Editorial

# Focusing in on radiation induced brain injury

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**Abstract:** Cognitive dysfunction following cranial radiotherapy remains a significant clinical problem, particularly in patients receiving whole brain radiation and in children. This has prompted development of therapeutic strategies that spare regions involved in learning and memory, particularly the hippocampus, which contains a radiation-sensitive population of neural precursor cells that actively divide in adulthood and contribute to hippocampal circuitry. Approaches that model localized brain irradiation in rodents can provide important insights into in-field and out-of-field changes that might contribute to cognitive dysfunction. Parihar and colleagues have used such an approach to ascertain cognitive and tissue changes in rats subjected to unilateral or bilateral hippocampal irradiation. Their findings have implications for the application of localized radiotherapy and provide insights for understanding brain radiation injury.

**Keywords:** Cognitive dysfunction; hippocampus; microglia; neurogenesis; stereotaxic radiosurgery

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In this issue of *Translational Cancer Research*, Parihar and colleagues describe a method for stereotaxic radiation exposure in rat brain that provides precise localization to specific brain regions (1). In this case, they focused their attention on radiation effects in the hippocampus, a structure that contains a population of radiosensitive adult neural precursor cells and is implicated in learning and memory deficits following clinical radiotherapy. In particular, Parihar *et al*. compared findings six weeks post exposure between rats that had received 10 Gy of whole brain irradiation and those receiving 10 Gy of localized bilateral or unilateral hippocampal irradiation. Specific endpoints examined included two measures of hippocampal-associated learning, neurogenesis, and expression of CD68, a marker of microglial activation.

Cranial radiotherapy is a key approach for the treatment of primary central nervous system (CNS) malignancies and brain metastases, as well as prophylaxis of metastatic and occult disease. Although there have been significant advances in limiting overt neurotoxicity, cognitive dysfunction is still a major concern, particularly in children receiving cranial radiotherapy and in adults subjected to whole brain radiation treatment for control of metastatic disease (2,3). Specific approaches have been developed that reduce radiation doses to normal brain tissue, including intensity modulated radiation therapy and stereotaxic radiosurgery, and these methods may reduce the incidence of cognitive dysfunction (4). Indications that adult neural stem cells in the subventricular zone and hippocampus play important roles in learning and memory as well as tissue recovery have led to the idea that cognitive dysfunction might be diminished or avoided if radiation doses to these areas are selectively reduced (5). Despite the demonstrated feasibility of developing approaches that spare the hippocampus and other critical brain regions, there are to date no reported results from randomized clinical trials testing this idea, though a phase II trial appears to be underway (5). The development of approaches in rodents that target radiation to specific brain regions is an important undertaking that provides opportunities for probing specific mechanisms underpinning radiation induced cognitive dysfunction and other CNS radiation effects.

Methodologically, the approach described by Parihar *et al*. will be of use to other investigators interested in irradiating select brain regions in rodent models. Such studies are of obvious importance in modeling focal human

radiotherapy procedures and in testing hypotheses about targeted and non-targeted effects in the CNS. Others have used focused irradiation protocols to expose limited areas of rodent brain, with the gamma knife most frequently cited. As pointed out by Parihar *et al*., a number of earlier studies utilized relatively high doses of radiation (e.g., 100 Gy) and described histological changes consistent with late radiation necrosis [e.g., (6)]. More recently, the gamma knife was used to unilaterally deliver a 10 Gy maximum dose to one hippocampus in Brown Norway rats. Similar to results presented in the current work, targeted irradiation of the hippocampus led to microglial activation and reduced neurogenesis measured 70 days post-irradiation (7). However, this earlier study did not include bilateral or total brain irradiation and no measures of cognitive function were performed. Parihar *et al*. found reduced contextual fear conditioned freezing behavior in rats receiving bilateral hippocampal irradiation or whole brain irradiation, but not in those receiving unilateral hippocampal irradiation. This is not surprising given the known dependence of this task on hippocampal function and the requirement that both hippocampi need to be inactivated to cause a deficit (8). In contrast, rats receiving unilateral or bilateral hippocampal irradiation showed essentially normal preference for exploring an object moved to a new location, whereas whole brain irradiated rats did not show a preference. Interestingly, the whole brain irradiated rats actually appeared to prefer spending time with the object that was not moved to a new location, rather than the anticipated "impaired" result of spending essentially equal time with both objects. One of the challenges in interpreting such results is the relatively small number of rats used in the hippocampal irradiated cohorts and the apparent testing of these animals in both tasks as opposed to independent cohorts of unirradiated and whole brain irradiated rats which were tested in each task. Nevertheless, the results are novel and worthy of follow up with larger numbers of experimental animals.

As expected, focal irradiation of the hippocampus with 10 Gy led to a decline in neurogenesis, revealed by substantial reduction in doublecortin (DCX) labeled cells in the dentate gyrus and more modest decreases in the percentage of BrdU positive mature dentate gyrus granule cells marked by NeuN immunostaining. Interestingly, in the contralateral hippocampus of rats receiving unilateral hippocampal irradiation, the percentage of BrdU positive mature neurons was increased and there was a trend for greater numbers of DCX positive cells. The authors suggest that these findings might represent a compensatory increase of neural stem cell proliferation in response to injury of the targeted hippocampus. Compensatory increases in adult neurogenesis have been described in several injury models, most notably proliferation and cell migration from the subventricular zone following ischemic injury, a response that depends on production of growth factors such as CNTF in response to injury (9). Greater demand on the intact hippocampus could also lead to activity-dependent changes in synaptic plasticity and neurogenesis (10). Another possibility alluded to by the authors is that the low dose received by the non-targeted hippocampus, calculated to be 1.5 Gy mean dose, stimulated proliferation. Formally, the observed changes could be due to radiation effects on cell survival rather than increased proliferation since BrdU labeling was carried out four weeks before tissue collection. Information about the total numbers of BrdU labeled cells and measures of proliferation at the time of sacrifice obtained using Ki67 labeling (11) would help to address this later issue.

In the work presented by Parihar *et al*. neuroinflammation was assessed by quantifying the number of ED-1 positive microglia in hippocampal subfields. They found clearly increased numbers of these activated microglial cells in hippocampi receiving 10 Gy and a modest increase over basal levels in the combined CA3/CA1 subfields of the contralateral, non-targeted hippocampus. These findings are consistent with the work of many others and demonstrate evidence of a sustained neuroinflammatory reaction following brain radiation injury that is localized within the field of radiation (12). The ED-1 antibody labels CD68, a component of inflammatory lysosomes that does not distinguish between resident microglia and infiltrating macrophages. Other investigators have found evidence of late cell infiltration in models of brain radiation injury. For example, Moravan *et al*. described increased numbers of MHC-II and CD11c positive cells as well at CD3 positive T cells in mice 30 days and later after whole brain radiation exposure, albeit at a slightly higher dose (15 Gy) (12). One caveat of the model used by Parihar *et al*. is that they carried out their experiments using athymic nude rats. Although these rats are clearly useful for transplantation studies (13), the neuroinflammatory response to radiation damage may be modified by the lack of T lymphocytes.

The connection between inflammation, neurogenesis and behavior has recently been reviewed (14) and is quite complex. There is ample evidence that neuroinflammation can reduce hippocampal neurogenesis and impact hippocampal cognitive function. For example, in our own

 

work, sustained hippocampal overexpression of interleukin-1 resulting in dramatic glial activation and expression of multiple inflammatory mediators was associated with deficits in hippocampal-dependent contextual fear conditioning (15) and greatly reduced neurogenesis (16). Although the degree of neuroinflammation following radiation exposure, particularly with doses of 10 Gy, is much lower than seen with cytokine overexpression, there have been multiple studies demonstrating an inverse relationship between levels of microglial activation and neurogenesis in the context of radiation exposure [e.g., (11,17)]. Importantly, some experiments using anti-inflammatory drugs suggest that suppressing neuroinflammation can partially restore radiation-induced deficiencies in neurogenesis (18) and cognitive performance (19). However, there are other studies demonstrating amelioration of radiation-induced cognitive dysfunction without effects on microglial activation (20,21) as well as instances where drugs that inhibit radiation-induced microglial activation don't restore neurogenesis or cognitive deficits (22). Such studies showing a lack of correlation between neuroinflammation, neurogenesis and cognitive capacity raise questions about their connection in radiation injury and suggest that other CNS radiation-related changes may contribute to cognitive deficits. Indeed, recent experiments from Dr. Limoli's group revealed dramatic alteration of neuronal hippocampal dendritic architecture following 1 and 10 Gy irradiation (23), an effect seen by others (24) that very likely contributes to radiation-associated changes in cognitive function. Although neuroinflammation might contribute to these changes, their appearance following 1 Gy of radiation suggests that other processes might be involved.

So how do the findings of Parihar *et al*. contribute to the use of stereotaxic radiotherapy in people? First, they demonstrate that direct hippocampal irradiation elicits deficits in a hippocampal-dependent learning task, contextual fear conditioning. This finding supports the idea that sparing the hippocampus could be beneficial, but does not exclude the possibility that radiation effects in other brain regions might also impact cognitive function. Future application of this stereotaxic method might include protocols that spare the hippocampus to test the contribution of other brain regions to cognitive function following radiation injury. The finding that deficits in behavioral task performance after radiation were correlated with increased microglial activation and decreased neurogenesis is similar to what has been demonstrated in whole brain irradiation paradigms. In this case, these changes were limited to the irradiated hippocampus, which confirmed that the targeting worked, and supports the utility of this approach as a way to model regional brain radiotherapy. Most interestingly, the study by Parihar *et al*. provides evidence of a compensatory response when one brain area is irradiated. These findings need to be reproduced and extended to include other measures of compensation such as dendritic spine density. This evidence of brain plasticity in non-targeted areas provides additional impetus for developing treatment strategies in people that spare normal tissue.

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