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Sensorimotor deficits associated with brain tumor progression and tumor-induced brain plasticity mechanisms

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Abstract

The objective of this study was to investigate functional deficits and reactive peri-tumoral brain plasticity events in glioma-bearing rats. 9L gliosarcoma cells were implanted into the forelimb region of the sensorimotor cortex in Fischer rats. Control animals underwent the same operation without tumor implantation. Sensitive tests for detecting sensorimotor dysfunction, including forelimb-use asymmetry, somatosensory asymmetry, and vibrissae-evoked forelimb placing tests, were conducted. We found that tumor-bearing animals exhibited significant composite behavioral deficits on day 14 post-tumor injection compared to surgical controls. With the assistance of magnetic resonance imaging, we demonstrated a significant correlation between tumor volume and magnitude of somatosensory asymmetry, indicating that the somatosensory asymmetry test can provide an effective and efficient means to measure and predict tumor progression. Histopathological assessments were performed after the rats were sacrificed 14 days following tumor implantation. Immunostaining revealed that densities of microtubule-associated protein 2, glial fibrillary acid protein, von Willebrand factor, and synaptophysin were all significantly upregulated in the peri-tumoral area, compared to the corresponding region in surgical controls, suggesting synaptic plasticity, astrocyte activation and angiogenesis in response to tumor insult. Understanding the behavioral and bystander cellular events associated with tumor progression may lead to improved evaluation and development of new brain tumor treatments that promote, or at least do not interfere with, functional adaptation.

Keywords: Brain tumor; Behavior; Sensorimotor; Neuroplasticity; MRI imaging

Introduction

Patients with brain tumors often develop focal neurological signs and symptoms, such as hemiparesis, dysphasia, visual problems, hearing loss, cognitive deficits, as well as seizures, dependent on tumor location. Other nonspecific symptoms, due to increased intracranial pressure (ICP), include headache, nausea and vomiting. Although the functional response to treatment of brain tumor is important for anticancer treatment development, the highest priority in the neuro-oncology field is on reduction of tumor size. Studies of behavioral changes associated with tumor and its treatment are sparse. However, certain anticancer treatments may impede brain plasticity and functional recovery. Irradiation-induced reduction of neurogenesis exacerbates ischemia-elicited functional deficits (Raber et al., 2004). Inhibition of angiogenesis can attenuate brain repair (Krum and Khaibullina, 2003). Local chemotherapy interferes with functional recovery after brain tumor-like mass compression (Yang et al., 2006c). Long-term systemic administration of an NMDA glutamate antagonist results in a reinstatement of functional deficits after complete recovery from mass compression (Yang et al., 2006a). Therefore, functional assessment of cancer therapy is essential in translational research and the first step is to establish a simple method for measurement of behavioral changes in animal tumor models.

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The present study employed an array of sensorimotor behavioral tests to determine focal functional deficits in rats following implantation of glioma cells into the forelimb region of the sensorimotor cortex (SMC). Due to the variabilities in both tumor growth and behavioral response, a closer analysis using magnetic resonance imaging (MRI) was conducted to examine the correlation between tumor size and functional impairment.

Both clinical observation and experimental research demonstrate the lack of severe functional deficits until the late stage when the tumor grows large, suggesting tolerance to tumor growth (Yang et al., 2007). Cerebral ischemia (Parent et al., 2002; Stroemer et al., 1992, 1993; Zhang et al., 2004a,b; Zhang et al., 2007a,b), mechanical compression (Kundrotiene et al., 2002, 2004; Moreira et al., 2005, 2006, 2007; Yang et al., 2006b), denervation (Gomez-Pinilla et al., 1992; Kadish and Van Groen, 2003) and excitotoxicity (Pollard et al., 1994) all induce brain plasticity that likely fosters functional recovery (Cramer and Chopp, 2000; Keyvani and Schallert, 2002). Brain plasticity may be most effective when the injury is minor and slowly increases over time, as observed in models of progressive neural degeneration (Fleming et al., 2005). Tumors likewise may gradually upregulate compensatory events, leading to their "stealth" nature. Normal brain cells, especially progenitor cells and oligodendrocytes, are more susceptible to chemotoxicity than cancer cells, and systemic administration of chemotherapeutic agents leads to reduced cell division and increased cell death in the adult mouse brain even long after drug exposure (Dietrich et al., 2006). Peri-tumoral plasticity may be vulnerable to tumor resection or anti-mitotic, anti-angiogenic, anti-growth factor and related treatments used to reduce brain tumor expansion. Understanding how peri-tumoral neurons resist dying is important, as protection of the surrounding normal tissue and preservation of function is a major goal and a great challenge in glioma management. To begin to address this issue, markers for neurons, astrocytes, endothelial cells, and synaptogenesis were used to examine reactive peri-tumoral plasticity in the rat glioma implantation model.

Materials and methods

Experiment 1

The goal of Experiment 1 was to examine forelimb sensorimotor function during the growth of tumors placed in the forelimb region of the sensorimotor cortex, and to investigate peri-tumoral plasticity-related events using immunohistochemistry.

Animals and housing

All of the surgical and experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of Henry Ford Hospital. A total of 18 adult male Fischer rats (Charles River Breeding, Wilmington, MA) weighing 180–250 g were used in Experiment 1. All animals were tamed by gentle handling before the experiments.

Experimental model

Animals were randomly assigned to either the tumor-bearing group ($n=9$) or the control group ($n=9$).

9L gliosarcoma cell culture. 9L gliosarcoma cells (ATCC, Manassas, VA, USA) were maintained in monolayer culture (37 °C, 5% CO_2, 95% O_2) in minimum essential media (MEM) with Eagle's salts, supplemented with 10% fetal bovine serum, penicillin and streptomycin (Gibco, Grand Island, NY). Cells were subcultured and used for implantation when they reached an exponential phase of growth. To harvest, cells were incubated with 0.05% trypsin EDTA (0.53 mM, Gibco) for 5 min, and then MEM was added to make a single cell suspension. After the suspension was centrifuged at 1000 rpm (4 °C) for 5 min, the media was removed and the cells were re-suspended in PBS. Cell viability was determined by trypan blue exclusion (non-viable cells stain blue). The number of unstained cells was counted using a hemocytometer under a microscope and then the suspension was diluted with PBS to a final concentration of 10^7 cells/ml (Chopp et al., 1996a,b; Jiang et al., 1997, 1998).

9L cell implantation in Fischer rats. Surgeries were all performed in the right hemisphere. Rats were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (13 mg/kg, i.p.) and then placed in a stereotaxic device. After the scalp was incised and the cranium was exposed, a circular craniotomy, 2 mm in diameter, was made over the parietal cortex, 1.0 mm anterior to the bregma, and 2.5 mm lateral to the midline. Fifty thousand 9L gliosarcoma cells in 5 μl PBS were injected intracerebrally with a 10-μl Hamilton syringe to a depth of 2.5 mm beneath the dura during a 5-min interval (Chopp et al., 1996a,b; Jiang et al., 1997, 1998). The needle was then retracted over a 6-min period. The craniotomy was covered with a piece of polyvinyl chloride film glued to the surrounding intact bone. The incision was closed with 4-0 silk sutures (Ethicon, Somerville, NJ).

Control animals were treated identically to the tumor-implanted rats, having scalp incision, skull removal, needle insertion and withdrawal, but without tumor cell injection.

Behavioral testing

Behavioral tests sensitive to focal insults to the sensorimotor cortex and the underlying striatum were carried out before and after surgical procedures by an investigator who was blinded to the experimental groups.

Forelimb-use asymmetry test. The experimenter placed the rat in a transparent cylinder (30 cm high and 20 cm in diameter) and recorded forelimb use during rearing and vertical weight bearing or weight shifting along the inner wall of the cylinder. Twenty consecutive behaviors were analyzed and the asymmetry score was calculated as the number of ipsilateral (unaffected) limb usages independent of "both" forelimb usages (simultaneous or alternating/stepping of the ipsilateral and contralateral forelimbs) plus 1/2 the number of "both" limb usages divided by the total number of independent ipsilateral, independent contralateral and both-limb-use behaviors. The score will be approximately 50% when there is no deficit. The higher the score, the greater the deficit (Schallert et al., 2000; Schallert and Woodlee, 2005; Yang et al., 2006b,c).

General activity was measured by recording the number of forelimb-use behaviors within the first 2-min observation period