Flow and video-imaging cytometry – usefulness of DNA ploidy measurements in diagnosis of malignant melanoma

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1. INTRODUCTION

DNA content detected by video-imaging and flow cytometry in malignant and premalignant cells becomes more and more often routine adjunct to clinical and histological diagnosis. It seems that the presence of abnormal DNA in many neoplasms is correlated with the poor clinical course (1,4,5). However, diploid cell population was found also in such malignant cell transformations as leukemia, lymphoma, and aneuploidy in such benign transformations as thyroid adenoma, mastopathy and premalignant cutaneous lesions. It is clear that DNA ploidy is not always an adequate parameter to differentiate benign from malignant lesions.

Recent results of DNA ploidy measurements in malignant melanoma cells are contradictory. There are relatively few conflicting studies, which describe DNA content of malignant melanoma and pigmented nevi.

The aim of this study is to present the results of DNA ploidy analysis in malignant melanoma cells in comparison with choosen prognostic clinical and histopathological factors and to present results of DNA ploidy analysis in randomly chosen nevi removed surgically. We compared both cytometric methods.

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2. MATERIAL AND METHODS

The study material contained 103 malignant melanomas and 61 nevi. In the group of pigmented nevi 21 Spitz nevi, 2 congenital nevi, 27 compound nevi including 3 with significant proliferation, 6 dysplastic, 4 intracutaneous and one junction nevi were identified.

Measurements of DNA ploidy in cell nuclei were done using two cytometric methods: flow cytometry and video-imaging cytometry. Suspension of cells from paraffin blocks, and for image cytometry, additionally fresh cells smears were stained for DNA. About 1000 cells of study population (image cytometry) or 10000 cells (flow cytometry) and 100 cells of human lymphocytes (internal control) were analysed. Results were achieved in form of histograms reflected percentage of cells in respective phases of cell cycle. 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%.

3. RESULTS

In whole population of malignant melanoma diploid DNA content was detected in 33 tumors (32,0%) and aneuploid DNA content in 70 tumors (68,0%) [$p<0,01$]. Correlation between aneuploidy rate, sex, age and localization did not show significant differences. Aneuploidy rate was higher in II and III clinical stage, significant correlation was shown between aneuploidy and tumor thickness greater then 1,50 mm and in Clark Level III,IV and V. There was no correlation between aneuploidy and clinical type, lymph node involvement, inflammatory infiltration, ulceration, cell type and regression.

In the whole population of nevi aneuploid or tetraploid DNA content was identified in 14 nevi (23,0%) and 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).

4. DISCUSSION

In whole group of malignant melanoma aneuploid DNA content was significantly greater then diploid DNA content. In examined group correlation between DNA ploidy and sex, age and localization of primary lesion was not observed. Female sex and localization of primary lesion in lower limbs prognoses better but in multivariate analysis (this features are occurring often connected), considering a fact that in women ulceration (worse prognostic factor) occurs rarely, prognostic meaning of both features is not so unequivocal. Higher clinical stage is connected with poor prognosis (higher tumor thickness according to Breslow, higher Clark level and positive lymph node status). Own results show higher aneuploidy rate in tumors with thickness greater then 1,50 mm, with Clark level III, IV and V. Difference in aneuploidy rate between clinical stages II-III and I was not statistically significant. Comparison of another histological factors with DNA ploidy did not show correlation. Small group of published results did not allowed to draw conclusions.

So far DNA ploidy results in melanocytic nevi have been contradictory (2,3). Some authors observed 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.

The present study confirmed the usefulness of video-imaging and flow cytometry method for the routine DNA content analysis (*table 1*). The results obtained from both methods were identical. Flow cytometry is a fast cytometric method, great number of cells and many of cell parameters can be measured at the same time. Defect of this method is lose of morphological identification of cells and complicated preparing of cell solution. Advantage of video-imaging cytometry is the possibility of morphological identification of cells using microscope and possibility to selection of artefacts and regular normal cells. Defect of this method is small group of measured cells and time-consuming measurement. By small population of obtained tumor cells video-imaging cytometry is a method of choice.

Table 1. Comparison of both cytometric methods

Flow – cytometry	Video - imaging cytometry
Advantages	Advantages
1. possibility of measurements with a speed of thousands of cells per second 2. possibility of detecting one atypical cell in thousands of normal cells 3. possibility of measurements even tens of parameters simultaneously	1. possibility of morphological identification measured cells 2. possibility of assessment cell fractions described using flow cytometry 3. makes possible selection of default cells and artifacts 4. makes possible use of additional measured parameters, like surface area, shape factor, cells circumference, etc.
Defects	Defects
1. lack of direct observation of measured cells 2. necessity of long-term preparation of cell suspension 3. necessity of achieving total dispersion of cell without disturbing of structure 4. possibility of false results arise from place more cells one on another or partial dispersion	1. small number of measured cells 2. time - consuming measurement 3. necessity of internal standard and standarization of measuring equipment

5. CONCLUSIONS

1. Both, the video-imaging cytometry and flow cytometry, can be employed for DNA ploidy measurements in cells.

2. The determination of cellular DNA content in cell does not differentiate between benign and malignant lesions.

REFERENCES

1. Barlogie B, Raber MC, Schumann J, Johnson TS, Drewinko B et al. Flow Cytometry in Clinical Cancer Research. Cancer Res 1983; 43: 3982-3997
2. Barnhill RL, Aguiar M, Cohen C, Kang S, Kennedy J, Schmidt B, Sober AJ, Solomon AR. Congenital Melanocytic Nevi and DNA Content. An Analysis by Flow and Image Cytometry. Cancer 1994; 74: 2935-2943
3. Bartkowiak D, Schumann J, Otto FJ et al. DNA Flow Cytometry in the Prognosis of Primary Malignant Melanoma. Oncology 1991; 48: 39-43
4. Buchner T, Hiddemann W, Wormann B et al. Differential pattern of DNA-aneuploidy in human malignancy. Pathol Res Pract 1985; 179: 310-317
5. Skowronek J, Adamska K, Filipiak K, Karaś Z, Krenz RM, Rutkowski R, Warchoł JB - DNA ploidy in malignant melanoma, skin cancer and pigmented nevi, Neoplasma 1997; 44: 282-288