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 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

Submitted Aug 01, 2013. Accepted for publication Aug 20, 2013.
doi: 10.3978/j.issn.2218-676X.2013.08.02
View this article at: http://www.thetcr.org/article/view/1497/2954
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 as 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.

**Acknowledgements**

Dr. O’Banion receives research grant support from the National Institutes of Health (NIH) and the National Aeronautics and Space Administration (NASA).

**Disclosure:** Dr. O’Banion is a member of the Medingen Group, LLC Scientific and Medical Advisory Board and reports no other biomedical financial interests or potential conflicts of interest.

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Cite this article as: O’Banion MK. Focusing in on radiation induced brain injury. Transl Cancer Res 2014;3(2):112-115. doi: 10.3978/j.issn.2218-676X.2013.08.02