

Trauma-induced tumorigenesis of cells implanted into the rat spinal cord

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Object. Findings in several clinical cases have suggested a correlation between tumor formation and previous injury to the central nervous system (CNS); however, the relationship between trauma and tumorigenesis has not been investigated well experimentally. In this study the authors provide evidence correlating tumorigenesis with trauma in the rat spinal cord.

Methods. A glial cell line, C6R-G/H, which expresses green fluorescent protein (GFP) and hygromycin phosphotransferase (HPT), was implanted into normal and injured rat spinal cords. In all rats in which the cells were implanted into an injured site, locomotor function deteriorated and histological analysis demonstrated glioblastoma multiforme by 6 weeks; tumorigenesis was correlated with a loss of both GFP expression and resistance to hygromycin treatment. In contrast, no evidence of tumor formation was found at 6 weeks in rats in which the cells were implanted into healthy tissue. When C6R-G/H cells were treated with contused spinal cord extract in culture before implantation, they lost GFP expression and hygromycin resistance, and later formed tumors after implantation into normal spinal cord.

Conclusions. The findings of this study indicate that trauma can induce tumorigenesis. Implantation of C6R-G/H cells into traumatized spinal cords resulted in their transformation, which was signaled by loss of GFP expression and hygromycin resistance accompanied by tumor formation. Exposure to extracts derived from injured spinal cord produced similar transformation and gene expression changes, as well as tumor formation after such cells were implanted into normal cords. Care, therefore, should be taken when cells are implanted into an injured CNS because of potential mutagenesis due to trauma-induced factors.

KEY WORDS • trauma • tumorigenesis • glioblastoma multiforme • cell implantation

THE relationship between trauma and the development of tumors in the CNS is still unclear. It has been suggested that trauma may induce certain tumors including gliomas.^{2,3,22,24} There is evidence that other biological insults such as radiation can induce glioma formation by causing genetic alterations;^{23,29} however, little is known about how trauma might induce the formation of gliomas. Trauma can induce the release of many factors including bFGF,^{14,18,19} which can transform cells and also promote glioma formation. Genetic alterations can also be induced chemically. Interestingly, trauma induced in rat pups following transplacental treatment with the carcinogen ethylnitrosourea yielded significantly more gliomas than those found in the nontraumatized brain of their siblings, which were given the same carcinogen.²⁰ This suggests that trauma can act to promote tumor formation in transformed cells.

In our previous study, we showed that C6R-G/H, a cell line derived from the radial glial cell line C6 glioma by

cotransfection of *GFP* and *HPT*, displays decreased tumor formation following implantation into the rat brain.¹¹ Hygromycin phosphotransferase exerts a tumor suppressive activity and GFP has been found to be a convenient indicator correlating with HPT activity in C6R-G/H cells. In the present study, we found that when C6R-G/H cells were implanted into injured rat spinal cords, they formed tumors and lost GFP expression, but when the same type of cells were implanted into normal spinal cords, they did not form tumors. In an additional *in vitro* experiment, C6R-G/H cells that were cultured with an extract derived from contused spinal cord lost GFP expression and hygromycin resistance and formed tumors in normal spinal cords after implantation, suggesting that trauma-induced factors caused alterations in the expression of various genes including the transfected ones.

Although cell implantation may be extremely useful as a therapeutic strategy to repair the injured CNS, one must consider whether implanted cells undergo neoplastic transformation in response to injury. Whereas C6R-G/H cells tend to transform because they are derived from the C6 glioma, the results indicate that trauma induces a release of factors into the injury site that can act as a potent mutagen. Therefore, it may be important to consider whether implanted cells carry a high risk to form tumors following trauma.

Abbreviations used in this paper: bFGF = basic fibroblast growth factor; CNS = central nervous system; DMEM = Dulbecco modified Eagle medium; FCS = fetal calf serum; GBM = glioblastoma multiforme; GFP = green fluorescent protein; HPT = hygromycin phosphotransferase; SEM = standard error of the mean.

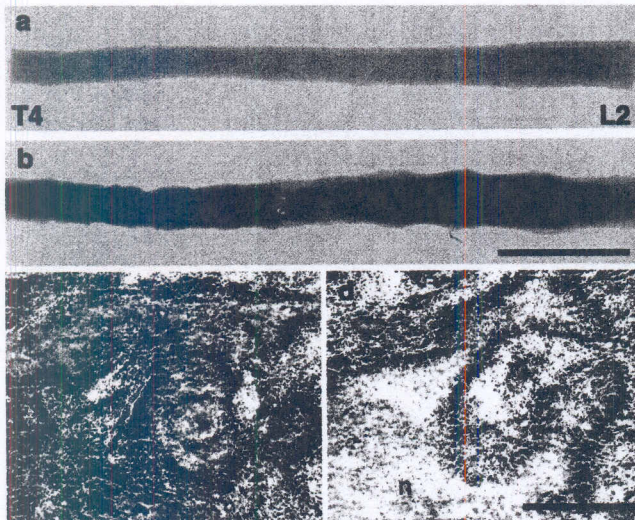


FIG. 1. Gross anatomical specimens of noncontused (a) and contused (b) spinal cord (T4–L2) 6 weeks after implantation of C6R-G/H cells and photomicrographs of cryosections. a: The C6R-G/H cells did not form tumors in noncontused spinal cord. b: The C6R-G/H cells formed prominent hemorrhagic tumors in contused spinal cord. c and d: Stained sections of the cord shown in b demonstrating features of GBM, including the presence of neovascularization (v), necrosis (n), and pseudopalisading (p). H & E (c and d). Scale bars = 10 mm (a and b) and 500 μ m (c and d).

Materials and Methods

Cell Lines

The C6R cell line was generated by transfection of C6 glioma cells with a construct encoding a mutant of the human *receptor protein tyrosine phosphatase β* gene, inserted into the pCDNA3 vector, as previously described.⁸ It lacks the phosphatase domains and contains a hemagglutinin tag in place of the two cytoplasmic domains. Cell lines were maintained in DMEM supplemented with 10% FCS, 2 mM L-glutamine, and 50 μ g/ml gentamicin (all of which were purchased from Gibco BRL, Grand Island, NY). The C6-R cells were intrinsically labeled with GFP by cotransfection with the plasmid pEGFP-N1, which codes for GFP, and plasmids conferring resistance to hygromycin were used for selection;⁸ neomycin resistance had already been conferred by the original transfection that generated the C6R cell line from C6 cells. The resulting cell lines in two independent transfections were called "C6R-G/H."

Animal Preparation and Surgical Procedure

Forty adult female Sprague-Dawley rats (Taconic Farms, Germantown, NY), each weighing between 200 and 250 g, were used in these experiments. All animal procedures were conducted with the approval of the Institutional Animal Care and Use Committee of the Rutgers University (Protocol No. 99-053) in accordance with the National Institutes of Health guidelines for the use of animals in research.

The animals were anesthetized with intraperitoneal injection of pentobarbital (35 mg/kg) and the spinal cord was exposed by laminectomy at T9–10. To prepare the cells for implantation, they were grown to approximately 75% confluence, and detached by a 3-minute incubation in 0.25% trypsin/1 mM ethylenediamine tetraacetic acid; DMEM/FCS was added to quench trypsinization. Cells were dissociated by vigorous pipetting, washed twice with DMEM, and counted (Coulter Counter; Beckman Coulter Inc., Fullerton, CA). A sterile glass tip (diameter 50 μ m) was connected to a 2.5- μ l Hamilton syringe, and 2 μ l of suspension containing 2×10^5 C6R-G/H cells or vehicle medium was injected slowly during the course of 10 minutes into an exposed spinal cord with or without contusion. Con-

TABLE 1

Summary of tumor formation at 6 weeks after implantation*

| Contents & Site of Injection | Rat No. | Macroscopic Findings | Necrosis | Pseudopalisading |
|------------------------------|---------|-----------------------------|----------|------------------|
| medium | | | | |
| contused spinal cord | 1 | shrinkage at injection site | — | — |
| | 2 | shrinkage at injection site | — | — |
| | 3 | shrinkage at injection site | — | — |
| | 4 | shrinkage at injection site | — | — |
| | 5 | shrinkage at injection site | — | — |
| C6R-G/H cells | | | | |
| noncontused spinal cord | 6 | normal | — | — |
| | 7 | normal | — | — |
| | 8 | normal | — | — |
| | 9 | normal | — | — |
| | 10 | normal | — | — |
| | 11 | normal | — | — |
| contused spinal cord | 12 | tumor | — | — |
| | 13 | hemorrhagic tumor | + | + |
| | 14 | hemorrhagic tumor | + | + |
| | 15 | hemorrhagic tumor | + | + |
| | 16 | hemorrhagic tumor | + | + |
| | 17 | hemorrhagic tumor | + | + |
| | 18 | hemorrhagic tumor | + | + |
| | 19 | hemorrhagic tumor | + | + |

* Medium or C6R-G/H cells were injected into rat spinal cords with or without contusion. Macroscopically, the spinal cords injected with medium showed evidence of shrinkage at the contusion site, whereas injection with C6R-G/H cells resulted in tumors often accompanied by hemorrhage. Noncontused cords injected with C6R-G/H cells appeared normal with no evidence of tumor formation. Sections were analyzed histologically for necrosis and pseudopalisading, which are characteristics of GBM. + = present; — = absent.

tused spinal cords were produced by dropping a 10-g rod onto the exposed spinal cord from a height of 25 mm by using the New York University standard impactor.⁷ After withdrawing the syringe, the muscles were sutured in layers and the skin was closed with metal clips. After surgery, the animals were kept on heating pads, closely observed until awake, and then returned to their home cages. The rats were maintained for up to 6 weeks after spinal cord injury. Cefazolin (25 mg/kg; Faulding Pharmaceutical Co., Elizabeth, NJ) was administered for 7 days after surgery to prevent postoperative infection.

Histopathological Analysis

The animals were killed with an intraperitoneal injection of pentobarbital (120 mg/kg) at 2, 4, or 6 weeks after surgery and perfused through the heart with 250 ml of normal saline followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The animals' spinal cords were dissected out and immersed in 0.1 M phosphate buffer at 4°C overnight, followed by cryoprotection in 25% sucrose in 0.1 M phosphate buffer for 3 to 5 days. Spinal cord specimens were embedded in optimal cutting temperature compound (Fisher Scientific, Pittsburgh, PA), and kept at -20° C before they were cut into 20- μ m-thick horizontal sections on a cryostat and mounted onto a Superfrost Plus microscope slide (Fisher Scientific). After the histological sections were analyzed for GFP labeling and nuclear fluorochrome bisbenzimidazole stained with Hoechst 33342 (Sigma-Aldrich, St. Louis, MO) by using a confocal laser scanning microscope (LSM 510; Carl Zeiss, Inc., Thornwood, NY), they were stained with hematoxylin and eosin. The sections were examined to assess hemorrhage, necrosis, and mass area by using either the confocal laser scanning microscope or a fluorescence microscope (Axio-phot 200M; Carl Zeiss, Inc.).

Images were acquired from every fourth cryostat section by using the confocal laser scanning microscope. The maximal cross-sectional area of the mass and GFP labeling was measured with the aid of imaging software (National Institutes of Health Image Software¹¹). The perimeter of the tumor mass, as determined using hematoxylin

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and eosin staining and GFP labeling, was traced and the area was measured in square millimeters by using a scale reference.

Behavioral Assessment

The Basso-Beattie-Bresnahan locomotor rating scale⁵ was used to evaluate changes in hindlimb locomotor function. The rats were first evaluated 1 week after surgery, and then once a week for 6 weeks by two observers under blinded conditions.

Isolation of Cells After Treatment With Spinal Cord Extract

Rats with spinal contusions were killed 1 day after injury without perfusion and 0.5-cm segments of spinal cords were homogenized in 500 μ l of phosphate-buffered saline containing Complete protease inhibitor (Roche Applied Science, Indianapolis, IN) by using a homogenizer (ULTRA-TURRAX Homogenizer; IKA Works, Inc., Wilmington, NC). The extracts were centrifuged at 15,000 rpm for 30 minutes at 4°C, and the supernatant was collected. Supernatants from normal (uninjured) spinal cords were also collected as controls. The C6R-G/H cells were cultured in defined medium (DMEM supplemented with 50 ng/ml bovine insulin, 50 μ g/ml transferrin, and 30 nM sodium selenite, each of which was purchased from Sigma-Aldrich) containing 6% (by volume) of contused or normal spinal cord extract. The cells were treated for 4 days, after which half the volume of medium was changed to fresh defined medium. After an additional 6 days in culture, the medium was replaced with DMEM containing 10% FCS. After we confirmed the loss of GFP expression by using the fluorescence microscope the cells were isolated for implantation.

The resistance of isolated cells to hygromycin was tested by applying hygromycin at a concentration of 300 μ g/ml for several days. Afterward these cells were implanted into normal spinal cords and the rats were maintained for 6 weeks after implantation.

Statistical Analysis

All data were analyzed using commercially available software (StatView [version 5.0.1]; SAS Institute, Inc., Cary, NC) and are expressed as means \pm SEMs. The Basso-Beattie-Bresnahan scores in rats injected with medium and in those injected with C6R-G/H cells were compared at each time point by using the Mann-Whitney U-test. The size of the mass and the amount of GFP labeling were compared between each time point by applying the Mann-Whitney U-test. Differences were deemed statistically significant at a probability value less than 0.05.

Results

Tumor Formation of C6R-G/H Cells

We compared tumor formation of C6R-G/H cells in contused spinal cords with that in noncontused spinal cords for 6 weeks following implantation. Pathological studies revealed that tumors developed in all rats in which C6R-G/H cells had been implanted into contused spinal cords (Fig. 1a and b, and Table 1), although the sizes of the tumors varied. At 6 weeks after implantation, seven of eight tumors were hemorrhagic and displayed characteristics of GBM, including the presence of necrosis and pseudopalisading (Fig. 1c and d). Implantation of C6R-G/H cells into noncontused spinal cords did not form tumors (Fig. 1a and Table 1). In control animals, which had received injections of medium into contused spinal cords, there was only shrinkage of the spinal cord in the vicinity of the contusion (Table 1). All animals injected with C6R-G/H cells into contused spinal cord exhibited significant bilateral hindlimb paralysis after 4 weeks postimplantation, and demonstrated no hindlimb movement at 6 weeks postimplantation (Fig. 2). There were significant differences in hindlimb locomotor function at 5 and 6 weeks postimplantation between rats injected with

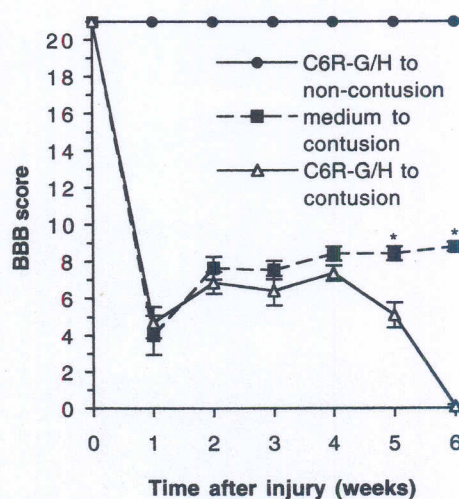


FIG. 2. Graph showing the time course of locomotor function as measured by Basso-Beattie-Bresnahan (BBB) locomotor scores. Animals in which C6R-G/H cells had been implanted in noncontused spinal cord displayed no evidence of hindlimb dysfunction. Animals in which C6R-G/H cells had been implanted in contused spinal cord exhibited significant bilateral hindlimb paralysis beyond 4 weeks postimplantation. Locomotor function at 5 and 6 weeks postimplantation was significantly different depending on whether medium or C6R-G/H had been injected into the contused spinal cords (* $p < 0.01$). Data are presented as means \pm SEMs.

C6R-G/H cells and those injected with medium into contused spinal cords ($p < 0.01$) (Fig. 2). When C6R-G/H cells were implanted into noncontused spinal cords, the animals did not exhibit any hindlimb dysfunction.

Relationship Between Tumor Formation and GFP Expression

Previous studies showed that when C6R-G/H cells lose GFP expression they form tumors in the brain, making loss of GFP a convenient marker of tumor formation for these cells. Two weeks after implantation into contused spinal cords, the injury site was filled with C6R-G/H cells and a slight bulge of the spinal cord was seen (Fig. 3a). The C6R-G/H cells dispersed at the injury site and some cells lost GFP expression (Figs. 3a, 3b, and 4). Cells at the edges of the mass were often GFP-negative, consistent with the idea that they may be more invasive and tumorigenic.¹¹ Four weeks following implantation into contused spinal cords, larger masses developed and the spinal cords became enlarged (Fig. 3c). At that time, however, the expression of GFP by C6R-G/H cells was significantly reduced (Figs. 3d and 4). By 6 weeks postimplantation C6R-G/H cells formed huge masses with little or no GFP expression (Figs. 3e, 3f, and 4). As the mass volumes and tumor formation increased with time, GFP expression of C6R-G/H cells was reduced (Figs. 3 and 4). Thus there was an inverse relationship between tumor formation and GFP expression (Fig. 4).

Effect of Contused Spinal Cord Extract in Culture

In view of previous results that C6R-G/H cells infrequently lose both GFP expression and resistance to hygromycin in the brain when they form tumors, we were interested to know whether factors induced by trauma could

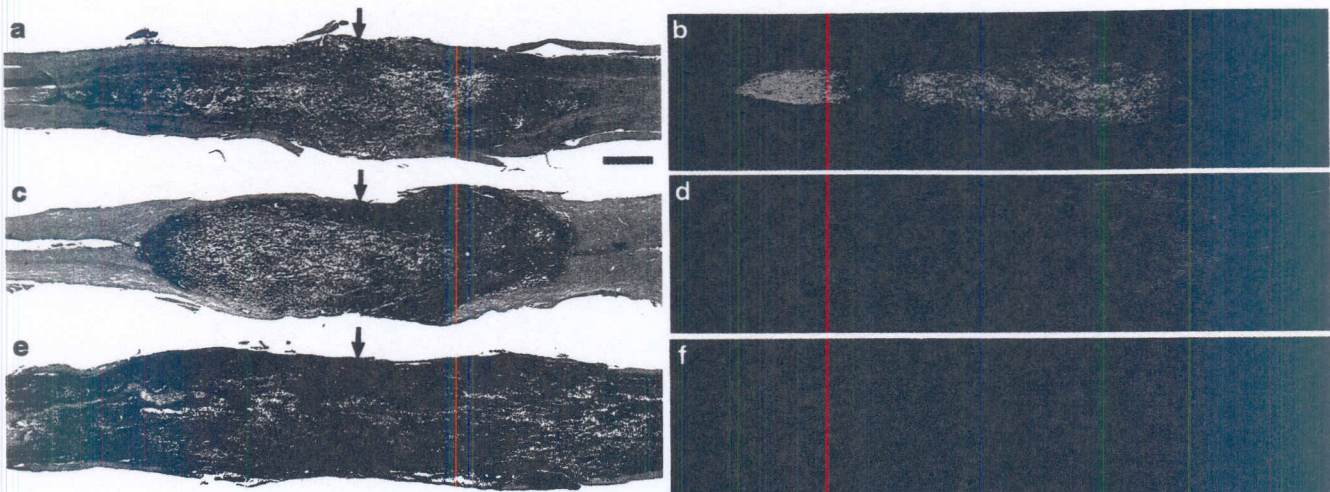


FIG. 3. Photomicrographs showing tumor growth (a, c, and e), and loss of GFP expression (b, d, and f), both of which increased with time after implantation of C6R-G/H cells into contused spinal cords. a and b: Mass formation at 2 weeks, with some C6R-G/H cells exhibiting a loss of GFP expression. c and d: Masses continue to develop and GFP expression of C6R-G/H cells is significantly reduced at 4 weeks. e and f: The C6R-G/H cells have formed huge masses with little or no GFP expression at 6 weeks. Arrows indicate the center of the contusion. The distal spinal cord is on the right side of each panel. H & E (a, c, and e). Scale bar = 1 mm.

induce changes in cells to promote tumorigenesis. We therefore tested the effects of extracts derived from contused spinal cords on C6R-G/H cells in culture.

When C6R-G/H cells were cultured for 4 days with 6% contused spinal cord extract, the long processes of these cells retracted and we could only find short or no processes (Fig. 5). In contrast, C6R-G/H cells treated with normal spinal cord extract had long processes similar to control cells maintained without extract. Treatment of cells with 6% contused spinal cord extract for longer periods of time

caused extensive cell death. After 4 days we therefore cultured the cells with only 3% extract for an additional 6 days. Then we replaced the medium with medium containing 10% FCS, which allowed the surviving cells to proliferate. Most cells treated with contused spinal cord extract lost GFP expression, whereas a minority of cells expressed GFP very weakly. In contrast, normal spinal cord extract did not have any effect on GFP expression (Fig. 5).

Tumor Formation of Isolated Cells Treated With Spinal Cord Extract

Two populations of cells that were isolated following treatment with contused spinal cord were called "C6R-C1" and "C6R-C2" (Table 2). Although both cell populations lost GFP expression and hygromycin resistance, their structures differed somewhat from that of C6R-G/H. The isolated cells treated with normal spinal cord extract were called "C6R-N" and they still maintained GFP expression and hygromycin resistance (Table 2). In four of six rats in which C6R-C1 or C6R-C2 cells were implanted into normal spinal cords, tumors developed within 6 weeks, whereas implantation of C6R-N cells, like that of the original C6R-G/H cells, did not produce tumors (Table 2). Although the size of the tumors generated by C6R-C1 or C6R-C2 cells was small, the histological characteristics of GBM, including presence of necrosis and pseudopalisading, were similar to those of C6R-G/H tumors in contused spinal cord (Fig. 6).

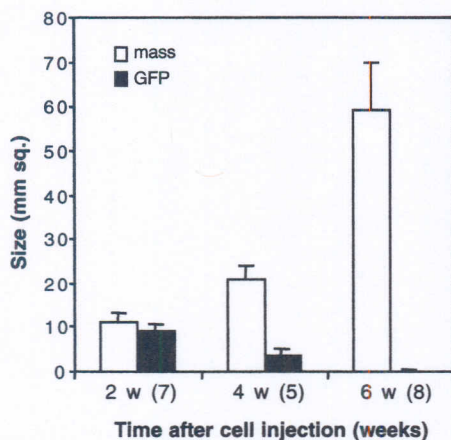


FIG. 4. Graph showing the relationship between the size of the mass and the amount of GFP labeling. Horizontal sections of rat spinal cord were analyzed and a maximal cross-sectional area displaying the transplanted mass and GFP labeling was used as a measure of size. As the area of the mass increased with time, the GFP expression of C6R-G/H cells was reduced. The difference in the size of the mass and the amount of GFP labeling between 2 and 4 weeks was statistically significant ($p < 0.05$). The difference in the size of the mass between 4 and 6 weeks was also statistically significant ($p < 0.01$). Data are presented as means \pm SEMs.

Discussion

The results of this study show that trauma can promote cell transformation in vitro and increases the frequency of tumor formation in vivo. Although there has been speculation on this idea in the literature, there have been few systematic studies that address this issue.

In this study, we have analyzed tumor formation for a cell

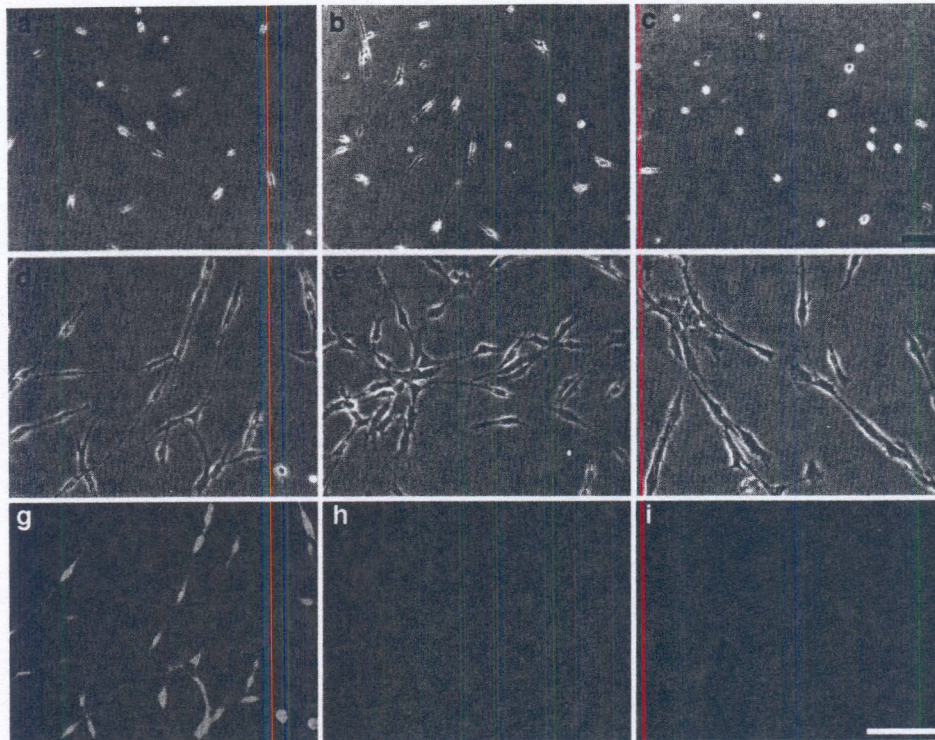


FIG. 5. Photomicrographs showing morphological changes and loss of GFP expression in C6R-G/H cells in culture following treatment with contused spinal cord extract. a–c: The C6R-G/H cells were cultured for 4 days without (a) and with (b and c) spinal cord extract. The cells maintain long processes without the presence of extract (a) and in the presence of normal spinal cord extract (b). The long processes of C6R-G/H cells retracted and only short or no processes could be observed in cultures containing contused spinal cord extract (c). d–i: After treatment with spinal cord extracts, the surviving C6R-G/H cells were cultured in medium containing 10% FCS. g–i: Green fluorescent images corresponding to the photomicrographs shown in d through f, respectively. Normal spinal cord extract did not have any effect on GFP expression (d and g). Most cells treated with contused spinal cord extract lost GFP expression (f and i), whereas a minority of cells expressed GFP very weakly (e and h). Scale bars = 100 μ m.

line derived from the C6 glioma. This is an interesting model system in which tumorigenesis in the rat brain has been reduced in a glioma cell line by introduction of foreign genes including *GFP* and *HPT* (Fig. 7),¹¹ the latter of which has been recognized to possess tumor-suppressive activity.^{15,25,27} Previous studies have demonstrated that the C6R-G/H cell line forms tumors in a delayed manner and at reduced frequency, compared with C6, which forms tumors rapidly and consistently.¹¹ The reduced tumorigenicity is believed to be due, at least in part, to expression of *HPT* and its tumor-suppressive activity. Loss of *HPT* expression, which confers hygromycin resistance, correlates with tumor formation (Fig. 7).

To analyze the potential role of trauma in tumor formation, we implanted C6R-G/H cells into the rat spinal cord either directly after laminectomy or following contusive injury. Whereas in both cases the cells migrated rapidly, tumor formation only occurred in the contused cords. The method of transplantation (see *Materials and Methods*) is quite gentle and probably does not induce sufficient trauma to promote transformation of C6R-G/H cells to any significant extent. Given the fact that, in previous cases of tumor formation from C6R-G/H cells, there was a loss of expression of both *GFP* and *HPT*,¹¹ we decided to use the *GFP* and *HPT* genes as reporters of the state of C6R-G/H cells. Genes introduced into cells by transfection are usually in-

serted into the same region of the genome and, therefore, may cosegregate during mutagenesis or be coregulated, for example by methylation.^{12,13,17} Expression of *GFP*, which can readily be detected by its intrinsic fluorescence, was dramatically reduced by 4 weeks following transplantation into contused spinal cords and was completely lost by 6 weeks when tumors were detected in all cases. These results suggest that the trauma induced changes in gene expression in the C6R-G/H cells and is tightly correlated with tumor formation.

We hypothesized that trauma induces the release of factors into the injury site and that these factors can alter gene expression in C6R-G/H cells. Evidence supporting this hypothesis was obtained by comparing extracts from normal and contused spinal cords. Exposure of C6R-G/H cells to extracts from contused spinal cords yielded cells that lost expression of *GFP* and *HPT*, and formed tumors at relatively high frequencies when transplanted into noncontused spinal cords. These results suggest that soluble factors released in the spinal cord during contusive injury have a mutagenic activity and that C6R-G/H cells can be used to assay for such factors. Extracts from contused spinal cord caused many C6R-G/H cells to die while surviving cells lost expression of *GFP* and *HPT*. It will be interesting to determine whether the cytotoxic factors overlap or differ from factors that induce alterations in gene expression.

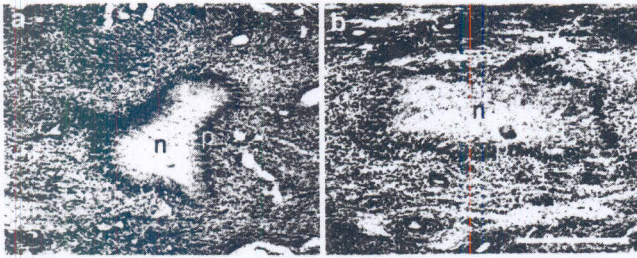


FIG. 6. Photomicrographs demonstrating sections of tumors arising from C6R-C cells. Cells isolated following treatment with contused spinal cord extract yielded GBMs in normal spinal cord 6 weeks following implantation. The histological characteristics, including the presence of necrosis (n) and pseudopalisading (p), were similar to the C6R-G/H tumors found in contused spinal cord. Scale bar = 500 μ m.

Within the injury site, the elevated expression of multiple factors has been demonstrated,¹⁹ including bFGF, which is overexpressed in injured brain¹⁴ and contused spinal cord.¹⁸ Expression of bFGF and the FGF receptor was also significant in gliomas^{21,28} and it has been reported that bFGF has been implicated in the neoplastic transformation of glioma cells.⁹ Basic FGF induced morphological changes in C6R-G/H cells from bipolar to more rounded shapes, but the cells did not lose GFP expression (data not shown). It is likely that other factors in contused spinal cords are associated with the rapid transformation of C6R-G/H cells. Recent studies have shown that there are rapid and dramatic changes following spinal cord injury in many other genes including cytokines.⁶ For example, messenger RNAs for interleukins 1 α and 1 β , and for tumor necrosis factor- α are rapidly induced following injury.⁴ These factors, as well as others including the chemokines, prostaglandins, leukotrienes, reactive oxygen species, and lipid oxidation products, are likely to play roles in the injury that follows spinal cord contusion. It will be interesting to determine which factors or combinations thereof are responsible for promoting transformation.

The evolution of glioma formation is believed to require multiple mutations in cells before they become tumorigenic.¹⁶ There is substantial experimental evidence to support this hypothesis and our data are consistent with it as well. The C6R-G/H cells have reduced tumorigenicity and can be considered to be partially transformed. Additional mutagenesis, for example that which results in loss of expression

TABLE 2
Tumor formation of isolated cells treated with spinal cord extract*

| Isolated Cells | GFP | Hygromycin Resistance | Tumor Incidence (%) |
|----------------|-----|-----------------------|---------------------|
| C6R-C1 | - | - | 2 of 3 (66.6) |
| C6R-C2 | - | - | 2 of 3 (66.6) |
| C6R-N | + | + | 0 of 3 (0) |

* Cells isolated from the C6R-G/H cell line following treatment with extracts of contused (C6R-C1 and C6R-C2 cells) and noncontused (C6R-N cells) spinal cord were analyzed for expression of GFP and resistance to hygromycin treatment. Tumor incidence was analyzed in three rats 6 weeks postimplantation for each group of isolated cells.

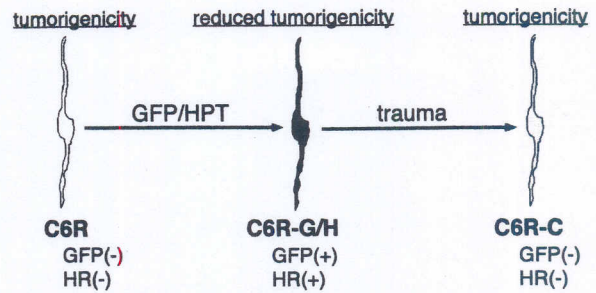


FIG. 7. Model correlating tumor formation with gene expression for the various C6R cell lines. Transfection of C6R cells with a combination of plasmids encoding for GFP and HPT reduced the tumorigenicity of the cells, which then exhibited GFP expression and hygromycin resistance (HR). Trauma induced tumorigenicity in the C6R-C cells, which in turn lost GFP expression and hygromycin resistance. The results indicate that the transfected genes can suppress tumor formation, but the suppression function is lost in response to trauma.

of GFP and HPT, can result in more rapid tumor formation. Interestingly, certain primary rat embryo brain cell lines isolated by immortalizing neural stem cells with a retrovirus encoding for *v-myc* expression²⁶ also showed increased tumor formation following trauma. One of two stem cell lines that we isolated recently (K Hasegawa, H Li, and M Grumet, unpublished observations) showed high rates of tumor formation following injection into contused spinal cords, but not after injection into normal spinal cords. This further suggests that factors released acutely following CNS trauma can be mutagenic for various types of cells and may pose increased risk of tumor formation. This may be an important consideration, given current excitement about the potential uses of stem cells for transplantation to treat various diseases. Although immortalized cells may have low oncogenic potential, their oncogenicity may be increased following trauma and, therefore, trauma represents a potential risk factor.

The C6R-C cell lines isolated from C6R-G/H cells following treatment with contused spinal cord extract (Fig. 7) formed tumors in normal spinal cord with histological characteristics that were similar to the C6R-G/H tumors in contused spinal cord, but their size was smaller. A possible explanation for the reduced size of these tumors is that, in normal spinal cord, transformed cells could not disperse or invade as widely as in contused spinal cord in which the injury causes tissue damage. After contusive injury,¹⁰ tissue damage may facilitate the dispersion of transformed cells to allow formation of large tumors. Trauma, therefore, may induce neoplastic transformation as well as provide transformed cells with better conditions in which to infiltrate.

The relationship between trauma and tumor formation has been suggested by several authors, usually regarding gliomas and meningiomas.^{2,3,22,24} Findings in various clinical case studies have been consistent with the hypothesis that trauma increases the risk of tumor formation, particularly for meningiomas. Meningeal lesions have been associated with implantation of foreign bodies or granulomatous reactions that are likely to facilitate development of meningioma.^{2,3} For gliomas it has been suggested in clinical cases that genetic alterations occur following injury.^{22,24} Although the mechanism of glioma formation may not be the same as

that for meningiomas, several authors have reported a high incidence of malignancy in cases of posttraumatic meningioma.^{1,2} Radiation induces mutagenesis, resulting in meningioma, sarcoma, and glioma formation.^{23,29} We propose that trauma promotes tumorigenesis for gliomas by altering patterns of gene expression.

In most clinical studies, outcomes have been assessed many years after injury, making it difficult to draw direct links between trauma and subsequent tumor formation. In our study in which we used the rat as a model, the development of tumors rapidly followed the traumatic events, making it likely that the trauma induced alterations that promoted tumor formation. Moreover, extracts from contused spinal cords rapidly altered gene expression in C6R-G/H cells and increased their tumorigenicity. In a study of chemically induced mutagenesis,²⁰ tumor formation following a stab wound was accelerated but was not as rapid as we observed with contusive spinal cord injury. In our study we used implantation of genetically modified cells, and the results suggest that the contusive injury provides sufficient factors to induce mutagenesis. Furthermore, formation of larger tumors may be facilitated because the implanted cells can easily infiltrate around the site of the contusion. In view of these observations, it will be important to consider the possibility of tumor formation when cells are implanted into traumatic lesions as a therapeutic intervention.

Acknowledgments

We thank Hock Ng and Eva Kucsma for technical assistance and Drs. David Zagzag, Wise Young, and Hedong Li for advice.

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Manuscript received September 13, 2002.

Accepted in final form January 9, 2003.

This study was supported by National Institutes of Health Grant Nos. NS38112 and NJCSCR02-3020a to Dr. Grumet.

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