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Heterotransplantation of Human Glioblastoma Multiforme and Meningioma to Nude Mice[1] (39750)

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A number of human tumors have been successfully xenografted in nude athymic mice. Once established, serial passage has always been feasible, and there has been no change in morphologic or biologic properties after as many as 56 passages. The karyotype and isoenzymes have remained human (1). The susceptibility of nude mice to xenograft survival and growth is presumably the result of deficiency in all thymus-derived immunologic activities. Kuga *et al.* (2) in 1975 reported that 27 of 31 cultured human tumor cell lines injected sc in nude mice resulted in solid tumor growth. Successful transplants included one neuroblastoma, but one of the four failures was a glioma. Direct transplantation of surgically removed tumor was successful in only 6 of 35 tumors. Histologic similarity to the human tumor or origin was usually, but not invariably found.

We report here the successful sc transplantation and growth of a human glioblastoma and a meningioma in nude mice, with retention of their unique histologic features in each case. We find no evidence that either of these tumors has previously been heterotransplanted to nude mice.

Materials and methods. Male nude mice with a BALB/c genetic background were obtained from the Charles River Laboratories. They were maintained, two per sterilized cage, at 80°F, in an air-tight glass door cabinet protected by laminar airflow, and given autoclaved Purina chow and water *ad libitum*. Sterile rubber gloves and a mask were worn by experimenters during transfer of mice to clean cages and other manipulations.

Ten mice, 6 weeks old, were injected sc with $2\text{-}5 \times 10^6$ highly anaplastic cells from a human glioblastoma line (T98) courtesy of Dr. S. Aaronson, made available by the Naval Biomedical Research Center, Oakland, California, and previously used by us for a chemotherapeutic study (3).

Six mice, 15 weeks old, were implanted sc in the right dorsal area with 0.025 ml of a non-necrotic portion of a human glioblastoma (Br7) minced finely enough to pass through a 15-gauge needle. The tumor tissue was injected about 2 hr after surgical removal at the Saint Louis University Hospital.

Eight mice, 8 weeks old, were implanted sc in the mid or lateral dorsal area with the same amounts of a similarly minced surgically removed benign human meningioma. In this case, however, the tumor tissue had remained intact, before mincing, for 3 days in a dry petri dish at refrigerator temperature (about 5°).

Results. T98 Cell line. Four of the ten mice became cachectic and died 6–8 weeks after injection, without sc tumors. Two of the mice which died showed massive amyloid deposition in the liver and spleen, and the other two could not be studied because of decomposition. The remaining six mice remained healthy, and did not develop visible tumors during an observation period of 7 months.

Human glioblastoma mince. Three of the six mice developed tumors, first noted 2 months after implantation. At $2\text{-}1/2$ months a mouse bearing a $7 \times 4 \times 3$-mm tumor was sacrificed for study.

At 3 months, the other two tumors had grown rapidly, and measured $2.0 \times 1.3 \times 1.3$ and $1.6 \times 1.4 \times 1.2$ cm. The tumors

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were moderately firm, well circumscribed, and freely movable in the sc tissue. Each was composed of 15–20 lobules, surrounded by connective tissue. There was no grossly visible necrosis. A segment of each of the larger tumors, approximately 1 cm^3, was removed surgically for histology and tissue culture. (These mice have remained well, but tumors about 1 cm in diameter have developed on both sides of the lines of excision.)

Figure 1A shows the histologic picture of the xenografted tumor, as compared with that of the human surgical specimen (Fig. 1B). All of the features unique to glioblastoma multiforme are noted in the xenograft pleomorphism, characteristic multinucleate cells, many mitotic figures, many thin-walled blood vessels, and proliferation of perivascular mesothelial cells. Noted, but not pictured, in both human and xenograft tumors are also the presence of pallisading around small areas of necrosis, many neuroglia fibers, and multiple mitotic spindles in single cells (the source of the giant cells). The degree of malignancy in the xenograft is comparable to that in the surgically removed human tumor. Staining by the Masson method shows bands of blue-staining collagen fibers in the capsule and interlobar septa, and a few thin fibers in the walls of the arterioles near the points of entry into the tumor. The tumor itself is practically free of collagen. The sc tissue is not invaded.

Of the remaining three mice receiving minced human tumor, one died after 7 weeks, and showed a rounded mass about 8 mm in diameter at the implantation site, as well as massive enlargement of the cervical, axillary, inguinal, and peritoneal lymph nodes. All masses histologically were lymphocytic lymphoma, type B. Another mouse developed similar enlargement of the cervical, axillary, and inguinal nodes, which regressed spontaneously in 10 days and this mouse has remained alive and well with no evidence of tumor for 5 months. (The significance of the lymphomas in these two mice will be discussed briefly.) The sixth mouse was found dead and decomposed at 10 weeks, without discoverable sc tumor.

Tissue cultures. Tissue cultures were established from the two large tumors excised from nude mice. The tissues were minced, and in some instances also trypsinized, and set out in 25-cm^2 plastic flasks, 5-cm plastic petri dishes, and 4-cm glass petri dishes, each container having 5 ml of BME × 2 with 10% fetal calf serum, and conventional

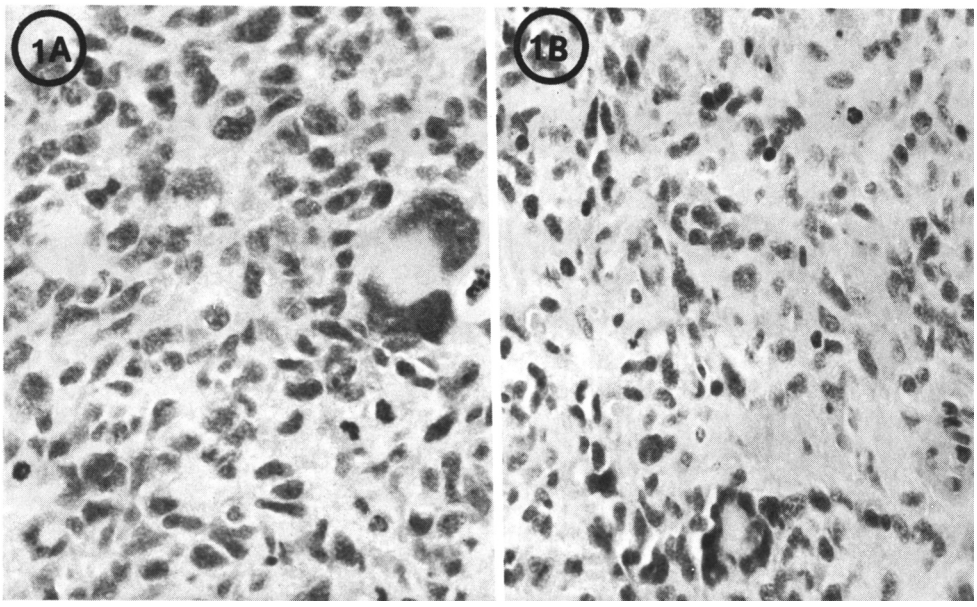

FIG. 1. Glioblastoma Br7. Xenograft in nude mouse (A) and human surgical specimen (B). H & E X 400.

antibiotics. The petri dishes contained coverslips to facilitate histologic study. The cultures grew fairly rapidly, and contained all of the cell types characteristic of glioblastoma multiforme, with many mitoses (Fig. 3). One culture is in its third transplant generation.

Human meningioma mince. Five of the eight mice xenografted with human meningioma developed small tumors 6 and 7 weeks later. These were fixed to the subcutaneous tissue. They grew at varying rates, but all enlarged slowly. At 8 weeks, one tumor (about 8 × 6 × 5 mm) was excised for histology and injection into fresh nude mice. Histologically, the xenograft (Fig. 2A) resembles the human tumor of origin (Fig. 2B). Both show whorls and the characteristic psammoma bodies. The xenograft, however, is somewhat less cellular than the surgical specimen. Invasion of fat and muscle is noted, as well as lysis of muscle fibers.

Serial passage. Both tumors were successfully passed to a second group of fresh mice, the glioblastoma first appearing after 28–31 days, and the meningiomas appearing within 45 days.

Ultrastructural features. Ultrastructural features of both types of xenografted tumors in the mouse were studied by conventional thin-section techniques. As noted above, the glioblastoma showed many neuroglia fibers (not shown). Both tumors contain scattered cytoplasmic A-type virus particles and mature C-type particles (see discussion).

Discussion. The presence of the unique histologic features of glioblastoma and meningioma in the xenografts emphasizes their identity to the human parental tumors. They may be useful models for etiologic, immunologic, and chemotherapeutic studies.

The virus bodies found in both types of tumors will be the subject of further study. The assumption is that they are a xenotropic virus of the mouse, that is, a virus which cannot be shown to multiply in mouse cells, but which grows freely in transplanted cells from another species (4). The possibility that the virus is of human origin is remote, in view of the eventual defection of RD 114, a virus recovered from a human tumor growing in kittens (5).

The nude mouse is of considerable immunologic interest. Although thymus-derived lymphocytes are generally accepted as important in immunosurveillance, spontaneous tumors appear to be rare, only one tumor, a lymphoma (reticulum cell type B), having apparently been previously reported (6). Lack of immunologic stimulation (im-

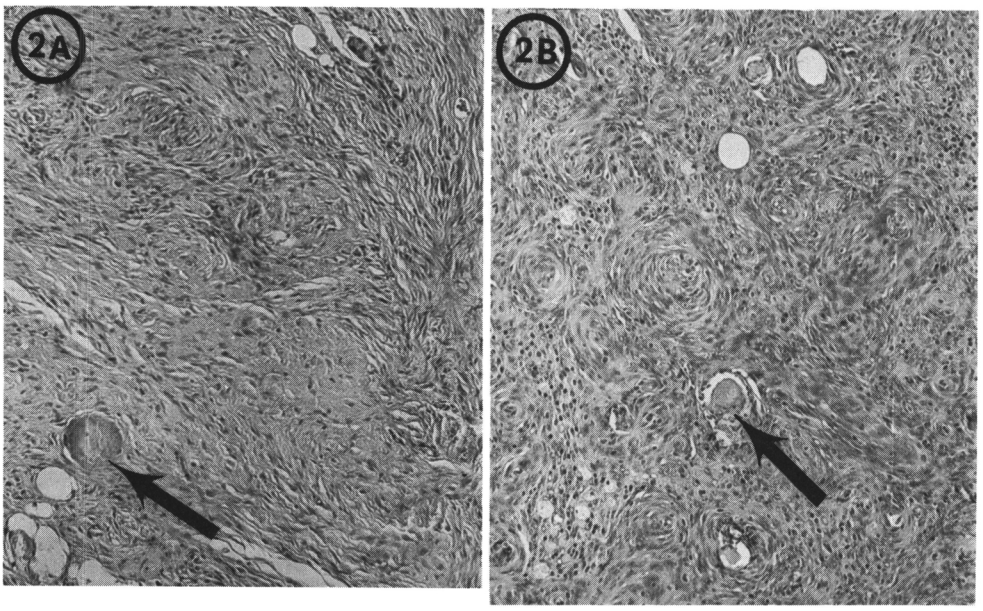

FIG. 2. Meningioma. Xenograft in nude mouse (A) and human surgical specimen (B). Arrows point to a psammoma body in each. H & E × 100.

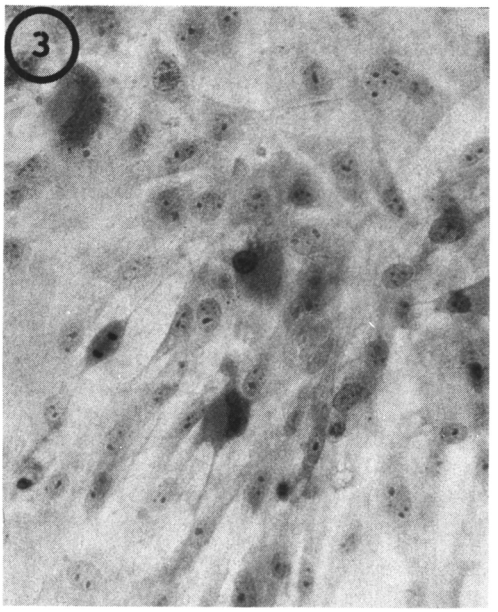

FIG. 3. Tissue culture of glioblastoma xenograft. Note cell types characteristic of glioblastoma and numerous mitotic figures. H & E × 400.

munologic enhancement) has been suggested as a possible explanation (6).

Summary. A human glioblastoma multiforme (Br7) and a meningioma have been successfully xenografted sc in nude mice. Both were successfully passed to a second generation. Complete retention of the unique histologic features was noted in each tumor xenograft. Actively growing cultures from the glioblastoma xenograft have been established; they are composed of the cell types characteristic of glioblastoma. Immature and mature C-type particles, presumably representing a xenotropic mouse virus, are present in both grafts. The growth of these two tumors in nude mice may provide a useful model for etiologic, immunologic, and chemotherapeutic studies. An attempt to produce glioblastoma in nude mice by the injection of cells cultured from human glioblastoma T98 was unsuccessful.

1. Spang-Thomsen, M., and Visfeldt, J., Amer. J. Path. **84,** 1 (1976).
2. Kuga, N., Seido, T., and Obosho, S., Gann **66,** 547 (1975).
3. Pinkerton, H., and Rana, M. W., Proc. Soc. Exp. Biol. Med. **151,** 532 (1976).
4. Levy, J. L., Science **182,** 1151 (1973).
5. McAllister, R. M., Nicolson, M., Gardner, M. B., Rongey, R. W., Sarma, P. S., Huebner, R. J., Hatanaka, M., Aroszlan, S., Gilden, R. V., Kabighting, A., and Vernon, L., Nature New Biol. **235,** 3 (1972).
6. Custer, R. P., Outzen, H. C., Eaton, G. J., and Prehn, R. T., J. Natl. Cancer Inst. **51,** 707 (1973).

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