Establishment and Characterization of Human Medulloblastoma Xenograft Line

Takashi MIKAMI, Kaoru KURISU, Keiichi KAWAMOTO, Katsuzo KIYA, Kazutoshi MUKADA, Takuhiro HOTTA, Hidenori OGASAWARA, Kazuhiko SUGIYAMA and Tohru UOZUMI

Department of Neurosurgery, Hiroshima University School of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734, Japan

ABSTRACT

A new xenotransplantable tumor line, MED-FU, was derived from a 6-year-old female patient with cerebellar medulloblastoma. This tumor was grown in nude mice as serially transplantable subcutaneous xenografts composed of small round cells with hyperchromatic nuclei and scant cytoplasm. Many rosettes and mitoses were observed. Immunohistochemically, glial fibrillary acidic protein (GFAP), S-100 protein, and neuron specific enolase (NSE) were not detected. The doubling time of the subcutaneous tumors was 6.8 days. Highly concentrated polyamines were detected in the tumor tissue and serum of tumor-bearing mice. This xenotransplanted tumor line, MED-FU, is considered to provide an available experimental model for the study of human medulloblastoma.

Key words: Medulloblastoma, Brain tumor, Xenograft, Nude mice

Medulloblastoma is one of the most malignant neoplasms in pediatric brain tumors[10]. The prognosis of such patients is extremely poor, and further advances in the therapy of medulloblastoma will require knowledge of its biological properties and response to chemotherapy. Although a number of investigators have attempted to cultivate human medulloblastoma to understand the biology of this tumor[5,19], only a small number of medulloblastoma cultures have survived for prolonged periods[6,8,11,16,20]. We have established several permanent lines of human glial tumors[12,13], and here we describe one successful serial xenograft line that was derived from human cerebellar medulloblastoma.

MATERIALS AND METHODS

Patient

The xenograft model was established from tumor tissue obtained at the time of surgery for recurrent disease in a 6-year-old girl with cerebellar medulloblastoma (Fig. 1). Initial therapy consisted of total surgical resection followed by external beam irradiation 40 Gy to the whole brain, 14 Gy to the posterior fossa and 26 Gy to the whole spinal cord. Eight months later the tumor recurred locally, and subtotal surgical resection was performed. She then received chemotherapy with ACNU, cis-dichlorodiaminoplatinum and interferon-β, but she died 8 months after the tumor recurred.

Experimental animal

The animals used in this series were female 5- to 7-week-old athymic nude mice (BALB/c nu/nu), 16–18 g each, purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan. All mice were kept under specific pathogen-free (SPF) conditions.

Fig. 1. Contrast-enhanced computerized tomography scan showing a high-density lesion in the cerebellar vermis.
Transplantation and passage
Tumor fragments obtained during surgery were washed in Dulbecco’s MEM with 10% fetal calf serum, cut with scissors 2–3 mm in diameter, and transplanted subcutaneously into the bilateral flank of mice by means of a trocar.

For subsequent passage, tumor-bearing mice were sacrificed by cervical dislocation, and the tumors handled as described above.

The shortest diameter (a, mm) and the longest diameter (b, mm) were measured every three to four days with a slide caliper. The relative tumor weight (RW, mg) was calculated by the formula: \( RW = a^2 \times b / 2 \). Tumor doubling times were calculated from sequential measurements once exponential growth began.

Pathological evaluation
Original tumor from surgical resection and subcutaneous tumors from each passage were fixed with 10% buffered formalin, embedded in paraffin, and stained routinely with hematoxylin and eosin (H & E). A immunohistochemical study was done using the avidin-biotin peroxidase complex (ABC) method. The antibodies to glial fibrillary acidic protein (GFAP), S-100 protein, and neuron specific enolase (NSE) were obtained from DAKOPATTS, Denmark.

For electron microscopy, subcutaneous tumors were fixed in cold 4% glutaraldehyde in 0.2 M sodium cacodylate buffer, post fixed in 1% osmium tetroxide, and embedded in epon. Sections were stained with uranyl acetate and lead citrate.

Polyamines determination
Concentrations of polyamines (putrescine, spermidine, spermine) in the subcutaneous tumor tissue and in the serum from tumor-bearing mice were measured by high performance liquid chromatography (HPLC).

Chromosomal analysis
Subcutaneous tumors in the tenth passage were dissected, minced, and enzymatically dissociated. The cell suspensions were plated in dishes, and incubated at 37°C in 5% CO₂ for 24 hours. Colcemide (1.0 µg/ml) was added to the dishes, and they were incubated for an hour. Then cells were fixed in methanol-acetic acid, and stained with Giemsa. Five adequate metaphase spreads were photographed and karyotyped.

RESULTS
Transplantation and growth
Initial transplantation resulted in large subcutaneous tumors in all the mice. The initial growth pattern and latencies to exponential growth were variable in the first three serial passages. Growth stabilization had taken place by the fifth passage. Then, at the 16th passage, progressive tumor growth occurred in 100% of the mice which had received tumor transplantation. The growth curves of the 12th passage of subcutaneous tumors are shown in Fig. 2. The latency to exponential growth was 18.6 ± 2.2 days and the doubling time, determined during initial exponential growth, was 6.8 ± 1.1 days.

Pathology
The original tumor removed from the patient was composed of small round uniform cells with hyperchromatic nuclei and scant cytoplasm (Fig. 3A). Mitotic figures were abundant and scattered areas of coagulation necrosis were present. The tumor cells formed numerous prominent Homer-Wright rosettes as well as perivascular pseudorosettes.

The transplanted tumor of the 12th passage demonstrated an essentially similar appearance to the original tumor (Fig. 3B). GFAP, S-100 protein, and NSE were negative in the original tumor cells as well as in the xenograft.

Polyamines
As shown in Table 1, serum putrescine and spermidine levels were significantly higher in tumor-bearing mice than in the control mice. The serum spermine level was also higher in tumor-bearing mice but there was no statistical difference. In the subcutaneous tumor tissue, polyamine levels were...
Fig. 3. Microscopic figures of the tumor. 
A: The original tumor is composed of small round cells with hyperchromatic nuclei and scant cytoplasm. Many rosettes and mitotic figures are observed. H & E, x320. 
B: The transplanted tumor (12th passage) shows a similar appearance to the original tumor. H & E, x320.

Fig. 4. Electron micrograph of the transplanted tumor (12th passage) shows primitive undifferentiated cells. No microvilli, junctional or synaptic complexes are seen. x8,900. Bar = 1 µm.

Fig. 5. G-banded Karyotype of the xenograft is 47, XX, -17, -19, +der (1) t (1;?) (p32;?), del (4) (p13), +der (13) t (13;?) (q32;?), +mar. Arrows indicate the karyotypic abnormalities.

Table 1. Serum and tissue polyamine levels.

<table>
<thead>
<tr>
<th></th>
<th>Putrescine nmol/ml (g)</th>
<th>Spermidine nmol/ml (g)</th>
<th>Spermine nmol/ml (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control mice (n=5)</td>
<td>1.6 ± 0.2a)</td>
<td>33.3 ± 5.5</td>
<td>4.1 ± 0.9</td>
</tr>
<tr>
<td>Tumor-bearing mice (n=5)</td>
<td>3.1 ± 0.8*</td>
<td>56.0 ± 14.5*</td>
<td>6.1 ± 4.4</td>
</tr>
<tr>
<td>Tumor tissue (n=3)</td>
<td>496 ± 74</td>
<td>687 ± 96</td>
<td>600 ± 110</td>
</tr>
</tbody>
</table>

a) Mean ± SD. *Statistically significant by Student's t-test as compared with that of control group (p<0.05).

Chromosomal analysis
The karyotype of the tenth xenograft passage (Fig. 5) was pseudodiploid and exhibited deletion of 4p and one marker chromosome [47, XX, -17, -19, +der (1) t (1;?) (p32;?), del (4) (p13), +der (13) t (13;?) (q32;?), +mar].

DISCUSSION
The study of human medulloblastoma has been significantly limited because this tumor grows poorly in culture and when directly transplanted into nude mice. This is in marked contrast with astrocytoma grade III or IV, from which many cell lines and xenograft models have been established. Only six cell lines (TE-671, Daoy, D283 Med, D341 Med, ONS-76 and ONS-81) and one xenograft line of human
medulloblastoma have been reported previously. We have attempted to establish a medulloblastoma xenograft line in nude mice\(^{18}\), but only one of five tumors was serially transplantable.

Some investigators have reported morphologic features suggesting a glial or neuronal differentiation of medulloblastoma.\(^{3,10}\) In our study, pathological examination showed that serially transplanted tumors have maintained primitive undifferentiated features as seen in the original tumor. No neuronal or glial elements were identified by electron microscopy. Immunohistochemically, GFAP and S-100 protein, as a marker protein of glial cells, were not detected in our transplantable tumor line nor in the other six reported medulloblastoma lines.\(^{5,6,8,11,20}\) On the other hand, NSE positive cells were present in all reported cell lines except for Daoy\(^{5}\). We could not detect NSE in MED-FU in the original tumor nor in the xenograft. Our immunohistochemical study was coincident with the ultrastructural study, and suggested no glial or neuronal differentiation in MED-FU.

Polyamines and their metabolic enzymes are present in normal\(^{18}\) and neoplastic brain tissue. In gliomas, a relation between tissue putrescine and its histological grading has been reported\(^{9}\). Moulinoux et al.\(^{14}\) studied polyamine levels in brain tumor patients, and the highest tumoral concentrations are found in medulloblastoma. We also detected a high concentration of tissue and serum polyamines in this xenograft line.

Chromosomal analysis of this transplanted tumor, MED-FU, showed diploid chromosome counts as seen in D283 Med\(^{5}\) and D341 Med\(^{6}\), while TE-671\(^{6}\) and Daoy\(^{5}\) demonstrated near tetraploid chromosome counts. No common profile of karyotypic abnormalities and no common marker chromosomes were seen in these cell lines including MED-FU. These karyotypic studies exclude the possibility of mouse-human hybridization or of induction of mouse stromal tissue tumors by the human xenograft\(^{20}\).

We have established and characterized a serially transplantable xenograft line of human medulloblastoma. The model system described here will allow further analysis of the biological properties and therapeutic efficacy of human medulloblastoma.

ACKNOWLEDGEMENTS

The authors gratefully thank Prof. N. Kamada (Dep. of Hematology, Res. Inst. for Nuclear Medicine and Biology, Hiroshima) for his advice and assistance with chromosomal analysis.

We also thank Drs. M. Takahashi, K. Ohta, and H. Yoshimoto (Matsue Red Cross Hospital, Shimane) for providing the tumor specimen.

(Received December 1, 1990)

(Accepted January 30, 1991)

REFERENCES


