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Heterotransplantation of malignant human gliomas in neonatal rats

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✓ Three human glioma cell lines (TE-671 medulloblastoma, U-87 MG glioblastoma, and U-373 MG glioblastoma) were transplanted to the quadrigeminal cistern of the brain in 37 newborn Sprague-Dawley rats and to the subcutaneous space in 30 of their siblings. Two of the three gliomas (the TE-671 medulloblastoma and the U-87 MG glioblastoma) grew both intracranially and subcutaneously. The U-373 MG glioblastoma did not grow in either site. The resulting tumors expressed unique morphological features characteristic of their tissue of origin. The newborn rat represents a model for the heterologous transplantation of human gliomas, providing a biological window for the study of these lesions.

KEY WORDS • **brain neoplasm** • **glioma** • **tumor transplantation** • **quadrigeminal cistern** • **rat**

SEVERAL experimental models are currently available for the study of malignant human gliomas, which can be grown either as solid tumors in immunodeficient animals[16] or *in vitro* using tissue culture techniques.[2] An experimental model, however, characteristically selects for and allows the expression of only some biological properties of a tumor. Therefore, if a variety of experimental settings can be used to study the same tumor, a better understanding of the tumor may emerge by comparing and contrasting its expression in the different settings.

Normal newborn rats, which have an incompetent but developing immune system, can become carriers of heterologous grafts, presumably due to the induction of immunological tolerance in the host.[17,18] Recently, mouse retinal neurons have been heterotransplanted to the quadrigeminal plate of newborn rats with about 90% graft survival and without evidence of rejection.[10,12] Based on these results, this model was utilized for the heterotransplantation of human glial tumors. This report describes a microsurgical technique for implanting human glial tumors in the quadrigeminal cistern of normal newborn rats. The model is characterized by a low operative mortality and consistent rates of tumor survival.

Materials and Methods

Tumor Lines

Three tumorigenic human glioma cell lines were used for these experiments: the TE-671 medulloblastoma,[5,11] the U-87 MG glioblastoma, and the U-373 MG glioblastoma.[1,3] The three cell lines* were received in frozen ampules and dispensed into tissue culture flasks according to the accompanying recommendations. They were grown in RPMI medium 1640 with L-glutamine, in a solution of 10% fetal bovine serum, penicillin (base) 100 U/ml, and streptomycin (base) 100 μg/ml in a humidified atmosphere of 5% CO_2 at 37°C.† The medium was changed every 2 to 3 days, and the cells were transferred as they became confluent (about once weekly) after detachment with trypsin 0.25% in Hanks' balanced salt solution without calcium and magnesium.

For the implantation of each litter, the cells were grown in three 150-sq cm flasks. They were incubated for 3 to 4 days after reaching confluence, with daily changes of the medium. At the time of implantation, the cells were trypsinized, the contents of the three flasks were combined, and the cells were centrifuged at 1000 rpm for 5 minutes. The supernatant fraction was completely aspirated and the cell pellet was kept on ice. This procedure usually yielded a 0.4-ml pellet. This cell suspension was then drawn into a 100-μl Hamilton syringe for intracranial (IC) or subcutaneous (SC) injection.

* Cell lines obtained from American Type Culture Collection, Rockville, Maryland.

† All products obtained from GIBCO Laboratories, Grand Island, New York.

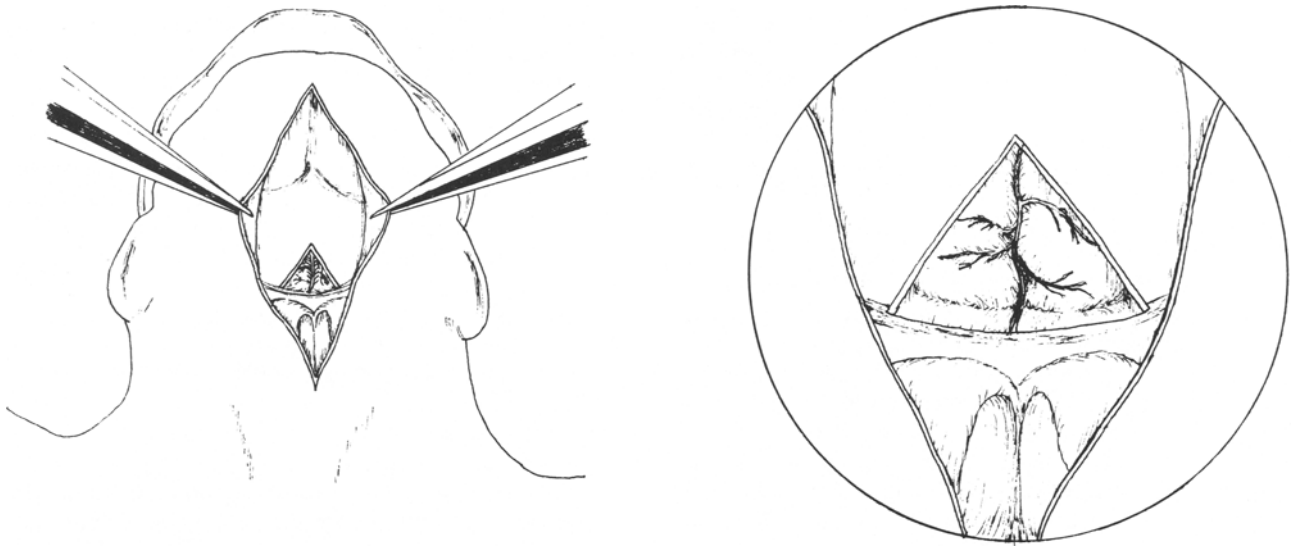

FIG. 1. *Left:* Operative drawing with the nuchal musculature detached from the occipital cartilagenous plate and reflected caudad. A triangular flap has been excised above the transverse occipital suture, exposing the quadrigeminal cistern and its contents. *Right:* High-magnification view of the quadrigeminal plate. A branching vessel is seen coursing between the inferior colliculi.

Study Animals

The newborn rats were obtained from timed-pregnant Sprague-Dawley dams.‡ The pregnant rats were kept in standard animal facilities, one rat per cage, with free access to food§ and Baltimore city water, and were followed closely to document the time of delivery. Litters 12 to 24 hours old were used for the implants. Only litters with a maximum of 10 newborns were selected. Smaller litters were usually associated with a high perinatal mortality rate due to either neglectful mothers or frail pups. The newborns were caged with their mothers and allowed to nurse freely. If the mothers were observed to neglect or harm their offspring, the mothers were replaced with more nurturing females that had delivered recently.

Induction of Anesthesia

The newborns were anesthetized by hypothermia. A 50-ml glass beaker was lined with gauze, capped with a glass Petri dish, and cooled to −30°C overnight. At the time of surgery, the pup was placed in the beaker and kept at −30°C for 20 minutes. Upon removal from the beaker, the animals remained anesthetized for about 10 minutes, which provided adequate time for the surgery or the subcutaneous injection. After completion of either procedure, the pup was rewarmed and returned to the mother when fully active.

Intracranial Tumor Implantation

The implantation procedures were carried out in a clean but nonsterile environment. The newborn rats were inoculated within 12 to 24 hours of birth. The head of the anesthetized rat was prepared with 70% ethyl alcohol and Prepodyne solution, and the animal was placed on an inclined stand with the head flexed at about 45°. Under microsurgical technique, the skin overlying the occipital region was incised with microscissors. The nuchal musculature attached on the occipital cartilagenous plate was detached and reflected caudad, exposing the transverse occipital suture (Fig. 1 *left*). A 1- to 2-mm incision was made with a microsurgical knife at the midpoint of the transverse occipital suture. Cerebrospinal fluid (CSF) flowed briskly when the subarachnoid space in the quadrigeminal cistern was penetrated. An equilateral triangular flap of the occipital plate was excised with the microscissors and saved, thus gaining wide access into the quadrigeminal cistern (Fig. 1 *left*). The CSF in the cistern was absorbed with a Weck-Cel surgical spear sponge.‖ As much CSF as possible was removed from the cistern to create a cavity into which the tumor cells could be deposited (Fig. 1 *right*).

A cell suspension was then drawn into a 100-μl Hamilton syringe* equipped with a blunted No. 22 needle. The tip of the needle was introduced into the cistern through the triangular window, and 10 to 15 μl of the cell suspension was dispensed into the cavity. This volume contained about one to two million cells by hemocytometer count. A 1- to 4-cu mm piece of Gelfoam was placed in the opening. The triangular flap

‡ Rats obtained from Harlan Sprague-Dawley, Inc., Indianapolis, Indiana.

§ Certified Rodent Chow No. 5002 obtained from Ralston Purina Co., St. Louis, Missouri.

‖ Surgical spear sponge manufactured by Edward Weck & Co., Inc., Research Triangle Park, North Carolina.

* Hamilton syringe manufactured by Hamilton Co., Reno, Nevada.

was inverted and placed under the edges of the triangular window to lie on top of the Gelfoam. In this arrangement, the triangular flap prevented the cell suspension and the piece of Gelfoam from being extruded through the hole when the animal emerged from hypothermia and the intracranial pressure increased. The skin was closed with a running 6-0 Dermalon suture.

Subcutaneous Tumor Implantation

Five animals in each litter received subcutaneous inoculations of the tumor as a control group for the intracranial implantation of the tumor in their siblings. The nuchal region of the anesthetized rat was prepared with 70% ethyl alcohol and Prepodyne solution. Under microsurgical technique, the skin of the nape was pierced with a No. 22 needle. The blunted No. 22 needle was then introduced through this defect into the subcutaneous space and tunneled caudad along the midline. Then 10 to 15 μl of cell suspension was injected into the subcutaneous space via a 100-μl Hamilton syringe, and the needle end was withdrawn slowly.

Histological Study

Each litter was sacrificed at 14 days, or earlier if any of the animals showed deterioration. The newborn rats were sacrificed by decapitation at the cervicothoracic junction. Under microsurgical technique, the brain of the animals with intracranial implants was exposed and the length and width of the tumor were measured with dial calipers. The tumor volumes were calculated by the following formula:[3,13] volume = (length $\times$ width^2) $\div$ 2. The specimens were fixed in 64% phosphate-buffered formalin for 10 to 14 days, embedded in paraffin, and stained with hematoxylin and eosin. The tumors in the subcutaneous space were measured, excised, and processed in the same manner as the brains.

Results

A total of 37 rats received IC implants and 30 received SC implants. The operative mortality rate was less than 10%. Each tumor line was implanted into two litters. Within each litter, five rats received SC implants and, depending on the size of the litter, five to seven received IC implants. The litters were sacrificed on the 12th to 14th day post-implantation. Only one early death (on Day 4) occurred in the series; postmortem examination of this animal was unremarkable except for the tumor growing on the quadrigeminal plate. No morphological evidence of rejection in the form of vasculitic changes or mononuclear cell infiltrates was observed in any of the IC or SC tumors.

The TE-671 medulloblastoma IC and SC implants produced large tumors with a calculated volume of about 500 cu mm. The animals with IC tumors were smaller and less active than were those with SC tumors, but were otherwise normal without any further evidence of neurological damage. The IC TE-671 medulloblastoma grew as a solid, disc-shaped mass overlying the quadrigeminal plate. Although it was well circumscribed, it invaded the mesencephalic tectum as well as the cerebellum, and displaced the cerebral hemispheres anteriorly. The SC tumor grew as a solid cylindrical mass.

The histological appearance of the IC and SC TE-671 medulloblastomas was virtually identical. The tumor was extremely cellular with minimal areas of necrosis. The IC tumor was noted to invade the brain, the brain stem (particularly through the Virchow-Robin spaces), and the cerebellum (Fig. 2 *left*). The SC tumor invaded fat (Fig. 2 *right*). The cells were small and uniform, with a high nuclear-cytoplasmic ratio and marked nuclear pleomorphism. The nucleoli were particularly prominent. There were numerous normal and abnormal mitoses, about 10 to 15/high-power field. No Homer Wright rosettes were noted.

The U-87 MG glioblastoma IC and SC implants produced large tumors with a calculated volume of about 300 cu mm. The animals with IC tumors were smaller and less active than were their siblings with SC tumors. The IC tumor grew as a solid, multilobulated, disc-shaped mass overlying the quadrigeminal plate (Fig. 3). Although it was well circumscribed, it invaded the mesencephalic tectum and displaced the cerebellum as well as the cerebral hemispheres. The SC tumor grew as a solid, multilobulated, cylindrical mass.

The histological appearance of the IC and SC U-87 MG glioblastomas differed in the degree of viability. Whereas the IC tumors exhibited less than 10% necrosis, the SC tumors were 40% to 80% necrotic (Fig. 4 *left*). Otherwise, the viable tissue of the IC and SC tumors was comparable. Numerous atypical astrocytic neoplastic cells with abundant cytoplasm and prominent processes were evident. In certain regions, the neoplastic cells were spindle-shaped and arranged in fascicles, giving the tumor a sarcomatous appearance. Only scattered mitoses were seen, about one or two/high-power field. The histological appearance of nonviable tumor ranged from individually necrotic cells to areas of linear necrosis with pseudopalisading to microcystic foci (Fig. 4 *right*). The cystic changes were interpreted as the sequelae of tumor necrosis since necrotic cells could often be identified at the interface between viable tumor and the cysts. Calcification was also noted within the necrotic regions. No endothelial proliferation was appreciated.

Neither the IC nor the SC implants of the U-373 glioblastoma produced tumors. The viability of the cells used for implantation was confirmed by the Trypan blue dye exclusion method. The IC implantation site had diffuse fibrosis with scattered areas of calcification and numerous lymphocytes and macrophages. In the midst of these changes, rare isolated neoplastic cells were identified.

Discussion

This report describes a microsurgical technique for the heterotransplantation of malignant human gliomas to the quadrigeminal cistern of normal newborn rats.

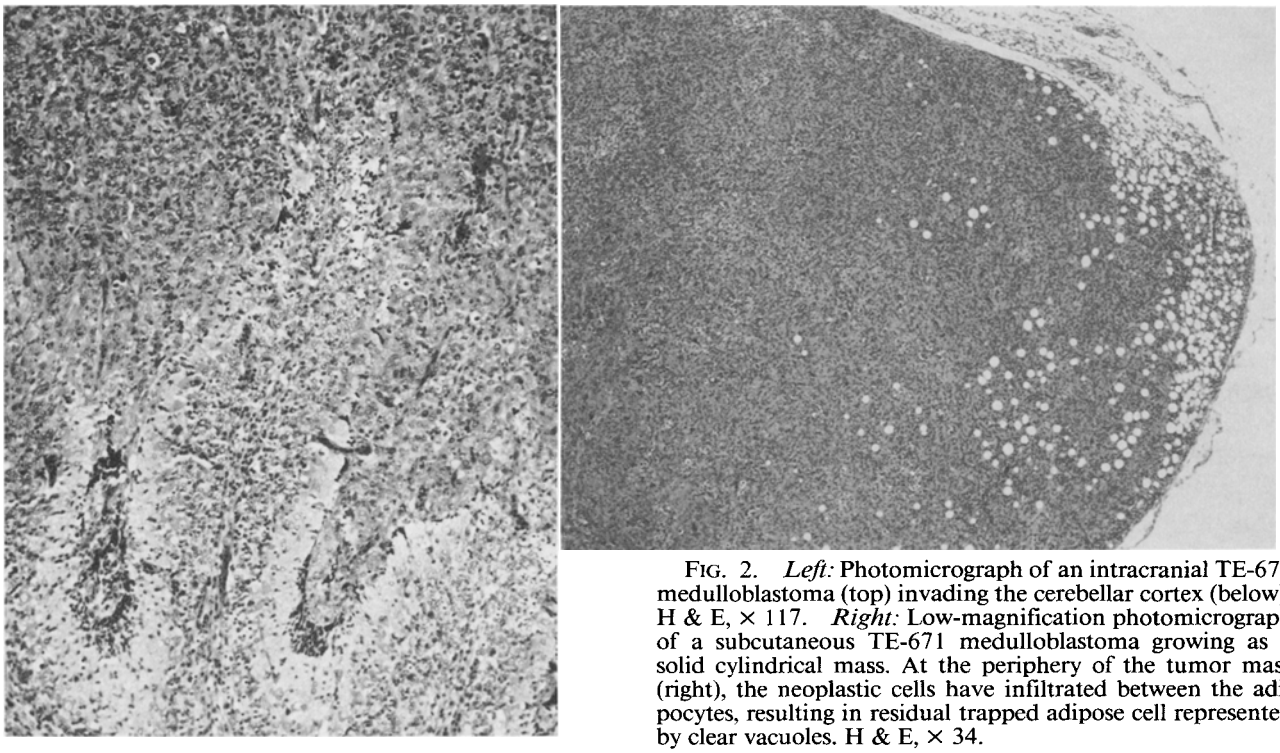

FIG. 2. *Left:* Photomicrograph of an intracranial TE-671 medulloblastoma (top) invading the cerebellar cortex (below). H & E, × 117. *Right:* Low-magnification photomicrograph of a subcutaneous TE-671 medulloblastoma growing as a solid cylindrical mass. At the periphery of the tumor mass (right), the neoplastic cells have infiltrated between the adipocytes, resulting in residual trapped adipose cell represented by clear vacuoles. H & E, × 34.

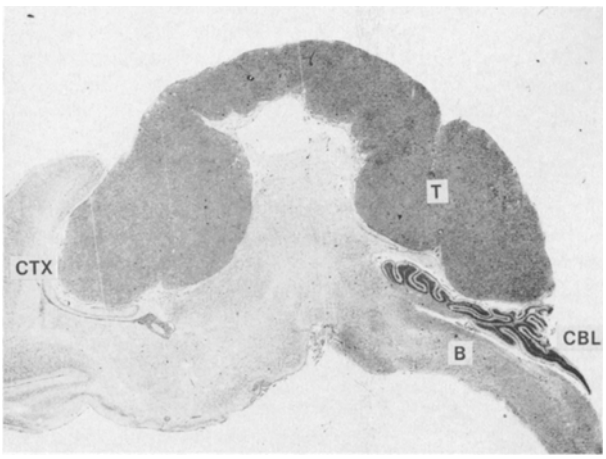

FIG. 3. Scanning magnification of U-87 MG glioblastoma overlying quadrigeminal plate. B = brain stem; CBL = cerebellum; CTX = cerebral cortex; T = tumor. × 5.

This simple intracranial technique allows for the inoculation of large numbers of animals with a low operative mortality rate and provides consistent results with respect to tumor implant survival. There was no histological evidence of rejection. The tumors were also grown subcutaneously, where tumor progression can be quantitated sequentially. The subcutaneous model may be preferred in experiments that test the sensitivity of neoplastic glial cells to chemotherapeutic agents.[7]

The neonatal rat model allowed each tumor line to express its unique morphological features as well as its malignant potential. Presumably, a state of immunological tolerance is induced by introducing the tumor in the rat while its immune system is still developing. The three tumors tested represent a spectrum, ranging from the aggressive TE-671 medulloblastoma, which grew equally well intracranially and subcutaneously, to the intermediate U-87 MG glioblastoma, which exhibited substantial necrotic regions subcutaneously but not intracranially, to the U-373 MG glioblastoma, which did not grow in either location.

The morphological features of these tumors grown in the newborn rat can be compared with those of the same lesions grown in the athymic mouse in other studies. The histological appearance of the TE-671 medulloblastoma in the newborn rat is similar to that described by Friedman, *et al.*,[5] in the athymic mouse: a highly cellular tumor made up of small cells with hyperchromatic nuclei, scant cytoplasm, and abundant mitoses. In the newborn rat, however, the perivascular pseudorosettes described in athymic mouse were not observed.

Of all intracranial tumors, the medulloblastoma is the most likely to metastasize outside the central nervous system.[9] Glioblastomas, on the other hand, rarely metastasize.[8] In this study, the comparable growth of the TE-671 medulloblastoma both intracranially and subcutaneously may reflect the ability of this tumor to become established extraneurally.

The U-373 MG glioblastoma has been reported as tumorigenic when implanted subcutaneously in athymic mice.[3] In that report, however, only large cysts

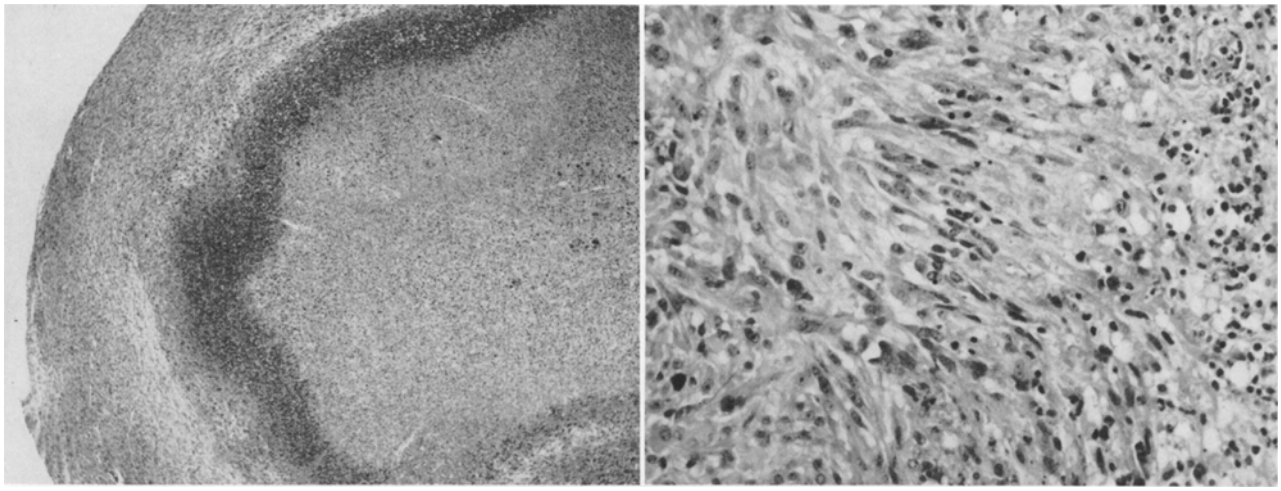

FIG. 4. *Left:* Photomicrograph of a subcutaneous U-87 MG glioblastoma with peripheral viable tumor (left), a dark band of recent cellular necrotic tumor (center), and less cellular necrotic tumor (right). H & E, × 30. *Right:* Higher magnification of a U-87 MG glioblastoma displaying linear necrosis lined by pseudopalisading tumor cells, characteristic of glioblastomas. H & E, × 260.

were produced; by contrast, the other tumorigenic lines yielded solid tumors. In the present study, the U-373 MG glioblastoma failed to produce tumors. Thus, this cell line seems to be poorly tumorigenic.

Many investigators have previously attempted to transplant human gliomas into the brains of immunocompetent animals. Greene[6] reported the successful heterotransplantation of eight of 11 glioblastomas into the brains of mice and guinea pigs; however, these results could not be duplicated.[16] Subsequent models were eventually replaced by that of the athymic mouse,[14] in which numerous brain-tumor samples have been grown.[16] Recently, the possibility of growing malignant human gliomas in ordinary laboratory animals has been reexamined.[19]

In conclusion, the quadrigeminal cistern and, to a lesser extent, the subcutaneous space of the newborn rat can be used as sites for the heterotransplantation of malignant human glioma cell lines. Important biological differences among the three neoplastic lines were identified, such as tumorigenicity, cellular morphology, invasiveness, mitotic rate, and degenerative features. Each tumor line expressed its unique morphological features as well as its malignant potential in both implantation sites. By exploring differences in tumor growth not only between the two sites in the newborn rat, but also between this model and others, the mechanism and control of brain tumor growth may be better understood.

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References

1. Beckman G, Beckman L, Pontén J, et al: G-6-PD and PGM phenotypes of 16 continuous human tumor cell lines. Evidence against cross-contamination and contamination by HeLa cells. **Hum Hered 21**:238–241, 1971
2. Bressler J, Smith BH, Kornblith PL: Tissue culture techniques in the study of human gliomas, in Wilkins RH, Rengachary SS (eds): **Neurosurgery.** New York: McGraw-Hill, 1985, pp 542–548
3. Bullard DE, Schold SC Jr, Bigner SH, et al: Growth and chemotherapeutic response in athymic mice of tumors arising from human glioma-derived cell lines. **J Neuropathol Exp Neurol 40:**410–427, 1981
4. Fogh J, Fogh JM, Orfeo T: One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. **JNCI 59:**221–225, 1977
5. Friedman HS, Bigner SH, McComb RD, et al: A model for human medulloblastoma. Growth, morphology, and chromosomal analysis *in vitro* and in athymic mice. **J Neuropathol Exp Neurol 42:**485–503, 1983
6. Greene HSN: The transplantation of human brain tumors to the brains of laboratory animals. **Cancer Res 13:** 422–426, 1953
7. Groothius DR, Fischer JM, Lapin G, et al: Permeability of different experimental brain tumor models to horseradish peroxidase. **J Neuropathol Exp Neurol 41:** 164–185, 1982
8. Hochberg FH, Pruitt A: Assumptions in the radiotherapy of glioblastoma. **Neurology 30:**907–911, 1980
9. Kleinman Gm, Hochberg FH, Richardson EP Jr: Systemic metastases from medulloblastoma: report of two cases and review of the literature. **Cancer 48:**2296–2309, 1981
10. Lund RD, Chang FLF, Hankin MH, et al: Use of a species-specific antibody for demonstrating mouse neurons transplanted to rat brains. **Neurosci Lett 61:** 221–226, 1985
11. McAllister RM, Isaacs H, Rongey R, et al: Establishment of a human medulloblastoma cell line. **Int J Cancer 20:**

206–212, 1977
12. McLoon SC, Lund RD: Specific projections of retina transplanted to rat brain. **Exp Brain Res 40:**273–282, 1980
13. Ovejera AA, Houchens DP, Barker AD: Chemotherapy of human tumor xenografts in genetically athymic mice. **Ann Clin Lab Sci 8:**50–56, 1978
14. Pantelouris EM: Absence of thymus in a mouse mutant. **Nature 217:**370–371, 1968
15. Pontén J, Macintyre E: Long term culture of normal and neoplastic human glia. **Acta Pathol Microbiol Scand 74:** 465–486, 1968
16. Schold SC Jr, Friedman HS: Human brain tumor xenografts. **Prog Exp Tumor Res 28:**18–31, 1984
17. Southam CM: Immunologic tolerance to human cancer transplants in rats. **Cancer Res 26:**2496–2502, 1966
18. Southam CM, Babcock VI, De Masi M: Growth of several human cell lines in newborn rats. **Cancer Res 24:** 345–355, 1964
19. Strömblad LG, Brun A, Salford LG, et al: A model for xenotransplantation of human malignant astrocytomas into the brain of normal adult rats. **Acta Neurochir 65:** 217–226, 1982

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