COMPARATIVE MICROSPECTROPHOTOMETRIC INVESTIGATION OF THE DNA CONTENT IN CELLS OF PRIMARY FOCI AND METASTASES OF MALIGNANT MELANOMA

G. G. Avtandilov and L. V. Chervonnaya

UDC 616.006.81.04-018.1-008.939.633.2-074:543.42

The DNA content and ploidy of cells of primary foci of malignant melanoma, metastases of melanoma, and intradermal pigmented nevi were studied by a microspectrophotometric method. If the primary foci of the melanoma and its metastases had general features such as heteroploidy and polyploidy, the cells of the metastases possessed high ploidy and a distinctive pattern of distribution with respect to DNA content as a proportion of the total DNA mass. It is postulated that superproduction of DNA and a change in the clonal profile are the principal quantitative characteristics of progressive malignant growth.

Progress in cytogenetics has led to the accumulation of information on genetic heterogeneity of the cell populations of malignant tumors. This fact means that a tumor focus must be regarded as a population of individual clones of tumor cells heterogeneous in their biological properties. Numerous investigations have shown that the degree of biological aggressiveness of malignant tumors is connected with the character of their ploidy [2, 3], and also that the potential ability of tumors to metastasize is dependent on the characteristics of the chromosome complement of their cells [5, 6]. Some workers [7] have found that only a few of the most viable tumor cells may be involved in metastasization; these cells usually have high polyploid DNA values and belong to particular clones of tumor cells.

In view of the facts described above, the comparative study of the ploidy of cells of primary tumors and their metastases is of great theoretical and practical importance, for because of differences in their cell profile for DNA content the metastases may have biological properties which differ from those of the original tumor. This fact has a very important bearing on treatment and prognosis.

No information on the comparative study of the DNA content in cells of primary and metastatic foci of melanoma could be found in the accessible literature.

A comparative study was made of the ploidy of primary melanomas and their metastases on the basis of results of microspectrophotometric analysis of the DNA content in the tumor cell nuclei.

EXPERIMENTAL METHOD

Histological specimens from 36 primary melanomas of the human skin, 12 metastases of melanomas in the regional lymph glands, and seven intradermal pigmented nevi (for comparison) were investigated. Sections 7 μ in thickness were stained by the Feulgen method (fixation in 10% neutral formalin, hydrolysis for 7 min). The DNA content in the nuclei of the tumor cells was determined with an integrating scanning microspectrophotometer [1]. The results of measurement of the optical density of the nuclei were expressed

Central Pathological Laboratory, Institute of Human Morphology, and Department of Pathomorphology of Tumors, P. A. Gertsen Moscow Cancer Research Institute, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 4, pp. 77-79, April, 1973. Original article submitted August 8, 1972.

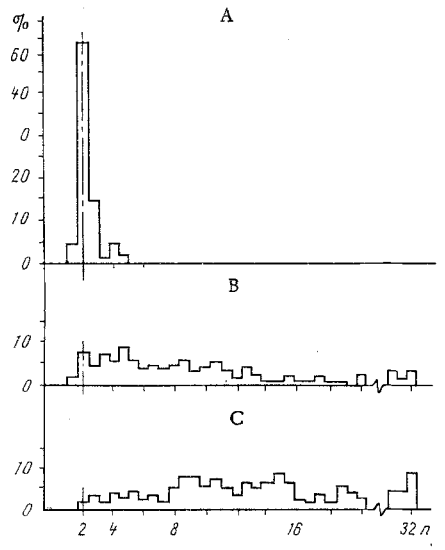

Fig. 1. Histogram of distribution of cells by DNA content: A) nevus; B) primary focus of melanoma; C) metastasis of melanoma. Abscissa, DNA content in ploidy units (n); ordinate, percentage of cells.

as ratios of their mean area, and data for the DNA content were obtained in relative units. The DNA content in the nuclei of 30 lymphocytes were determined in the same specimens and by the same method, and the averaged values of the measurements were taken as a standard of reference for a diploid cell; this was later used to convert the DNA content in the nuclei of the tumor cells into ploidy units. Histograms of distribution of the cells of nevi and primary and metastatic foci of melanoma by the character of their ploidy were plotted from these results. The cell ploidy was studied only in completely untreated neoplasm in view of information that chemotherapy or radiotherapy may lead to changes in the DNA content in the nuclei.

EXPERIMENTAL RESULTS

The results revealed a clearly defined modal class of diploid cells (Fig. 1A) in the benign pigmented neoplasms, while the primary foci (B) and metastases (C) of the melanoma exhibited marked heteroploidy and polyploidy.

The mean values for the primary foci of the melanoma showed that 10% had normal ploidy values, 45 and 40% of cells contained from 4 to 8 n and from 8 to 16 n, respectively, while only 5% of cells had high polyploid DNA values (over 16 n).

In metastatic foci of melanoma these proportions were considerably modified to give an increase in polyploidization of the cells: the number of cells containing from 8 to 16 n rose to 70%, the index of high polyploid cells rose to 15%, but the percentage of cells in the range from 4 to 8 n fell to 15%.

The results of these experiments show marked differences in the character of ploidy of the benign nevi and malignant melanomas. Primary and metastatic foci of melanoma in turn differ in their distribution profile of the tumor cells on the ploidy scale: the metastases have more marked polyploidization of the tumor cells.

This higher ploidy of cells of the metastases can be explained by the hypothesis [4 et al.], based on the marked resistance of polyploid cells to the action of unfavorable factors and the clonal structure of the tumor focus, that metastasization of malignant tumors is effected by certain polyploid clones of tumor cells with a high potential capacity for malignant growth.

To quantify the process of malignant transformation, the total mass of DNA was calculated in 100 cells of the structures studied (the sum of the products of the number of cells and their corresponding ploidy units). The calculations showed that for 100 cells of the nevi there were on the average 250 conventional units of DNA, while in the primary foci of melanoma the total DNA content was increased by almost 1.5 times and in metastases of melanoma by 4 times. In other words, the total DNA content in metastatic foci in a standard number of cells was almost twice that in the primary tumor.

Superproduction of DNA and a change in the clonal profile can thus be regarded as the principal quantitative features of progressive malignant growth.

It can be concluded from the results of these investigations that while primary and metastatic foci of malignant melanoma share common features of heteroploidy and polyploidy, primary and metastatic foci of malignant melanoma have distinctive profiles of cell distribution by DNA content as well as in their total mass of DNA, evidently reflecting differences in the karyotype of these neoplasms and responsible for differences in their biological activity.

LITERATURE CITED

1. G. G. Avtandilov, Yu. I. Blagoveshchenskii, A. S. Dolmatov, et al., Arkh. Pat., No. 10, 70 (1970).
2. G. G. Avtandilov and V. S. Pashkova, Byull. Éksperim. Biol. i Med., No. 4, 82 (1971).
3. T. Hauschka and A. Levan, Exp. Cell Res., 4, 457 (1953).

4. E. Meek, J. Path. Bact., 82, 167 (1962).
5. G. Moore and A. Sandberg, Acta Cytol. (Philadelphia), 9, 175 (1965).
6. W. Sandritter, Acta Cytol. (Philadelphia), 10, 26 (1966).
7. A. Tavares, Europ. J. Cancer, 3, 449 (1968).