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Orthotopic transplantation of v-*src*–expressing glioma cell lines into immunocompetent mice: establishment of a new transplantable in vivo model for malignant glioma

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Object. The aim of this study was to develop and characterize a new orthotopic, syngeneic, transplantable mouse brain tumor model by using the cell lines Tu-9648 and Tu-2449, which were previously isolated from tumors that arose spontaneously in glial fibrillary acidic protein (GFAP)-v-*src* transgenic mice.

Methods. Striatal implantation of a 1-μl suspension of 5000 to 10,000 cells from either clone into syngeneic B6C3F1 mice resulted in tumors that were histologically identified as malignant gliomas. Prior subcutaneous inoculations with irradiated autologous cells inhibited the otherwise robust development of a microscopically infiltrating malignant glioma. Untreated mice with implanted tumor cells were killed 12 days later, when the resultant gliomas were several millimeters in diameter. Immunohistochemically, the gliomas displayed both the astroglial marker GFAP and the oncogenic form of signal transducer and activator of transcription–3 (Stat3). This form is called tyrosine-705 phosphorylated Stat3, and is found in many malignant entities, including human gliomas. Phosphorylated Stat3 was particularly prominent, not only in the nucleus but also in the plasma membrane of peripherally infiltrating glioma cells, reflecting persistent overactivation of the Janus kinase/Stat3 signal transduction pathway. The Tu-2449 cells exhibited three nonrandom structural chromosomal aberrations, including a deletion of the long arm of chromosome 2 and an apparently balanced translocation between chromosomes 1 and 3. The GFAP-v-*src* transgene was mapped to the pericentromeric region of chromosome 18.

Conclusions. The high rate of engraftment, the similarity to the high-grade malignant glioma of origin, and the rapid, locally invasive growth of these tumors should make this murine model useful in testing novel therapies for human malignant gliomas.

Key Words • glioma • transplantation • v-*src* transgene • immunogenicity • syngeneic mouse model

Malignant gliomas are the most common primary brain tumors found in adults. Despite intensive research, the long-term prognosis for patients with GBM remains dismal. Thus, new, more effective therapies are urgently needed.

Preclinical trials for brain tumors have been conducted largely using human glioma xenografts implanted in nude mice, although xenografts often do not possess the genetic heterogeneity and invasive neuropathological characteristics of spontaneous gliomas. Furthermore, the use of immunocompromised murine hosts may obscure important immune mechanisms involved in tumor growth or elimination. Therefore, we have produced a germline insertion of a transgene expressing v-*src* under the control of the GFAP promoter, resulting in the formation of astrocytomas.[30] The v-*src* activates several signal transduction pathways[8] that are also activated in human gliomas; for example, pathways involving epidermal growth factor, IL-6, and nonreceptor tyrosine kinases, which promote uncontrolled growth and survival through dysregulation of gene expression.[14] These GFAP-v-*src* gliomas closely match characteristic features of human malignant gliomas; that is, astrogliosis, dysplastic changes, transition from low-grade to high-grade astrocytomas, diffuse infiltration, endothelial proliferation, and necrosis.[26,30]

Tumor incidence in GFAP-v-*src* animals is low (15–

Abbreviations used in this paper: FISH = fluorescence in situ hybridization; GBM = glioblastoma multiforme; GFAP = glial fibrillary acidic protein; IL-6 = interleukin-6; PNST = peripheral nerve sheath tumor; pY-Stat3 = tyrosine-705 phosphorylated signal transducer and activator of transcription–3; SSC = standard saline citrate; TBS = Tris-buffered saline.

20%), however, and stochastic tumors develop in mice between 4 and 50 weeks of age, a fact that precludes the use of these mice for most therapeutic trials. Thus, to render those GFAP-v-*src* tumors more accessible for preclinical studies, we have derived and used two cell lines from tumors that arose spontaneously in GFAP-v-*src* transgenic mice to develop a new orthotopic, syngeneic, transplantable mouse tumor model.

Materials and Methods

Cell Lines and Cell Culture

The tumor cell lines Tu-9648, Tu-9668, and Tu-251 were derived from spontaneously arising intracerebral tumors in GFAP-v-*src* transgenic mice.[21,30] The Tu-2449 cell line was derived from a subcutaneous malignant PNST, but it could not be distinguished by any means, including its morphological features in vivo or its in vitro growth pattern, from the cell lines derived from the intracerebral gliomas.[21,30] Cells were maintained in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 1% L-glutamine (1.46 mg/ml), 1% penicillin–streptomycin, and 1 μg/ml Fungizone, removed from tissue culture flasks with trypsin/ethylenediaminetetraacetic acid solution (medium, drugs, and trypsin solution all obtained from GIBCO), and used between passages 5 and 10.

Intracerebral Implantation of Tu-9648 and Tu-2449 Cell Lines in Syngeneic Mice

The B6C3F1 hybrid mice (Taconic Farms), each weighing approximately 18 to 20 g, were anesthetized by intraperitoneally injecting approximately 0.09 ml of a Telazol/xylazine mixture containing 3.5 ml phosphate-buffered saline, 0.25 ml Telazol solution (20 mg/ml, Lederle Parenterals, Inc.), and 0.4 ml xylazine solution (20 mg/ml, Ben Venue Labs). Tumors were initiated in deeply anesthetized mice by inoculating 1 μl of culture medium containing the indicated number of cultured Tu-9648 or Tu-2449 cells into the left striatum 2.5 to 3 mm deep at a point along the (serrated) coronal suture approximately halfway between the midline and the cranial insertion of the left temporalis muscle (~ 5 mm posterior to the interocular plane). At that point, a 0.5-mm bur hole was drilled through the skull, and a27-gauge needle (fitted with a depth-limiting plastic collar to ensure cell injection 2.5–3 mm beneath the skull) that was connected to a 1-μl Hamilton microsyringe by flexible tubing was then inserted into the bur hole. Following a 30-second infusion of the cells, another 30 seconds was allowed to pass for the cells to settle before removing the inoculation needle from the brain. This technique resulted in a locally expanding and partially infiltrating tumor of the striatum and around the needle track, with no evidence of blood- or cerebrospinal fluid–borne metastases.

Subcutaneous Implantation of Tu-2449 and Tu-9648 Cells

For the initiation of subcutaneous tumors, Tu-2449 or Tu-9648 cells (80–90% confluence) were removed from T75 or T175 flasks (Sarstedt) by trypsinization. Cell clumps were disrupted by gentle hydrodynamic shearing through a Pasteur pipette; up to 10×10^6 cells were suspended in 0.1 ml of medium and injected subcutaneously into the left flank of manually restrained mice through a 26-gauge needle.

Subcutaneous Injection of Gamma-Irradiated Tu-2449 and Tu-9648 Cells

The Tu-2449 or Tu-9648 cells were removed from culture flasks by trypsinization. Cell clumps were disrupted by gentle hydrodynamic shearing through a Pasteur pipette. A suspension of 25 to 50×10^6 cells/ml was exposed to a 50-Gy dose (0.765 Gy/minute) in a ^{137}Cs irradiator (Gamma Cell 40, Nordion). Doses of 2.5 to 5×10^6 irradiated, clonogenically disabled cells in 0.1 ml of medium were injected subcutaneously through a 26-gauge needle into the left flank of manually restrained mice in approximately 5 seconds. The series of four injections was repeated every week for 1 month.

Immunohistochemical Evaluation

Paraffin sections from tumor-bearing mice were probed with polyclonal antibodies against latent Stat3α (Santa Cruz Biotechnology) or pY-Stat3 (Cell Signalling Technology), GFAP (monoclonal antibody 6F2), and neurofilament protein (monoclonal antibody 2F11, both from Dako). Slides were dewaxed, rehydrated, and pretreated by boiling either in a pressure cooker for 5 minutes (Stat-3α in citrate buffer; pH 6.0) or in a microwave oven three times for 5 minutes each (GFAP and neurofilament protein in citrate buffer and pY-Stat3 in 5% urea; pH 9.5). Sections were then washed in TBS, as they were after all subsequent steps, and incubated with the primary antibody diluted in TBS with 0.5% casein and 5% normal goat serum for 60 minutes at room temperature (Stat3α, GFAP, and neurofilament protein) or overnight at 4°C (pY-Stat3). Sections were then incubated with biotinylated goat–antimouse immunoglobulin or goat–antirabbit immunoglobulin serum, and thereafter with avidin–biotin complex/alkaline phosphatase (1:200 in TBS, all from Dako). Finally, sections were developed in the new fuchsin–naphthol solution (AS-BI, Sigma-Aldrich), counterstained with hematoxylin, and mounted.

Performance of G-Banding. Confluent tumor cell cultures were harvested with colcemid (0.1 μg/ml) and slides were prepared according to standard protocols. The G-banding was performed by partial digestion of the chromosome slide preparations in a 0.005% trypsin solution in phosphate-buffered saline (pH 7.0), followed by staining in 5% Giemsa in Gurr pH 6.8 buffer.[4]

Performance of FISH Analysis. The FISH analysis was performed using Mouse Rainbow FISH chromosome paints (Cambio) for each of the 40 mouse chromosomes in three hybridization experiments (chromosomes 1–7, 8–14, and 15–19 with X and Y). The plasmid containing the v-*src* transgene[2] was labeled with digoxigenin by using the nick-translation method according to the manufacturer's protocol (Roche 1745816), precipitated with ethanol, and rehydrated in Hybrisol VII (Qbiogene). Slides were pretreated with 0.1 mg/ml RNase in 2XSSC, rinsed in 2XSSC, and then serially dehydrated in 70, 90, and 100% ethanol. Chromosomal denaturation was performed at 70°C in 70% formamide/2XSSC followed by serial dehydration at −20°C in 70, 90, and 100% ethanol. Mouse Rainbow FISH paints were denatured for 10 minutes at 65°C and preannealed at 37°C for 90 minutes. The v-*src* transgene was denatured at 75°C for 10 minutes. Hybridization under a sealed coverslip was performed at 37°C in a humid chamber for 20 hours. Posthybridization washes were done at 45°C in 50% formamide/2XSSC and 0.5XSSC.

After blocking with 5% bovine serum albumin in 0.2% Tween 20/4XSSC, detection was performed using fluoresceinated avidin (Vector Laboratories) for the Rainbow FISH paints, fluorescein-labeled antidigoxigenin (Roche) for the v-*src* transgene, and avidin–Texas red (Molecular Probes) for single mouse chromosome paints at 37°C in a humid chamber for 20 minutes. Excess detection reagents were washed at 45°C in 0.2% Tween 20/4XSSC. Slides were mounted in VECTASHIELD mounting medium with DAPI (Vector Laboratories). Images were captured on an Olympus AX70 fluorescence microscope equipped with appropriate filters and a charge-coupled device camera. Images were analyzed using the Applied Imaging System and Genus Software (Applied Imaging).

Statistical Analysis

The difference between Kaplan–Meier survival plots was analyzed for statistical significance with the log-rank and chi-square tests.[12]

Results

Growth of Tumor Cell Lines

The Tu-9648 and Tu-2449 cell lines formed progressively growing tumors when implanted intracerebrally in syngeneic B6C3F1 mice. Four cell lines established from spontaneously arising tumors in B6C3F1 GFAP-v-*src* transgenic mice (Tu-251, Tu-9648, Tu-9668, and Tu-2449)

were grown in cell culture and injected back into syngeneic B6C3F1 mice. Only cells from Tu-9648 and Tu-2449 formed progressively growing tumors when implanted intracerebrally and subcutaneously in such mice.

Macroscopic Findings

For the Tu-2449 cell line, tumors were present in all but one of 10 mice 12 days after implantation of 10,000 (~ 7000 viable) Tu-2449 cells. Relatively large tumors, partly with extracerebral extensions (maximum diameters between 3 and ~ 8 mm, including extracerebral extensions) were apparent in eight mice. The tumors were beige to brown. Most tumors were located in the striatal region. A few extended along the needle track, showed exophytic growth at the brain surface, and were adherent to the skull. Foci of hemorrhage and necrosis were present in two of nine tumors. For the Tu-9648 cell line, intracerebral tumors were present in seven of the 10 mice 19 days after the intracerebral implantation of 5000 (~ 1000–2000 viable) Tu-9648 cells, with localizations similar to those noted in mice bearing Tu-2449 tumors. Tumor diameters ranged from 3 to 7 mm. In several instances, the tumors or parts thereof were exophytic and adherent to the skull. In one mouse only a subcutaneous tumor was noted; in the remaining two, extracerebral tumor nodules were noted.

Histopathological Findings

Intracerebral implantation of Tu-2449 cells yielded tumors that consisted of dense accumulations of mostly fusiform to plump, often bipolar, medium-sized cells arranged in intersecting bundles and fascicles. The cells contained considerable amounts of slightly eosinophilic cytoplasm and few cytoplasmic processes; round to rather polymorphous nuclei of quite variable size, with coarse chromatin structure and large nucleoli; and focally displayed features reminiscent of gemistocytic astrocytes. There were up to 10 typical or atypical mitotic figures per high-power field, and fewer cells with condensed and/or fragmented nuclei. Binucleated to multinucleated large to giant cells were occasionally present. Focal necrosis was apparent in most of the larger tumors, sometimes in a serpentine shape and, in some tumors, focally bordered by pseudopalisading cells. Spotty hemorrhage was occasionally seen within or near the tumor. Many small vessels were noted within the tumor, but glomeruloid tufts arranged in festooned patterns (referred to as microvascular proliferation) were not seen.

The margins of the tumors were often infiltrative (Fig. 1 A–C), with single cells or cell clusters forming satellites and extending into perivascular spaces, leptomeninges, subependymal regions, and along myelinated tracks. Some tumors infiltrated and partially destroyed the parietal bone. Other border regions were well delineated and expansive. Most tumors displayed a marked perifocal astrogliosis. In some sections, the implantation canal was recognized, devoid of neoplastic tissue. The tumor cells, including many of the multinucleated giant cells, were immunoreactive for GFAP (Fig. 1D), Stat3α (Fig. 1A), and activated oncogenic pY-Stat3 (Fig. 1E and F). The pY-Stat3 immunoreactivity was often strongly associated with the plasma membrane of the tumor cells. These features are consistent with a high-grade, poorly differentiated ("anaplastic") malignant glioma with astrocytic differentiation; microvascular proliferation, a criterion required for a diagnosis of GBM, was not detected. The implanted tumors were morphologically similar to the spontaneous ones encountered in GFAP-v-*src* mice, including the prominent plasmalemmal immunoreactivity for pY-Stat3 (Fig. 1E and F). In the seven of 10 mice bearing intracerebral Tu-9648 tumors with intracerebral localizations, tumor type, grade, and other morphological features were similar to those noted in the first series, except for the margins of the tumors, which were more frequently well delineated than infiltrative. Perifocal round cell infiltrates, mostly lymphocytic, were seen in some tumors, or in their vicinity, occasionally located around small blood vessels. Both Tu-2449 and Tu-9648 tumor cells showed immunohistochemical staining for GFAP and both latent and constitutively activated Stat3.

Karyotype Analysis

The G-band analysis of cell line Tu2449 revealed a hypotetraploid female karyotype with a modal chromosome number of 75 with random and three nonrandom structural chromosomal aberrations. Nonrandom chromosomal aberrations included a deleted chromosome and two structurally rearranged ones for which the chromosome composition could not be completely identified on G-banding (Fig. 2 *upper*). Twenty cells from each of the three Rainbow FISH hybridization experiments were analyzed. There were no clonal chromosome abnormalities observed with the aid of Rainbow FISH hybridizations that included whole chromosome paints for mouse chromosomes 8 through 14, 15 through 19, and the sex chromosomes. Analysis of chromosomes 1 through 7 revealed an apparently balanced translocation between chromosomes 1 and 3 as well as a deletion of the distal long arm of chromosome 2 (2A1) in all cells examined (Fig. 2 *lower*). The T(1;3) was present in two copies in all cells examined.

*Mapping of the v-*src *Transgene*

The v-*src* transgene has been found to map to the pericentromeric region of chromosome 18 of Tu-2449 cells (Fig. 3). The band location on the chromosome is 18A1.

Cell Line Tu-9648

The Tu-9648 cells are immunogenic but will form progressive intracerebral tumors in untreated mice. Figure 4 *upper* shows that 16 of 17 B6C3F1 mice injected intracerebrally with 5000 Tu-9648 cells (1000–2000 viable cells) in 1 μl of medium 2.5 to 3 mm below the surface of the striatum developed tumors. They either died or had to be killed 16 to 28 days after tumor cell implantation (median Day 20). Most of these mice (~ 10 of 17) developed a noticeable subcutaneous scalp nodule as the tumors became advanced (> Day 14). If mice were preinjected with approximately 5×10^6 irradiated (50 Gy) Tu-9648 cells at intervals 25, 18, 11, and 4 days prior to viable Tu-9648 cell implantation, all the mice survived. By definition, therefore, Tu-9648 cells are immunogenic.

Cell Line Tu-2449

The Tu-2449 cells are immunogenic but will form progressive intracerebral tumors in untreated mice. Figure 4 *lower* shows that all 10 B6C3F1 mice injected intracere-

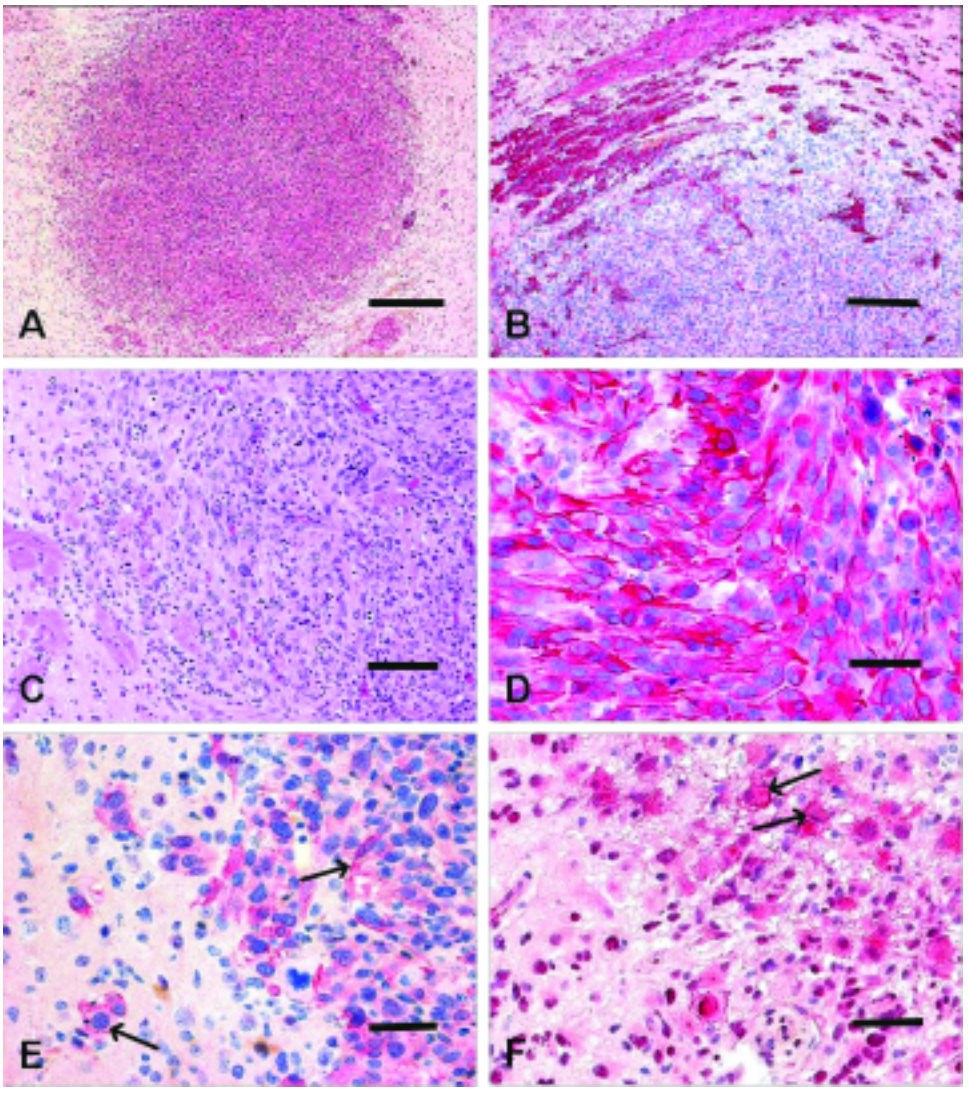

FIG. 1. Photomicrographs showing sections used for histopathological examination of formalin-fixed, paraffin-embedded malignant gliomas, which was performed 12 days after implantation of 10,000 cells of the clone Tu-9648 or Tu-2449 (shown) into the striatum of syngeneic B6C3F1 mice. A: Transplanted tumor located in the striatum. Immunohistochemical labeling (red) for Stat3α is shown. The tumor nodule (red area), which is immunoreactive for Stat3, is not sharply delineated and has several small satellites. Bar = 250 μm. B: Axon bundles of the internal capsule, which are immunoreactive for neurofilaments (red areas), are diffusely infiltrated by the implanted tumor. Bar = 150 μm. C: Diffusely infiltrating, polymorphic cells of a transplanted tumor. H & E, bar = 100 μm. D: The tumor cells show moderate to intense cytoplasmic GFAP immunoreactivity. Bar = 50 μm. E: Distinct, plasma membrane–associated immunoreactivity for activated pY-Stat3 of neoplastic cells is seen at the margin of a transplanted tumor *(arrows)*. Nuclear pY-Stat3 labeling is minor to undetectable in this image's field, but was strong in other tumors derived from the transplanted cell lines (not shown). Bar = 50 μm. F: Margin of a glioma that spontaneously developed in a GFAP-v-*src* transgenic mouse. As in the transplanted tumors, the neoplastic cells show distinct pY-Stat3 immunoreactivity of the plasma membrane *(arrows)*. In addition, nuclear labeling of neoplastic cells is prominent in this field. Bar = 50 μm.

brally with 10,000 Tu-2449 cells (~ 7000 viable cells) in 1 μl of medium 2.5 to 3 mm below the surface of the striatum developed tumors. They died or had to be killed 14 to 40 days after tumor cell implantation (median Day 19.5). Most of these mice (seven of 10) developed a noticeable subcutaneous nodule as the tumors became advanced (> Day 14). If mice were preinjected with approximately 5×10^6 irradiated (50 Gy) Tu-2449 cells at intervals 25, 18, 11, and 4 days prior to viable Tu-2449 cell implantation, intracerebral tumors did not develop, subcutaneous nodules were not seen, and all the mice survived. Thus, by definition, Tu-2449 cells are immunogenic.

Discussion

The tumors that arise in GFAP-v-*src* transgenic mice mimic hallmarks of human high-grade malignant gliomas;[30] that is, intersecting bundles of atypical cells, often bipolar, focally reminiscent of astrocytes, with a high mitotic and apoptotic index, presence of multinucleated giant

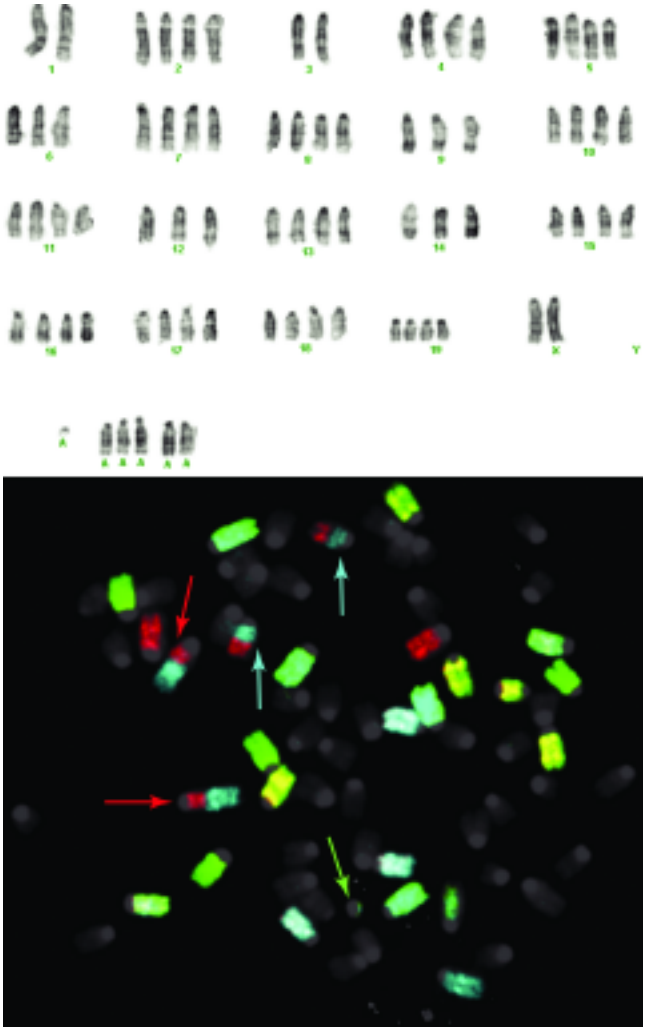

FIG. 2. Karyotype of a G-banded metaphase cell from a Tu-2449 lesion. *Upper:* Structurally rearranged chromosomes that could not be confidently identified using G-band analysis are indicated by the letter A. *Lower:* Fluorescence in situ hybridization with Cambio Mouse Rainbow FISH paint set 1 (whole chromosome paint probes 1–7) to a metaphase cell from a Tu-2449 lesion. Translocation between chromosomes 1 and 3 is indicated by *blue* and *red arrows*, respectively. Deletion of chromosome 2 (2A1) is marked with a *green arrow*.

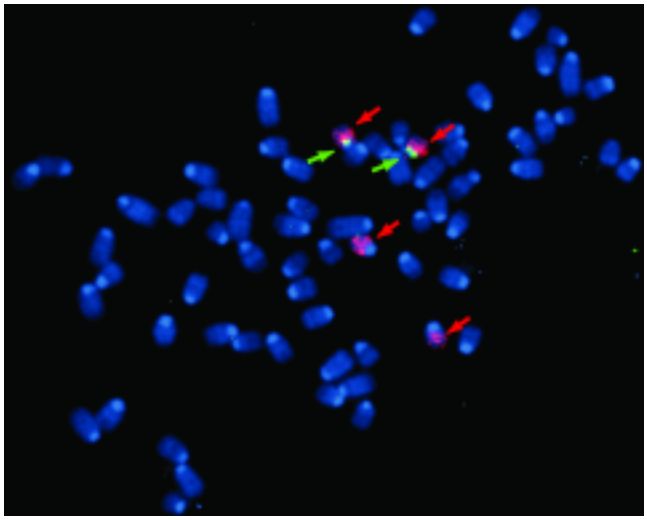

FIG. 3. An FISH study showing mapping of the GFAP-v-*src* transgene to the chromosome 18A1 region. Cohybridization of Cambio whole chromosome mouse paint for chromosome 18 and the GFAP-v-*src* transgene to Tu-2449 metaphase chromosome is shown. *Red arrows* point to chromosome 18, which is labeled with avidin and detected with avidin–Texas Red. *Green arrows* point to the v-*src* transgene probes labeled with digoxigenin and detected with antidigoxigenin fluorescein. Metaphase chromosomes are blue.

cells, focal necrosis, sometimes serpentine shape, and occasionally bordered by pseudopalisading cells. The tumor cells, including many of the multinucleated giant cells, were immunoreactive for GFAP (Fig. 1D), Stat3α (Fig. 1A), and activated oncogenic pY-Stat3 (Fig. 1E and F). Immunoreactivity for pY-Stat3 was often strongly associated with the plasma membrane of the tumor cells. Therefore, we have developed a transplantable brain tumor model in which we use cell lines derived from such tumors. The transplantation model has several advantages over the GFAP-v-*src* transgenic mouse model, as follows: 1) approximately 100% tumor penetrance compared with approximately 15 to 20%; 2) rapid tumor formation (advanced tumors are present within 2 weeks); 3) most of the tumors that form follow a similar time course (70–80% of the mice die within a 10-day window); 4) immunocompetent syngeneic recipient B6C3F1 mice are commercially available; and 5) the transplanted tumors retain many of the features of the parental tumor.

Aberrant cell signaling is a salient feature of oncogenesis. Most of the aberrant signaling pathways in GBM include those mediated by downstream signal transduction elements of oncogenes and growth factors such as c-jun N-terminal protein kinase, Stats, and protein kinase C. Persistently active Stat3 promotes uncontrolled growth and survival by dysregulation of gene expression,[28] including *cyclin D1, c-Myc, Bcl-xL, Mcl-1, survivin*, and *VEGF*. Upregulation of *VEGF* induces angiogenesis.[19] It is likely that the targeting of signaling components downstream of the initiating mutation and the use of genetically engineered mouse models of cancer will be useful for the development of novel therapeutic strategies.[11] For example, small molecular inhibitors were used to target the sonic hedgehog pathway in a mouse model of medulloblastoma.[23]

All transformed v-*src*–expressing cells, including our GFAP-v-*src* cell lines, have persistently active Stat3. In fact, Stat3 is required for v-*src*–mediated transformation.[6,27] A recent fingerprint study revealed that the mRNA expression profiles for *cyclin D1, Bcl-xL, SOCS3*, and *VEGF* in cells transfected with either a constitutively active mutant form of Stat-3, Stat3-C,[7] or v-*src* are virtually identical.[20] Recently we have shown that IL-6 is required for glioma development in the GFAP-v-*src* model and that IL-6 directly upregulates *VEGF* in astrocytes in vivo through Stat3.[15,29] The GFAP-v-*src* transplantation model should therefore be useful for the study of Stat3-mediated oncogenesis and tumor therapy by either blocking Stat3 activity or restoring physiological inhibition capacity. Many strategies will be

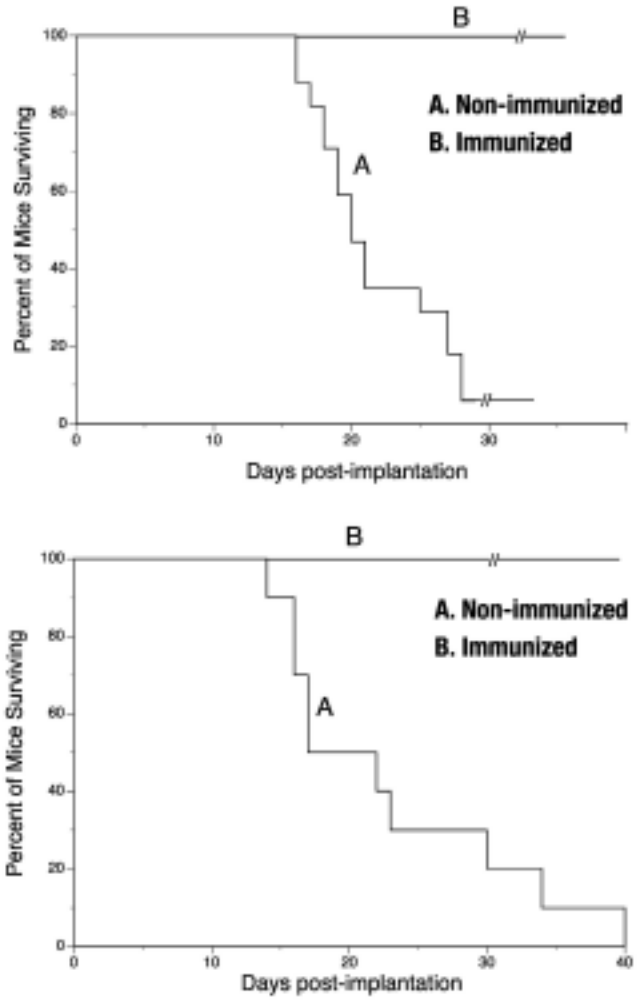

FIG. 4. Kaplan–Meier plots showing the fraction of surviving mice according to days after intracerebral implantation of tumor cell lines. The Tu-9648 *(upper)* and Tu-2449 *(lower)* tumor cells were implanted with and without prior immunotherapy (immunized and nonimmunized). The B6C3F1 hybrid mice were challenged with either 5000 cultured Tu-9648 or 10,000 cultured Tu-2449 cells in the left striatum (Day 0). Immunized mice had received a series of four weekly subcutaneous injections of irradiated (50 Gy) Tu-9648 or Tu-2449 cells before the intracerebral challenge.

possible, including the use of the in vivo model for the discovery of new small-molecule Stat3 inhibitors.[27,34]

Furthermore, Src itself is involved in several pathways that have been shown to be important for glioma development and progression; it is downstream of the epidermal growth factor receptor[32] and activates phosphatidylinositol 3–kinase.[3] Human c-Src and specifically Lyn kinase, the predominantly cellular Src kinase activity in GBM, is overactive and implicated in human gliomagenesis.[25] Reduced glioma infiltration has been shown in Src-deficient mice.[17] Recent in vitro experiments have shown that the inhibition of *Src* family activity is a potential therapeutic approach to treat highly invasive malignant glioma,[2] because Src regulates actin dynamics and invasion of malignant glial cells; thus the GFAP-v-*src* transplantation model should be useful for the study of the role of *src* in glioma invasion.

Our data revealed that Tu-2449 and Tu-9648 are immunogenic. Previous work on v-*src*–induced sarcomas in chickens[9] (and later in mice[10]) showed that inoculation with DNA constructs possessing v-*src* but lacking viral replication–specific genes leads to the formation of sarcomas. Depending on the chicken cell line, these sarcomas either progressed or spontaneously regressed. Regressor lines are protected from subsequent sarcomagenic challenge. This inducible antisarcoma response, which is believed to be mediated by *Src*-specific tumor antigen,[33] is protective against subsequent sarcomagenic challenge and underlies the regression of v-*src*–induced primary sarcomas. It is possible that v-*src* confers immunogenic properties to the Tu-9648 and Tu-2449 cell lines as well, because subcutaneous leg inoculations with irradiated Tu-9648 and Tu-2449 cells also protect mice against subsequent intracerebral cell challenge. These results are in sharp contrast to results obtained with the F98 rat glioma cell line, in which similar subcutaneous injections of irradiated cells provided no protection to viable cell challenge (Smilowitz, unpublished results). The results we have obtained with the Tu cell lines are generally similar to the results obtained with the 9L gliosarcoma[24] and the GL261 mouse glioma cell line (Smilowitz, unpublished results). Further studies would be needed to quantify the relative immunogenicities of the Tu-2449 and Tu-9648 cell lines relative to those of various mouse and rat glioma cell lines. Nevertheless, the data presented here show clearly that although the Tu-9648 and Tu-2449 cell lines are immunogenic, they form intracerebral tumors reliably when implanted in the striatum of syngeneic mice, and are suitable for study.

The substantial similarity of the central nervous system glioma-derived tumor cell line Tu-9648 to the malignant PNST-derived Tu-2449 cell line is of interest. Both lines were strongly immunoreactive for GFAP and activated Stat3 and yielded similarly invasive tumors after intracerebral implantation. The malignant PNST-derived cell line used in our study differs from the cells of other non–central nervous system–derived tumors, which show a pattern of mass-like expansion after implantation into brain tissue.[2] Our results support the suggestion that a phenotypic overlap between a v-*src*–promoted malignant glioma and malignant PNST occurs.

To characterize the two cell lines in more detail, we also analyzed one of them, Tu-2449, for chromosomal alterations. It is likely that the observed translocation between chromosomes 1 and 3 was present in the original Tu-2449 tumor, because it was found in two copies in all hypotetraploid cells examined. The status of the origin of the deletion of the distal long arm of chromosome 2, del(2A1), is less certain. Although the deletion was noted in all cells examined, del(2A1) was not present in more than one copy per cell and there were at least three copies (often four copies) of an apparently normal-looking chromosome 2. In addition to these alterations, which were present in all cells, there were several abnormalities that were present in subsets of cells examined. These abnormalities may be random and arise during cell culture, because human high-grade gliomas such as GBM show a wide variety of numerical and structural chromosome aberrations and rapid karyotype evolution. The most common chromosome changes associated with human GBM are trisomy 7 and total or partial monosomy for chromosome 10q.[1,16] However, cytogenet-

ics, spectral karyotyping, and comparative genomic hybridization of high-grade gliomas have shown clonal and nonclonal as well as primary and secondary numerical and structural chromosomal aberrations for each of the 24 human chromosomes.[5,13,16,18,22,31] The hypotetraploid cell count, the numerical changes, balanced translocations, complicated unbalanced translocations, and marker chromosomes observed in the mouse tumor indicate that the model system is consistent with what is seen in the karyotypes of human high-grade gliomas. Thus, Tu-9648 and Tu-2449 are suitable in vivo models for malignant gliomas.

Conclusions

The Tu-9648 and Tu-2449 cell lines, which are derived from spontaneously arising tumors in two different GFAP-v-*src* transgenic mice, can be used to generate high-grade malignant gliomas in the brains of syngeneic mice following intracerebral implantation. The implanted tumors are both macroscopically and immunohistochemically similar to the original tumors; they stain for GFAP and both latent and constitutively activated Stat3. Karyotyping and Rainbow FISH analysis of Tu-2449 cells revealed an apparently balanced translocation between chromosomes 1 and 3 (T[1;3]) as well as a deletion of the distal long arm of chromosome 2 (2A1) in all cells examined. Furthermore, both tumor lines are demonstrably immunogenic; prior subcutaneous inoculation of mice with approximately 5×10^6 irradiated tumor cells prevented subsequent intracerebral tumors from forming in all of the inoculated mice following an intracerebral challenge with viable tumor cells. The high rate of engraftment, the similarity to the malignant glioma of origin, and the rapid, locally invasive growth of these cell lines should make these murine models useful for the dissection of the Stat3 and other signal transduction pathways relevant for glioma development as well as the development and testing of novel therapies for human malignant gliomas.

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Drs. Smilowitz and Weissenberger contributed equally to this work.

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