Abstract

Using a neural transplantation model and retrovirus-mediated gene transfer, we have introduced the oncogenes \( \text{v-Ha-ras} \) and \( \text{v-myc} \) into the developing rat brain. Upon insertion of a construct encoding \( \text{v-Ha-ras} \) and the \( \text{Escherichia coli} \ \beta \)-galactosidase marker gene, the retroviral vector was found to be expressed in neurons, astrocytes, and endothelial cells of the graft. After latency periods of several months, fascicular neoplasms with expression of S-100 protein were observed in 50% of the transplants. The foreign genes were shown to be highly expressed in the tumors and in intact donor cells, by 5-bromo-4-chloro-3-indolyl-\( \beta \)-D-galactopyranosidase histochemistry, indicating that an activated \( \text{Ha-ras} \) oncogene has the potential to initiate neoplastic transformation of glial cells. Introduction of the \( \text{v-myc} \) oncogene into 15 grafts resulted in only a single primitive neuroectodermal tumor. However, simultaneous expression of the \( \text{v-Ha-ras} \) and \( \text{v-myc} \) genes yielded highly malignant, polyclonal neoplasms in all recipient animals, as early as 13 days after transplantation, from which cell lines could be easily derived. In addition, neoplastic transformation was also observed in vitro following introduction of \( \text{ras} \) and \( \text{myc} \) into embryonic forebrain cultures and into newborn cerebellar cultures. These data indicate a powerful complementary transforming effect of \( \text{ras} \) and \( \text{myc} \) on neural progenitors in vivo and in vitro. Coexpression of \( \text{ras} \) and \( \text{myc} \) may, therefore, provide a highly efficient tool for transforming neural precursor cells in distinct segments of the central nervous system at different stages of development.

Introduction

It is commonly accepted that neoplastic transformation of primary cells proceeds as a multistep process which involves the combined activation and/or inactivation of several genes. A paradigm model for cooperation of oncogenes was established by Land et al. (1, 2), using primary rat embryo fibroblasts. Simultaneous introduction of activated \( \text{ras} \) and \( \text{myc} \) genes into these cells results in malignant transformation, whereas each oncogene alone does not induce full transformation. According to their functional activity in this system, oncogenes have been allocated to two cooperation groups (reviewed in Refs. 3–5).

Members of the first group include genes encoding membrane-associated gene products such as \( \text{ras} \), \( \text{src} \), and the middle \( \text{T} \) antigen of polyoma virus. Upon introduction into embryo fibroblasts, these genes cause morphological features of transformation but are unable to immortalize. Oncogenes of the \( \text{myc} \) family, adenovirus E1A, polyoma large \( \text{T} \) antigen, and papilloma virus E7 induce immortalization without a significant change in cell morphology.

Recently, cooperation of \( \text{ras} \) and \( \text{myc} \) genes has also been demonstrated in vivo. Using retrovirus-mediated gene transfer in a prostatic gland reconstitution model, Thompson et al. (6) were able to induce carcinomas with a combination of \( \text{v-Ha-ras} \) and \( \text{v-myc} \), while the single oncogenes caused hyperplasia and dysplastic development of prostatic cells but failed to elicit prostatic neoplasms. Also, cross-breeding of transgenic mice expressing high levels of \( \text{ras} \) and \( \text{myc} \) in the mammary gland was shown to enhance mammary tumorigenesis dramatically (7, 8). Clynes et al. (9) reported a high incidence of plasmacytomas in Pristane-pretreated mice following infection with a retroviral vector encoding both \( \text{ras} \) and \( \text{myc} \) oncogenes, whereas each oncogene alone did not induce neoplasms.

We have developed a retrovirus-mediated gene transfer model which allows us to introduce and express foreign genes in neuroectodermal and mesodermal cells of fetal brain transplants (10–12). Introduction of viral oncogenes with an intrinsic or associated protein tyrosine kinase activity into neural transplants resulted in cell type-specific tumor induction in the grafts. While the polyoma middle \( \text{T} \) gene rapidly and selectively interfered with the development of endothelial cells, expression of \( \text{v-src} \) produced glial and mesenchymal neoplasms, following multistep kinetics. We have now generated neural transplants carrying replication-defective retroviral vectors encoding \( \text{v-Ha-ras} \), \( \text{v-myc} \), or a combination of both oncogenes. The results demonstrate a powerful cooperative transforming effect of \( \text{ras} \) and \( \text{myc} \) in the brain and indicate that gene transfer into CNS5 transplants provides a unique opportunity for studying oncogene cooperation in the nervous system.

Materials and Methods

Retroviral Vectors. Replication-defective retroviral vectors encoding the \( \text{v-Ha-ras} \) gene, the \( \text{v-gag/myc} \) gene of the MC29 avian myelocytomatosis virus, or both oncogenes together were generously provided by Hartmut Land (Imperial Cancer Research Fund Laboratories, London). Fig. 1 illustrates the structure of the retroviruses. All constructs are derived from the Moloney leukemia virus and have been described elsewhere (6). A retrovirus that encodes only the neomycin phosphotransferase gene (neo) was used for control transplants. Packaging cell lines which produce the appropriate virus were grown in Iscove’s modified Dulbecco’s medium containing 10% newborn calf serum. Supernatants were collected when cells were >80% confluent and were filtered. Titer and transforming activity were tested in a focus-forming assay on NIH-3T3 cells.

Preparation of Fetal CNS Donor Cells. Timed pregnant Fischer F344 rats were anesthetized at day 13.5 to 15.5 of gestation, the embryos were removed, and the entire fetal brain was carefully dissected using a stereomicroscope. Special care was taken to avoid contamination with leptomeninges. Approximately 10 fetal brains were then enzymatically dissociated (0.25% trypsin, 0.1% DNase in phosphate-buffered saline; 10 min at room temperature) and gently triturated, in order to obtain a single-cell suspension. For retroviral infection, the cells were incubated with tissue culture supernatant of the respective packaging cell line, in the presence of polybrene (3 μg/ml), for 4 h at 37°C and 5% CO2, at a multiplicity of infection of 1–2. After adsorption of the virus, the cells were washed with Hanks’ balanced salt solution, to remove free retroviral particles. Cells were then pelleted and immediately used for transplantation. Small aliquots of these pellets were resuspended and kept in culture, to assess cell viability and to test potential in vitro effects on neural cells of the respective oncogene. Organotypic cultures
ONCOGENE COMPLEMENTATION IN BRAIN TRANSPLANTS

Fig. 1. Structure of the retroviral vectors used for oncogene transfer into brain transplants. In neo SV(X), expression of the neo gene is driven by the Moloney virus long terminal repeat. In DoK v-myc, v-gag/myc is controlled by the long terminal repeat and neo is controlled by an internal SV40 early promoter. ras/myc and ras/lacZ are based on the zip vector, in which coexpression of the two genes relies on a partial mRNA splice mechanism. The 0.7-kilobase ras comple

of postnatal day 2 rat cerebellum were prepared using a protocol described by Trenkner and Sidman (13).

Stereotaxic Transplantation. Adult male F344 rats (150-200 g body weight) were used as recipients, and a stereotaxic injection system (Narishige, Tokyo, Japan) was used for transplantation. Host animals were anesthetized with pentobarbital (20 mg/kg body weight) and received a single injection of 5 µl of cell suspension, containing approximately 10^5 cells, into the center of the left caudoputamen (coordinates: bregma +0.8 mm, left +2 mm).

Microscopic and Immunocytochemical Analysis of the Grafts. Postoperatively, the host animals were closely monitored for signs of neurological impairment. Animals with severe neurological symptoms were anesthetized with diethyl ether and sequentially perfused with 70% ethanol. Brain slices were then dehydrated and embedded in paraffin without prior paraformaldehyde fixation. Proliferating cultures were obtained from selected tumors and kept in vitro for several passages. One of these cultures (TZ 263) was found to be clonal, by Southern blot analysis, and was used for retransplantation and assayed for oncogene expression.

Proviral Integration and Oncogene Expression. For detection of proviral DNA and oncogene transcripts, genomic DNA and total RNA were extracted from tumor cells and subjected to Southern and Northern analyses following standard protocols. DNA probes for the ras and myc oncogenes were a generous gift of Jurgen Muser (Department of Microbiology, University of Basel). NIH-3T3 cells transformed with the ras/myc retroviral vector were used as a positive control.

Escherichia coli β-galactosidase activity was visualized by X-gal histochemistry, as described (6, 16). Briefly, animals were perfused sequentially with 4% buffered paraformaldehyde, permeabilization buffer containing MgCl2, sodium deoxycholate, and Nonidet P-40, and finally with a substrate solution containing 1 mg/ml X-gal (Boehringer). The brain was removed and cut in slices, and the reaction was allowed to proceed for up to 72 h. The tissue was then processed for paraffin histology. In some cases, tissues were fixed in Delaunay’s solution and frozen sections were prepared, which yielded equivalent staining results.

Exon 1 of v-Ha-ras and a junction fragment of the v-gag/myc sequence were amplified from paraffin sections of tumor tissue, using the PCR. Briefly, tumor-bearing transplants were excised from dewaxed 5-µm sections, treated overnight with proteinase K, and subjected to the PCR reaction, following a protocol provided by the manufacturer (Cetus Corporation). Amplification products were directly sequenced by a modified Sanger method (17). NIH-3T3 cells transformed by infection with the ras/myc retrovirus and normal Fischer rat brain served as positive and negative controls, respectively.

RESULTS

Development and Microscopic Appearance of Control Transplants. Donor cell suspensions were prepared from fetal rat brains at 13.5 days of gestational age, as described (12). They were mock-infected or incubated with the neo SV(X) retrovirus encoding the neomycin resistance (neo) gene (Fig. 1) and were grafted intracerebrally into syngeneic host animals. Two months after stereotaxic implantation, the grafts showed the characteristic histological appearance of fetal brain transplants, with an irregular distribution of neurons and glial cells and formation of pseudocortical patterns (Fig. 2, A and B). Immunohistochemically, all major neuroectodermal and non-neural cell types of the CNS were identified in the grafts, using antibodies to NSE, synaptophysin, neurofilament protein, GFAP, and myelin basic protein (Fig. 2). No evidence of developmental defects or tumorigenesis was found in these control transplants. Since retroviruses are believed to integrate only into proliferating cells, we determined the proliferation potential of the transplanted fetal CNS cells by labeling the recipient animals with BrdU and counting labeled cells on histological sections stained with an antibody to BrdU. A significant fraction of donor cells was shown to be proliferating 7 days after implantation (Fig. 2C).

Identification of Target Cells for Retroviral Infection. The retroviral vector ras/lacZ, encoding both the v-Ha-ras oncogene and the E. coli β-galactosidase (lacZ) gene, was used to identify target cells prone to infection and capable of expressing the transferred genes in E13.5 CNS grafts. By double-labeling tissue sections with a chromogenic β-galactosidase substrate (X-gal) and with antibodies to synaptophysin or to GFAP, we could demonstrate expression of the retroviral construct in all major cell types of the graft, including neurons, astrocytes, and endothelial cells (Fig. 2D). The experiments established that it is possible to study the effects of oncogenes in neuroectodermal and non-neuroectodermal cells by using this gene transfer model.

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Tumor Induction in Transplants Exposed to the v-Ha-ras Gene. A total of 10 transplants were generated with the ras/lacZ retrovirus and fetal donor cells of embryonic days 13.5 and 14.5. The recipient animals were observed for periods of up to 12 months. Of eight animals with a vital graft, four developed large neoplasms in the transplant within 3–6 months after implantation (Fig. 3A). The remaining four grafts were morphologically normal 12 months after transplantation. Histologically, the tumors were characterized by a spindle cell morphology and a fascicular growth pattern (Fig. 3, C and D). Extensive immunohistochemical analyses revealed uniform expression of the neuroectodermal calcium-binding protein S-100 in these neoplasms but failed to demonstrate GFAP, neuronal marker proteins (NSE, neurofilament protein, and synaptophysin), or vimentin (Fig. 3C). Therefore, we have tentatively diagnosed the tumors as immature gliomas which have not advanced to the stage of expression of GFAP; however, an alternative histogenesis cannot be ruled out.

Expression of the retroviral vector was studied by X-gal histochemistry. It was shown previously that NIH-3T3 cells infected with the ras/lacZ retrovirus reliably coexpress both genes (6). A strong X-gal stain was detectable in tumors and in early neoplastic foci of the transplants (Fig. 3, A and B). In addition, phenotypically normal cells within the residual graft were labeled intensely, indicating that these cells tolerate the activated oncogene without necessarily advancing to malignant transformation. The remaining four transplanted animals did not develop lesions.

Aliquots of the donor cell populations were established as organotypic cultures and propagated in vitro. During an observation period of several months, no significant differences were noted, compared to control cultures; none of the cultures developed transformed foci.

Transplants with the v-gag/myc Retroviral Vector. E13.5 donor cell populations exposed to the v-gag/myc retroviral vector were transplanted into 13 recipient animals. Twelve of the recipients were followed for periods of 12–18 months without signs of neurological impairment. Neuropathological analysis revealed phenotypically normal CNS grafts, without evidence for abnormal development or tumor formation. One recipient animal developed a progressive hemiparesis 49 days after transplantation. A large tumor mass was found at the implantation site in the left hemisphere. Microscopically, this neoplasm exhibited features of a primitive neuroectodermal tumor, with extensive areas of differentiation containing hypertrophic neurons and neoplastic glial cells (data not shown). The retroviral vector was identified in paraffin sections of the tumor by PCR amplification of a gag/myc fusion fragment, using oligonucleotide primers to a 3′-gag/5′-myc primer of the virus (data not shown). Suitable tumor tissue for expression analysis was not available. Aliquots of the infected donor cell populations were established as organotypic cultures and were grown in selection medium containing G418 sulfate (0.5 mg/ml), starting 7 days after primary seeding. G418-resistant colonies derived from this experiment did not develop features of malignant transformation.

Oncogene Cooperation in Fetal Brain Transplants. Donor cells were prepared at days 13.5, 14.5, and 15.5 of embryonic development, incubated with the retroviral vector ras/myc, which encodes both v-Ha-ras and v-gag/myc oncogenes, and stereotactically implanted into the brains of 13 adult recipients. Two of the recipients did not carry vital grafts. The remaining 11 host animals presented with severe neurological impairment after latency periods of 13–60 days. Numerous tumor nodules were found in these transplants. A representative example of a graft with multiple neoplastic foci is shown in Fig. 4. This animal survived for only 13 days following transplantation. A detailed histopathological evaluation of these neoplasms revealed rapidly proliferating, highly malignant tumors, without morphological evidence for differentiation or formation of spe-
integrated retroviral construct (Fig. 5B). By PCR amplification with a pair of primer oligonucleotides derived from the first exon of Ha-ras, and subsequent sequence analysis, we were also able to identify, in TZ 363 cells and in paraffin blocks of brain transplants harboring ras/myc-induced primary tumors, the C-T mutation of codon 12 specific for v-Ha-ras (Fig. 6).

Northern blot analysis of the transgenic transcripts was carried out with total cellular RNA, using DNA probes specific for the Ha-ras and myc genes (Fig. 7). NIH-3T3 cells before and after infection with the ras/myc vector served as negative and positive controls, respectively. Both oncogenes were found to be highly expressed in TZ 363 cells. High levels of v-gag/myc and of c-myc mRNA were detectable in TZ 363 cells, indicating that these transcripts are coexpressed in these cells. Expression of Ha-ras was also examined immunohistochemically, using an antibody to p21. The reaction revealed a strong signal in all tumor cells, without significant heterogeneity (data not shown).

Oncogene Cooperation in Vitro. In contrast to the single oncogenes, the combination of v-Ha-ras and v-gag/myc was also found to transform primary neural cells in vitro. For these experiments, aliquots of the fetal donor cells were exposed to

Fig. 3. Induction of gliomas in brain transplants exposed to the v-Ha-ras oncogene. A, frontal whole-mount section through the brain of a rat which survived 5 months after transplantation, after X-gal staining. A large malignant tumor has arisen from the graft in the left hemisphere and has led to a considerable mass effect, with shift of the midline to the right and destruction of the surrounding structures. The tumor tissue expresses large amounts of β-galactosidase. B, X-gal staining for β-galactosidase of a ras-harboring graft, × 100. Three neoplastic foci are intensely labeled. Single, scattered, morphologically normal cells with a strong cytoplasmic signal are detectable also in the nontransformed part of the transplant (arrows). C, detail of a ras-induced tumor, showing moderately dense neoplastic cells embedded in a fibrillary matrix with uniform expression of S-100 protein. Immunocytochemical staining with a polyclonal antiserum to S-100 protein, × 400. D, detail of a neural transplant 2 months after stereotaxic injection of ras-exposed donor cells. H&E, × 400. Note a preneoplastic nodule of tightly packed cells with morphological features of astrocytes.

specific growth patterns. Immunocytochemical reactions with antibodies for neural, glial, mesenchymal, and epithelial marker proteins failed to identify the histogenetic origin of the tumor cells.

Three of these tumor-bearing grafts were mechanically dissociated and explanted in vitro. Rapidly proliferating transformed cultures were obtained from such explants, subcloned, and propagated for several months. A subclone termed TZ 363 was selected for further characterization. Fig. 5A shows a phase-contrast photomicrograph of TZ 363. Intracerebral transplantation of the cells into syngeneic rats gave rise to poorly differentiated neural tumors, some of which were found to express GFAP (Fig. 4D). This indicates that neural precursors capable of astrocytic differentiation represent targets for neoplastic transformation by the combined action of ras and myc.

Genomic DNA extracted from TZ 363 cells was used to demonstrate the presence of viral DNA, by Southern hybridization analysis. Compared to DNA from F344 host brain, the tumor cells displayed an additional band, corresponding to the

Fig. 4. Oncogene cooperation in the graft. A, section through a graft exposed to the ras/myc virus. H&E, × 100. Several tumor nodules are visible. The recipient animals survived for 13 days following stereotaxic implantation of fetal donor cells. B, section parallel to A, stained with an antiserum to GFAP. Expression of GFAP is restricted to entrapped reactive astrocytes. × 100. C, detail of a ras/myc-induced tumor, showing a poorly differentiated, malignant neoplasm consisting of small round cells with hyperchromatic nuclei and scant cytoplasm. A small necrosis, characteristic of highly malignant gliomas, is visible in the center. H&E, × 200. D, secondary transplant of a tumor cell line derived from a ras/myc-induced graft tumor. The expression of GFAP in most tumor cells indicates an origin from cells with a potential for glial differentiation. GFAP immunostain, × 200.
the retroviral vector and then propagated in culture. Within 10–14 days, multiple transformed foci appeared in these cultures and started to replace the organotypic brain reaggregates (Fig. 5A). Such foci were never observed in control dishes exposed to a retroviral vector encoding only neo.

The newborn rodent cerebellum harbors an expanding population of proliferating neuronal precursor cells in the external granular cell layer. In order to examine oncogene cooperation in this tissue, organotypic cultures obtained from postnatal day 2 cerebellum were infected with the ras/myc retrovirus and propagated in vitro. Numerous transformed foci developed in these cultures within 2–4 weeks. The neoplastic colonies did not exhibit cytological or immunocytochemical features of neuronal or glial maturation. Upon intracerebral implantation into F344 rats, aggressive neoplasms, with morphological features indistinguishable from those of the neoplasms induced in forebrain grafts, developed within 2–3 weeks. Transcripts of both oncogenes were readily detectable in total RNA prepared from these transplantation tumors and from in vitro transformed cells (CBP2; Fig. 4). It was also possible to demonstrate v-Ha-ras and v-gag/myc genomic sequences by PCR amplification (data not shown). These findings indicate that both embryonic forebrain and perinatal cerebellum are highly susceptible to neoplastic transformation by the combined action of ras and myc.
DISCUSSION

The present work was initiated by experiments which demonstrated cell type-specific transformation in brain transplants exposed to replication-defective retroviruses encoding either polyoma middle T or v-src (10-12). These studies also revealed that all major cell types in the brain can be stably targeted with retroviral constructs and can express the transferred genes. Using retrovirus-mediated gene transfer into CNS transplants, we have now examined the effects of the v-Ha-ras and v-gag/myc oncogenes on the developing and adult nervous system in vivo.

Recipient animals with transplants harboring an activated v-Ha-ras gene developed solid tumors in the graft at an incidence of approximately 50%. Based on the histopathological features and immunocytochemical reactivity pattern, with no expression of the examined markers except for S-100 protein, we tentatively classified these neoplasms as immature gliomas of astrocytic origin (18). Because S-100 protein is not a specific marker for glial cells, however, an alternative histogenesis cannot be excluded. The tumors induced by v-Ha-ras exhibited a uniform morphological appearance. This suggests that astrogial precursors are susceptible to ras-initiated transformation. Other cell types in the graft were not visibly affected, although X-gal histochemistry demonstrated expression of the retroviral construct in neurons, glia, and endothelial cells. Similar lesions have not been previously described, to our knowledge, in any of the neoplasms induced by ras or by ras/myc in our experiments.

Special attention was given to the neuronal compartment in the graft, since many observations point to a role for ras genes in neuronal differentiation pathways (22-24). Introduction of activated ras into PC12 pheochromocytoma cells induces features of neuronal differentiation (25), and nerve growth factor-induced neurogenesis in PC12 cells can be abolished by microinjection of antibodies to p21WAF1. Introduction of p21WAF1 in primary neurons may obviate the requirement for neurotrophic factors (26). In Schwann cell cultures, an activated ras gene can inhibit cell proliferation (27). These observations indicate that ras proteins act in a highly cell type-specific fashion. A careful histopathological and immunocytochemical analysis of the transplants failed to identify phenotypic changes in X-gal-labeled neurons, suggesting that expression of activated p21WAF1 is tolerated in CNS neurons without causing morphological signs of abnormal neurogenesis.

The latency period of several months after transplantation and the detection of β-galactosidase activity in phenotypically normal neuro-glial cells within the grafts suggest that v-Ha-ras initiates an early stage of neoplastic transformation, which requires additional genetic alterations for progression into macroscopic tumors. Complementing spontaneous mutations have also been proposed in mammary carcinomas and skin tumors of transgenic mice, with targeted expression of an activated ras gene in the respective tissue (8).

In contrast to oncogenes of the ras family, which so far have not been associated with human brain tumors, myc genes appear to be frequently involved in the pathogenesis of neuroectodermal tumors. Prominent examples include amplification of the N-myc gene in neuroblastomas, an event which correlates with a poor clinical outcome for the individual patient, and c-myc amplification in glioblastoma multiforme and medulloblastoma cell lines (28, 29). Recently, we have demonstrated overexpression of N-myc in neuroblastomas of transgenic mice expressing the polyoma middle T antigen (30). Thus, myc was a particularly interesting candidate for introduction into CNS transplants. Intense efforts with a high titer retroviral vector failed to induce neoplastic transformation in the graft, with the exception of a primitive neuroectodermal tumor in a single recipient. The integrated provirus could be demonstrated in this tumor by PCR analysis. Since additional tumors have not been observed in the same and subsequent experiments, we cannot exclude the possibility that positional effects of the retroviral integration, perhaps in combination with myc expression, might be responsible for tumorigenesis in this particular graft (31, 32). Evidence for insertional mutagenesis was not found in any of the neoplasms induced by ras or by ras/myc in our experiments.

No evidence of grossly impaired neurogenesis was seen in transplants harboring the v-gag/myc gene, suggesting that myc overexpression is generally tolerated by transplanted CNS neurons. However, preliminary observations suggest that fetal CNS cells pretreated with alkylating carcinogenic agents become highly susceptible to tumorigenesis when infected with the v-gag/myc retrovirus. A similar observation was recently made in transgenic mice containing the pim-1 oncogene (33).

Fetal brain transplants harboring both v-Ha-ras and v-myc demonstrated a dramatic cooperative transforming effect of these oncogenes in transforming neural cells in vivo. All recipient animals with vital transplants displayed multiple tumors in the graft within 2-6 weeks of stereotactic implantation. These poorly differentiated, malignant tumors most likely originated from glial precursor cells in the fetal CNS preparation, as indicated by the finding of GFAP immunoreactivity in retrans-
planted cell lines derived from these neoplasms. Since integration and expression of zip retroviral vectors occur in a variety of cell types in the graft, the predominant induction of malignant neuroectodermal tumors indicates that these progenitor cells are highly susceptible to the transforming potential of the v-Ha-ras and v-gag/myc oncogene combination. The observation of rapid induction of multiple tumors in the grafts strongly suggests that the combination of ras and myc may be sufficient to transform primary neural cells in vivo. Although we are not able to show clonality of single neoplasms in the graft, the nodular appearance of the tumors suggests that the single nodules are monoclonal.

In contrast to other transforming genes which we have introduced into brain transplants, the coexpression of ras and myc also elicits transformed foci in organotypic CNS cultures in vitro. The short latency period required for focus formation in vitro would, again, be compatible with a single-step transformation event. Upon cytological and immunocytochemical analysis, it was shown that these colonies were derived from immature neural cells, without evidence for neuronal or glial differentiation. Stereotaxic implantation into the brain of syngeneic recipients resulted in malignant neoplasms indistinguishable from ras/myc-induced primary tumors. This in vitro transformation potential was not restricted to the embryonic brain, since cultures of postnatal rat cerebellum also gave rise to rapidly proliferating and tumorigenic transformed colonies after introduction of the ras/myc retroviral vector. We, therefore, conclude that neural precursor cells are a major target cell population for ras/myc oncogene cooperation. Coexpression of ras and myc provides a rapid and efficient tool to transform these cells in distinct segments of the CNS and at different stages of neurogenesis.

Following injection of the ras/myc retroviral construct into mouse embryos, Compere et al. (19) observed a high incidence of malignant neoplasms in the brain and in other tissues, with a median latency period of only 3 weeks. Based on their histopathological appearance, the intracranial tumors were classified as vascular meningeal neoplasms; gliomas were not observed. The authors proposed that additional genetic lesions may be required to complete the transformation event initiated by ras and myc; introduction of either ras or myc alone did not induce a pathological phenotype in the nervous system. An alternative approach for transfer of oncogenes into the brain was chosen by Corallini et al. (21). Plasmids encoding an activated ras gene, the BK virus early region including BK large T antigen, or a combination of both were injected into the brain of newborn hamsters. Inoculation of the construct harboring both transgenes led to the development of intracranial sarcomas within 2–6 weeks, whereas the single genes did not induce tumor formation. Neuroectodermal tumors were not observed. Although these findings provide evidence for in vivo cooperation in the brain between an activated ras gene and the BK virus large T antigen, the differences in the histogenetic origin of intracranial tumors following introduction of complementing oncogenes into either neural transplants, midgestation mouse embryos, or newborn hamster brains suggest that distinct target cells are preferentially transformed at different developmental stages.

Retrovirus-mediated oncogene transfer into fetal brain transplant provides a novel approach to study neoplastic transformation pathways in the nervous system. In contrast to transgenic animals, in which all cells contain exogenous DNA, target cells for the retroviral vector develop in an environment of unmodified normal cells, thus mimicking important features of sporadic tumorigenesis in the brain. Using this transplantation model, it will be possible to evaluate the transforming potential of alternative oncogene combinations in the CNS. Candidate genes include the N-myc oncogene and the epidermal growth factor receptor, which have recently been implicated in the pathogenesis of human brain tumors (28, 29). Finally, gene transfer into neural transplants can also be applied to the study of the molecular pathogenesis of other neurological disorders (34).

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