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Cytokeratin Expression Correlates with Aneuploidy in Cytological Specimens of Melanoma Metastases

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Abstract. *It has been postulated that the high malignancy of melanomas could be connected with an increased cytokeratin (CK) expression. In order to define the relationship between CK expression and genetic instability of melanoma metastases, ploidy-related parameters were compared in cytological specimens of CK-positive and CK-negative melanoma metastases. CK expression was investigated immunohistochemically in 35 melanoma liver metastases and in 52 melanoma lymphatic metastases. Ploidy-related parameters were evaluated on Feulgen-stained specimens with a CAS200 image analyzer. Cytokeratin was detected in 14 out of 35 melanoma liver metastases and in 24 out of 52 melanoma lymphatic metastases investigated. Significant differences between CK-positive and CK-negative melanoma metastases were found for the percentage of diploid cells, percentage of tetraploid cells, percentage of aneuploid cells between 4c and 8c, as well as for 5c exceeding rate. Our results confirmed that CK is present in more advanced (unstable), clearly aneuploid forms of melanoma metastases.*

The cytokeratin (CK) pattern of many tumour types, other than melanomas, is constant and assists in tumour progression from the very early stages until late metastasis.

Melanomas are generally vimentin-positive and mostly CK-negative. Certain research groups, however, have demonstrated CK expression not only in primary melanomas but also in their metastases (1-3). A direct connection between CK expression and melanoma genomic instability, as well as increased migration and invasivenes of melanoma cell lines *in vitro,* have been postulated (4, 5).

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In human tumours, genomic instability most frequently expresses itself in changes of chromosome number (DNA aneuploidy). Translations, deletions, amplifications, as well as more discrete changes of DNA content such as point mutations, deletions and insertions, can influence genomic instability (6, 7).

An aneuploid cell has either a total number of chromosomes, which is not an exact multiple of the number of chromosomes in normal haploid cells, or the change in the total amount of DNA is sufficiently disturbed to be detected by quantitative DNA analysers. Modern image analysis systems are able to detect differences in the total amount of DNA in an abnormal cell when this difference is greater than 2% of that of a normal cell (8, 9).

The main objective of this study was to relate the CK expression to ploidy-related parameters in melanoma metastases. Melanoma metastases were selected because of the high genomic instability of these lesions as well as for their changed and increased CK pattern.

Materials and Methods

The material investigated consisted of 52 melanoma lymphatic and 35 liver metastases. Of the patients, 42 were female and 45 were male, and their ages ranged between 22 and 96 years (mean 59.5 years). Cytological specimens were taken from the Archive of the Department of Cytopathology, University of Göttingen, Germany, and were collected during the period 1996-2001. The ethical aspects of this study were verified at the Ethical Center of the University of Poznan, Poland.

To demonstrate cytokeratin expression, KL1 antibody (Immunotech, Hamburg, Germany) directed against pancytokeratin

Table I. *Cytokeratin positivity in melanoma metastases in the liver and lymph nodes.*

Location	CK-positive cases	High positivity (>50% of cells)
Liver	14/35	7
Lymph nodes	24/52	11
All metastases	38/87	18

(CK 1, 5, 6, 8, 14, 18, 19) was applied. Cytological specimens, after acetone fixation, were incubated for 2 h with KL1 antibody diluted 1:2 in TRIS. To demonstrate the reaction product, bridge antibody (Dako, Hamburg, Germany), PAAP complex (Quartett, Berlin, Germany) and new fuchsin as chromogen were applied.

To evaluate the ploidy status, the cytological specimens were destained from MGG and were treated for 60 min with 5N HCl. The sections were then stained with the Feulgen kit according to the manufacturer's instructions (CAS DNA dye kit for cell analysis; Becton-Dickinson, Erembodegen, Belgium). Following dehydration in an ascending alcohol series, the sections were transferred to xylol and covered with a synthetic medium. Feulgen-stained rat hepatocytes were used as control cells. Stained specimens were examined with the CAS200 image analysis system (Becton-Dickinson) and Quantitative DNA analysis software. The DNA content of the tumour cell was quantified by allocating the optical density to each pixel of the sectional image and adding the optical densities for each nucleus. The image system was calibrated by measuring the DNA content of rat hepatocytes. Ploidy analysis involved the measurement of 200 tumour cells and determination of the following ploidy-associated parameters:

- percentage of diploid cells,
- percentage of aneuploid cells between 2c and 4c,
- percentage of tetraploid cells,
- perentage of aneuploid cells between 4c and 8c,
- percentage of octaploid cells,
- 5c exceeding rate, defined as the percentage of cells with DNA content higher than 5c.

Ploidy status was determined on the basis of the Auer scheme (10).

The Mann-Whitney *U*-test was applied for statistical evaluations.

Results

Cytokeratin expression. Among the melanoma liver metastases investigated, CK expression was found in 14/35 cases. In 7 metastases CK expression was found in more than 50% of melanoma cells.

CK presence was found in 24/52 melanoma lymphatic metastases. In 11 cases CK was present in more than 50% of malignant cells (Table I, Figure 1).

Ploidy-related parameters in CK-positive melanoma metastases. The ratio of diploid cells in CK-positive melanoma metastases ranged between 3.98% and 12.70%. The percentage of aneuploid cells between 2c and 4c oscillated between 28.90 and 31.80%, and the percentage of tetraploid cells reached values between 14.90 and 28.20%. The percentage of aneuploid cells between 4c and 8c in CK-positive melanoma metastases ranged between 27.30 and 46.80%, while that of octaploid cells did not exceed 2.99%. The 5c exceeding rate ranged between 27 and 54% (Table II).

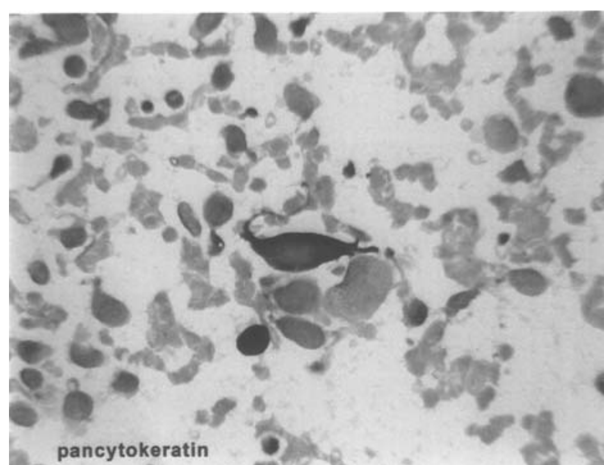

Figure 1. *Cytokeratin positivity in single melanoma metastatic cells in cytology.*

Ploidy-related parameters in CK-negative melanoma metastases. In CK-negative melanoma metastases the ratio of diploid cells ranged between 0 and 85.5%. The percentage of aneuploid cells between 2c and 4c oscillated between 11 and 83.2% and the ratio of tetraploid cells ranged between 0 and 31%. The percentage of aneuploid cells between 4c and 8c reached values between 0 and 36%, while the ratio of octaploid cells did not exceed 4%. The 5c exceeding rate values ranged between 0 and 49% (Table III).

Comparison of ploidy-related parameters in CK-positive and -negative melanoma metastases. Comparison of ploidy-related parameters between CK-positive and -negative melanoma metastases demonstrated statistically significant differences for the percentage of diploid cells ($p<0.05$), percentage of tetraploid cells ($p<0.05$), percentage of aneuploid cells between 4c and 8c ($p<0.05$) as well as for the 5c exceeding rate ($p<0.05$).

Ploidy status in CK-positive and negative melanoma metastases. Among the 38 CK-positive melanoma metastases, 3 cases were euploid (Auer II). Fourteen were suspected as aneuploid (Auer III) and 21 were clearly aneuploid (Auer IV).

Table II. *Values of ploidy-related parameters in cytokeratin-positive melanoma metastases.*

Parameters	Minimum	Maximum	Median
Diploid cells	3.98%	12.70%	8.35%
Aneuploid cells between 2c-4c	28.90%	31.80%	30.30%
Tetraploid cells	14.90%	28.20%	21.60%
Aneuploid cells between 4c-8c	27.30%	46.80%	37.00%
Octaploid cells	0.00%	2.99%	1.50%
5cER	27.00%	54.00%	40.50%

Table III. *Values of ploidy-related parametes in cytokeratin-negative melanoma metastases.*

Parameter	Minimum	Maximum	Median
Diploid cells	0.00%	85.50%	35.60%
Aneuploid cells between 2c-4c	11.00%	83.20%	23.40%
Tetraploid cells	0.00%	31.00%	2.31%
Aneuploid cells between 4c-8c	0.00%	36.00%	2.40%
Octaploid cells	0.00%	4.00%	0.00%
5cER	0.00%	49.00%	4.00%

Table IV. *Auer classification of DNA histograms in cytokeratin-positive and -negative melanoma metastases.*

Auer type	CK-positive metastases	CK-negative metastases
I	0/38	0/49
II	3/38	6/49
III	14/38	17/49
IV	21/38	26/49

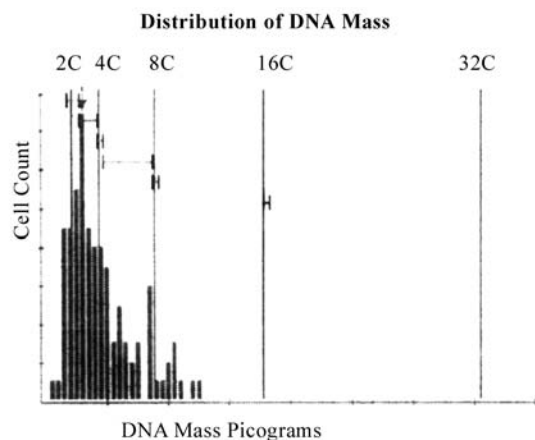

Figure 2. *Clearly aneuploid DNA histogram typical of the majority of melanoma metastases.*

Six of the CK-negative melanoma metastases showed an euploid (Auer II) histogram, 17 were suspected as aneuploid (Auer III) and 26 were clearly aneuploid (Auer IV) (Figure 2, Table IV).

Discussion

To our knowledge, the statistical relationship between CK presence and ploidy status has not been previously demonstrated in either melanomas or in other tumours.

In this study, the statistically significant differences between CK-positive and -negative melanoma metastases concerning the ratio of diploid cells, of tetraploid cells, of aneuploid cells between 4c and 8c, of octaploid cells, and the 5c exceeding rate have been demonstrated. Such detailed analysis was possible with a CAS200 image analysis system. The CAS200 image analyser makes possible the measurement of additional parameters defining single subfractions of cells, such as the ratios of diploid, aneuploid, tetraploid, octaploid and 16-ploid cells. The prognostic significance of these parameters for malignant melanomas and malignant fibrous histiocytomas has been demonstrated (11-13). Aneuploidy status is regarded as a mirror of genetic instability of tumours, resulting from different mutations or other genetic abnormalities. Quite often these mutations lead to the patients' poor outcome.

Primary malignant tumours contain cell populations, which are heterogenous for different biological characteristics, such as metastatic potential. This diversity in metastatic behaviour within the same tumour, as well as the phenotypic instability of cloned tumour cells in culture and the increased motility of cell lines prepared from melanoma metastases, can be explained by the high aneuploidy grade found in melanoma metastases.

Cell motility is necessary for tumour cells to traverse many stages in the complex cascade of invasion and metastases. Such stages may include the detachment and subsequent infiltration of cells from the primary tumour

into adjacent tissue, the migration of the cells through the vascular wall into the circulation (intravasation) and the extravasation of the cells to a secondary site. The movements of cells through biological barriers such as the endothelial membrane could also be connected with the activation of intermediate filaments such as cytokeratin in melanomas.

All these speculations could account for the relatively high expression of CK in melanoma metastases (higher than previously reported for primary tumours), as well as the connection between aneuploidy and CK expression. All our observations confirmed that the presence of CK is connected with higher malignancy and genetically more unstable forms of melanoma metastases.

The possibility of evaluating ploidy status and ploidy-related parameters on cytological specimens of melanoma metastases, destained from MGG-cytological routine staining, was also investigated. This method may be used for the evaluation of ploidy status when only very few cytological specimens are available.

References

1 Gatter KC, Ralfkiaer E, Skinner J, Brown D, Heryet A, Pulford KAF, Hou-Jensen K and Mason DY: An immunocytochemical study of malignant melanoma and its differential diagnosis from other malignant tumors. J Clin Pathol *38*: 1353-1357, 1985.

2 Achilles E and Schroeder S: Zytokeratin-Positivität bei malignen Melanomen. Fallgrube bei der immunologischen Differentialdiagnose okkulter Neoplasien. Pathologe *15*: 235-241, 1994.

3 Ben-Izhak O, Stark P, Levy R, Bergman R and Lichig C: Epithelial markers in malignant melanoma. A study of primary lesions and their metastases. Am J Dermatopathol *16*: 241-246, 1994.

4 Zarbo RJ, Gown AM, Nagle RB, Visscher DV and Crissman JC: Anomalous cytokeratin expression in malignant melanoma: one and two-dimensional Western-blot analysis and immunohistochemical survey of 100 melanomas. Mod Pathol *3*: 494-501, 1990.

5 Hendrix MJC, Seftor EA, Chu YW, Trevor KT and Seftor REB: Role of intermediate filaments in migration, invasion and metastasis. Cancer Metastasis Rev *15*: 507-525, 1996.

6 Almasan A, Linke SP, Paulson TG, Huang LC and Wahl GM: Genetic instability as a consequence of inappropriate entry into and progression through S-phase. Cancer Metastasis Rev *14*: 59-73, 1995.

7 Tlsty TD, Briot A, Gualberto A, Hall I, Hess S, Hixon M, Kuppuswamy D, Romanov S, Sage M and White A: Genomic instability and cancer. Mutat Res *337*: 1-7, 1995.

8 Haroske G, Dimmer V, Meyer W and Kunze KD: DNA histogram interpretation based on statistical approaches. Anal Cell Pathol *15*: 157-173, 1997.

9 Auer G: DNA: a clinical parameters. Becton Dickinson immunocytometry system. Frembodegem-Aalst, Belgium 1990.

10 Auer GU, Casperson TO and Wallgren AS: DNA content and survival in mammary carcinoma. Anal Quant Cytol *2*: 161-165, 1980.

11 Korabiowska M, Ruschenburg I, Brinck U, Jahns A, Berger H and Droese M: Comparison of ploidy status of melanoma metastases in different locations. Int J Mol Med *2*: 113-116, 1998.

12 Korabiowska M, Brinck U, Brinkmann U, Berger H, Ruschenburg I and Droese M: Prognostic significance of newly defined ploidy related parameters in melanoma. Anticancer Res *20*: 1685-1690, 2000.

13 Brinck U, Korabiowska M, Buschmann N, Stachura J, Ruschenburg I, Cordon-Cardo C and Schauer A: Relevance of ploidy related parameters in prognosis of malignant fibrous histiocytomas. Anticancer Res *19*: 5211-5216, 1999.

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