Development and Characterization of Human Ependymoma Xenograft HxBr5

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ABSTRACT

We describe the successful heterotransplantation of a human ependymoma in CBA/CaJ mice immune deprived by infant thymectomy and whole-body irradiation. The xenograft, HxBr5, was established from a fourth ventricular ependymoma, locally recurrent in an 11-yr-old girl who had been treated with radiation therapy to the posterior fossa. HxBr5 retains histological and ultrastructural fidelity to the tumor from which it was derived as does the DNA content, as confirmed by flow cytometric analysis. The karyotype of the xenograft, which is pseudodiploid and exhibits trisomy 1q and deletion of 1p, is the first human ependymoma banded karyotype to be reported. Growth rates of the xenograft tumors are similar to the primary tumor as clinically observed with a doubling time of approximately 42 days. Cell kinetic parameters indicate that this slow-growing tumor has a relatively high growth fraction of 70.8% with a high cell loss of approximately 91%. We anticipate that HxBr5 may be useful as one component of a more complex model for studying the biology and differentiation of human ependymoma.

INTRODUCTION

Ependymomas originate from the ependymal cells that form the lining of the ventricular surfaces of the brain and the central canal of the spinal cord (1). Although ependymomas may occur at sites throughout the central nervous system, they are more common at sites adjacent to the ventricles. Supratentorial ependymomas occur throughout the first four decades of life; infratentorial ependymomas are most common in the first decade of life (2). The mean age at diagnosis for all patients with ependymoma is 5 to 6 yr. Ependymomas represent approximately 9% of childhood brain tumors (3). Currently, surgery and radiotherapy are the standard treatments resulting in an overall 5-yr survival of 20 to 30% (4). There are few reports of results of chemotherapy for the treatment of this tumor. Responses in tumors recurring after irradiation have been reported for cisplatin (5), dibromodulcitol (6), and the nitrosoureas (4). Because of their rarity, developing more effective therapies for many of the solid tumors that occur in children has been very difficult. To address this problem, we have taken the approach of establishing models of specific tumor types that may parallel the metabolic characteristics of human cancer in situ. Data are accumulating that human tumors heterografted into mice may be useful models for studying tumor biology and metabolism (13) and, as such, present appropriate systems for developing new therapeutic strategies (14). To develop a representative model of specific histiotypes, we have attempted to heterograft brain tumors into CBA/CaJ mice. We describe herein the successful heterotransplantation of an ependymoma and its characterization.

MATERIALS AND METHODS

Patient. The xenograft model was established from tumor tissue obtained at the time of surgery for recurrent disease in an 11-yr-old girl with an ependymoma of the fourth ventricle. Fig. 1 details her clinical course. The tumor was first diagnosed when she was 9 yr old. Initial therapy consisted of surgical resection followed by 50 Gy of external beam irradiation to the posterior fossa. Twenty mo later the tumor recurred locally, and retreatment included three monthly courses of chemotherapy (nitrogen mustard, vincristine, prednisone, and procarbazine) with no evidence of response, as determined by CT. A subtotal surgical resection was performed, at which time tumor tissue was obtained for heterografting and flow cytometry studies. She then received cis-dichlorodiaminoplatinum(II) (90 mg/m²) monthly for 7 mo until a contrast-enhancing posterior fossa tumor was observed on CT. No additional chemotherapy was given, and she died 4 mo later.

To measure growth rate, tumor volumes were calculated from serial contrast enhanced CT scans performed at diagnosis of recurrent tumor and monthly thereafter. The maximum perpendicular diameters of the contrast enhanced lesion were measured by a radiologist, and the following formula was used to calculate volume.

\[ V/6 \times \bar{d}^3 \]

where \( \bar{d} \) is the mean of two diameters.

Immune Deprivation. Four-wk-old, female, CBA/CaJ mice were immune deprived by thymectomy followed, 3 wk later, by i.p. administration of 1-β-arabinofuranosylcytosine (200 mg/kg) 48 h before whole-body irradiation (950 rad of 137Cs at 170 rad/min) (15). Tumor fragments obtained during surgery were placed in RPMI-1640 medium with 10% fetal calf serum and, within 3 h, placed s.c. in the dorsal flanks of three mice. Tumor diameters were measured at 7-day intervals using Vernier calipers. Tumor volumes were calculated by the formula

\[ V/6 \times \bar{d}^3 \]

where \( \bar{d} \) is the mean diameter.

Percentage of Labeled Mitosis. [methyl-³H]Thymidine (specific activity, 50 Ci/mmol) (Moravek Biochemicals, Brea, CA) was injected i.p. into mice bearing 0.5- to 1.0-cm-diameter, bilateral flank tumors (passage 3) at a dose of 1 µCi/g body weight. Whole tumors were excised at various times up to 72 h after injection. Autoradiographs were prepared using the technique of Houghton and Taylor (16). A mitotic cell was considered labeled if it contained at least 5 grains, and at least 100 mitotic figures were scored per slide. For each time point, four

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3 The abbreviations used are: CT, computerized tomography; GFAP, glial fibrillary acidic protein; NSE, neuron-specific enolase.
tumors were examined. Cell cycle kinetic parameters were analyzed by the method of Steel and Haines (17).

A labeling index was obtained from the 2-h postinjection tumors by scoring at least 500 cells in each of 4 fields in both flank tumors. Cells were considered labeled if there were at least 5 grains present. The labeling index was calculated in the following manner.

Labeling index = labeled cells/total cells counted

Pathology. Tissue from both the primary tumor and xenograft passages 2 and 5 was submitted for routine histological examination in 10% buffered formaldehyde and stained with hematoxylin:eosin and phosphotungstic acid:hematoxylin. Xenograft tissue (passage 2) was also immersed in cold 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded series of alcohols, and embedded in Spurr resin. Semithin sections were cut and stained with toluidine blue, and suitable blocks were selected for thin sections. Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 301 electron microscope. Material was not available from the original tumor for electron microscopy.

Immunoperoxidase staining of sections from both the primary tumor and the xenograft (passage 2) was performed by using polyclonal antiserum to GFAP, S100 protein, and NSE. GFAP and S100 staining were by the peroxidase:antiperoxidase method using commercial kits (Dakopatts; DAKO Corp., Santa Barbara, CA); NSE staining was by the peroxidase:antiperoxidase method using rabbit anti-bovine NSE (DAKO) at a 1:200 dilution, swine anti-rabbit immunoglobin (DAKO) at 1:200 dilution, and rabbit anti-peroxidase (DAKO) at 1:40 dilution, and rabbit antiperoxidase:peroxidase complex (DAKO) at a 1:200 dilution. Negative controls were created by substituting similar dilutions of normal rabbit serum for the primary antiserum and yielded essentially no staining. Positive controls consisted of brain (NSE, GFAP) and cartilage (S100) and stained appropriately.

Cytogenetics and Flow Cytometry. The karyotype of the secondary xenograft passage (Fig. 4) was pseudodiploid and exhibited trisomy 1q and deletion of 1p [46XX,-6,-15,-17,-21,inv(1)(p34q44), t(1;15)(p12;q11), del(3)(q12), +der(6) i(6q21);q21], del(10)(q24), +del(12)(p11), +mar]. The karyotype of the fifth passage was the same except for lack of an extra 14 and the addition of an acrocentric marker which may represent +del(3)(p11). This is consistent with the DNA index of 1.0 obtained by flow cytometry. Although no cytogenetic analysis of the original tumor material was made, the DNA index was also 1.0 as determined by flow cytometry, and the percentage of cells in S-phase was 14.2% in the original tumor and 14.4% in the xenograft.
Fig. 2. Light photomicrographs. A, section of the original tumor showing prominent central perivascular pseudorosette. H & E, × 125. B, section of the original tumor demonstrating ependymal rosettes. H & E, × 125. C, section of xenograft tumor (passage 2) containing elongated ependymal rosette with gland-like configuration. H & E, × 125. D, section of the xenograft tumor (passage 2) containing central perivascular pseudorosette and true rosette (arrow). H & E, × 250.

13 recipient mice, tumor growth was progressive and approximately exponential. Tumor volume doubling time was from 41 to 48 days with the growth rate of tumors in different mice being quite consistent.

Because the growth rate of this tumor was similar in both the patient and in mice it was of interest to determine in more detail cell kinetic parameters that dictated the slow rate of growth. Cell cycle parameters as determined by the technique of labeled mitosis are presented in Table 1 and Fig. 8. The marked damping of the second peak of mitosis indicates considerable heterogeneity of cell cycle times. Such damping also renders estimates of the cell cycle time unreliable. However, the computed cell cycle time (mean) was 78.7 h, which yielded calculated estimates of growth fraction of 70.8%, with a high cell loss factor (90.1 to 91.8%).

DISCUSSION

We have established and characterized a xenograft that may be a useful resource for studying the biology and treatment of ependymoma. This xenograft line, HxBr5, maintained with considerable fidelity the histology of the original tumor. It has ultrastructural features that are characteristic of ependymoma (1). Immunoperoxidase stains showed the xenograft to be similar to the tumor from the patient. The karyotype established that the mouse-grown tumor is of human origin, and flow cytometric analysis demonstrated that the DNA content of the xenograft and the original tumor is the same. The xenograft tumor growth is reproducible, the histological and cytogenetic features are stable, and the successful transplant rate is high.

The histological features of the xenograft tumor are those of a well-differentiated ependymal neoplasm. True rosettes are diagnostic of ependymoma and found only in the better differentiated examples (1). The electron microscopic findings of cilia, junctional complexes, and complexly folded basal surfaces are also supportive of a diagnosis of ependymoma (20). The immunohistochemical findings, although relatively nonspecific, are consistent with ependymoma as S100-positive examples have been reported (21) as have GFAP-negative examples (22, 23).

Distinction between this tumor and certain variants of ependymoma should also be considered. Anaplastic ependymoma is characterized by marked pleomorphism, a high mitotic count, vascular changes, necrosis, and the development of giant and multinucleated cell forms (24). Neither the primary tumor nor the xenograft contained pleomorphic or multinucleated cell forms, although the xenograft contained a moderate number of mitoses, and the primary tumor showed focal necrosis. These tumors also differ from ependymoblastomas which contain rosettes composed of cells forming multiple layers and juxtaluminal mitoses (12). The rosettes in our samples contained only one or two layers of cells and were generally devoid of mitoses.

Cytogenetic studies of eight cases of ependymoma were reported before the advent of chromosomal banding (25); however, we have been unable to locate any reports of karyotyping of ependymomas by chromosomal banding. Abnormalities of chromosome 1 are frequent in both adult (26) and pediatric (27) malignancies; trisomy 1q, as in this case, is also common. These changes probably represent secondarily acquired chro-
Fig. 3. Electron micrographs. A, perivascular pseudorosette composed of complex intertwining of ependymal cell processes abutting basal lamina of capillary (arrowheads). × 2,500. B, ependymal rosette composed of tall columnar cells containing basal nuclei and apical microvilli with occasional cilium (arrow). × 1,900. C, apical portion of ependymal cells containing microvilli, cilia (arrow), and junctional complex (arrowhead). × 19,000.

Fig. 4. The karyotype of the xenograft was pseudodiploid and exhibited trisomy 1q and deletion of 1p [46XX, -6,+14,-15,-17,-21, inv(1)(p34q44), t(1:15)(p12;p11), del(3)(q12), + der(6)(t:15)(p21;q21), del(9)(q24), + del(12) (p11), +mar1]. Arrows indicate the karyotypic abnormalities.
Fig. 5. CT of the brain demonstrating enhancing tumor of the posterior fossa which was measured (cross-bars) to obtain the tumor growth rate. A is from the CT obtained at 31 mo (see Fig. 1) and corresponds to Day 0 in Fig. 6. B is from the CT obtained at 34 mo (see Fig. 1) and corresponds to Day 110 in Fig. 6.

Table 1  **Cell cycle parameters**

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<tr>
<td>Cell cycle time</td>
<td>mean = 78.7 h</td>
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<td></td>
<td>S phase = 11.9 ± 1.9 h</td>
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<tr>
<td></td>
<td>G2 = 8.8 ± 3.9 h</td>
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<td></td>
<td>Labeling index = 10.4%</td>
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<tr>
<td></td>
<td>Calculated growth fraction = 70.8%</td>
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<td>Calculated cell loss = 90.1-91.8%</td>
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Fig. 6. *Growth curve* of the tumor in the patient from 31 to 34 mo calculated from the CT scan measurements (see text). Actual calculated volumes are plotted.

Fig. 7. Growth of the s.c. xenograft tumors bilaterally in 13 mice. A and B display the *growth curve* for each of the 26 tumors.

mosomal rearrangements which may confer an increased growth advantage. Determination of the influence of other chromosomal changes and rearrangements associated with this tumor will require additional studies.

An interesting finding of this study was the similarity between the growth rate of the tumor in the patient and the xenograft. To our knowledge, there is no other direct information available about the growth rate of infratentorial ependymoma. Yamashita and Kuwabara (28) estimated the doubling times of medulloblastoma in three patients by volume changes on CT scans and found them to be 19.2, 19.4, and 20.9 days. That medulloblastoma grows twice as rapidly as the ependymoma is consistent with what is known about the median survival of patients with these tumors. While survival curves for medulloblastoma plateau at 2 to 3 yr from diagnosis, those for ependymoma do not plateau until 5 to 7 yr (7, 8). The *in vivo* doubling time of medulloblastoma xenograft models was much more rapid; Schold and Friedman noted that the medulloblastoma xenograft line TE-671 doubled in 3.2 days (29).

Analysis of the percentage of labeled mitoses experiments...
gives us an explanation for the ependymoma's slow growth rate. Although the cell cycle time is relatively long, the growth fraction is quite high as compared to colon, lung, and rhabdomyosarcoma xenografts (30). This high growth fraction is not translated into a rapid tumor doubling time because of a cell loss factor of about 90%. The implication of a high cell loss factor in a tumor with scant necrosis is that this histologically well-differentiated tumor contains a large population of cells which have ceased to proliferate and may thus be end cells or G0 cells. The HxBr5 xenograft should therefore be a valuable tool for studying modalities which may recruit cells into cycle.

The utility of xenograft model systems for studying the biology of and therapy for human cancer is well documented (13, 14). Few xenograft models of the pediatric central nervous system tumors exist. Rahman et al. (31) recently reported the establishment of a primitive neuroectodermal tumor cell line from an ependymoma. The xenograft model established from this cell line grows as discrete masses of poorly differentiated, uniform, round cells with numerous mitotic figures and shows neuron-specific enolase staining which is very different from the classical well-differentiated ependymoma that we report. Friedman et al. (32) have extensively characterized a medulloblastoma line and found it useful for chemotherapy and radiotherapy investigations.

We anticipate that ependymoma xenograft HxBr5 will prove a useful tool given its close fidelity to the histological, biochemical, and growth characteristics of the original tumor. However, it must be emphasized that the xenograft was derived from a patient previously treated with both radiotherapy and chemotherapy, and studies using it must be interpreted in that light. Further, it is reasonable to assume that no single tumor will be representative of ependymoma. The HxBr5 must be considered an early component of a more comprehensive model we are currently developing.

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