Hiroshima J. Med. Sci.
Vol.40, No.1, 41~45, March, 1991
HIJM 40-7

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[15]. 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[9,19], only a small number of medulloblastoma cultures have survived for prolonged periods[5,6,8,11,16,20]. 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.

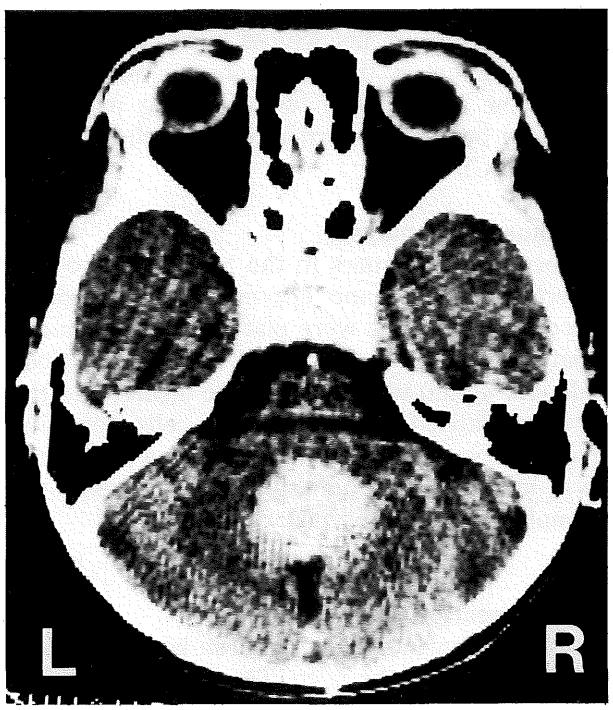


Fig. 1. Contrast-enhanced computerized tomography scan showing a high-density lesion in the cerebellar vermis.

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 folmalin, 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% CO_2 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.

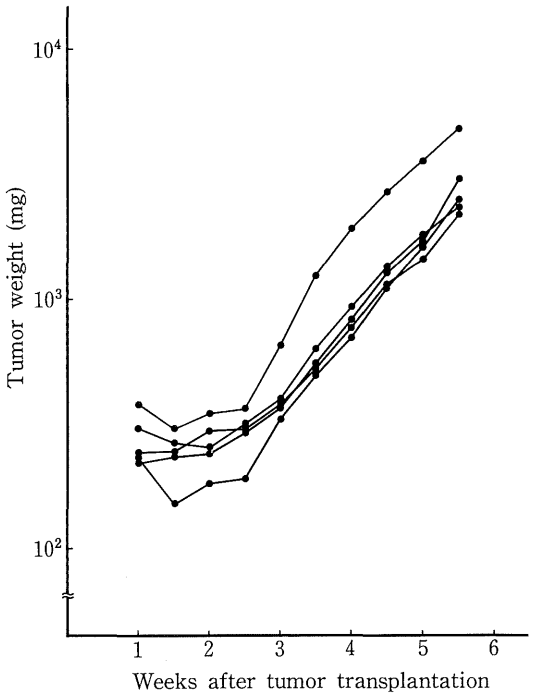


Fig. 2. Growth curves of the 12th passage of tumor (MED-FU) transplanted subcutaneously into nude mice.

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.

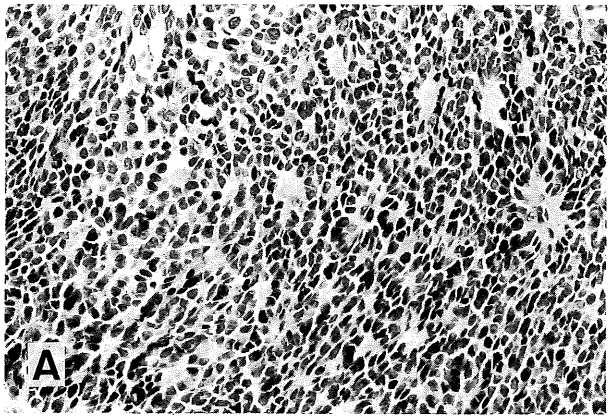
The transplanted tumor of the 12th passage demonstrated an essentially similar appearance to the original tumor (Fig. 3B).

GFAP, S-100 protein, and NSE were negative in the original tumor cells as well as in the xenograft.

Ultrastructurally, the 12th passage of subcutaneous xenograft was composed of primitive undifferentiated cells. No microvilli, junctional or synaptic complexes were noted (Fig. 4).

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



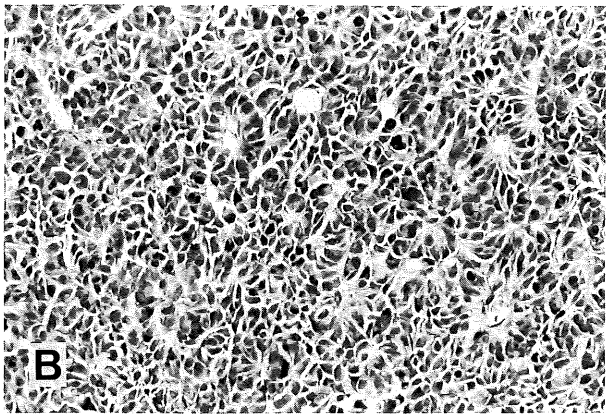


Fig. 3. Microscopic figures of the tumor.
A: The original tumor is composed of small round cells with hyperchromatic nuclei and scant cytoplasm. Many rosettes and mitotic figures are observed. H & E, ×320.
B: The transplanted tumor (12th passage) shows a similar appearance to the original tumor. H & E, ×320.

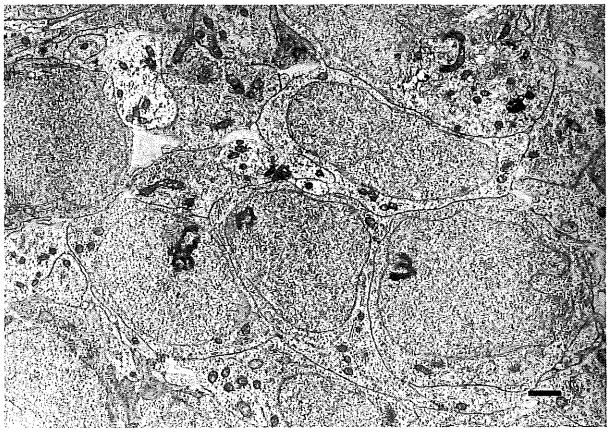


Fig. 4. Electron micrograph of the transplanted tumor (12th passage) shows primitive undifferentiated cells. No microvilli, junctional or synaptic complexes are seen. ×8,900. Bar = 1 μm.

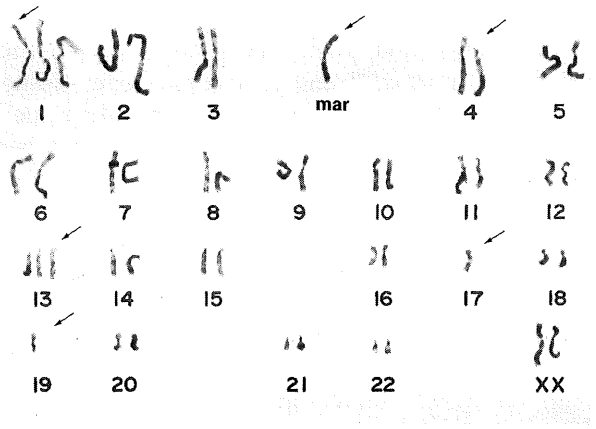


Fig. 5. G-banded Karyotype of the xenograft is 47, xx, −17, −19, +der (1) t (1;?) (p32;?), del (4) (p13), +der (13) t (13;?) (q32;?), +mar. Arrows indicate the karyotypic abnormalities.

Table 1. Serum and tissue polyamine levels.

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

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

high as compared with previous reports[14].

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[11]. This is in marked contrast with astrocytoma grade III or IV, from which many cell lines and xenograft models have been established[1,12,17]. Only six cell lines (TE-671[11], Daoy[8], D283 Med[5], D341 Med[6], ONS-76 and ONS-81[20]) and one xenograft line[16] of human

medulloblastoma have been reported previously. We have attempted to establish a medulloblastoma xenograft line in nude mice[13], 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, pathlogical 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 line[5,6,8,11,20]. On the other hand, NSE positive cells were present in all reported cell lines except for Daoy[8]. 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[7]. In gliomas, a relation between tissue putrescine in the tumors and their histological grading has been reported[7]. 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[4] and Daoy[8] 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[2].

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

1. **Abernathey, C.D., Kooistra, K.L., Wilcox, G.L. and Laws, E.R.Jr.** 1988. New xenograft model for assessing experimental therapy of central nervous system tumors: Human glioblastoma in the intrathecal compartment of the nude mouse. Neurosurg. **22**: 877–881.
2. **Beattie, G.M., Knowles, A.F., Jansen, F.E., Baird, S.M. and Kaplan, N.O.** 1982. Induction of sarcomas in athymic mice. Proc. Natl. Acad. Sci. USA **79**: 3033–3036.
3. **Camins, M.B., Cravioto, H.M., Epstein, F. and Ransohoff, J.** 1980. Medulloblastoma: An ultrastructural study - evidence for astrocytic and neuronal differentiation. Neurosurg. **6**: 398–411.
4. **Friedman, H.S., Bigner, S.H., McComb, R.D., Schold, S.C.Jr., Pasternak, J.F., Groothuis, D.R. and Bigner, D.D.** 1983. A model for human medulloblastoma. Growth, morphology, and chromosomal analysis in vitro and in athymic mice. J. Neuropathol. Exp. Neurol. **42**: 485–503.
5. **Friedman, H.S., Burger, P.C., Bigner, S.H., Trojanowski, J.Q., Wikstrand, C.J., Halperin, E.C. and Bigner, D.D.** 1985. Establishment and characterization of the human medulloblastoma cell line and transplantable xenograft D283 Med. J. Neuropathol. Exp. Neurol. **44**: 592–605.
6. **Friedman, H.S., Burger, P.C., Bigner, S.H., Trojanowski, J.Q., Brodeur, G.M., Wikstrand, C.J., Kurtzberg, J., Berens, M.E., Halperin, E.C. and Bigner, D.D.** 1988. Phenotypic and genotypic analysis of a human medulloblastoma cell line and transplantable xenograft (D341 Med) demonstrating amplification of c-myc. Am. J. Pathol. **130**: 472–484.
7. **Harik, S.I. and Sutton, C.H.** 1979. Putrescine as a marker of malignant brain tumors. Cancer Res. **39**: 5010–5015.
8. **Jacobson, P.F., Jenkyn, D.J. and Papadimitriou, J.M.** 1985. Establishment of a human medulloblastoma cell line and its heterotransplantation into nude mice. J. Neuropathol. Exp. Neurol. **44**: 472–485.
9. **Manuelidis, E.E.** 1969. Experiments with tissue culture and heterologous transplantation of tumors. Ann. N. Y. Acad. Sci. **159**: 409–431.
10. **Markesbery, W.R., Walsh, J.W. and Frye, M.D.** 1979. Ultrastructural study of the medulloblastoma in tissue culture. J. Neuropathol. Exp. Neurol. **39**: 30–41.
11. **McAllister, R.M., Isaacs, H., Rongey, R., Peer, M., Au, W., Soukup, S.W. and Gardner, M.B.** 1977. Establishment of a human medulloblastoma cell line. Int. J. Cancer **20**: 206–212.
12. **Mikami, T., Kurisu, K., Kiya, K., Mukada, K., Kawamoto, K., Hotta, T. and Uozumi, T.** 1989. Antitumor effect of recombinant human lymphotoxin on a tumor line of human malignant glioma. Hiroshima J. Med. Sci. **38**: 103–107.
13. **Mikami, T., Kurisu, K., Kiya, K., Mukada, K., Kawamoto, K., Hotta, T., Sugiyama, K., Ogasawara, H., Uozumi, T., Fujioka, Y. and Saito, Y.** 1989. Heterotransplantation of human malignant brain tumors into nude mice. Tumor growth and clinical malignancy. The Clinical Report® **23**: 7077–7079.

14. **Moulinoux, J.P., Quemener, V., Calve, M.L., Chatel, M. and Darcel, F.** 1984. Polyamines in human brain tumors. A correlative study between tumor, cerebrospinal fluid and red blood cell free polyamine levels. J. Neuro-oncol. **2**: 153–158.
15. **Russell, D.S. and Rubinstein, L.J.** 1989. p.251–279. Pathology of tumors of the nervous system, 5th ed. Edward Arnold, London.
16. **Sawa, H., Takwshita, I., Kuramitsu, M., Egami, H., Fukui, M. and Sato, Y.** 1986. Neuronal and glial proteins in medulloblastomas. II. Heterotransplantation of human medulloblastoma. Anticancer Res. **6**: 911–916.
17. **Schold, S.C.Jr., Bullard, D.E., Bigner, S.H., Jones, T.R. and Bigner, D.D.** 1983. Growth, morphology, and serial transplantation of anaplastic human gliomas in athymic mice. J. Neuro-oncol. **1**: 5–14.
18. **Seiler, N.** 1981. Polyamine metabolism and function in brain. Neurochem. Int. **3**: 95–110.
19. **Westermark, B., Pontén, J. and Hugosson, R.** 1973. Determinants for the establishmant of permanent tissue culture lines from human gliomas. Acta Path. Microbiol. Scand. **81**: 791–805.
20. **Yamada, M., Shimizu, K., Tamura, K., Okamoto, K., Matsui, Y., Moriuchi, S., Park, K., Mabuchi, E., Yamamoto, K., Hayakawa, T. and Mogami, H.** 1989. Establishment and biological characterization of human medulloblastoma cell lines. Brain and Nerve (Tokyo) **41**: 695–702.