PROTEIN CONTENT OF CELL NUCLEI IN BRAIN TUMORS OF MAN

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E. D. Tomina and L. Z. Pevzner

Institute of Physiology, USSR Academy of Sciences, Leningrad
(Presented by Academician V. N. Chernigovskii)
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Advance in the methods of quantitative cytochemistry and, above all, cytospectrophotometric and microinterferometric methods, have made it possible to estimate the protein contents of individual tumor cells in situ. It has been found [16, 17, 18] that protein content is increased in various types of tumor, and similar changes have even been demonstrated in cells in the precancerous state. It has also been established that the protein content of the cytoplasm of tumor cells is generally proportional to the increased DNA content of their nuclei [18]. Increased content of solids (i.e., mainly protein) has also been observed in various types of tumor cells [12, 20].

While protein metabolism has been studied in considerable detail in quite a number of tumors [3, 17], similar studies on brain tumors are only just beginning, mainly because of the difficulty of obtaining tumor material. This difficulty has only been overcome in recent years, with the advance of neurosurgical technique.

The available evidence [11, 14] indicates that the total content of soluble proteins is considerably higher in brain tumor homogenates than in normal brain tissue. Some authors [5, 9, 13] have been able to observe certain differences in the protein fraction composition of brain tumor tissue. The changes observed have been mainly reduced contents of "albumins" and, in some cases, increase of "globulins;" the albumin/globulin generally bore some relationship to the degree of malignancy.

These investigations were, however, carried out with total tumor tissue homogenates, and it must be remembered that brain tumors contain other structural elements as well as the actual tumor cells; furthermore, the density of the arrangement of tumor cells (and consequently the extent to which they determine the biochemical characteristics of the homogenate as a whole) varies considerably in tumors of different types, and in tumors as compared with normal brain tissue.

It was, therefore, thought of some importance to compare the protein contents of individual cells in brain tumors of different types and normal macroglial cells.

This part of the investigation deals with the protein content of the nuclei of tumor cells, as histone, a protein of considerable importance in the mechanism of cancerogenesis [10, 19, 21], constitutes the bulk of the nuclear proteins.

METHOD

Histological sections from 27 tumors of human brain were examined. The pathological features of these tumors and the methods employed for preparation of the sections have already been described [8]. The tumor tissue was obtained from patients operated on for cerebral tumor in the Leningrad Neurosurgical Research Institute.

The protein contents of the nuclei of tumor cells were assessed from the optical density of 280 mμ sections of nuclei. As the values obtained might be increased by reason of the presence of nucleic acids in the cells, the latter were removed from the sections by extraction with 5 per cent trichloroacetic acid at 90°C for 5-6 min [1, 7]. Optical density was measured with a UV cytospectrophotometer, the optical arrangement and actual form of which have been described by Argoskin et al. [1]. Protein contents were expressed in arbitrary units as the product of D_P and V_N, D_P being optical density for 280 mμ after removal of nucleic acids, and V_N the average nuclear volume.

Protein Contents of Nuclei of Cells of Brain Tumors

Type of tumor	No. of cases	No. of cells	Nuc. vol. μ^3	D_{DNA}	D_P	D_{DNA}/D_P	Protein cont. (arbitrary units)
Astrocytes (control)	3	77	173	0,28	0,31	0,90	$57 \pm 2,0$
Astrocytomas	10	261	234	0,65	0,33	1,97	$81 \pm 5,7$
Multiform glioblastomas	4	101	187	0,82	0,39	2,10	$69 \pm 4,8$
Arachnoi endotheliomas	8	217	200	0,80	0,35	2,29	$75 \pm 5,4$
Ependymomas	2	54	330	0,88	0,39	2,25	$127 \pm 5,4$
Other brain tumors	3	76	135	0,90	0,32	2,86	$45 \pm 3,7$

Note. D_P - optical density of nuclei for 280 mμ after extraction of nucleic acids. D_{DNA} - optical density of DNA for 280 mμ as measured earlier [8] by two-wave UV cytospectrophotometry.

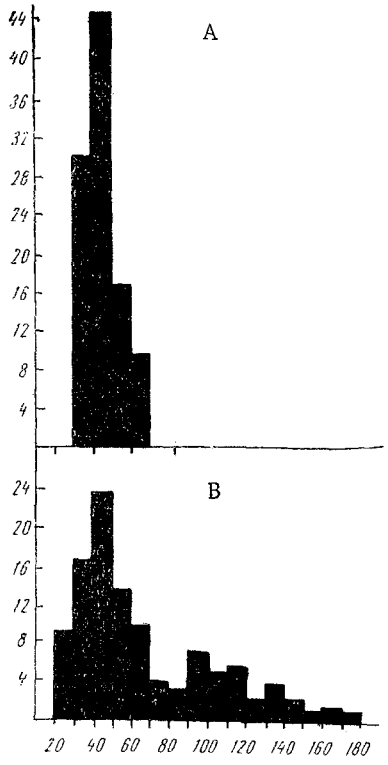

Fig. 1. Distribution of tumor cells by nuclear content of protein. Ordinate - percentage of tumor cells. Abscissa - protein content per nucleus. A) Normal astrocytes; B) cells of all brain tumors examined.

The formula for a sphere or ellipsoid was used to calculate nuclear volume from the measurements of the nucleus, made with a micrometer eyepiece.

Controls were provided by the examination of astrocytes from 3 patients operated on for different kinds of malignant brain tumors. Areas of unchanged tumor tissue were found to be present in the tissues removed at operation from these patients. Although the possibility of biochemical changes in these areas could not be excluded, only astrocytes presenting no signs of pathological change were selected. As was shown earlier [8], the DNA contents of these astrocytes were between 6 and 6.5 μμg, which would correspond to the diploid level of DNA content, observed in cortical cells [7, 15].

RESULTS

The results of the protein estimations in the nuclei of brain tumor cells are shown in the table, together with the control values for astrocytes. The quantities of protein in the nuclei of tumor cells were generally larger than the quantities found in the nuclei of normal astrocytes.

Protein contents varied considerably in different individuals with similar types of tumor. Similar variations had been noted in the DNA contents of nuclei of cerebral tumor cells [8]. In both cases, these individual variations could probably be ascribed to different degrees of differentiation in the cells of the tumors examined.

In the case of one patient adjoining cells were found to have different nuclear protein contents. Figure 1 shows the distribution of nuclear protein contents for all the brain tumor cells examined and for the astrocyte controls. The values for astrocytes showed considerably less scatter than those for tumor cells and had one maximum, as compared with several for the tumor cells (Fig. 1), the latter distribution being very similar to the DNA distribution for the same tumors. The first maximum would appear to represent tumor cells with diploid DNA contents, the second, cells with tetraploid contents, the third, cells with octaploid DNA contents and so on.

The average increase in the protein content of the nuclei of tumor cells was more or less proportional to increase of nuclear DNA (Fig. 2). Comparison of the slopes of the graphs for normal astrocytes and tumor cells showed, however, that DNA content increased much more than protein content in the same cell nucleus. In a

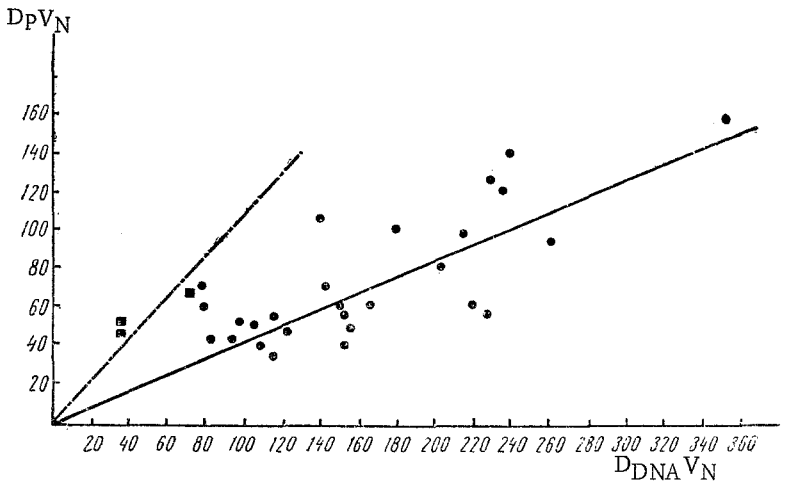

Fig. 2. DNA/protein content ratio for astrocytes and cerebral tumor cells. Ordinate) protein content; Abscissa) DNA content arrived at by similar method. Black squares) values for astrocytes in the different patients. Black circles) values for tumor cells in the different patients. Interrupted line) mean value of ratio for astrocytes. Continuous line) mean value for tumor cells.

comparative study of astrocytomas and multiform glioblastomas, which may be regarded as representing different stages of astrocytic dedifferentiation, it was found (table) that in astrocytomas, in which nuclear volume was only slightly increased, the optical density of DNA was 130%, and the optical density of protein less than 10% higher than in normal astrocytes, whereas in glioblastomas (in which nuclear volumes are almost the same as those of astrocytes) the optical density of DNA was 210% and that of protein 25% higher than in astrocytes.

It would appear that, in cerebral tumors of man, the increase in the total mass of nuclear proteins is almost proportional to the increase in nuclear volume, so that the protein concentration in the nuclei is only slightly increased, whereas the total increase of DNA is relatively much greater than the increase in nuclear volume, and the DNA concentration is therefore much higher than in normal brain cells.

These findings thus prove that the nuclei of the tumor cells examined generally contained more protein than the nuclei of normal glial cells or, more particularly, the nuclei of astrocytes. The protein content per nucleus in the cells of such well differentiated malignant tumors as astrocytomas, was considerably greater than the content of astrocyte nuclei, but in more malignant, less differentiated tumors (multiform glioblastomas) it was only slightly higher than in astrocytes. An interesting point is that similar relationships have been demonstrated in the case of early stages of epidermal carcinoma [2].

Absorption at 280 mμ is effected solely by the aromatic amino acid radicals, tryptophan, tyrosine, and phenylalanine, and not by the entire protein molecule; it is therefore, not impossible that the changes demonstrated in these investigations may apply only to these aromatic amino acids, and not to the total protein content. Published reports [6] indicate, however, that the amino acid composition of quite a number of tumors does not differ essentially from the composition of the homologous tissues.

While, in this investigation, only total contents of nuclear proteins were determined, there must undoubtedly be numerous patterns of tumor growth which are linked with changes in the proportions of the various fractions of the proteins in the nuclei of tumor cells [3, 4]. A detailed analysis of protein fractions in the nuclei of brain tumor cells by quantitative cytochemical methods is obviously a matter for future research.

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