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Melanoma in situ (MIS) adjacent to an invasive nodular melanoma ("SSM/NM") and its metastases – DNA-cytophotometry, mitotic index, and anisokaryosis

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Summary. Malignant melanomas of the superficial spreading type usually have an intraepidermal tumour component in their periphery which frequently displays the morphological features of a melanoma in situ (adjacent MIS). It is thus comparable to exclusively epidermal melanomas; melanoma in situ (MIS). Taking 10 superficial melanomas with a nodular component ("SSM/NM") 31 adjacent MIS regions and 36 nodular melanoma components were analysed in serial tissue slides. Planimetric estimation of the nuclear areas was employed as a measure of anisokaryosis. DNA-Feulgen-cytophotometry was applied to obtain an objective variable in judging malignancy in the DNA-histographs (paraffin material). Furthermore we investigated 8 metastases of one of the malignant melanomas applying the methods described. A comparison of the epidermal with the invasive tumour components revealed an increase in the nuclear area which, however, decrease from the superior to the inferior nodular regions and which are further reduced in melanoma metastases. Anisokaryosis is evidently less in metastases compared with all primary melanomas. The nuclear DNA-content increases from the epidermal to the invasive tumour compartments and is lower in the inferior nodular regions when compared with the superior ones. No further significant differences are, however, established in the metastases. The coefficients of variability of the DNA-contents, being a potential indicator of DNA-heterogeneity reflect higher values in the epidermal tumour components compared with the nodular regions, decreasing from the superior to the inferior nodular parts of the tumour. All metastases have smaller values than the respective primary melanoma. In the DNA-histographs 75% of the intraepidermal tumour components have obvious signs of malignancy including tumour cell stem lines in 19% of the cases. 85% of the nodular regions investigated have clear signs of malignancy, 33% of which also have uneuploid stem lines. All metastases have obvious signs of malignancy and tumour cell stem lines in 50% of the cases observed. The following conclusions can be drawn from our findings: DNA-Feulgen-cytophotometry and nuclear planimetry are additional feasible methods for judging the epidermal component of a melanocytic lesion as malignant (adjacent melanoma in situ) on paraffin material. Furthermore these methods give different results in invasive nodular versus epidermal (in situ) melanoma components. Both the DNA-histographs and our immunohistochemical investigations (monoclonal antibody *P* 3.58) indicate the malignant potential of adjacent MIS. Great DNA-heterogeneity both in primary melanomas and their metastases are of particular interest with regard to the possible predictibility of probability of metastases. This cannot be derived from our present findings.

Key words: Melanoma in situ (MIS) – Adjacent MIS – Invasive melanoma – Melanoma metastases – Topographical DNA-cytophotometry

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Introduction

The term "superficial spreading melanoma" implies that malignant cells spread horizontally within the epidermis and the upper dermis (Clark et al. 1969; 1977). In fact, there are often intraepidermal melanocytic cells singly or grouped in atypical nests to be found in the marginal zones of malignant melanomas. The question arises as to whether intraepidermal malignant melanoma cells in the

periphery of the tumour derive from the tumour center or whether they are possibly autochthonous primary melanoma cells of a different cell clone (heterogeneity). Secondly, it can be questioned if they are malignant at all, since they sometimes possess the pattern of a so-called dysplastic nevus (Ackerman 1980; 1982; Crucioli and Stilwell 1982; McGovern 1983). Whether these marginal melanocytes are transformed to malignant cells "in situ" by the same tumour progression mechanisms as in the tumour center and whether the cells of the tumour center which have already been transformed have any transforming effects on the adjacent melanocytes is unclear. In addition whether or not there is centrifugal tumour cell migration, can all only be matters of discussion or mere speculation at present (Clark et al. 1977; Ackerman 1982; Rhodes et al. 1983; Van der Kamp and Jaspers 1984).

When comparing strictly intraepidermal melanocytic lesions such as junctional nevus, dysplastic nevus, and melanoma in situ with the intraepidermal margins of invasive melanomas, in order to find criteria for judging an intraepidermal melanocytic lesion as benign or malignant, we have to consider the possibility that malignant transformation might assume a different form in exclusively epidermal (melanoma in situ = MIS) and in the intraepidermal melanocytic components adjacent to invasive melanoma (adjacent MIS). Morphological comparison of the histological pattern, nuclear planimetry, DNA-Feulgen-cytophotometry (Schmiegelow et al. 1986b), and topographical immunohistochemistry using monoclonal antibodies (Nüßgen et al. 1986) in adjacent MIS is of great interest as the adjoining invasive tumour center demonstrates a malignant potential for the lesion (Schmiegelow et al. 1986a).

In 10 superficial melanomas with a nodular component ("SSM/NM"), 17 intraepidermal tumour regions and 10 melanoma nodules were investigated with respect to anisokaryosis (nuclear planimetry) and aneuploidy in the DNA-histographs (Feulgen-cytophotometry) analyzing adjacent intraepidermal melanocytic components (adjacent MIS) in 31, and nodular regions in 36 serial tissue slides from the 10 cases.

In comparing intraepidermal, nodular, and the 8 metastatic tumour regions which derived from one of the 10 melanomas investigated, we tried to ascertain if there were unequivocal signs of malignancy in the DNA-histographs. We also sought rules of increasing or decreasing anisokaryosis or changes in the degree of nuclear DNA-uneuploidy in these melanocytic neoplasms.

Material and methods

10 melanomas, each composed of a central tumour nodule with 1 or 2 intraepidermal marginal zones, were clinically diagnozed and excised (E.W.B.) in the Dermatological Clinic of the University of Hamburg. The histological diagnoses of the melanomas were made (M.J., P.S.) according to the classification evolved by Clark and his colleagues; tumour thickness in mm was evaluated according to Breslow.

The tissue was fixed in 4% neutrally buffered formalin for 48 h at a temperature of 4° C. After dehydration it was embedded in paraffin wax. 7.5 µm thick sections were cut using a Reichert-Jung microtome and Feather® disposable microtome blades. The sections were mounted on slides and deparaffined. After hydrolysis in 1 n HCl (pH 1.2) for 15 min at 60° C they were stained with Schiff's reagens for 30 min at 25° C in the dark and then put into $Na_2S_2O_5$ for 30 min. Following dehydration the cover-glass was fixed with Eukitt® (refractive index: 1:1.495).

A section thickness of 7.5 µm appeared to be appropriate to our research, as these slides contained a sufficient number of complete nuclei and relatively few overlapping nuclei, factors which are prerequisites for cytophotometrical analysis. By focussing the nucleus it was made sure that the entire nucleus was located within the slide. The transmission measurements were carried out at the level of maximum length and breadth of the nuclei. This enabled us to determine the DNA-content and also served as a parameter for the anisokaryosis (variation of nuclear size).

The measurements were carried out (T.O., R.S.) with a microscope-photometer MPV 3 (Leitz, Wetzlar) using the plug-method at a wave-length of 587 ± 9.5 nm under constant optical and electronic conditions. The length and breadth of each measured nucleus was determined. The nuclear area was calculated according to the formula $F = \pi \times a \times b$. The proper application of this formula is normally confined to those nuclei which are of a perfectly oval shape. However, since the heterogeneity of nuclear shape of the cells which were investigated was relatively small, this formula nevertheless appeared to serve our purpose adequately. The transmission T of the Feulgen-stained nucleus was determined and employed for the calculation of the extinction e according to the formula $e = \log 1/T$. The multiplication of extinction and nuclear area gave as a result an approximate value for the DNA-content of the nucleus in arbitrary units (au). One of the computers in our Calculation Center, which is under the direction of J. Berger, was employed for this calculation as well as for the printout of the DNA-histographs (programme by Jens Bahnsen, cf. Schmiegelow et al. 1986b).

Due to the fact that one of the patients died shortly before the begin of these studies, 8 metastases of the autopsy material were investigated using the same methods (case 9).

The interpretation of the DNA-distribution patterns was carried out applying the usual criteria relating to DNA-histographs. We divided the DNA-histographs into 3 ploidy levels (diploid/hyperdiploid and tetraploid/hypertetraploid).

For the purpose of statistical evaluation (A.N., T.O., P.S.) we compared the intra-individual differences of the nuclear areas as well as of the coefficients of variability of the nuclear areas in the epidermal, invasive nodular and metastatic melanoma regions. The inter-individual comparison was performed using only the intra-individual differences between epidermal and nodular or nodular and metastatic melanoma regions respectively.

Besides the statistical evaluation of the average nuclear DNA-contents and the respective coefficients of variability of the DNA-contents, each DNA-histograph was interpreted according to the usual criteria for benign, proliferative, suspect

and malignant DNA-distribution. A $2n$ distribution is typical for a benign lesion. A $2n$ distribution with additional number of nuclei between the diploid and the tetraploid range, i.e. with a greater fraction of cells in the S-phase of the cell cycle, was regarded as an expression of an increased proliferative activity: "P". A loss of the $2n$ peak without further unequivocal signs of malignancy as described below was interpreted as suspect: "(M)". A loss of the $2n$ peak combined with broad DNA-distribution patterns beyond $4n$ and possibly with uneuploid tumour cell stem lines "(St)" represent signs of malignancy in terms of Feulgen-cytophotometry: "M". In order to obtain more topographical information about the variability of the parameters determined, each case was analyzed in 2 section levels separated by 10 sections which were not included in our investigations.

The nuclear areas of the topographically defined regions were compared using their average values.

"Anisokaryosis" was linked to the coefficient of variability of nuclear areas of each region.

The nuclear DNA heterogeneity was determined by the coefficient of variability of the DNA-contents and furthermore by the variability of the DNA-histographs of each region, the latter taking particular account of the number of tumour cell stem lines and cells above $6n$.

Results

The average values of the *nuclear areas* in the nodular, as compared to the intraepidermal, tumour regions were seen to be significantly higher in 6 out of 10 cases (Table 1). The superior parts of the nodular tumour regions have significantly higher average nuclear areas in 7 of the 10 SSM/NM than the inferior nodular regions (Table 5).

In contrast to the above, the SSM/NM with widespread metastases (case 9) reflected significantly higher values in the inferior nodular region than in the superior nodular parts (cf. Table 5a).

The average nuclear areas of the 8 metastases of SSM/NM case 9 have significantly higher values than the intraepidermal or the nodular regions in the corresponding and all other primary SSM/NM which were investigated (Table 2).

In case 10 there is a superficially invasive tumour part in the upper dermis which shows significantly smaller nuclear areas than the adjacent intraepidermal tumour part.

The coefficient of variability (c.v.) of the nuclear areas, called anisokaryosis, shows variable values in the intraepidermal and in the nodular tumour regions with no uniform tendency being reflected by a comparison of regional differences (Table 1).

It is surprising to note that the coefficient of variability of the nuclear areas of all 8 metastases of case 9 shows significantly lower values than all regions of all primary melanomas which were the subject of investigation (Table 2).

In comparison with the intraepidermal tumour regions, the average nuclear *DNA-content* with regard to nodular components is either higher or remains constant in 8 of the 10 cases with significant differences in 5 cases; in case 3 the reverse situation occurs (Table 3). Compared with the

Table 1. Nuclear areas in 10 "SSM/NM". $\bar{X}$ = mean values; c.v. = coefficient of variability (in brackets); ← = evident difference

Nuclear areas ($\bar{X}$ & c.v.) in SSM/NM			
	Intraepitermal		Nodular
1. (79.572 I)	40.03 (30.85)		39.07 (29.89)
2. (76.943 I)	40.10 (32.99)		42.86 (35.51)
3. (77.024 A)	54.99 (32.76)		56.16 (33.58)
4. (77.798)	34.16 (28.07)	←	37.24 (33.29)
5. (82.687)	37.10 (33.20)	←	43.49 (36.16)
6. (77.019)	39.44 (27.70)	←	43.36 (31.7)
7. (76.430)	26.31 (38.80)		27.71 (28.40)
8. (78.091 I)	42.03 (25.57)	←	50.43 (27.00)
9. (80.480)	36.91 (47.30)	←	44.38 (41.39)
10. (81.261)	66.28 (50.85)	←	71.11 (29.30)

Table 2. Nuclear areas in 8 metastases compared to the respective primary melanoma (case 9). $\bar{X}$ = mean values; c.v. = coefficient of variability (right side)

Nuclear areas ($\bar{X}$ & c.v.) in Metastases		
I	46.73	26.51
II	57.67	28.59
III	55.05	23.05
IV	54.37	30.20
V	47.59	25.36
VI	58.29	25.15
VII	62.12	27.25
VIII	61.79	25.00
prim. intraep.	36.91	47.30
nod.	44.38	41.39

Table 3. DNA-content in 10 "SSM/NM". $\bar{X}$ = mean values; c.v. = coefficient of variability (in brackets); ←, → = evident difference

DNA-content ($\bar{X}$ & c.v.) in SSM/NM			
	Intraepidermal		Nodular
1. (79.572 I)	2.56 (44.36)		2.52 (36.33)
2. (76.943 I)	2.41 (36.91)	←	2.84 (59.89)
3. (77.024 A)	3.28 (39.09)	→	2.99 (31.94)
4. (77.798)	2.39 (33.72)		2.48 (34.39)
5. (82.687)	2.16 (34.41)		2.34 (38.50)
6. (77.019)	4.57 (36.65)	←	4.93 (37.3)
7. (76.430)	3.21 (38.5)		3.48 (43.3)
8. (78.091 I)	2.37 (42.61)	←	3.28 (39.01)
9. (80.480)	3.56 (45.34)	←	4.32 (42.46)
10. (81.261)	3.12 (53.26)	←	3.66 (34.53)

Table 4. DNA-content ($\bar{X}$ = mean value), DNA-histograph (M = malignancy, St = stem lines), and coefficient of variability (= c.v.) of DNA in 8 metastases compared to the respective primary melanoma (case 9)

DNA-content ($\bar{X}$ & c.v.) in Metastases			
	Nuclear DNA		
	$\bar{X}$	Histographs	c.v.
I	4.34	M (St)	37.58
II	3.39	M	35.38
III	4.01	M (St)	35.96
IV	4.35	M (St)	33.66
V	4.22	M (St)	23.89
VI	5.23	M	43.90
VII	3.64	M	31.29
VIII	4.25	M	35.06
Prim. epid.	3.56	M (St)	45.34
nod.	4.32	M (St, St)	42.46

other SSM/NM, case 6 and case 9 have the highest absolute values in the intraepidermal and the nodular tumour regions. In all but two of the 8 metastases, the DNA-content is about the same as in the nodular parts of the corresponding case 9 (Table 4). In 8 out of 10 cases, the inferior parts of the nodular melanoma regions either have similar or lower values than the superior nodular parts (Table 5). However, in case 9 we find significantly higher DNA-contents in the inferior as compared to the superior nodular tumour region (Table 5).

In 7 out of 10 cases, the coefficient of variability (c.v.) of the DNA-content in the epidermis is higher or equivalent to the nodular parts. The remaining 3 cases (cases 2, 5 and 7) reflect an inverse relation with significant differences being established in some instances.

In 9 out of 10 cases, the upper parts of the tumour nodules have higher or equivalent values as compared to the lower nodular parts, but an inverse relation is established with regard to case 6 (Table 5c). The c.v. of the DNA-content in all but one (case 6) of the 8 metastases is significantly smaller than in the corresponding primary melanoma (Table 4). The 6th metastasis (lung) has an average DNA-content which is higher than all other metastases and all other primary melanoma regions as well as a higher DNA-heterogeneity than the other metastases which is equivalent to the corresponding nodular melanoma regions of case 9 (Table 4).

The two different serial sections mentioned above enabled us to draw up two *DNA-histographs* of each tumour region which was analyzed. Well defined signs of malignancy by means of DNA-histographs were established in 12 of the 16 intraepidermal tumour margins and in 17 of the 20 superior and inferior nodular melanoma regions (Table 6). In case 1 one intraepidermal region shows malignancy whilst the other does not. In 6 intraepidermal regions only in one of the two slide sections are clear signs of malignancy found but not in the other serial slide (Table 6).

DNA-histographs with values above $6n$ are found in 18 of the 31 intraepidermal (i.e. 58%) and in 24 of the 36 nodular tumour parts (i.e. 67%) which were investigated. Tumour cell stem lines were found in 6 of the 31 intraepidermal (i.e. 19%) and in 12 of the 36 nodular tumour regions (i.e. 33%) which were investigated (Table 6). In one case a tumour cell stem line was found at the same

Table 5. Nodular components of 10 "SSM/NM": Mean values of nuclear areas, DNA-content and DNA-heterogeneity (= c.v. of DNA-content) comparing the superior and the inferior portion. ←, → = evident difference

SSM/NM: Nodular components									
	a Nuclear areas			b DNA-content			c DNA-heterogeneity		
	Superior		Inferior	Superior		inferior	superior		Inferior
1. (79.572 I)	41.08	→	37.08	2.52		2.52	37.89	→	34.59
2. (76.943 I)	47.49	→	38.28	3.57	→	2.11	49.22	→	40.86
3. (77.024 A)	67.26	→	45.06	3.22	→	2.77	31.46	→	26.71
4. (77.798)	41.57	→	32.92	2.61		2.34	36.88	→	29.22
5. (82.687)	52.09	→	34.90	2.72	→	1.98	34.77		34.45
6. (77.019)	50.62	→	36.1	5.43	→	4.42	32.6	←	42.0
7. (76.430)	27.42		28.0	3.37		3.58	45.8	→	40.7
8. (78.091 I)	51.51		49.36	3.28		3.28	41.21	→	36.33
9. (80.480)	36.80	←	51.96	3.98	←	4.68	48.75	→	33.83
10. (81.261)	75.82	→	66.41	3.42		3.91	33.54		31.85

Table 6. DNA-histographs of 10 "SSM/NM" comparing the both epidermal with the nodular component (superior & inferior portion). IV, V = Clark Level IV resp. V. 1.29 ... = vertical tumour diameter acc. to Breslow. P = signs of proliferation; (M) = suspect; M = obvious signs of malignancy; (St) = tumour cell stem line

DNA-histographs in SSM/NM				
	Epidermal		Nodular	
	Left	Right	Superior	Inferior
1. (79.572)	P	M	M	M
IV/1.29	P	M	M	M
2. (76.943)	M	M	M (St)	(M)
IV/1.30	(M)	M	M	M
3. (77.024)	(M)	M	M	(M)
IV/1.42	P	M (St)	(M)	(M)
4. (77.798)	(M)	M (St)	(M)	(M)
IV/1.68	(M)	M	M	M (St)
5. (82.687)	(M)	M	M (St)	(M)
IV/2.75	P	P	M	P
6. (77.019) IV/2.00	M	M (St)	M (St)	M
7. (76.430) IV/1.10	M (St)	–	M	(M)
8. (78.091)	P		(M)	M (St)
IV/4.34	M	–	M	M
9. (80.480)	M		M	M (St)
IV/7.01	M (St)	–	M (St)	M
	epid.	early invas.		
10. (81.261)	M	M	M (St)	M (St, St)
V/4.12	M (St)	M	M (St)	M (St)

point in both the intraepidermal as well as the nodular part of the same case (case 6). In case 10 unequivocal signs of malignancy were found in the intraepidermal, the superficial invasive as well as in the upper and the lower nodular tumour parts; all nodular regions of this case have tumour cell stem lines (Table 6). Comparison of the intraepidermal and the nodular tumour parts revealed interesting divergences: In cases 2, 3 and 7, all of the intraepidermal regions showed clearer signs of malignancy than the nodular regions (Table 6). More obvious signs of malignancy in the nodular as compared with the intraepidermal tumour parts can be found in cases 4 and 5, whereas totally malignant DNA-histographs were established in cases 6, 9 and 10 (Table 6), the latter two cases being metastatic.

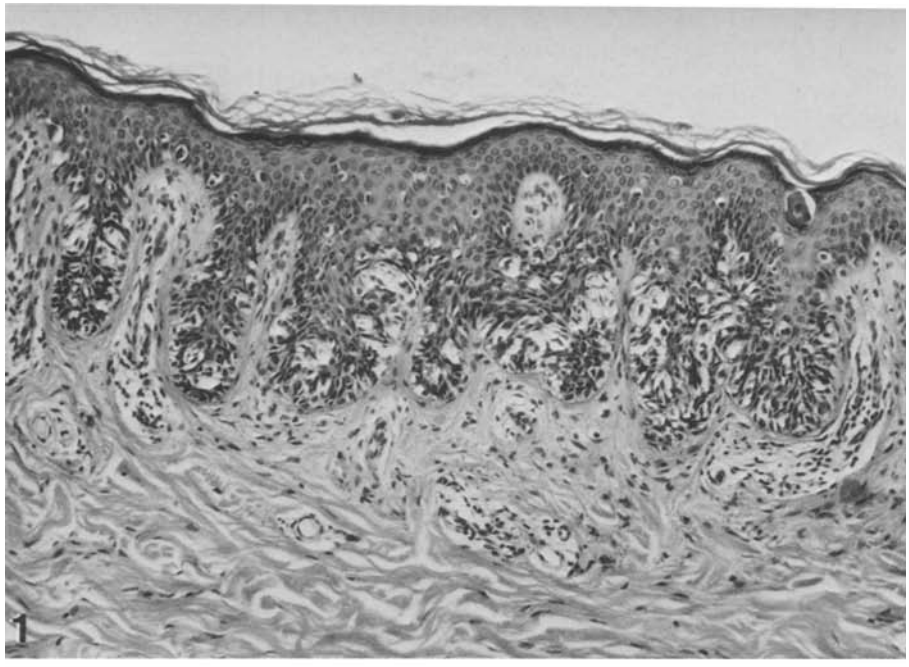
The DNA-histographs of the 8 metastases of case 9 showed very heterogeneous distribution patterns even if they derived from metastatic regions of the same organ such as the lung (metastases 4–6). All 8 histographs have DNA-values above 6*n*. Two tumour cell stem lines were found in both the lung as well as in lymph nodes, these being the predominant metastatic locations in this case (Table 4). None of the tumour cell stem lines which were found in the metastases had been observed in the histographs of their respective primary melanoma, but the histographs of the 3rd metastasis (lymph node) resembles that of the superior nodular region of the primary melanoma. Thus, with regard to the tumour cell stem lines in the DNA-histographs, there is a great heterogeneity in all primary and metastatic melanoma regions which were investigated. A mathematical analysis of the DNA-histographs did not seem appropriate to the authors.

Discussion

Hence the treatment of metastasising malignant melanoma is a frustrating experience for both the patient as well as the physician, current medical interest is concentrated on a diagnosis of early cases of melanoma (melanoma in situ) and on the recognition of (putative) precursors of malignant melanoma (Ackerman 1980; Rhodes et al. 1983; Schmiegelow et al. 1986a). We are in need of objective and reproduceable indicators for judging whether a questionable melanocytic precursor lesion is malignant or benign.

In addition to the histological pattern there are qualitative cytological aspects, such as nuclear hyperchromatism and cellular pleomorphism, which separate a melanoma in situ (MIS) from a presumably benign melanocytic lesion (Clark et al. 1969; Ackerman 1982; McGovern 1983). As far as objective cellular parameters for the appraisal of a questionable intraepidermal melanocytic lesion are concerned, DNA-Feulgen-cytophotometrical investigations of 15 so-called dysplastic nevi (DN) revealed almost normal DNA-histographs with additional signs of activated proliferation (Schmiegelow et al. 1986b) whereas in this study the intraepidermal margins (referring as adjacent MIS) of malignant melanomas showed obvious signs of malignancy in their DNA-histographs in 12 of the 16 cases investigated. The nuclear areas and their coefficients of variability (anisokaryosis) also had very different values when comparing the so-called dysplastic nevi and intraepidermal margins of malignant melanomas (Schmiegelow et al. 1986b).

A topographical comparison was made of the intraepidermal tumour margins with the superior and inferior regions of the nodular tumour components. 8 metastases of one of the 10 melanomas were investigated using nuclear planimetry and



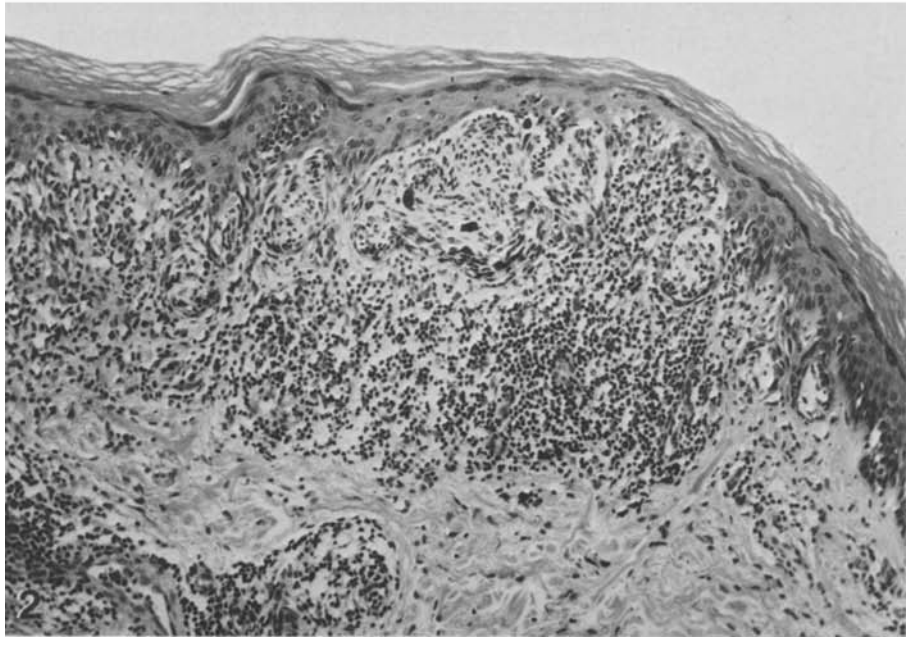
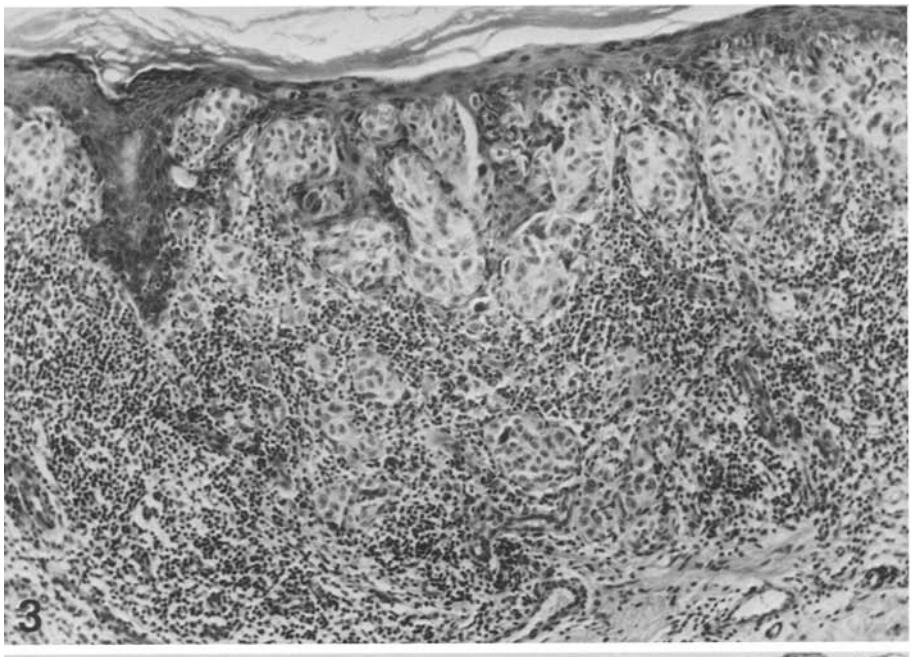


Fig. 1. Epidermal periphery of a melanoma with a nodular component with morphologically questionable dignity (melanoma in situ? so-called dysplastic nevus?); unequivocal malignancy in the DNA-histograph (100 ×)

Fig. 2. Questionable in situ-malignancy of a superficial component of a "SSM/NM" with strong inflammatory reaction; unequivocal malignancy in the respective DNA-histograph (100 ×)

DNA-Feulgen-cytophotometry. The quantitative results are presented in Tables 1–5. In order to provide a summary of the most important data as well as the tendencies shown by our findings, a schematic diagram of the same has been drawn up (cf. Fig. 6):

The nuclear areas seem to increase from the epidermal to the invasive nodular tumour compartments with higher values being recorded in the superior than the inferior nodular regions and the largest areas are found in the melanoma metastases. Anisokaryosis is evidently smaller in all metastases compared to the 10 primary melanomas including their primary tumour. Nuclear DNA-content likewise increases from the epidermal to the invasive nodular tumour compartment and also decreases from the superior to the inferior nodular regions without further significant differences to the metastases. The coefficients of variability of the DNA-contents, being one possible marker of DNA-heterogeneity, are higher in the epidermal tumour components than the nodular



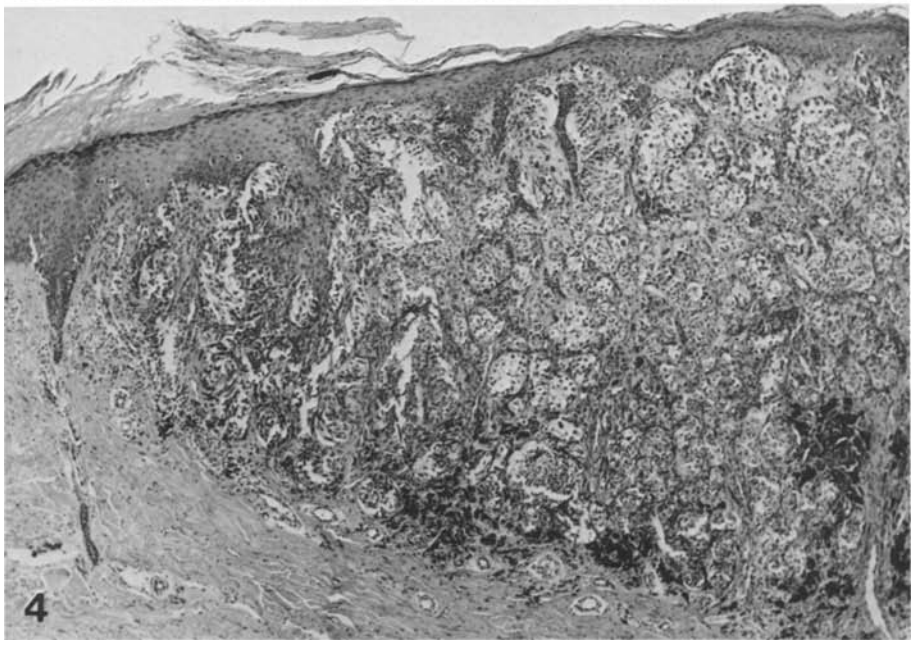


Fig. 3. Invasive melanoma with strong inflammatory infiltration throughout the tumour (100 ×)

Fig. 4. Nodular periphery of a "SSM/NM" without an epidermal component on this side but on the other side there was an adjacent melanoma in situ (MIS) with proven malignancy (40 ×)

regions in most cases. They diminish in the lower nodular tumour parts when compared with the upper regions. The DNA-contents of all metastases have smaller coefficients of variability than those of the equivalent primary melanoma. 75% of the intraepidermal tumour components have obvious signs of malignancy, 58% of them have DNA-contents above 6*n*, and 19% have tumour cell stem lines, whereas 85% of the nodular regions have obvious signs of malignancy including values above 6*n* with regard to 67% and tumour cell stem lines 33% thereof.

All metastases have clear signs of malignancy including DNA values of approximately 6*n* in all cases, 88% thereof with DNA values in excess of 6*n* and 50% thereof revealing tumour cell stem lines.

In contrast, to these general tendencies, metastatic melanoma in case 9 behaves differently: nuclear areas and average DNA-contents are greater in the inferior nodular regions than in the superior ones. Whilst there is a decrease of anisokaryosis in the metastases, the average DNA-contents of the primary and the metastatic melanoma compo-

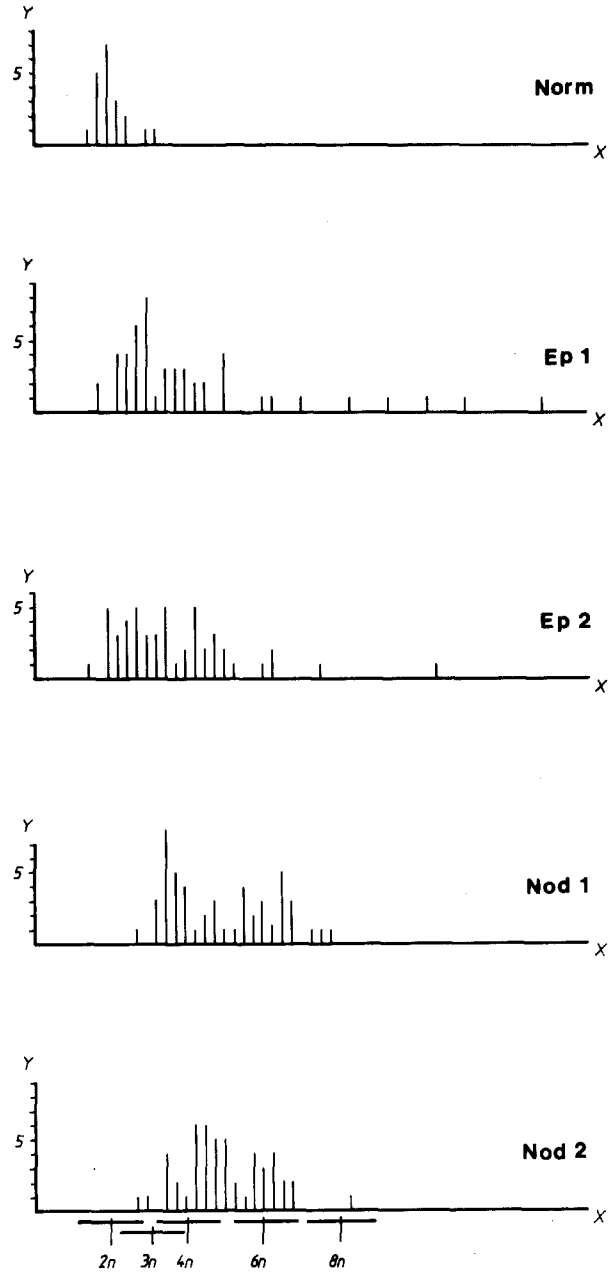


Fig. 5. DNA-histographys (Feulgen-cytophotometry) of a melanoma in situ (MIS) adjacent to an invasive nodular melanoma ("SSM/NM")–topographic analysis of normal epithelium (Norm), intraepidermal MIS-components on both sides of the lesion (Ep 1+2), and of the nodular component distinguishing the upper (Nod 1) from the lower portion (Nod 2). All 4 lesional components are obviously malignant. X-axis: DNA-distribution in arbitrary units; Y-axis: no. of cells

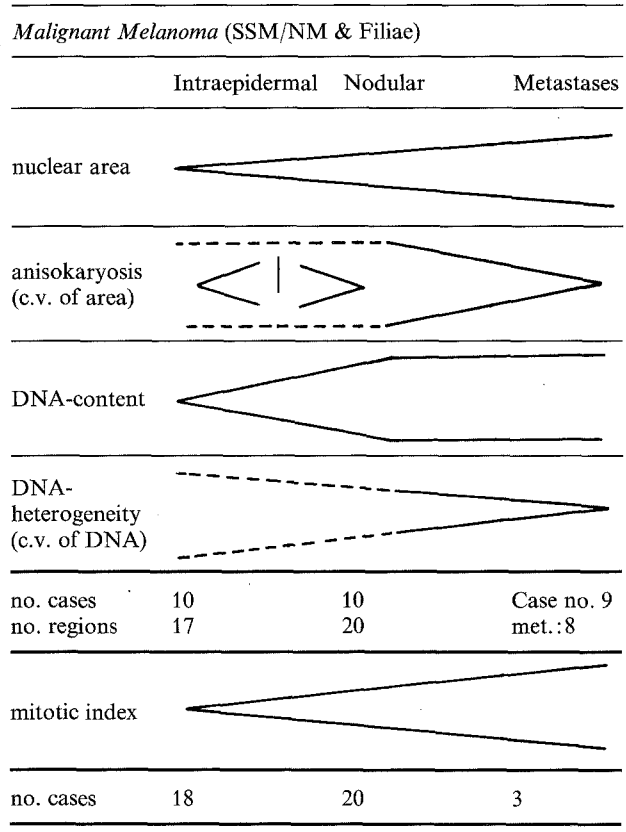


Fig. 6. Schema of the tendencies summarizing our results concerning 10 melanomas with an (epidermal) in situ component (MIS) adjacent to a tumour nodule; additionally 8 metastases of one of the cases. Mitotic index of 41 more cases (referred to Virchows Arch A 409:48–59 (1986))

nents are almost equivalent and all DNA-histographs of this case show a large degree of variability with different tumour cell stem lines, a fact which can be interpreted in terms of a large degree of DNA-heterogeneity. Thus, the nuclei of the metastases are larger and more uniform than those of the primary melanomas, but they have a DNA-heterogeneity which is as great as in the corresponding primary melanoma.

By analogy with the concept that most skin melanomas begin in the epidermis (Clark et al. 1977; Ackerman 1980; Curcioli and Stilwell 1982; Rhodes et al. 1983) our investigations are directed, in particular, to melanomas composed of an (intra)epidermal margin and an invasive nodular component ("SSM/NM"). For this purpose, nuclear planimetry, DNA-Feulgen-cytophotometry (Schmiegelow et al. 1986a, 1986b) and the monoclonal antibody *P* 3.58 were employed. The fact that this antibody produced positive reactions in only 4 of 23 cases of benign nevi as opposed to 36 out of 38 cases of malignant melanomas (cf. Nüßgen et al. 1986) was even more interesting in view of the positive reaction which was also registered with this antibody by the marginal epidermal melanoma components (adjacent MIS) of individual SSM/NM (Figs. 1–4).

Topographical DNA-cytophotometry and immunohistochemistry thus provide new ways of

reaching conclusions as to the diguity of the epidermal component of melanomas, which may, in turn, allow for differential diagnosis of the so-called dysplastic nevus and the melanoma in situ (MIS) (Fig. 5).

From our results we conclude that malignant epidermal lesions (i.e. MIS) may become demonstrable earlier by applying the afore mentioned methods (Boehm and Sandritter 1975). However, theoretically, there is no objective way of proving the existence of a putative precursor lesion (Clark et al. 1977; Ackerman 1982; Rhodes et al. 1983; Schmiegelov et al. 1986b).

The melanoma metastases with their primary melanoma which have hitherto been the subject of our investigations have revealed a large degree of DNA-heterogeneity, in conformity with other observations (cf. Nielsen et al. 1984). Although the primary melanomas of the clinical stage III (cases 9 and 10) appear to have more tumour cell stem lines than the others, the investigations which have hitherto been carried still do not allow for any conclusions to be drawn with regard to the prediction of metastatic behaviour.

Summing up our results, the following *conclusions* can be drawn:

DNA-Feulgen-cytophotometry and nuclear planimetry are additional methods, feasible on paraffin material, for judging whether an epidermal melanocytic component ("adjacent MIS") adjoining an invasive melanoma is malignant.

Nuclear area, average DNA-content, the number of tumour cell stem lines, and mitotic index are greater in invasive nodular than in adjacent epidermal (in situ) melanoma components.

Both melanoma metastases and primary melanomas with proven wide-spread metastases have a large degree of DNA-heterogeneity (e.g. number of tumour cell stem lines); but a predictive value for these methods with respect to the biological behaviour of primary invasive melanoma (probability of metastases) cannot be derived from our results.

In comparing epidermal (in situ) melanoma components with the corresponding nodular (invasive) ones, both DNA-histographs and the application of the monoclonal antibody *P* 3.58 may indicate a malignant potential of adjacent melanoma in situ.

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