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Hippocampal dysfunctions in tumor-bearing mice

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ABSTRACT

Individuals with cancer are particularly susceptible to depression and cognitive impairment. However, the precise mechanisms underlying cancer-induced hippocampal dysfunction are poorly understood. We investigated the effects of a peripheral tumor on emotional behavior, hippocampus-dependent memory and associated molecular and cellular features using an experimental animal model. Behavioral alterations were examined; stress-related parameters measured; hippocampal neurogenesis evaluated; and the levels of pro-inflammatory cytokines, brain-derived neurotrophic factor (BDNF) and cyclooxygenase-2 (COX-2) assayed, 2 weeks after inoculation of adult BALB/c mice with cells of a colon carcinoma cell line (CT26). As the tumors developed, CT26-inoculated mice showed significant increases in the depression-like behavior (measured using the tail suspension test) and memory impairment (in terms of object recognition) compared with vehicle-inoculated controls. The presence of a peripheral tumor significantly elevated the hippocampal levels of mRNAs encoding interleukin-6 (IL-6) and tumor necrosis factor-α, as well as plasma IL-6 and corticosterone levels. Additionally, the adrenal glands became enlarged, and the numbers of Ki-67-positive proliferating hippocampal cells and doublecortin-positive immature progenitor neurons, as well as the constitutive levels of mRNAs encoding BDNF and COX-2 were significantly reduced. Therefore, a peripheral tumor alone may be sufficient to induce hippocampal dysfunction, possibly by reducing the rate of neurogenesis and the levels of BDNF and COX-2 in that tissue and also by increasing stress-related parameters and the circulating levels of pro-inflammatory cytokines.

1. Introduction

Mounting evidence suggests that individuals suffering from cancer have a higher prevalence of mood symptoms and cognitive dysfunction than the healthy population (Bower, 2008; Braun et al., 2012). Furthermore, cancer-related memory deficits have been reported in several patient populations prior to the administration of antineoplastic agents (Wefel et al., 2004a, 2008). Cognitive and emotional states may influence quality of life and long-term survival (Brown et al., 2003). Several studies have explored the effects of chemotherapy and other forms of cancer treatment on memory and emotional wellbeing (Kim et al., 2008; Yang et al., 2010, 2011). However, little is known about the effects of the peripheral tumor per se on brain functions including mood and cognition.

Although inflammation is primarily a protective response intended to eliminate an injury-inducing agents (Das, 2006), it is also involved in the etiology and pathophysiology of several brain disorders (e.g., Alzheimer's disease, post-stroke depression, cognitive aging) (Baune et al., 2012; McAfoose and Baune, 2009; Morales et al., 2010). The immune system becomes hyper-activated in some cohorts of depressed patients who suffer from certain conditions including cancer, hepatitis, or multiple sclerosis (Maes, 1995), leading to development of cognitive impairment and neurobehavioral sequelae (Kohman and Rhodes, 2012; Wefel et al., 2008). As inflammatory stress is the primary activator of the hypothalamus-pituitary-adrenal (HPA) axis, it has been suggested that the development of brain dysfunction is closely linked to glucocorticoid up-regulation in response to stress, triggered, in turn, by the

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activation of the HPA axis (Baune et al., 2012; McAfoose and Baune, 2009; Morales et al., 2010). Recent studies have shown that peripheral tumors caused by carcinogens affect systemic and brain cytokine levels and neurobehavioral sequelae (Pyter et al., 2009, 2010). However, it is unclear whether the observed alterations in cytokine levels and the neurobehavioral changes (e.g., mood changes and development of cognitive disorders) are attributable to carcinogens or to the tumor per se. It is thus important to explore whether a peripheral tumor can elevate stress response levels and alter cytokine concentrations both in peripheral locations and in the central nervous system (CNS).

The hippocampus, a major limbic system structure, has been extensively studied in individuals with learning and memory deficits, and its role in the regulation of emotion, such as depression, has been investigated (Kempermann et al., 1997). Hippocampal neurogenesis is central to hippocampal integrity and plasticity (Kempermann et al., 2002), and alterations in neurogenesis affect an individual's ability to perform in hippocampus-dependent tasks. Reduced hippocampal neurogenesis related to aging, oxidative stress and neuroinflammation has been shown to cause cognitive impairment and/or depression in experimental models (Goshen and Yirmiya, 2009; Kim et al., 2008; Seo et al., 2010; Yang et al., 2010). Brain-derived neurotrophic factor (BDNF) is involved in hippocampal neurogenesis and plasticity and plays a role in mood regulation and memory processes (Dinel et al., 2011; Li et al., 2008). Alterations in BDNF levels affect the proliferation of hippocampal neural precursor cells, synaptic plasticity, and spatial memory (Korte et al., 1995; Linnarsson et al., 2006; Scharfman et al., 2005). Hippocampal BDNF expression is modulated by various promoters (e.g., calcium responsive elements), pro-inflammatory cytokines and stress-related hormones (Barrientos et al., 2004; Marmigère et al., 2003; Tao et al., 1998, 2008). Moreover, cyclooxygenase-2 (COX-2) is closely related to synaptic plasticity and cognitive outcomes under normal condition (Minghetti, 2004; Rall et al., 2003; Teather et al., 2002). Recent studies have shown that down-regulation of COX-2 activity triggered development of mood disorders and memory deficits via a mechanism involving BDNF (Shaw et al., 2003).

Although several factors involved in hippocampal function have been identified, it is not known whether a peripheral tumor itself has an impact on hippocampus-dependent behaviors and the mechanisms that would mediate such an effect. In the present study, we sought to develop an animal model that could be used to determine the effects of peripheral tumors on brain function. Specifically, peripheral tumors were induced by inoculation of cells of a colon carcinoma cell line (CT26) into female BALB/c mice. We sought to explore how fully developed tumors affected depression-like behavior and hippocampus-dependent learning and memory. We sought to identify possible mechanisms underlying tumor-induced brain dysfunction by measuring the peripheral levels of stress hormones and cytokines and by evaluating changes in neurogenesis and the expression of plasticity-related genes (such as BDNF and COX-2) in the hippocampus.

2. Materials and methods

2.1. Cell culture

CT26 murine colon carcinoma cells syngenic to BALB/c mice, were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) with 10% heat-inactivated fetal bovine serum. The medium was supplemented with 100 units/mL penicillin, 0.1 mg/mL streptomycin and 0.5 mM glutamine (Invitrogen, Grand Island, NY, USA) and incubated at 37 °C in a humidified atmosphere of 5% CO_2. The cells were maintained in an exponential growth phase for periods of 3 days. To develop homografts, CT26 cells cultured *in vitro* were harvested by exposure to trypsin–EDTA, washed three times with phosphate-buffered saline (PBS, pH 7.4), and resuspended in serum-free RPMI. Trypan blue exclusion was performed to insure cell viability.

2.2. Animal experiments

Female BALB/c mice aged 5 weeks were purchased from Central Laboratory Animals, Inc. (Seoul, Korea) and used after 1 week of quarantine and acclimatization. We chose female mice because male mice often bit tumor sites. The animals were housed in a room maintained at 23 ± 2 °C, with a relative humidity of 50 ± 5%, artificial lighting from 08:00 to 20:00 h, and 13–18 air changes per hour. All mice received tap water and commercial rodent chow (Samyang Feed, Seoul, Korea) *ad libitum*. After acclimatization, mice were randomly divided into two groups (n = 24 mice/group), which were inoculated either with PBS or CT26 cells. Cells were subcutaneously injected into the right flanks of 6-week-old female mice at a dose of 1×10^7 CT26 cells dissolved in 1 mL of PBS per animal, to initiate tumor growth. Tumor-free mice were injected with PBS using the same procedure.

Fourteen days after inoculation, the mouse groups were subdivided (1–3 experiments, Fig. 1A): (1) Six mice per group were subjected to open-field test, followed by hippocampal collection for mRNA extraction 4 h later. (2) Eight mice from each group were sacrificed, and tissues were collected. Serum was stored prior to measurement of corticosterone and cytokine levels. The adrenal glands were collected for evaluation of stress-related parameters, and hippocampi were collected to explore neurogenesis. (3) Ten mice from each group were subjected to the tail suspension test (TST), followed 4 h later by the object recognition memory test (ORM). The Institutional Animal Care and Use Committee at Chonnam National University approved the protocols used in the present study (CNU IACUC-YB-R-2009-14), and the animals were cared for in accordance with the internationally accepted principles for laboratory animal use and care as found in the National Institutes of Health Guidelines (USA).

2.3. Behavioral tests

Behavioral dysfunction was measured using the open-field test (n = 6 mice/group), TST (n = 10 mice/group) and ORM (n = 10 mice/group) 2 weeks after CT26 or PBS inoculation (Fig. 1A). All behavioral testing was conducted between 10 a.m. and 4 p.m.

2.3.1. Open-field test

The open-field test was used to measure the activity of vehicle (PBS)- and CT26-inoculated mice in a novel environment. Ambulatory count, total moving distance, ambulatory movement time and resting time were determined over a 5-min period using the TruScan Photo Beam Activity System (Coulbourn Instruments, Whitehall, PA, USA). Furthermore, some parameters in the center arena (e.g., center time, center distance and center entries) were evaluated to assess anxiety-related phenotypes.

2.3.2. TST

The TST performed here was similar to that described previously (Seo et al., 2010; Steru et al., 1985). Briefly, mice were suspended from a plastic rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape. Time spent immobile during a 6-min period was measured using the SMART video-tracking system (Panlab, Barcelona, Spain).