Intracranial germ-cell tumors

Immunohistochemical study of three autopsy cases

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Histological and immunohistochemical findings in three cases with primary intracranial germ-cell tumors are reported. Based on histological examination, they were diagnosed, respectively, as an endodermal sinus tumor with immature teratoid differentiation, teratoma and embryonal carcinoma, and choriocarcinoma and germinoma. The first case was presumed to be a combination of endodermal sinus tumor and germinoma. An immunohistochemical examination showed positive reactions to alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) in the endodermal sinus tumor, and to human chorionic gonadotropin (HCG) in the choriocarcinoma, but showed no reaction in the embryonal carcinoma. Human chorionic gonadotropin was demonstrated in syncytiotrophoblastic cells and CEA in a gland-like structure. The value of measuring CEA in cases of germ-cell tumors, in addition to AFP and HCG assays, is stressed, and the characteristics of CEA-positive tissue are discussed.

KEY WORDS • germ-cell tumor • alpha-fetoprotein • human chorionic gonadotropin • carcinoembryonic antigen

Case Reports

Case 1

This 14-year-old boy was admitted on December 18, 1980, with a 6-day history of headache and vomiting.

Examination. The patient was drowsy and had left hemiparesis, hemihypesthesia, and Horner's syndrome. Computerized tomography (CT) revealed a contrast-enhancing mass in the posterior portion of the third ventricle and the head of the left caudate nucleus (Fig. 1 left). The serum level of AFP was elevated (560 ng/ml), but HCG and CEA were within normal limits. A presumptive diagnosis was germ-cell tumor.

Course. The patient received neuraxis radiation therapy. After a total of 2400 rads had been delivered to the left caudate head, a CT scan revealed that the enhanced mass had become a smaller low-density area, while the pineal mass remained unchanged. After a total irradiation dose of 5200 rads was delivered to the posterior portion of the third ventricle, the size of that mass was also reduced markedly (Fig. 1 right) and the serum level of AFP fell to within normal limits (1.5 ng/ml). The patient was discharged on March 13, 1981, with the only neurological sign being slight anisocoria.
H. Naganuma, et al.

FIG. 1. Case 1. Contrast-enhanced computerized tomography scans. Left: At admission, showing enhanced masses in the posterior portion of the third ventricle and the head of the left caudate nucleus. Right: After delivery of 5200 rads to the tumor area, the mass in the head of the caudate nucleus has disappeared. The third ventricular mass is reduced in size.

A month after therapy, the pineal tumor was again enlarged on CT scanning, and serum levels of AFP and CEA rose to 1440 ng/ml and 13.0 ng/ml, respectively. The patient received a second course of radiation therapy with chemotherapy: 2000 rads to the pineal region; two courses of vincristine (0.85 mg intravenously) and ACNU (1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride) (30 mg intravenously) for 2 successive days; and two doses of methotrexate (5 mg intrathecally). In May, 1981, he developed tetraparesis and hypesthesia below the C-5 spinal level. He received additional radiation therapy to the spinal axis, two courses of cis-platinum (20 mg intravenously for 5 successive days), and bleomycin (30 mg intravenously once a week for 5 consecutive weeks). During the course of the treatment, serum levels of AFP decreased, but CEA remained at abnormally high levels (between 6.3 and 13.3 ng/ml), and the pineal tumor gradually enlarged with marked hydrocephalus on CT scan. The patient died without further therapy on July 20, 1981.

Postmortem Examination. Autopsy was limited to the brain. A hemorrhagic and necrotic tumor was located in the pineal region, 5 × 3.5 × 5 cm in size (Fig. 2). The tumor predominantly extended to the right hemisphere, compressing the basal ganglia and the thalamus, partly penetrating into the right lateral ventricle with a small implantation metastasis on the ependymal surface. A small implantation was seen in the inferior medullary velum. A semitransparent area of cystic degeneration was observed in the head of the left caudate nucleus. The testes were normal in appearance.

Papillary structures consisting of pale nucleated tumor cells, cuboidal in shape, were seen on microscopic examination (Fig. 3). Mitosis was frequent, and multinucleated cells were occasionally found. Glandular structures composed of a layer of cuboidal or cylindrical cells were rarely observed. Periodic acid-Schiff (PAS)-positive hyaline droplets after diastase digestion were visualized in the cytoplasm of tumor cells. The head of the left caudate nucleus showed demyelination and formation of cyst, but no tumor cells. The histological diagnosis was endodermal sinus tumor.

Case 2

This 13-year-old boy was admitted on August 9, 1973, with a 6-month history of headache, nausea, and vomiting.

Fig. 2. Case 1. Midsagittal section of the brain showing a hemorrhagic and necrotic tumor in the third ventricle and the pineal region. Rule is in centimeters.

Fig. 3. Case 1. Photomicrograph of tumor cells showing an epithelial arrangement forming a papillary structure. H & E, × 88.
Intracranial germ-cell tumors

**FIG. 4.** Case 2. Sagittal section of the brain showing a round tumor with capsule in the pineal region. A necrotic tumor protrudes into the third ventricle. A hemorrhagic tumor is located in the anterior portion of the medulla.

Examination. On admission he was alert, but had choked discs and a bilateral Stewart-Holmes sign. Precocious puberty was noted. Cerebral angiography revealed hydrocephalus and a space-occupying lesion in the pineal region. A CT scan was not available at that time. A ventriculoperitoneal shunt was placed on August 23, 1973.

Course. With the presumptive diagnosis of a pineal tumor, radiation therapy was started on August 27; 5350 rads was delivered to the pineal region. On October 20, the patient had lumbar pain, urinary retention, and paresis of the lower extremities. A lumbar puncture revealed a blockage of cerebrospinal fluid (CSF) flow and a high protein content (2800 mg/ml). He received additional radiation therapy; 4000 rads to the whole spinal axis, and, 20 days later, 2500 rads to the T-9 through L-1 spinal level. During the course of the treatment, his neurological symptoms deteriorated. In December, 1973, he had paresis of the left arm and left facial paresis of the infranuclear type, and dissemination of tumor cells to the medulla was suspected. In January, 1974, his disturbed level of consciousness worsened, and he had intestinal bleeding and pneumonia. He died on January 11, 1974, about 11 months after the onset of the symptoms.

Postmortem Examination. In the sagittal section of the brain, a round, multicystic tumor, 2 cm in diameter, was found in the pineal region, covered with a fibrous capsule (Fig. 4). Anterior to the cystic tumor, there was a yellowish-brown necrotic tumor, 1.5 cm in diameter, protruding into the third ventricle. The tumor had completely obliterated the cerebral aqueduct. A dark reddish soft tumor, 4.5 x 4.5 cm in size, was located in the anterior portion of the medulla. Below the level of the L-3 vertebral segment, a dark-brown arachnoid membrane thickened and partly entrapped the cauda equina. The testes appeared normal.

On microscopic examination, the pineal tumor consisted mostly of stratified squamous epithelium which was highly keratinized (Fig. 5 left). Cylindrical epithelium similar to intestinal epithelium and small cysts lined by ciliated epithelium were found. The tumor, which protruded into the third ventricle, was composed of necrotic tissue. The tumor in the medulla consisted of large cells that had one or two prominent nucleoli and pale and oval nuclei. An epithelial arrangement of the tumor cells was seen, with some clustering (Fig. 5 right). Mitotic figures were frequent. Glycoprotein materials positive for PAS were visualized inside and outside the tumor cell cytoplasm after diastase digestion. No tumor cells were seen in the lumbar spinal cord. In the testes, seminiferous tubules were well developed, but only Sertoli cells were seen. The histological diagnosis was a mature teratoma combined with embryonal carcinoma.

**FIG. 5.** Case 2. **Left:** Photomicrograph of the cystic tumor in the pineal region, showing stratified squamous epithelium with keratinization. H & E, x 60. **Right:** Photomicrograph of the tumor in the medulla. Large cells with prominent nucleoli show an epithelial arrangement. H & E, x 95.
H. Naganuma, et al.

The tumor compressed the midbrain and the infundibulum, and extended to the pineal region. No metastatic lesion was seen.

On microscopic examination, the tumor was composed mostly of cytotrophoblastic cells with prominent nucleoli and pale nuclei, and syncytiotrophoblastic giant cells (Fig. 7 left). Round or polygonal cells with round nuclei were seen mixed with lymphocytic cells (Fig. 7 right). Mature follicles and corpus luteum were seen in the ovary, and the endometrium of the uterus exhibited proliferation. The histological diagnosis was choriocarcinoma combined with germinoma.

Immunohistochemical Study

An immunohistochemical study was performed by the peroxidase-antiperoxidase (PAP) method with minor modification. Immunostain preparations for AFP, HCG, and CEA were developed on paraffin-embedded tissue sections. At least two different parts of each tumor were examined. Rabbit anti-CEA serum absorbed with nonspecific cross-reacting antigen (NCA) was also applied. As a negative control, tissue sections were treated with normal rabbit serum in place of the primary antibody. As a positive control for CEA, a section of adenocarcinoma of the colon was used.

In Case 1, a weak but definite reaction for AFP was obtained in the cytoplasm of tumor cells in a limited area (Fig. 8a). A positive reaction for anti-CEA serum was seen in tumor cells forming a gland-like structure which revealed no characteristics of an endodermal sinus tumor (Fig. 8b). For anti-CEA serum absorbed with NCA, a positive reaction was also shown in the same portion of tumor (Fig. 8c). Tumor tissue was negative for HCG. In Case 3, HCG was demonstrated in syncytiotrophoblastic cells (Fig. 8d), and AFP and CEA were not. In Case 2, no reaction for AFP, HCG, or CEA was seen in the part of embroyal carcinoma.

These results are summarized in Table 1.
Intracranial germ-cell tumors

Discussion

The view is held that germ-cell tumors are characteristically multipotential. Kleinsmith and Pierce\textsuperscript{13} noted that single embryonal carcinoma cells differentiated into yolk sacs, trophoblasts, and embryonal carcinoma. In Case 1, the tumor in the head of the left caudate nucleus was a diffusely contrast-enhancing mass on CT scanning, and disappeared after delivery of a total of 2400 rads. Histological examination showed demyelination, but no tumor cells. The tumor was highly sensitive to radiation, and was assumed to be a germinoma. Several authors support this assumption. Bito,\textit{ et al.},\textsuperscript{3} reported a patient in whom a pineal tumor was irradiated; the patient later had a left frontal lobe tumor which was verified histologically as a germinoma, and an autopsy showed no tumor in the pineal region. Fowler,\textit{ et al.},\textsuperscript{7} reported a similar case.

The pineal tumor in our Case 2 was a teratoma, and the necrotic tumor in the third ventricle was presumed to be an embryonal carcinoma. This tumor might have metastasized to the medulla and the lumbar spinal cord. In Case 3, a combination of choriocarcinoma and germinoma was seen histologically. Therefore, the tumors in Cases 1, 2, and 3 were considered to be of a mixed germ-cell type; that is, endodermal si-

<table>
<thead>
<tr>
<th>Case No.</th>
<th>AFP</th>
<th>HCG</th>
<th>CEA</th>
<th>Histological Diagnosis</th>
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<tr>
<td>1</td>
<td>+</td>
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<td>endodermal sinus tumor with immature teratoid differentiation</td>
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<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-†</td>
<td>teratoma &amp; embryonal carcinoma</td>
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<td>3</td>
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*AFP = alpha-fetoprotein; HCG = human chorionic gonadotropin; CEA = carcinoembryonic antigen (anti-CEA serum absorbed with nonspecific cross-reacting antigen was used). + = positive findings; - = negative findings.† The teratoma samples were not examined.

FIG. 8. Immunohistochemical demonstration in tumor sections prepared with hematoxylin counterstain. a, b, and c: Case 1. a: A positive reaction for alpha-fetoprotein is seen in the cytoplasm of tumor cells. × 270. b: A positive reaction to anti-carcinoembryonic antigen (CEA) serum is shown in the tumor cells which form a gland-like structure. × 135. c: Section adjacent to b. A positive reaction to anti-CEA serum absorbed with nonspecific cross-reacting antigen is also seen. × 135. d: Case 3. Human chorionic gonadotropin is demonstrated in syncytiotrophoblastic cells. × 135.
nus tumor and germinoma, teratoma and embryonal carcinoma, and choriocarcinoma and germinoma, respectively. The combination of two or more subtypes of germ-cell tumors has been observed by a number of workers.\textsuperscript{2,4,6,9,11,12,20,29,32}

Specific types of germ-cell tumors produce AFP and/or HCG. Embryonal carcinoma may produce AFP and/or HCG; endodermal sinus tumor, AFP; choriocarcinoma, HCG; however, germinoma and teratoma produce neither.\textsuperscript{14} There are many reports on the immunohistochemical demonstration of AFP and HCG in primary intracranial germ-cell tumors.\textsuperscript{1,8,11,19,22,26-29,33} but to our knowledge only one report has revealed CEA in intracranial germ-cell tumors: Irie, et al.,\textsuperscript{10} detected CEA in primitive glands of a mediastinal teratocarcinoma.

In our Case 1, a positive reaction to anti-CEA serum absorbed with NCA was seen in the tumor cells forming a gland-like structure. Several CEA-related antigens are known, including NCA,\textsuperscript{8} NCA-2,\textsuperscript{1} normal fecal antigen (NFA),\textsuperscript{17} and biliary glycoprotein (BGP).\textsuperscript{5} In our examination, CEA-related antigens other than NCA might have been detected. Mori, et al.,\textsuperscript{18} noted that, using anti-CEA serum absorbed with NCA, positive reactions were obtained in malignant endodermal tissues. It is considered that a gland-like structure in our case of endodermal sinus tumor may exhibit an immature teratoid differentiation. In Cases 1 and 3, AFP and HCG were visualized in the tumor cells, respectively, and these observations were compatible with the histological diagnosis. However, in the embryonal carcinoma (Case 2), neither AFP nor HCG was demonstrated. Kurman, et al.,\textsuperscript{14} also noted that embryonal carcinoma cells were negative for AFP and/or HCG in some cases. These results suggest that embryonal carcinoma may not produce AFP and HCG, and the parts of the tumor that differentiated into yolk sac and choriocarcinoma may produce AFP or HCG.

Serum levels of AFP and CEA were measured serially throughout the course of the disease in Case 1. Serum levels of AFP decreased during radiation therapy, but the high levels of CEA were not influenced by the therapy. Irie, et al.,\textsuperscript{10} also noted that serum levels of CEA remained high during chemotherapy and radiation therapy in a case of mediasinal teratocarcinoma. It is considered that some tumor cells producing CEA may be resistant to radiation and chemotherapy.

Serum or CSF levels of CEA are rarely elevated in primary brain tumors. Suzuki\textsuperscript{24} reported that serum levels of CEA were elevated in three of 67 cases of glioma, in one of 12 cases of acoustic neurinoma, in one of eight cases of germinoma, and in one case of embryonal carcinoma. Lee, et al.,\textsuperscript{16} found an elevated CEA level of CEA in an intracranial endodermal sinus tumor. The diagnostic value of measuring CEA may not be significant at present because CEA is not yet specific for a tumor type, but CEA examination in serum or CSF should be added because some elements of germ-cell tumor may produce CEA or CEA-related antigens.

Precocious puberty can be caused by dysfunction of the hypothalamus that normally suppresses gonadotropin secretion,\textsuperscript{31} or by a high serum level of HCG secreted by neoplasms.\textsuperscript{27} In Case 2, the tumor was negative for HCG, and invaded the hypothalamus. Therefore, hypothalamic dysfunction was considered to be the cause of precocious puberty. In Case 3, although precocious puberty was not observed, histological examination showed maturity of the ovary and the uterus. It is concluded that gonadotropin secretion induced by the hypothalamic dysfunction might have affected the ovary because maturation of the ovarian follicle may be induced mostly by follicle-stimulating hormone and not by HCG, which possesses weak luteinizing hormone activity.\textsuperscript{21}

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References

Intracranial germ-cell tumors


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