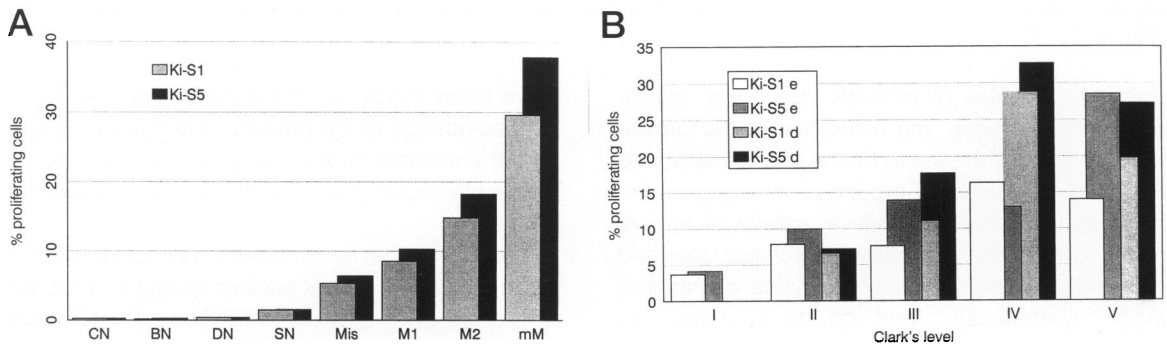

Figure 3. a: *Average proliferation rates in 314 cases of melanocytic tumors as assessed by Ki-S1 and Ki-S5. CN, common nevi; BN, blue nevi; DN, dysplastic nevi; SN, Spitz's/Reed's nevi; Mis, melanoma* in situ*; M1, thin melanoma; M2, thick melanoma; mM, metastatic melanoma.* b: *Average proliferation of malignant melanomas in relation to Clark's level as assessed by Ki-S1 and Ki-S5. e, epidermal; D, dermal compartment.*

Table 3. *Expression of HMB-45 in Melanocytic Tumors*

Diagnosis	+++	++	+	−
Junctional nevus	2/25	4/25	5/25	14/25
Compound nevus (epidermal compartment)	21/43	6/43	11/43	5/43
Compound nevus (dermal)	0/33	4/33	17/33	22/33
Dysplastic nevus (junctional type)	3/7	2/7	2/7	0/7
Dysplastic nevus (epidermal)	21/43	14/43	7/43	1/43
Dysplastic nevus (dermal)	0/43	6/43	18/35	19/35
Dermal nevus	0/34	1/34	2/34	31/34
Blue nevus	4/32	7/32	12/32	9/12
Spitz's nevus (epidermal)	2/22	11/22	9/22	0/22
Spitz's nevus (dermal)	0/22	0/22	15/22	7/22
Reed's nevus (epidermal)	2/4	1/4	1/4	0/4
Cutaneous melanoma (epidermal)	31/60	18/60	8/60	3/60
Cutaneous melanoma (dermal)	11/65	29/65	23/65	2/65
Metastatic melanoma	12/18	3/18	2/18	1/18

antigens in all tumor subgroups. The percentage of Ki-S1-positive cells was constantly lower compared with Ki-S5 values, with the single exception of level V melanomas. This is readily explained by the fact that the Ki-67 antigen is expressed during the whole cell cycle, whereas topoisomerase IIα becomes first detectable in late G_1 phase and disappears soon after the cells exit from mitosis. The comparatively higher expression in level V melanomas may be a result of a lesser sensitivity of the Ki-S1 antigen to fixation artifacts that are likely to occur in larger tumor masses.

We found no significant difference between the growth fractions in common, dysplastic, and blue nevi, whereas an abrupt increase occurred in melanoma *in situ* ($P < 0.01$). These findings are in accordance with the well-founded observations of Slater and colleagues.[35] Spitz's/Reed's nevi occupied an intermediate position, as already documented by earlier investigations.[36,37] However, the percentage of proliferating cells assessed by immunohistochemistry in the current study was considerably lower than the values described in the aforementioned papers and, contrary to their data, proliferation was more elevated in the epidermal than in the dermal compartment of compound-type lesions. This discrepancy may be explained by the use of proliferating cell nuclear antigen as a marker of proliferation. Although proliferating cell nuclear antigen (cyclin) is indubitably associated with cellular proliferation,[63] it is also involved in DNA repair, and its expression may be induced by any kind of DNA damage even in quiescent cells.[64–66] In the case of nevi, this would be congruent with the concept of an increased DNA fragility and vulnerability by UV irradiation.[67] Whether the dermal fraction of nevocytes is more sensitive, or whether the second divergence is merely a result of the fact that we regarded nest-shaped melanocytic compounds immediately contiguous to the epidermis as junctional unless they were clearly separated by a band of collagen, is a matter of conjecture.

We also found a consistently higher proliferative activity in the epidermal compartment of thin invasive melanomas (Clark's level II) than in the dermal compartment, whereas at later stages a burst of proliferation became apparent in the dermal tumor cell fraction. This phenomenon could be explained by the tumor regression that is frequently associated with the transition from radial to vertical growth phase, as observed by Barnhill and colleagues.[68] Indeed, the cells of radial growth phase melanomas appear to be sensitive to growth-inhibitory cytokines secreted by endothelial cells, such as interleukin-6, whereas further tumor progression seems to require the selection of cytokine-resistant cell variants.[69] In this way, the release of angiogenic factors by melanoma cells may produce an anti-proliferative, or even suicidal, effect on the tumor cells at early stages of the disease, resulting in histologically appreciable regressive change. On the other hand, once the tumor cells have become refractory to inhibitory signals, angiogenesis may further promote tumor growth by providing an adequate blood supply.[69] In this view, it could be of interest to examine the proliferative activity of tumors in relation to their vascularity.

Despite a statistically significant correlation with Breslow's thickness and to some extent with Clark's level, a strong inter-tumor heterogeneity was noted in invasive (vertical growth phase) melanomas. Similar results have been reported by Kaudewitz and coworkers[70] already in 1989, intimating the possibility of an alternative concept for the classification of melanoma. In fact, the traditional prognostic models[71,72] may soon be replaced by alternative criteria, eg, a rapid and precise evaluation of the proliferative activity. In this study, an average growth fraction was assessed for all tumors, regardless of highly prolif-

erating foci. The phenomenon of intra-tumor heterogeneity has been pointed out in other studies,[62,70] and it remains to be determined whether these areas of actively dividing cells would influence the clinical course rather than the mean proliferation index, which also takes into account the central parts of tumor tissue, where many cells may have undergone regressive change, occasionally resulting in pronounced cellular anaplasia unrelated to proliferation.

Contrary to the hypothesis that malignant transformation of melanocytic tumors occurs in a stepwise manner, reflected by increasing architectural and cytological atypia, our findings provide no evidence of a continuous evolution into malignancy in terms of a correspondingly augmenting cellular growth. The former conclusions are deduced from the concept of progradient dysplasia in squamous epithelium, for which a concomitant increase of the proliferative activity in keratinocytes could be verified.[73] Keratinocytes, however, grow in tight complexes and are subject to a rapid turnover, whereas melanocytes are essentially migratory cells[74] that occasionally may assemble in nest-shaped compounds but are not known to proliferate under normal conditions. Squamous epithelium proliferates, matures, and undergoes senescence with eventual apoptosis in a genetically regulated manner, whereas melanocytes are likely to represent long-lived cells that may be stimulated by UV irradiation. This activation seems to be reflected by an increased expression of the HMB-45 antigen that, in our study, was unrelated to cellular proliferation. Thus, an analogy between these two cell populations regarding their biological behavior is not compelling.

Despite the improvement of clinical diagnosis,[75] the final decision has to be made on the basis of histopathological examination. The gross diagnosis of atypical nevi may be poorly correlated with the histomorphological features,[76] and even the prognostic impact of the microscopic finding of dysplasia in a nevus remains debatable. A large number of investigations using a variety of methods have failed so far to provide definitive proofs of a stepwise evolution into malignancy, and the relevance of so-called dysplastic nevus remnants in contiguity with malignant melanoma has already been contested[2] as these changes could be secondary to malignancy. Our findings, revealing an increase of proliferative activity in the squamous epithelium adjacent to larger melanocytic lesions, consistent with the often observed epidermal hyperplasia, support the theory that the secretion of growth factors by melanocytes, ie, melanocytic growth-stimulatory activity,[77,78] may be responsible for an increased proliferation of surrounding tissues. Consequently, we suggest that, at least for isolated lesions, the histopathological diagnosis might as well be restricted to whether a melanocytic lesion is nevus or melanoma. In cases of equivocal histology, the immunohistochemical assessment of proliferation may prove to be helpful.

In our terminology concerning melanocytic proliferations we have endeavored in maximal simplicity, limiting ourselves to the initial nomenclature of so-called precursor lesions to determine their biological significance. This is why the terms of common nevus, dysplastic nevus, melanoma *in situ*, and invasive melanoma were used exclusively. We come to the conclusion that sporadic dysplastic nevi might as well, if this designation seems too implicating, be named Clark's nevi as proposed by Ackerman,[10] as no proof of premalignancy could be established and similar histological features are likely to occur also in common nevi of compound type.[79]

We conclude further that the overwhelming majority of melanomas probably arise *de novo* and originate from a cell population that may be wholly different from common nevocytes. The proliferative activity correlates well with Breslow's thickness and to some extent with Clark's level. However, on account of the marked heterogeneity between different tumors, the immunohistochemical assessment of the growth fraction, being far more reliable than the mitotic count, may prove to have a stronger bearing on prognostic considerations than the hitherto applied criteria.[72,80] Extensive retrospective studies will be needed to establish its exact significance. These will be facilitated by the now available antibodies suitable for archival material.

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