Pol J Pathol 1997, 48, 1, 37-41 PL ISSN 1233-9687

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Examination of DNA-Ploidy in Melanocytic Nevi Cells Using Video-Imaging Cytometry

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DNA content in 61 different types of melanocytic nevi was determined by video-imaging cytometry. The nevi were selected for this study because of melanoma risk associated with each nevus, which is 20 - 50% according to different authors. DNA ploidy was detected in paraffin embedded and fresh tissue material of each patient and the results were comparable. The sample preparation process and video-imaging method were presented in this study. 47 (77.0%) lesions exhibited diploid cell populations, 13 (21.3%) aneuploid and 1 (1.7%) tetraploid cell population. A significant correlation was observed between DNA ploidy measured in fresh tissue and retrospective material. The results indicate the presence of abnormal DNA content in some of the lesions.

Introduction

DNA content detected by video-imaging cytometry in malignant and premalignant cells becomes more and more often routine adjunct to clinical and histological diagnosis [1, 5, 7, 15]. Many nevi are thought to be associated with a potential risk for developing malignant melanoma - especially dysplastic and congenital nevi [9, 12, 13, 23, 24, 25]. Compound nevi, blue nevi or melanosis Dubreuilh can also give rise to malignant melanoma [19]. According to different authors the potential risk for developing malignant melanoma in various nevi ranges from 4 to 80%. The most often reported numbers are 20-50% [6, 8, 18, 26]. It seems that the presence of abnormal DNA in many neoplasms is correlated with the poor clinical course [7, 11, 15]. However, diploid cell population was found also in such malignant cell transformations as leukemias, lymphomas, and aneuploidy in such benign transformations as thyroid adenoma, mastopathy and premalignant cutaneous lesions [4].

It is clear that DNA ploidy is not always an adequate parameter to differentiate benign from malignant lesions [19].

The aim of this study is to present the results of DNA ploidy analysis in randomly chosen nevi removed surgically on the out patients basis. The video-imaging method, process of fresh and retrospective paraffin tissue block preparation for DNA detection are described.

Material and Methods

The study material contained 61 nevi of different types preventively removed in the Wielkopolski Center of Oncology between 01.01.93 - 31.12.93. Histopathology was assessed in the Department of Oncologic Pathology of Medical Academy in Poznań. In this group 21 Spitz nevi, 2 congenital nevi, 27 compound nevi including 3 with significant proliferation, 6 dysplastic, 4 intracutaneous and one junction nevi were identified.

Sample preparation

Fresh cell smears delivered from the operation theatre and retrospective - paraffin blocks were evaluated by video-imaging cytometry. The smears were supplied on the microscopic glass covered with gel and then with human lymphocytes as a positive diploid internal control tissue. The microscopic glass was rinsed in Carnoy's solution which consisted of absolute alcohol (15 parts), formalin (4 parts), acetic acid (1 part) which, as it was proved in other studies is a sufficient chromatine fixer and permits examination of DNA content [22]. Fixation time was 3 min. So prepared sample was stained with Feulgen method.

Preparation of paraffin blocks was more complicated and consisted of the following stages:

a) 2 - 3 50μm sections were cut from each paraffin embedded block,

b) excessive tissue was removed with the scalpel,

c) sections were dewaxed in xylene baths for 1 - 2h,

d) tissue was rehydrated in 96% alcohol and xylene (1:1) for 1h, alcohol 96% for 0.5h, alcohol 85% for 0.5h, alcohol 70% for 24h,

e) rinsed with destillated water,

TABLE 1

DNA content in melanocytic nevi

Types of nevi	n	2n		2n		4n	
		n	%	n	%	n	%
congenital	2	1	50.0	1	50.0		
compound	24	21	87.5	3	12.5		
compound with proliferation	3	2	66.7	1	33.3		
compound dysplastic	6	3	50.0	3	50.0		
intracutaneous	4	4	100.0	0	0.0		
junction	1	1	100.0	0	0.0		
Spitz	21	15	71.4	5	23.8	1	4.8
	61	47	77.0*	13	21.3	1	1.7

* $p<0.001$

TABLE 2

Summary of studies analyzing DNA content in dysplastic nevi

author	n	method	abnormal DNA content
Schmiegelow (1986)	15	image	none
Bergmann (1988)	29	image	slight atypia: 33.3%; moderate atypia: 67.9%; marked atypia: 87.5%
Fleming (1990)	24	image	none
Schmidt (1994)	35	image	slight atypia: none; moderate atypia: 22.0%; marked atypia: 75.0%
Newton (1988)	20	flow	30% of cases aneuploid; no correlation between grading of atypia and aneuploidy
Winokur (1990)	41	flow	24% of cases aneuploid; no correlation between grading of atypia and aneuploidy
Slater (1991)	38	flow	none
Sangueza (1993)	38	image	none

Acc. Schmidt et al. (1994)

f) digested with 0.5% pepsin (SIGMA) in 0.9% NaCl at pH=1.5 and temp. 37°C (0.5h),

g) centrifuged 3x10min in 0.9% NaCl. Cell suspension was placed on the microscopic glass and stained with Feulgen method.

DNA measurement

DNA content in the consecutive phases of the cell cycle determined by video-imaging cytometry was referred to the cells of control population with diploid (2n) standard DNA content. To do this the DNA index was defined :

$$DNA\ INDEX = \frac{DNA\ content\ in\ G_0/G_1\ study\ population}{DNA\ content\ in\ G_0/G_1\ control\ population}$$

For the normal diploid population the DNA index was 1. The DNA index higher or lower than 1 was classified as aneuploid (the index of 2 was interpreted as tetraploidy). The results were received as histograms. The histograms were scaled using diploid references where diploid is equal 2n and is defined by the presence of cells differing from diploid by less then 10% . The tumor was considered as aneuploid when the histograms showed a distinctive peak differing from standard by at least 10% .

Image cytometry quantitation was performed using Magical (Joyce-Loebl) analyzer. 1000 nevi cells and 100 control cells - human lymphocytes were measured each time. GENIAS and RESULTS programs were used. The examinations were carried out in constant conditions of: magnification 640x, individually provided light, analyzed area - 0.04 mm^2, mistake range - 2.58.

Figures 1 and 2 show the histograms of diploid melanocytic compound nevi, figures 3 and 4 present the histograms of aneuploid melanocytic dysplastic nevi.

Results

In the whole population of nevi aneuploid or tetraploid DNA content was identified in 14 nevi (23.0%) and

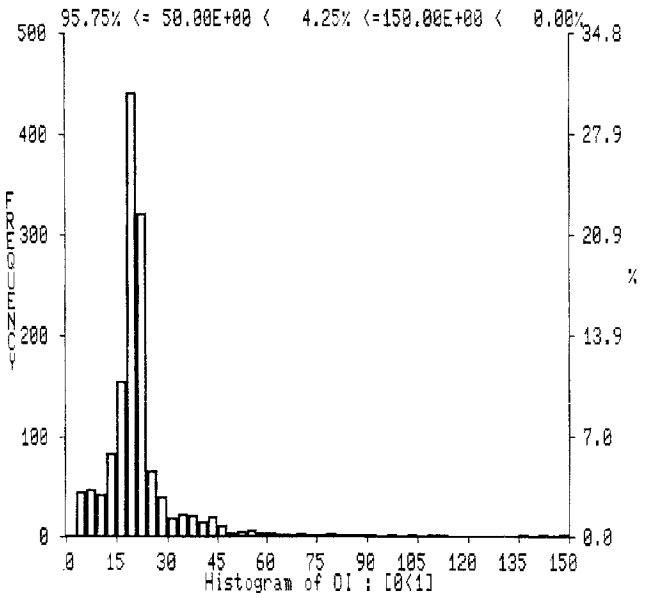

Fig. 1. Diploid histogram of compound nevi - correlation between DNA content (OI) and frequency.

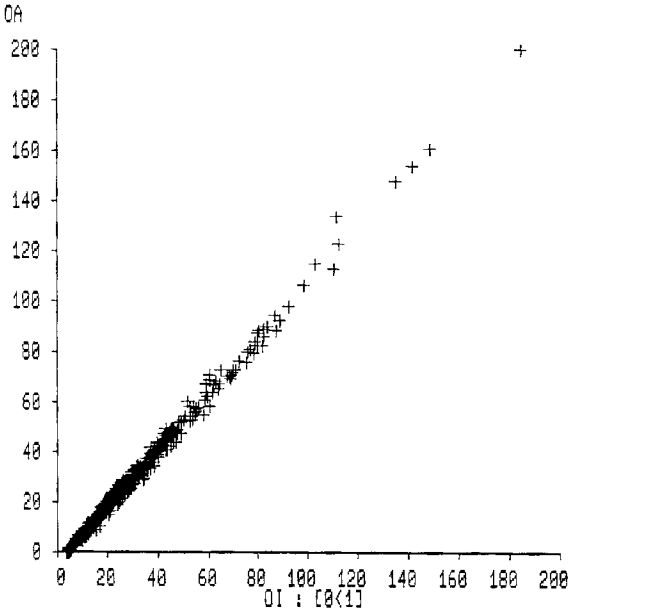

Fig. 2. Diploid histogram of compound nevi - correlation between DNA content (OI) and cell superficial area (OA).

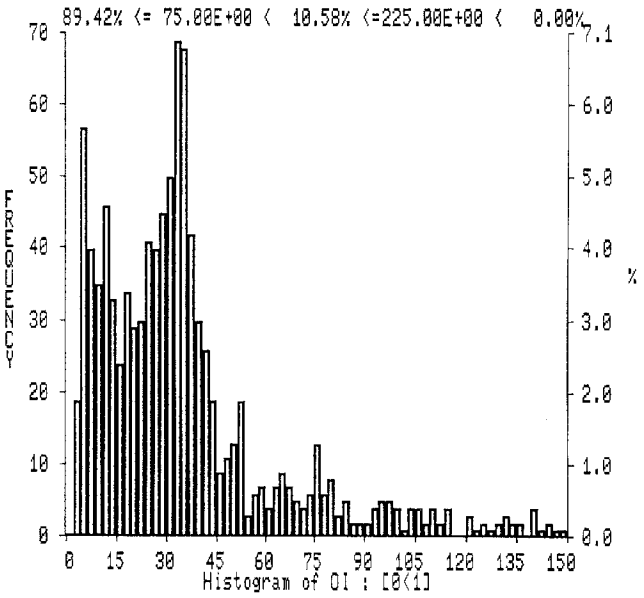

Fig. 3. Aneuploid histogram of dysplastic nevi - correlation between DNA content (OI) and frequency.

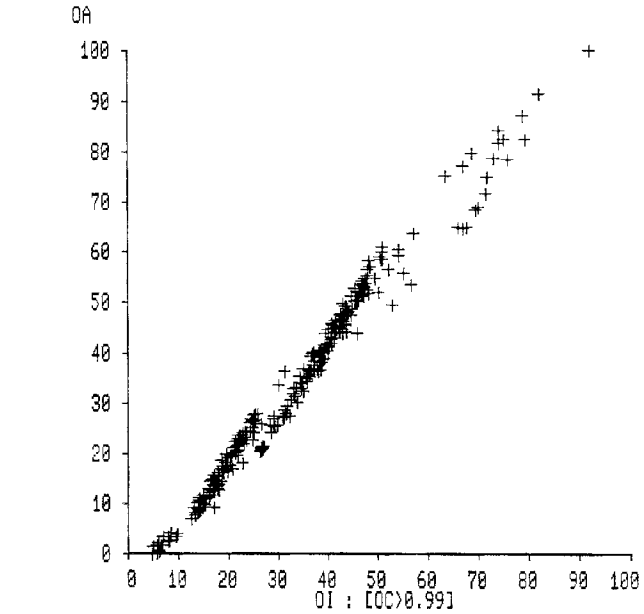

Fig. 4. Aneuploid histogram of dysplastic nevi - correlation between DNA content (OI) and cell superficial area (OA).

diploid in 47 nevi (77.0%) ($p<0.001$). Aneuploidy was found in one of 2 congenital nevi, in 3 of 24 compound nevi, in one of 3 compound nevi with proliferation, in 3 of 6 dysplastic nevi, in 5 of 21 Spitz nevi. In one Spitz nevi the tetraploid profile of DNA was found. The results obtained from fresh tissue material and retrospective material from the paraffin embedded blocks were identical (Tab. 1).

Discussion

The present study confirmed the usefulness of video-imaging cytometry method for the routine DNA content analysis in melanocytic nevi cells. The technique of preparing samples from paraffin blocks for DNA ploidy assessment described earlier by Hedley et al. was tested and modified [14]. The method is relatively simple, reproducible and objective. It may be employed to study archival material and for retrospective prognostic analysis of the pathological material. The preparation of cells smears from fresh tissue material supplied directly from the operation theatre appeared to be a simple, fairly fast method and the results of DNA determination correlated with those obtained from the paraffin blocks. Hedley et al.'s conclusions were pretty similar [14]. It is very important

for this method to remove carefully excessive parts of paraffin sections not belonging to the nevi (for instant subcutaneous tissue) because of the possibility of measuring DNA content in unwanted tissue.

So far DNA ploidy results have been contradictory. In congenital melanocytic nevi aneuploidy range was between 8.7% and 24.9% [20]. Stenzinger et al. [32] and Newton et al. [20] did not find a significant correlation between histological degree of atypia and DNA content. On the other hand, Branhill et al. [2] observed such a correlation. In dysplastic nevi with varying degree of atypia the range of aneuploidy percentage was quite wide 22.0% - 87.5% (Tab. 2).

In all 376 acquired melanocytic nevi Buchner et al. [5] confirmed the diploid DNA content. In contrast, Sondergaard et al. [31] in 16 nevi found only 4(25%) with the aneuploid amount of DNA. LeBoit et al. [17] noticed different DNA content in the consecutive lesion levels - mostly diploid cells in the upper levels and a mosaic of diploid and hyperdiploid lines in the lower levels. These authors and other investigators observed a certain percentage of aneuploidy and tetraploidy in benign melanocytic lesions. It can indicate a higher risk of proliferation and melanoma development.

Both our own results and the results reported by many other authors concerning the DNA ploidy in the cells of different nevi, which are benign, nonneoplastic lesions, indicate the occurrence of a certain amount of aneuploidy and tetraploidy. It may indicate an increased tendency towards proliferation and associated increased risk of malignant transformation. It suggests the need for measuring DNA ploidy in nevi considered as premalignant and should qualify such patients for more frequent prophylactic examinations. The nevi cannot be used as diploid control in DNA ploidy determination.

Conclusions

1. A certain percentage of cells with abnormal DNA content occurs in pigmented nevi.

2. The determination of cellular DNA content does not differentiate benign from malignant lesions.

3. The video-imaging technique enables measurement of DNA content with the microscopic visualization, which minimizes the possibility of measuring DNA content in other cells.

4. The preparation of smears for DNA ploidy determination is simple, fast and exact.

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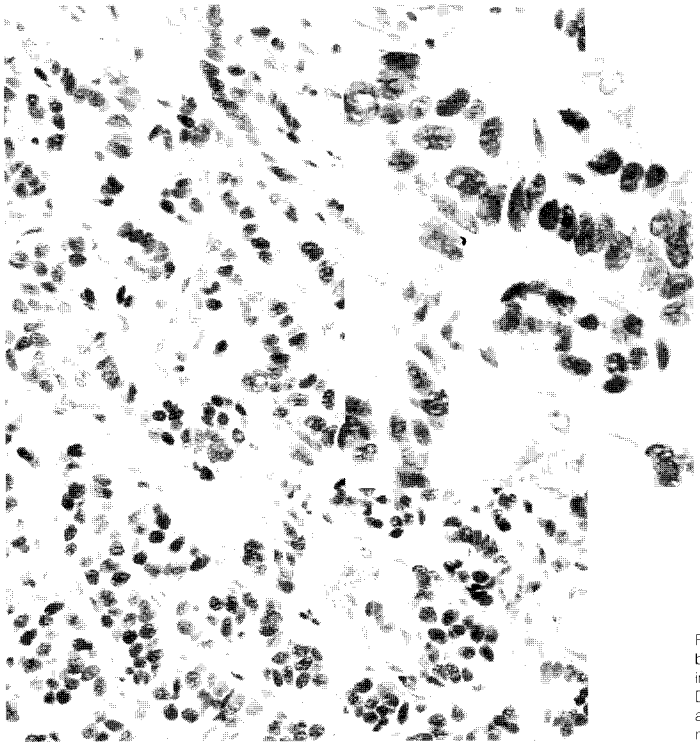

Formalin-fixed, paraffin-embedded breast carcinoma tissue. Pretreated in microwave oven. Stained with DAKO-ER, 1D5, code No. M 7047 and streptABComplex/HRP Duet, mouse/Rabbit code No. K 492.

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