Glial Fibrillary Acidic Protein in Medulloblastomas

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Summary. Two cases of medulloblastoma are described which show positive immunostaining for glial fibrillary acidic protein (GFAP) in many cells. The surgical and autopsy specimens were examined by the indirect immunoperoxidase method. Positive staining for GFAP was demonstrated in the small, round to polygonal cells in both surgical specimens and in the small spindle cells in the autopsy specimen of one case. In the small, round to polygonal cells positive GFAP was shown as a perinuclear brown rim or intracytoplasmic brown droplet. In the small spindle cells, the cytoplasm and the polar processes were stained. Except for GFAP staining, these positive cells were morphologically indistinguishable from the accompanying unstained cells, indicating that GFAP was expressed in the medulloblastoma cells. Considering that GFAP is specific for astrocytes, these findings suggest the potential of astrocytic differentiation in the neoplastic cells of these medulloblastomas. The findings obtained in other 28 medulloblastomas examined in parallel are also discussed briefly.

Key words: GFAP — Intermediate filament protein — Medulloblastoma — Immunohistochemistry

Introduction

It has long been assumed that the medulloblastoma of the cerebellum is composed of cells with the potential of differentiation toward glial cells [1, 15]. Actually, astrocytic cells are sometimes found in the medulloblastoma, suggesting differentiation of the tumor cells [6, 7, 9, 13, 14, 16, 21]. However, it is usually difficult or impossible to determine whether or not these coexisting differentiated cells are really derived from the medulloblastoma cells. A possibility that these cells are pre-existing, non-neoplastic cells is hardly ruled out. Thus, the demonstration of the astrocytic nature of the actual medulloblastoma cells seemed to be of value in considering the potential of differentiation in this tumor.

The present paper describes two medulloblastomas showing in their main constituent cells glial fibrillary acidic protein (GFAP), which is specific for astrocytes [3, 10, 19]. The findings in 28 other medulloblastomas examined in parallel are discussed briefly.

Materials and Methods

Antibodies

Rabbit antibody to bovine GFAP was supplied by Drs. H. Mannoci and I. Takeshita, Dept. of Neurosurgery, Kyushu University (Japan). Monospecificity of the antibody was described elsewhere [13]. Horseradish peroxidase (HRP)-labeled goat anti-rabbit γ-globulin was purchased from E. Y. Laboratory (USA).

Immunoperoxidase Method

Tumor tissues were fixed routinely in 10% formalin and embedded in paraffin. Sections, 6 μm thick, were deparaffinized in xylene and pretreated with 0.3% H2O2-methanol for 1 h and with normal goat serum for 16 h to block non-specific reaction [12]. The sections were then incubated with rabbit anti-GFAP diluted 1:40 for 1 h in the primary reaction and with HRP-labeled goat anti-rabbit γ-globulin diluted 1:20 for 1 h in the secondary reaction. Each antibody was diluted with normal goat serum. Sections were incubated in 3,3'-diaminobenzidine tetrahydrochloride-H2O2 solution [11] for 20 min to visualize peroxidase reaction. In the control, normal rabbit serum was used instead of the primary antibody. In each experiment, sections counterstained lightly with hematoxylin and cosin (HE) were also prepared.
Table 1. Clinical course of two patients with a medulloblastoma

<table>
<thead>
<tr>
<th></th>
<th>Age* (years)</th>
<th>Sex</th>
<th>Site of original tumor</th>
<th>Time* of Surgery operation</th>
<th>Recurrence</th>
<th>Death</th>
<th>Postoperative therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>4</td>
<td>Male</td>
<td>Cerebellar vermis</td>
<td>4</td>
<td>10</td>
<td>16</td>
<td>Irradiation, Chemotherapy</td>
</tr>
<tr>
<td>Case 2</td>
<td>6</td>
<td>Male</td>
<td>Cerebellar vermis</td>
<td>1</td>
<td>9</td>
<td>10</td>
<td>Irradiation, Chemotherapy</td>
</tr>
</tbody>
</table>

* Age of which first neurological symptoms appeared  
* Months after the onset of illness

Results

Clinical Course of Patients

The clinical course of two patients with a medulloblastoma is summarized in Table 1. The tumors were found originally in the cerebellar vermis. Surgical removal was performed 4 months (case 1) and 1 month (case 2) after the onset of the illness. The patients received irradiation and chemotherapy after the surgical operations. Recurrence occurred, about 6 months in case 1 and 8 months in case 2. In case 1, clinical examination revealed tumor in the 4th ventricle and basal cisterns. The patient became progressively worse despite further irradiation and chemotherapy and died 6 months later, 16 months after the onset of illness. In case 2, tumors recurred in the lateral and the 3rd ventricles. The patient became rapidly worse and died 1 month later, 10 months after the first neurologic complaint. During the last month, the patient received only conservative therapy because of his poor condition.

Histological and Immunohistochemical Findings

In case 1, the biopsy revealed a highly cellular tumor composed of small, round to polygonal cells with hematoxyphilic nuclei and sparse cytoplasm (Figs. 1 A, B). No distinct processes were recognized in these cells. Mitotic figures were frequently observed. Most cells showed no special arrangement as seen in Fig. 1 A, though, in some fields, cells were arranged in narrow bands between connective tissue fibers (Fig. 1 B). Positive staining for GFAP was observed in many of these cells, as a perinuclear brown rim or as a droplet (Figs. 1 C, D). Morphologically, the positive cells were similar in shape and size to the coexisting unstained cells, but differed apparently from astrocytes which occurred sporadically and were presumed to be reactive (Fig. 1 D). Postmortem examination: extensive dissemination of tumor cells was observed, especially in the 4th ventricle, the basal cisterns and both Sylvian fissures. As in the biopsy, the tumors were highly cellular and composed of small cells. However, most fields of the autopsy tumor were composed predominantly of small spindle cells which were not evident in the biopsy. Figure 1E shows these cells in tumor from the left Sylvian fissure. The fields of the small, round to polygonal cells similar to those in the biopsy specimen were observed only occasionally. On immunohistochemical examination, GFAP staining was observed in the cytoplasm and polar processes of the small spindle cells (Fig. 1F), though the proportion of stained cells varied from one field to another. No distinct staining was observed in the occasional fields of small, round to polygonal cells in the autopsy specimens.

In case 2, the biopsy revealed a highly cellular tumor (Fig. 2A), the main cells being small, round to polygonal in shape and somewhat larger in size as compared with those of case 1. In some fields, small spindle cells were also noted. Many of these cells were in mitotic division. Positive staining for GFAP was observed in the small, round to polygonal cells. As shown in Fig. 2B, fine and delicate staining was observed in the perinuclear cytoplasm of many cells. These cells showed no morphological difference from the coexisting unstained cells. The small spindle cells showed no positive staining. Autopsy specimens revealed tumor throughout the ventricular system. Microscopic dissemination of tumor cells was observed in the leptomeninges of the entire central nervous system (CNS). The histological findings were identical to those of the biopsy. However, no staining for GFAP was observed in the small, round to polygonal cells or in the small spindle cells in the autopsy specimens.

Discussion

In the course of the immunoperoxidase examination of 30 medulloblastomas, we found positive staining for GFAP in the main constituent cells of five cases. Two of the cases showed positive staining in many cells, whereas the other three cases showed a few posi-