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SINGLE CELL DNA CYTOPHOTOMETRY IN CLINICAL STAGE I MALIGNANT MELANOMA

Relationship to prognosis

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Abstract

Histological sections of 50 clinical stage I cutaneous melanomas were analyzed by single cell DNA cytophotometry. Forty-two percent of the melanomas had diploid modal values. Ploidy was not significantly related to the level of invasion, melanoma thickness or prognosis. These results are contradictory to published data from flow cytophotometry which, however, differ inter alia concerning patient materials and follow-up times. Mean nuclear area was in our study significantly correlated to the prognosis.

Key words: Malignant melanoma, skin, DNA content, single cell cytophotometry, prognostic factors.

The prognosis of malignant melanomas is a subject that has been extensively studied in recent years. Clinical and histopathological parameters, such as age, sex, anatomical location, melanoma thickness, Clark's level of invasion and occurrence of ulceration, all have prognostic significance (1). Relatively few studies about DNA cytophotometry of malignant melanomas have been published yet (2–9). The aim of the present study was to analyze histological sections of cutaneous malignant melanomas by DNA cytophotometry and to evaluate whether these findings could provide prognostic information. Nuclear areas were also measured which made it possible to analyze karyometric data in parallel to the DNA cytophotometry results.

Material and Methods

From the period 1972 to 1974 karyometry was done in 82 cutaneous malignant melanomas in clinical stage I and the results were related to the prognosis (10). The same material was used in the present study for single cell DNA cytophotometry of histological sections. Thirty-two melanomas were excluded since there was lack of material (n=15), the earlier GMA (glycolmethacrylate)-embedded material was too brittle for sectioning (n=5), it was not possible to measure (n=8), and there were too few nuclei (n=4). Thus, 50 cases remained for analysis. In 17 cases the specimens were embedded in GMA resine and in 33 cases in paraffin.

From available formalin-fixed and GMA- or paraffin-embedded specimens, two consecutive 5 μm thick sections were prepared. One section was stained with hematoxylin-eosin for orientation purpose. The other section was Feulgen-stained by the method of Duijndam & Van Duijn (11, 12) after acid hydrolysis in 5 N HCl for 1 h. After dehydration in graded ethanols and clearing in xylene, the sections were mounted in a mixture of fluoromount and Cargille oil (RI 1.54) as described by Van der Ploeg & Van Duijn (13). Computerized stage scanning photometry was performed using the Histoscan program (14) with a Leitz MPV system. The Histoscan program was run on a Monroe 8880 computer.

Measurements were made without prior knowledge of the patient data. Within the relevant area in each specimen as a rule 100 consecutive nuclei were measured. Cases with a fewer number of apparent nuclei were accepted if at least 65 nuclei could be measured (n=13).

The nuclear area (μm^2) and the integrated absorbance (DNA content AU) for each nucleus were measured. We used the mean integrated absorbance of 10 lymphocytes as an internal control. A DNA value of 1.25 times that of lymphocytes was regarded as borderline between diploid

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and hyperdiploid nuclei. From other studies, it is known that the DNA value of lymphocytes is underestimated by about 10% in Feulgen absorbance measurements (15, 16). Our borderline should then represent about 2.25 cDNA. For each melanoma the main modal peak(s) of the DNA-histogram was evaluated if located in the diploid or the hyperdiploid region or both. Melanomas with only one modal peak in the diploid region were considered as diploid; otherwise the melanoma was classified as hyperdiploid. The mean nuclear areas and the percentage of nuclei with an area of more than 100 μm^2 were analyzed in the 33 paraffin-embedded melanomas. The 17 GMA-embedded melanomas were not comparable and, therefore, excluded from the analysis of karyometry. In 13 cases material was available for flow cytophotometry. The melanomas were desintegrated by the method described by Schutte et al. (17) and stained according to Vindeløv et al. (18). Flow cytophotometry was performed using a FACS scan analyzer (Becton-Dickinson FACS Systems Mountain View CA) and the found data were compared with the results of single cell cytophotometry.

Statistics. Influence of ploidy on clinical and histopathological data was tested using the χ^2-test and Fischer's exact test respectively. Crude and corrected survival rates were estimated according to Kaplan-Meier for the total patient material. The corrected survival rates were calculated for Clark's level of invasion, melanoma thickness, nuclear area and ploidy and differences were tested with log rank test (19). When the calculation of corrected survival rates was done, the nine patients dying of intercurrent disease were also included in the analysis but considered as censored observations.

Results

Clinical and histopathological data. The 50 patients whose specimens were available for single cell cytophotometry had a median age of 63 years (21–92). There were 18 males with a median age of 55 years (29–80) and 32 females with a median age of 63 years (21–92). Seven melanomas were located in the head–neck region, 20 on the trunk and 23 on the extremities. Twenty-nine of the melanomas had superficial spreading type, 17 nodular type and 4 lentiginous or unclassified type.

The distribution of the melanomas in different Clark's level of invasion was: II:9; III:24; IV:15 and V:2. Ten melanomas were 0–0.79 mm, 13 were 0.8–1.99 mm, 8 were 2–3.49 mm and 19 were ≥3.5 mm thick.

The total 5- and 10-year crude survival rates were 60% and 49.8% respectively and the corresponding corrected survival rates 64.9% and 60.5%. No association between corrected survival rate and age, sex and location was observed in this material while both Clark's level of invasion and melanoma thickness were significantly correlated to the corrected survival rate ($p=0.02$ for Clark's level and $p=0.002$ for melanoma thickness).

Table 1

DNA cytophotometric findings related to clinical data

Clinical parameter	Frequency of diploid melanomas	
Total material	21/50 (42%)	
≤50 years	7/16 (44%)	NS
>50 years	14/34 (41%)	
Males	8/18 (44%)	
Females	13/32 (41%)	
Head and neck	3/7 (43%)	
Trunk	11/20 (55%)	NS
Extremities	7/23 (30%)	

Table 2

DNA cytophotometric findings related to histopathological data

Clinical parameter	Frequency of diploid melanomas
SSM-melanomas	13/29 (45%)
NM-melanomas	6/17 (35%)
Clark's level II	3/9 (33%)
Clark's level III	9/24 (38%)
Clark's level IV	8/15 (53%)
Clark's level V	1/2
Thickness 0–0.79 mm	3/10 (30%)
Thickness 0.8–1.99 mm	4/13 (31%)
Thickness 2–3.49 mm	4/8 (50%)
Thickness ≥3.5 mm	10/19 (53%)

Karyometry data. The 5- and 10-year corrected survival rates for melanomas with a mean nuclear area of <65 μm^2 were both 79.7% and the comparable rates for melanomas with mean nuclear area of ≥65 μm^2 were 48.6%, the differences between the two nuclear size groups being statistically significant ($p=0.035$). The percentage of melanoma nuclei above 100 μm^2 was not significantly related to survival.

DNA cytophotometry data. Twenty-one (42%) of the melanomas were classified as diploid while 29 (58%) were hyperdiploid. Ploidy related to clinical and histopathological data is presented in Tables 1 and 2. No significant differences in ploidy were observed that could be related to age, sex, melanoma location, histological type of melanoma, Clark's level of invasion or melanoma thickness. Nor were any significant differences demonstrated between Clark's level II+III and Clark's level IV+V or between melanoma thickness 0–1.99 mm and ≥2.0 mm. However, the examined subgroups in this study were small.

Five- and 10-year corrected survival rates were for the diploid melanomas 59.4% and 59.4% and for the hyperploid melanomas 68.6% and 61.4% respectively, the differences between the two ploidy groups being non-significant. Corrected survival curves for different combinations of melanoma thickness and ploidy are shown in the Fig-

Table 3

Melanoma thickness related to ploidy (proportion of diploid tumors)

Thickness (mm)	Büchner et al. (3)	Søndergaard et al. (6)	Von Roenn et al. (8)	Kheir et al. (9)	Thickness (mm)	Our material
0–0.74	44/64 (69%)	2/3 (67%)	12/12 (100%)	56/61 (92%)	0–0.79	3/10 (30%)
0.75–1.49	27/57 (47%)	2/4 (50%)	17/21 (81%)		0.8–1.99	4/13 (31%)
1.5–2.99	20/51 (39%)	3/12 (25%)	10/14 (71%)	45/60 (75%)	2.0–3.49	4/8 (50%)
≥3.0	16/58 (28%)	2/16 (13%)	1/6 (17%)	23/38 (61%)	≥3.5	10/19 (53%)

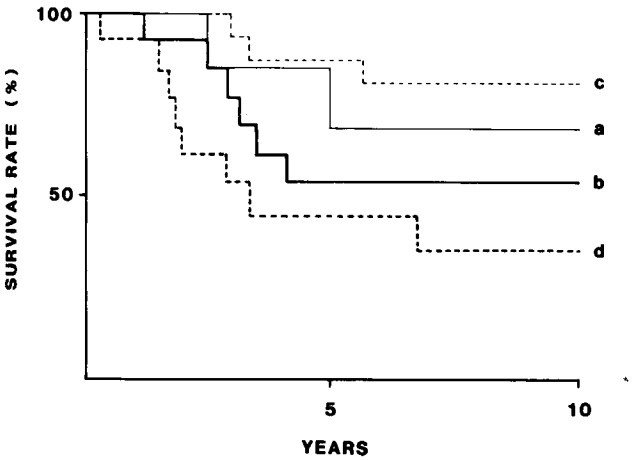

Figure. Corrected 5- and 10-year survival rates for different combinations of ploidy type and thickness. a) Diploid melanomas <2.0 mm thick (n=7). b) Diploid melanomas ≥2.0 mm thick (n=14). c) Hyperdiploid melanomas <2.0 mm thick (n=16). d) Hyperdiploid melanomas ≥2.0 mm thick (n=13). Log-rank test a) versus b) p=0.31. Log-rank test c) versus d) p=0.007.

ure. Neither for thin (<2 mm) nor for thick (≥2 mm) melanomas was the type of ploidy significantly associated with survival. No significant survival difference was observed between diploid melanomas <2 mm and ≥2 mm thick whilst a significant difference was found for the hyperdiploid melanomas in this respect (p=0.007).

Flow cytophotometry data. Among the 13 melanomas examined by both single cell and flow cytophotometry, 9 were considered diploid according to the single cell method. Eight of these were considered diploid also by flow cytometry while one was evaluated as hyperdiploid. Three melanomas were considered as hyperdiploid by the static method. Two of these were judged as hyperploid also by flow cytometry while one was considered as diploid.

Discussion

All previous reports on DNA content in melanomas have been based on flow cytometry. It seems probable, however, that static and flow cytometry should give rather similar evaluations, as was confirmed in the melanomas, which we could evaluate by both methods. Only in 2 out of 13 cases was there a discrepancy concerning the distinction diploid–hyperdiploid.

In our material the proportion of diploid melanomas was 21/50 (42%) which is similar to the finding of Büchner et al. (3), when studying 'metastasis forming melanomas' 107/230 (47%) but a higher proportion than found by Wass et al. (7) among 'primary melanomas' 5/14 (36%) and by Søndergaard et al. (6) in a study of 'primary melanomas' 9/35 (26%). Our proportion of diploid cases was lower than reported by von Roenn et al. (8) 40/53 (75%) and by Kheir et al. (9) 124/162 (77%). The two latter studies included only stage I cutaneous melanomas. The differences between these reports can be due to different composition of patient materials but also to different selection of borderline between diploidy and hyperploidy. For instance Büchner et al. (3) used 2.09 cDNA as borderline while we used 2.25 cDNA.

More important, however, is that the materials differ when looking at thickness related to ploidy. Several authors (3, 6, 8, 9) have thus reported that thin melanomas more often are diploid than thick ones, a difference that we could not confirm (Table 3). The discrepancy between our results and the mentioned reports is difficult to explain. One reason could be random variations as all materials are rather small. Another factor might be the different methods used for evaluation of DNA ploidy.

In our study no obvious relation could be found between ploidy type and prognosis while Søndergaard et al. (6) reported a tendency towards lower 2-year survival rates of heteroploid melanomas and Büchner et al. (3) a higher frequency of metastases in aneuploid tumours. Von Roenn et al. (8) and Kheir et al. (9), have also reported similar findings, and in the latter study the mean follow-up was 4 years. However, our study is the only one in which all patients were followed for 10 years. Further studies on larger series and with long observation times are needed. With our results as a background, DNA ploidy does not seem likely, however, to make a major contribution to prognostic prediction in malignant melanomas.

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