NEUROSPECIFIC PROTEINS IN MALIGNANT TUMORS OF THE HUMAN BRAIN

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Difficulties in the differential diagnosis of malignant neuroglial brain tumors are due to the marked morphological anaplasia of these neoplasms [2] and a fall in the level of functional differentiation of the tumor cells, namely a decrease in the content of neurospecific proteins S-100 and GFAP [10-12, 15].

The aim of the present investigation was to study the content, not only of S-100 and GFAP, but also of the specific protein of nerve cell adhesion (glycoprotein D_2 [1]) in malignant brain tumors and to assess the comparative diagnostic importance of these three neurospecific markers.

EXPERIMENTAL METHOD

Samples of human brain tumors were obtained in the form of biopsy material from the Kiev Research Institute of Neurosurgery, Ministry of Health of the Ukrainian SSR. The tumors were classified on the basis of the results of light-optical investigation of fragments from the same regions of the neoplasm as were used for the immunochemical analyses. The content of proteins S-100, GFAP, and D_2 was determined quantitatively in extracts (0.025 M Tris-buffer, pH 7.4, containing 2% Triton X-100) of the tumors by methods of rocket or rocket-linear electrophoresis [4] in agarose gel (from "Litex"), using monospecific antisera. Monospecific rabbit antiserum against human brain protein D_2 was otained by immunization into the popliteal lymph nodes with a purified preparation of D_2 with Freund's incomplete adjuvant. Protein D_2 was isolated and purified successively by extraction of brain membrane proteins with Triton X-100, chromatography with hydrophobic interaction on phenylsepharose [1], and chromatography on concanavalin A-sepharose, lysine-sepharose, and hydroxyapatite [16]. Monospecific rabbit antiserum against GFAP was obtained by subcutaneous immunization with the purified protein preparation with Freund's complete adjuvant. GFAP was isolated and purified by a modified method in [3]. Monospecific antiserum against protein S-100 was generously provided by Senior Scientific Assistant L. S. Cmerchinskaya, Institute of Biochemistry, Academy of Sciences of the Ukrainian SSR. Crossed immunoelectrophoresis of preparations of protein D_2 from normal brain and tumors was carried out by the method in [9].

EXPERIMENTAL RESULTS

The group of neuroglial tumors studied were characterized as a whole by a decrease in the content of S-100 and variability of the GFAP content: in three cases the latter protein was not found at all, but in two cases its content exceeded 200% compared with that in the white matter of the hemispheres (Table 1). This agrees with data in the literature [10, 11]. Weak positive correlation ($r = +0.25$) was observed between the content of S-100 and GFAP in neuroglial tumors. The proteins studied were not present in tumors of connective-tissue or epithelial origin, except in one metastasis of a carcinoma, in which the content of S-100 and GFAP was exceedingly small. This fact can be explained by the presence of a small quantity of nerve tissue in the sample.

Comparative analysis of the content of specific proteins in malignant glial (glioblastoma) and malignant connective-tissue (sarcoma) tumors is of the greatest interest, for a correct diagnosis based on morphological criteria is particularly difficult. Determination

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TABLE 1. Content of Neurospecific Proteins in Human Brain Tissue

No. of tumor sample	Histological diagnosis	Content of neurospecific proteins		
		S-100	GFAP	D_2
1	Glioblastoma	38,0	87,0	177,0
2	Glioblastoma	15,4	0	183,0
3	Glioblastoma	19,5	81,0	36,0
4	Glioblastoma	78,2	121,2	98,8
5	Glioblastoma	19,7	0	67,2
6	Glioblastoma	26,6	0	28,9
7	Glioblastoma	11,5	255,0	134,4
8	Glioblastoma	7,5	17,3	45,1
9	Glioblastoma	5,8	15,3	11,2
10	Glioblastoma	18,2	233,4	16,9
11	Glioblastoma	29,3	67,8	86,3
12	Glioblastoma	11,5	56,1	114,5
13	Medulloblastoma	5,4	16,0	121,2
14	Medulloblastoma	15,6	7,3	168,0
15	Medulloblastoma	0	10,1	138,4
16	Medulloblastoma	83,8	98,9	135,3
17	Sarcoma	0	1,3	0
18	Sarcoma	0	0	0
19	Meningioma	0	0	0
20	Sarcomatous meningioma	0	0	0
21	Metastasis of carcinoma	3,7	1,0	0

Legend. Content of neurospecific proteins (μg/ml of soluble protein [15] of fraction) expressed as a percentage of their content in normal brain. Sample No. 15 was an undifferentiated desmoplastic medulloblastoma. Tumors whose morpholigical diagnosis was difficult because of marked anaplasia are indicated by an asterisk.

of neurospecific proteins in tumors with a similar degree of morphological anaplasia and a similar histological structure, enabled them to be classed as different nosological entities (specimens Nos. 7 and 18, Fig. 1). A study of five highly anaplastic brain tumors (specimens Nos. 6, 7, 12, 15, and 18) led to the conclusion that immunochemical detection of neurospecific markers is an adequate criterion for the differential diagnosis of intracerebral gliomas, sarcomas, and metastases of carcinoma. Combined determination of S-100 and GFAP enables the histogenesis of the tumor to be judged more reliably than investigation of each protein separately. The most stable specific marker was found to be protein D_2, although its content varied considerably in the glial tumors studied.

Data on neurospecific proteins in medulloblastomas, which are relatively undifferentiated tumors belonging to the group of dysontogenetic neoplasms [5, 7], are of great interest. A high content of D_2, found in all four medulloblastomas (on average 140% of its relative content in the brain), including in a desmoplastic medulloblastoma (specimen No. 15), in our opinion, is undisputed evidence of the neuroepithelial origin of medulloblastomas.

Protein D_2 in embryonic brain is known to have higher electrophoretic mobility than in the adult brain, due to a higher content of sialic acids [1, 13]. Besides membrane D_2, its soluble derivatives also exist [14].

The study of the content of D_2 in soluble and membrane fractions of several glioblastomas showed that the fraction of soluble D_2 in them was 2 to 3 times greater than in brain tissue, namely 23.2% (specimen No. 1), 36.9% (No. 2), and 38.5% (No. 4), compared with 12.3% in brain. Crossed immunoelectrophoresis of the phenylsepharose fraction [1] of adult human brain (the membrane form of D_2) and of the total Triton extract of glioblastoma (specimen No. 7) revealed two forms of D_2 with different electrophoretic mobility in the tumor (merging on immunodiffusion, Fig. 2). Further immunoelectrophoretic analysis of the fetal form of D_2, and also of the soluble and membrane form of tumor D_2 showed that the soluble form corresponds in mobility to D_2 from adult brain, whereas the membrane form corresponds to D_2 of fetal brain. This is a very interesting fact, which calls for special study, because the fetal form of D_2, which

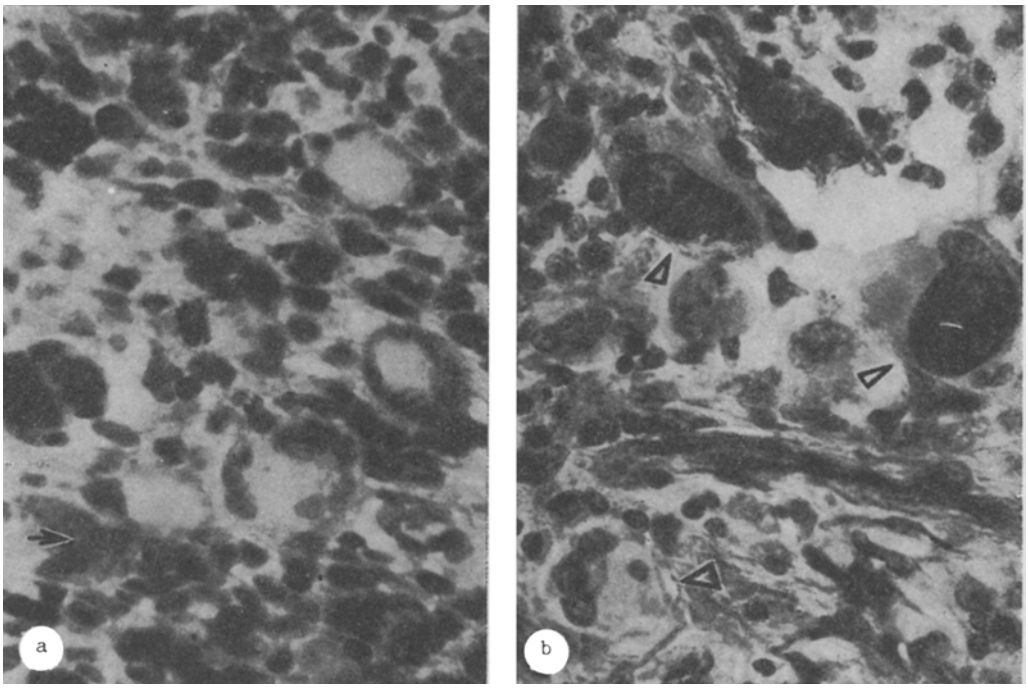

Fig. 1. Histological structure of highly anaplastic human malignant brain tumors containing (a) and not containing (b) neurospecific proteins. a) Specimen No. 7, glioblastoma; b) specimen No. 18, sarcoma. Mitoses (arrow) and atypical giant cells (triangles) present in field of vision. Hematoxylin and eosin, 240×.

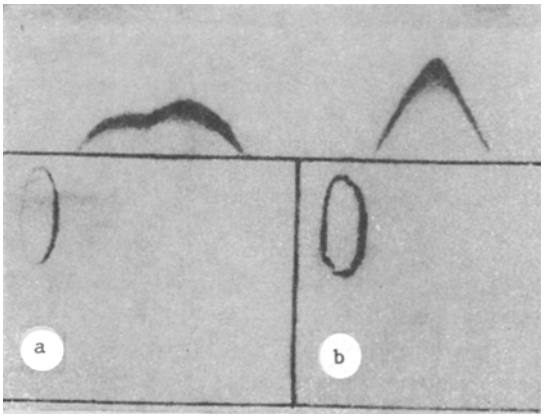

Fig. 2. Crossed immunoelectrophoresis of protein D_2 from brain and glioblastoma. a) 20 µl (156 µg protein) of Triton extract of glioblastoma No. 7 (Table 1); b) 20 µl (275 µg protein) of phenylsepharose fraction of adult human brain. Antibodies — monospecific antiserum (14 µl to 1 ml of agarose gel) against protein D_2 of adult human brain.

contains sialic acid residues (by contrast with D_2 from adult brain) is characteristic of nerve cells whose synapses are not yet established (during synaptogeneis) [13]. No such phenomenon was observed previously in a study of the other neurospecific proteins, GFAP isolated from a glioblastoma was identical with the protein from adult brain [10].

The results of these experiments confirm Khominskii's concept of divergent anaplasia of intracerebral neuroectodermal (neuroepithelial) tumors [6, 8]. In particular, in a group of medulloblastomas definite functional differentiation toward astrocytic type was observed in one case (specimen No. 16), as shown by the high content of S-100 and GFAP, whereas three

other tumors contained only small amounts of these proteins. Divergent anaplasia also was exhibited in the group of glioblastomas.

The results indicate the need for combined determination of several specific markers and they demonstrate the advantage of immunochemical determination of protein D_2 over that of proteins S-100 and GFAP in the differential diagnosis of malignant brain tumors.

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STRAINS OF TUMORS OF THE HUMAN GASTROINTESTINAL TRACT AND UTERUS TRANSPLANTABLE INTO NUDE MICE AND RATS

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Nude mice or rats are nowadays used as experimental models of human tumors. Transplantation of human tumors into nude mice has been carried out on quite a wide scale since 1969 [5]. Recently papers have been published on heterografting of human tumors into nude rats also [1-6].

In the investigation described below 30 strains of human tumors transplantable into nude mice and 30 strains transplantable into nude rats were obtained. It is therefore possible to study the same human tumor in different laboratory animals. The strains obtained will be described in a series of communications. This paper describes strains of carcinoma of the colon (RTK-1, RTK-2, RTK-7), carcinoma of the stomach (RZh), chorionepithelioma of the uterus (KhÉ), carcinoma of the cervix uteri (RShM), and carcinoma of the body of the uterus (RTM), transplantable into nude mice and rats.

EXPERIMENTAL METHOD

Nude mice were used at the age of 6-8 weeks and nude rats at the age of 4-6 weeks respectively. Human tumors used for transplantation were obtained during surgical operations. The tumors were transplanted subcutaneously in fragments into the mice. Strains RTK-1, RTK-2, RTK-7, RZh, KhÉ, and RTM were obtained in this way. Strain RShM was obtained by transplanta-

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