DNA CONTENT IN ATYPICAL GLIO-MESENCHYMAL CELLS DURING CHEMICAL CARCINOGENESIS IN THE ALBINO RAT CEREBELLUM

I. A. Kazantseva and L. Ya. Yablonovskaya

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The DNA content was determined by comparative microspectrophotometry in atypical glio-mesenchymal cells around the villous capsule surrounding the DMBA pellet, and in cells of microastrocytomas and microglioblastomas induced by DMBA. A characteristic feature of the late stages of this type of chemical carcinogenesis in the albino rat cerebellum was found to be the preservation of a well-marked diploid modal class, except in cases when the pathological process developed along malignant lines in the early stages of tumor progression (microglioblastoma).

KEY WORDS: cytophotometry; glio-mesenchymal cells; chemical carcinogenesis; DNA content.

An increase in genetic heterogeneity of cells with a tendency toward their DNA content to increase is known to be a characteristic feature of growth of tumors, including many mainly malignant tumors of the neuroectodermal series found in the brain of man and experimental animals [5, 6, 9, 12]. Cytophotometric and cytogenetic investigations have also shown that heteroploidy may also be a feature of the cells of pretumor hyperplastic foci of proliferation [1, 8, 10, 11]. The distinctive characteristics of these stages of pretumor changes in the brain, including their proliferative phase, call for comprehensive and combined investigations of the population kinetics of the glio-mesenchymal cells at various stages of experimental carcinogenesis.

Avtsyn and Yablonovskaya [2-4] showed that during carcinogenesis induced in the cerebellum by DMBA, features of pretumor hyperplasia of glio-mesenchymal cells with the appearance of the first atypical cells occur on the 50th-100th day after insertion of the pellet of carcinogen. After the 100th day these pretumor changes are found as foci of proliferation of large astrocyte-like cells with irregularly shaped nuclei and enlarged nucleoli, located among the fibrinoid masses of the pathologically changed blood vessels close to the "villous" capsule surrounding the pellet. Mitoses of atypical cells are either extremely rare (2-3 per specimen) or are not observed at all in histological specimens. The suggestion has been made that these foci of glial proliferation directly precede the development of gliomas (most frequently, astrocytomas) of the cerebellum.

In the present investigation a comparative cytomorphometric study was made of the DNA content in the cells of a preglioma in seven albino rats of the SHK strain at various times after the beginning of the experiment (from 63 to 362 days). After a careful histological study, foci of microblastoma, assessed in two cases as the beginning of a cytoplasmic astrocytoma and in one animal as a glioblastoma, were found in three animals. Cells of an inflammatory focus of glio-mesenchymal proliferation around pellets of paraffin wax were investigated as the control (in 2 rats on the 82nd and 119th days after the beginning of the experiment).

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TABLE 1. Distribution of Cells (in %) by DNA Content Measured in Conventional Ploidy Units (c)

Character of pathological process	Time from beginning of expt. (in days)	1 c	2 c	3 c	4 c	5 c	6 c	7 c
Inflammatory proliferation of glio-mesenchymal cells around paraffin pellet	82	4,0±2,0	90,8±2,9	5,2±2,1				
	119	2,1±1,4	66,3±4,7	27,7±4,5	2,9±1,7			
Foci around DMBA pellet	63		68,0±4,6	22,0±4,1	8,0±2,5	2,0±1,4		
	296	1,1±1,0	54,8±5,0	36,1±4,8	8,0±2,5			
	297	8,5±2,5	65,5±4,7	22,0±4,1	2,0±1,4	2,0±1,4		
	362		86,2±3,5	11,8±3,2	2,0±1,4			
Microastrocytoma	98	4,0—2,0	51,7±5,0	36,2±4,8	4,1±2,0	2,0±1,4	2,0±1,4	
	339		60,0±4,9	30,0±4,6	10,0±3,0			
Microglioblastoma	196	8,0±2,5	34,0±4,7	40,0±4,9	16,0±3,6			2,0±1,4

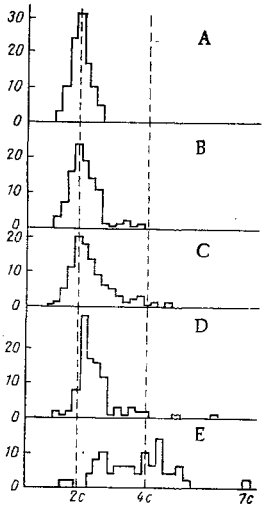

Fig. 1. Histograms of distribution of cells by DNA content. Ordinate: number of cells (in %); abscissa: DNA content (in c); A) small granule cells from the cerebellar cortex; B) glio-mesenchymal cells from focus of inflammatory proliferation around paraffin pellets; C) atypical glio-mesenchymal cells around DMBA pellet (preglioma); D) microastrocytoma; E) microglioblastoma.

EXPERIMENTAL METHOD

The Feulgen reaction was carried out on series of paraffin sections, 5 μ in thickness (fixation of the brain with 12% neutral formalin, hydrolysis with 5 N HCl at room temperature for 50 min, staining with Schiff's reagent for 1 h). Cytophotometry was carried out on a digital integrating microphotometer, made at the Institute of Chemical Physics, Academy of Sciences of the USSR [7]. As the standard for the diploid quantity of DNA small neurons, consisting of granule cells from the cerebellar cortex, were chosen [13, 14]. Comparative cytophotometric analysis of granule cells in films from the tissue of the rat cerebellar cortex and of small lymphocytes (in films from a suspension of rat spleen in bovine serum) showed that the DNA content in these cells in every case corresponded to diploid and near-diploid values. In each preparation the measurements were made in 100 cells from foci of glio-mesenchymal proliferation (altogether 900 cells) and in 5-60 small granule cells (altogether 375 cells). The results of cytophotometry (in conventional ploidy units — c) for each observation are given in Table 1 and are summarized as histograms (Table 1, Fig. 1).

EXPERIMENTAL RESULTS

The focus of glio-mesenchymal proliferation around the paraffin pellet was characterized by the overwhelming predominance (83%) of diploid and near-diploid cells, a comparatively few (about 15%) cells with the DNA content corresponding to triploid and near-tetraploid values (Fig. 1B). The fraction of cells with ploidy levels of 3c and 4c, most of which were probably in the S and G_2 periods of the cell cycle, thus did not exceed $1/5$ (about 22%) of the number of diploid cells. This evi-

dently indicates the comparatively low level of DNA synthesis in the glial and connective-tissue cells of the brain substance during productive chronic inflammation around a foreign body (Fig. 1C).

A distinct predominance of diploid and near-diploid cells (69.5%) was observed in the atypical foci of preglioma proliferation, but compared with the control, there was an increase in the number of cells in the near-triploid region (24%) as well as a small increase in the number of paratetraploid cells (6.5%), and also there were a few hypertetraploid nuclei (1%). Altogether the fraction of paratriploid cells during pretumor proliferation increased to 40% of the number of diploid cells, possibly evidence mainly of activation of DNA synthesis.

Compared with the control and with the foci of preglioma proliferation, in foci of cytoplasmic astrocytoma (Fig. 1D) the number of paradiploid cells was reduced to 59% whereas the number of paratriploid cells rose to 32%; however, the number of cells with tetraploid and hypertetraploid nuclei remained about the same as in the preglioma (9% of cells altogether). A further increase in the number of cells synthesizing DNA was evidently observed in the microblastomas, as shown by the greater increase in the fraction of paratriploid and paratetraploid cells than in the preglioma, and amounting together to about 70% of the paradiploid cells, the peak of which on the histogram of the microastrocytomas is shifted a little to the right.

Only in the case of the microglioblastoma (Fig. 1E) could a considerable degree of aneuploidy and polyploidy be found; the modal class was evidently formed by paratetraploid cells, the fraction of which increased to 40%, and there was also a considerable increase in the number of cells with ploidy levels in excess of 4 c (amounting altogether to 18%), but in this case the maximal level of polyploidy corresponded to paraoctaploid cells.

The results are evidence that the early stages of carcinogenesis induced by DMBA in the rat cerebellum are characterized by a well-marked diploid modal class; differences between the histograms of atypical cells from foci of preglioma proliferation and microastrocytomas and the histograms of inflammatory proliferation of glio-mesenchymal cells around the paraffin pellets can be interpreted as the result of an increase in the number of cells synthesizing DNA and with a fairly low yield of polyploidy. Considerable heteroploidy and polyploidy are observed only when the process of carcinogenesis turns toward malignancy in the early stages. In the earlier stages of pretumor changes and in the initial stages of growth of a benign glioma (cytoplasmic astrocytoma) no significant changes in karyotype are evidently observed. This is in agreement with the established view of the diploid nature of cytoplasmic astrocytomas in man and experimental animals [5, 6, 9, 12]; as the results of the present investigation show, this is also a characteristic feature of these tumors in the initial stages of tumor growth.

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