ELSEVIER
SAUNDERS

Neurosurg Clin N Am 18 (2007) 115–127

NEUROSURGERY
CLINICS
OF NORTH AMERICA

Radiation Response of Neural Precursor Cells

John R. Fike, PhD[a,*], Radoslaw Rola, MD, PhD[b], Charles L. Limoli, PhD[c]

[a]*Department of Neurological Surgery and Radiation Oncology, University of California, San Francisco, San Francisco General Hospital Building 1, Room 101, 1001 Potrero Avenue, San Francisco, CA 94110–0899, USA*
[b]*Departments of Neurology and Neurological Sciences, F. Skubiszewski Medical University, 8 Jaczewskiego Street, Lublin 20950, Poland*
[c]*Department of Radiation Oncology, University of California, Irvine, Medical Sciences I, B-149 Irvine, CA 92697–2695, USA*

The brain is exposed to ionizing irradiation during the management of specific disease states, particularly cancer, and the amount or dose of irradiation that can be administered is largely dictated by the tolerance of normal tissues surrounding the tumor [1]. Radiation injury has a variable character involving multiple regions and cell/tissue types, and a large number of physical and biologic factors influence the expression and extent of radiation injury [2,3]. In patients, overt tissue injury generally occurs after relatively high doses (>60 Gy, fractionated), and the morphologic consequences of such exposures are well documented in human beings and experimental systems [3–8]. Less severe morphologic changes can occur after relatively lower doses, resulting in variable degrees of cognitive impairment, particularly in children [9–12]. Although considerable descriptive information is available regarding radiation injury after high and low radiation doses, the pathogenesis of these changes remains uncertain. It has been suggested that the cellular events leading to radiation injury can be explained in terms of a loss in the reproductive capacity of cells regarded as targets within the central nervous system (CNS) [7]. In the past, those targets were primarily restricted to oligodendrocytes and vascular endothelial cells, two cell types in the mature brain capable of division [7,8,13]. Based on clinical and laboratory data, there is considerable support for both of these targets as critical players in the development of radiation injury; however, it seems unlikely that radiation injury can be attributed to only a single cell target [3]. In fact, current studies of radiation effects in the brain involve the assessment of factors not specifically associated with assays of clonogenic cell survival [3,14]. Regardless, an understanding of the response of potential target cell populations within the CNS still forms an important strategy for studying the development and possible treatment of radiation injury.

In the mammalian forebrain, two sites of active neurogenesis have been identified: the subventricular zone (SVZ) adjacent to the lateral ventricles and the dentate subgranular zone (SGZ) in the hippocampus [15–17]. Multipotent stem/precursor cells exist in these regions and produce cells that migrate away and differentiate into neurons and glia [15,18–20]. It has been suggested that cells of the SVZ may act as a reserve population of undifferentiated cells that can be recruited after tissue injury [21–23]. In the dentate gyrus, newly born cells become functionally integrated into the local circuitry [24,25] and have passive membrane properties, action potentials, and functional synaptic inputs similar to those found in mature granule cell neurons [26]. A positive correlation has been established between dentate neurogenesis

This work was supported in part by National Institutes of Health grant R01 NS46051 and National Aeronautics and Space Administration grants NNJ05HE33G, NNA04CK56G, and NNJ04HC90G.

* Corresponding author.
E-mail address: john.fike@ucsf.edu (J.R. Fike).

doi:10.1016/j.nec.2006.10.010

and behavioral performance in animals [26,27], and it has been hypothesized that depletion of neurogenic cells in the SGZ by ionizing irradiation may contribute to the cognitive impairments seen after such treatment [28–33]. Understanding how neural precursor cells respond to irradiation and determining if and how that response can be changed may provide critical information regarding strategies and approaches to ameliorate or treat radiation-induced cognitive injury.

Radiation response of neural precursor cells: subventricular zone

It has been proven that populations of multipotent cells exist in the SVZ and SGZ, although whether they should be referred to as stem, precursor, or progenitor cells is still being debated [34]. Regardless of the particular nomenclature used, in both neurogenic regions, new cells are born, migrate away, and have the potential to differentiate into mature neural phenotypes. The production of new cells takes place in specific regions or niches [35–38], and it has been speculated that microenvironmental cues ultimately control the tissue-specific fate of the newly born cells [35]. Accordingly, these microenvironments should also have an impact on precursor cell fate and function when those cells are subjected to irradiation or other adverse stimuli.

The first studies of the radiation response of neural precursor cells were performed more than 30 years ago, when it was hypothesized that cell depletion within the mitotically active SVZ (referred to then as the subependymal plate) could play a role in radiation-induced white matter injury [39,40]. Without the methods and approaches currently available, those investigators concluded that white matter necrosis might be attributable to a suppression of cell proliferation in the SVZ, along with a gradual neuroglial cell loss. Subsequently, those investigators specified three morphologically distinct cells types within the SVZ: (1) cells with small dark-staining (SD) nuclei that were mitotically active and that the authors thought represented "stem cells," (2) cells with small light-staining (SL) nuclei that were considered to be neuroglial precursors, and (3) cells with large light-staining (LL) nuclei that were neuroglial precursors or astrocytes [41]. All three cell types decreased with animal age as well as with radiation dose, with the SD cells being the most sensitive. The authors concluded that their study provided evidence of cell transformation and cell migration, suggesting that SD nuclei transformed into SL nuclei and that losses of SL nuclei were accompanied by a corresponding rise in LL nuclei. When these data are considered in the context of the elegant morphologic study of the SVZ reported by Doetsch and colleagues [21] 25 years later, the SD cells seen by Hubbard and Hopewell [41] likely represent neuroblasts or type A cells; the LL cells correspond to the type B cells, which have astrocytic properties; and the SL cells are the type C or putative precursor cells.

The early radiation studies of Hopewell and his colleagues [40,41] were not followed up for more than two decades. Then, Bellinzona and coworkers [42] demonstrated that in rats, the early cell loss observed after irradiation was attributable to programmed cell death, or apoptosis, and that the process peaked 6 hours after exposure and seemed to be dose dependent. Although that study was the first to show that radiation-induced acute cell loss in the SVZ could be attributed to a specific type of cell death, no information was provided regarding which cell type(s) in the SVZ were affected. Shinohara and colleagues [43] later presented a more complete dose response for apoptosis (Fig. 1) and showed that most of the cells undergoing apoptosis were undifferentiated and that many of them were in the constitutively proliferating cell population that made up approximately 43% of the cells of the SVZ. This suggested that the radiosensitive population in the SVZ was largely made up of putative neural precursor cells.

Shinohara and colleagues [43] also showed that 24 to 72 hours after the x-ray–induced apoptosis, there was an increase in 5-bromo-2′deoxyuridine (BrdU) labeling in the rat SVZ. This was similar to results seen in mouse brain irradiated using tritiated thymidine (3H-dThd), an isotopic source that produces low-energy β particles of short range [23]. In the latter study, it was shown that that there was a relatively quiescent stem cell population residing in the SVZ that was capable of regenerating the constitutively proliferating population. Based on the study by Morshead and coworkers [23] and on their own data, Shinohara and colleagues [43] concluded that the radiation response of SVZ stem cells, rather than the apoptotic-sensitive population per se, might be the most critical elements in the response of the brain after radiation exposure.

Tada and coworkers [44] hypothesized that x-rays would influence the SVZ stem/precursor

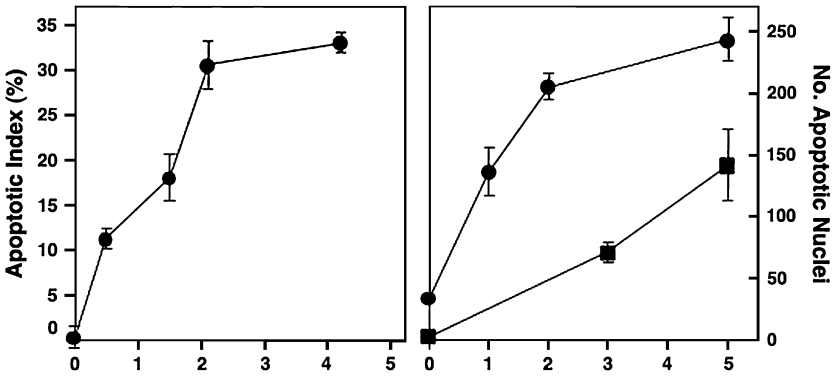

Fig. 1. Low doses of x-rays induce apoptosis in the neurogenic zones of the rodent forebrain. Apoptosis was detected in the rat SVZ (*left panel*) and in the rat and mouse dentate SGZ (*right panel*) using terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) and morphologic criteria [43]. Given differences in the morphology of the two neurogenic regions, different counting procedures were used. In the SVZ, apoptotic cells were expressed as an apoptotic index or percentage of total SVZ cells that had characteristics of apoptosis [43]. In the SGZ of mice (*solid circles*) and rats (*solid squares*), total numbers of apoptotic nuclei for a specified counting region were assessed [33]. Regardless of the area or species, these data show that cells in neurogenic zones are extremely sensitive to clinically relevant doses of x-rays. Each datum point represents at least four animals; error bars are standard errors of the mean (SEMs). (*Data from* Refs. [29,33,43].)

cells in a dose-dependent fashion and that repopulation would occur as a function of time after irradiation. This study differed from that of Morshead and coworkers [23] in several important ways, not the least of which was the use of external beam x-irradiation. In contrast to 3H-dThd, external beam x-rays not only affected proliferating cells but any noncycling precursor cells. Because of the stochastic nature of x-irradiation, the probability of inducing damage in any given cell increases with dose. Therefore, assuming that precursor cells were responsible for maintaining the normal cellularity or mitotic character of the SVZ [21,23,45,46], it was possible to estimate indirectly the extent of stem cell death by quantifying the cellular composition of the SVZ as a function of radiation dose and time after irradiation. Using this approach, Tada and coworkers [44] showed the following:

1. Twenty-four hours after 2 to 15 Gy, there were significant reductions in total cell number as well as numbers of proliferating cells and immature neurons in the SVZ.
2. Nestin and vimentin immunoreactivity was decreased, supporting the contention that most of the cells lost early after irradiation were undifferentiated.
3. Cell proliferation increased between 1 and 7 days after irradiation, and there was an apparent delay in the onset of proliferation with increasing radiation dose.
4. At 7 days, there was a clear radiation dose response in terms of reduced numbers of proliferating cells and immature neurons. This last result was consistent with the idea that more stem cells were killed as the radiation dose was increased.

To address the longer term ability of surviving stem cells to repopulate the SVZ after irradiation, Tada and coworkers [44] quantified cellular changes up to 6 months after single doses of 2 to 15 Gy. After peaking at 7 or 14 days after irradiation, numbers of proliferating cells then decreased to levels that were maintained for up to 180 days, and the ultimate extent of cell loss was dose dependent. Those data also showed that there was no appreciable repopulation of the SVZ for at least 6 months after irradiation with higher doses (>2 Gy) of irradiation (Fig. 2).

Transient changes in cell number after irradiation, such as those reported by Tada and coworkers [44], are also seen in other renewing tissues, such as the gut [47], and may reflect a variety of factors, including abortive cell divisions, changes in proliferation rate, or a change from asymmetric to symmetric division within the stem cell population [48]. Although Tada and coworkers [44] did not specifically address the mode of cell division after irradiation, the chronic

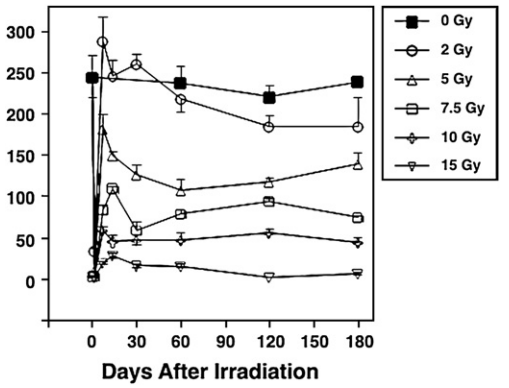

Fig. 2. Numbers of proliferating cells in rat SVZ stay relatively constant after x-irradiation. Proliferating cells were detected using an antibody against the cyclin-dependent kinase $p34^{cdc2}$. Twenty-four hours after irradiation, there were significant reductions in the number of proliferating cells attributable to cell loss from apoptosis. The magnitude of subsequent changes was radiation dose dependent, and there did not seem to be any apparent recovery in proliferation up to 180 days after exposure. Each datum point represents a mean of four rats; error bars represent standard errors of the mean (SEMs). (*From* Tada E, Yang C, Gobbel GT, et al. Long-term impairment of subependymal repopulation following damage by ionizing irradiation. Exp Neurol 1999;160:72; with permission.)

depletion of cells from the SVZ, accompanied by decreased numbers of proliferating cells and immature neurons, suggested that sufficient numbers of symmetric divisions did not occur to repopulate the SVZ. This conclusion was in agreement with those of others [23,46], who concluded that SVZ stem cells self-renewed in an asymmetric mode even during repopulation after irradiation. Therefore, these data suggested that under conditions in which stem cells were damaged or lost, there was no apparent compensatory shift to symmetric division and that the dose-related loss of SVZ stem cells seemed to be permanent [44]. Given that SVZ stem cells apparently have the ability to undergo symmetric divisions when exposed to the right conditions in vitro [49,50] or in vivo [51], the data from Tada and coworkers [44] and others [46] suggested that the surviving stem cells may not have received the "proper" signal(s) to initiate a change in mode of division. Alternatively, inhibitory factors might have existed in vivo to limit symmetric self-renewal [46], or perhaps stem cell recovery partially depends on surrounding supportive elements (eg, glia). Data are now becoming available showing that a variety of factors and conditions stimulate cell proliferation, migration, and differentiation of cells in the normal SVZ [51–55]. How or if these or other factors may be involved in the radiation response of the SVZ is not yet clear. Recent data regarding hippocampal neural precursor survival and differentiation after irradiation [29,32,56,57] show that local microenvironmental factors, such as vascular integrity, oxidative stress, or inflammation, may also play inhibitory roles in postirradiation precursor cell function and recovery, however.

Radiation response of neural precursor cells: dentate subgranular zone

More radiation studies have focused on the cells of the dentate SGZ than on those of the SVZ, probably because of the greater potential significance of SGZ neurogenesis. Although neural precursor cells in the SGZ exist in a different morphologic or physiologic niche than cells in the SVZ, it has been clearly shown that they also undergo rapid cell death after exposure to ionizing irradiation [29,33,58–62]. Regardless of species (mice or rats) and independent of dose and manner of exposure (brain only or whole body), cells of the dentate SGZ are extremely sensitive to irradiation, undergoing apoptosis after doses ranging from 0.4 to 18 Gy (see Fig. 1). The process is rapid, usually peaking within 12 hours of exposure, and at least in one study that used whole-body irradiation, was independent of p53 status [61]. A recent study of whole-brain irradiation also associated radiation-induced apoptosis in the hippocampal formation with the activation of the c-JUN NH2-terminal kinase (JNK) pathway [62]. The sensitive cell populations in the SGZ include proliferating cells [29,33,59–61] as well as cells showing characteristics of immature neurons [29,58,59] or oligodendrocytes (Fig. 3) [60]. Most of these studies dealt with the early time course of cell loss after a single dose of irradiation, and they did not address the dose response characteristics of the sensitive cell population. Two studies do exist, one in rats [33] and one in mice [29], that show the dose response for apoptosis as well as for specific cell loss at times when apoptosis is complete, however (Fig. 4). Based on these dose-response studies, it is fair to say that in the SGZ, neural precursor cells (proliferating cells) and their progeny (immature neurons) are extremely sensitive to irradiation, undergoing apoptosis after clinically

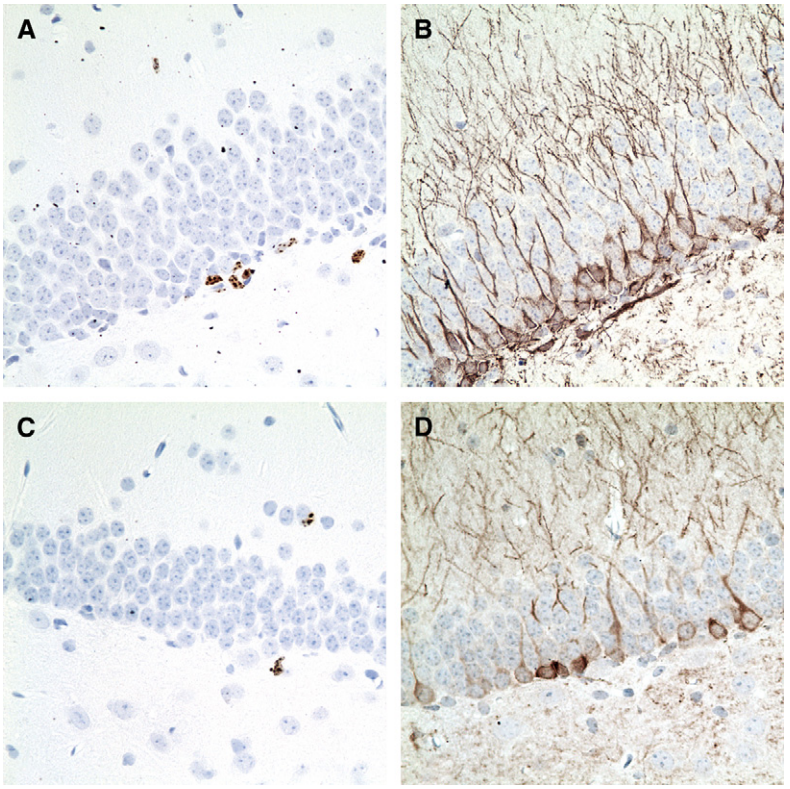

Fig. 3. Cellular changes in the dentate SGZ after exposure to x-irradiation include losses of proliferating cells (*A, C*) and their progeny as well as immature neurons (*B, D*). Proliferating cells (*brown nuclei in A and C*) were detected using an antibody against Ki-67 and were restricted to a narrow area in the granule cell neurons and the hilus. Immature neurons (*brown cells with or without processes in B and D*) were detected using an antibody against doublecortin and were located in the SGZ and in the lower third of the granule cell layer. All micrographs are at original magnification ×400. (*From* Rola R, Raber J, Rizk A, et al. Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. Exp Neurol 2004;188:320; with permission.)

relevant doses. Furthermore, the overall acute sensitivity of SGZ precursor cells is similar to what is seen in the SVZ, suggesting that at least in terms of acute radiation sensitivity, there is a common response among neural precursor cells in the rodent forebrain.

Although these acute studies as well as some longer term assessments of indicators of neurogenesis [63–65] provide useful insight into the inherent radiation sensitivity of neural precursor cells in the SGZ, most of them did not address whether surviving precursor cells were able to proliferate or if or how irradiation affected the fate of the progeny of the proliferating cells. It is possible to determine long-term survival and fate of newly born cells by giving multiple injections of BrdU, waiting a few weeks after the last injection before perfusing animals, and then using cell-specific antibodies to determine what fraction of BrdU-positive cells expresses markers of mature cell phenotypes. Recent investigations in rats [28,56], mice [29,32], and gerbils [66] have used this approach to show that overall BrdU labeling after irradiation was reduced in irradiated animals and that the fraction of newly born cells differentiating in to neurons was significantly affected. This effect on neurons was dose dependent (Fig. 5) [29] and persistent [32] and did not seem to be the same for glia [29,32,56]. Whether the latter observation represented a relative resistance of glial progenitor cells, an aberrant regulation of differentiation, or alterations in the microenvironment that could adversely affect fate decisions was not known [29]. Whatever the mechanisms(s) responsible, the available data suggest that the production of new neurons is more sensitive to low-moderate

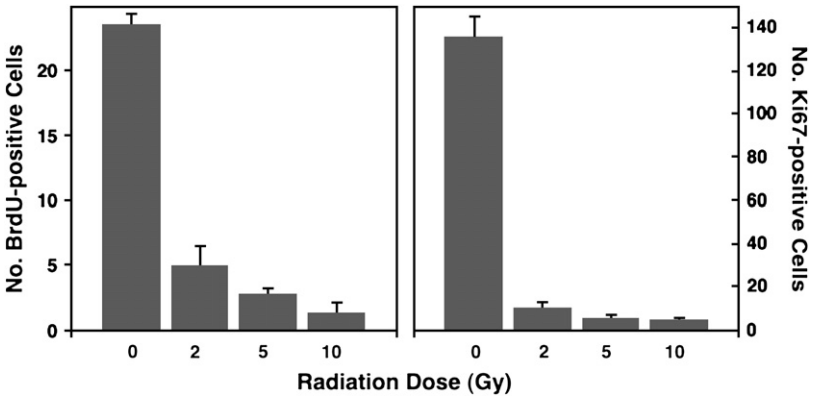

Fig. 4. Proliferating cells in the rat (*left*) and mouse (*right*) dentate SGZ decrease shortly after x-irradiation. In these studies, cell proliferation was detected after apoptosis was complete (24–48 hours after irradiation), using a pulse label of BrdU or an antibody against Ki-67, a nuclear antigen expressed during all stages of the cell cycle, except G_o [83,85]. Numbers of proliferating cells were detected in a standardized counting region and show clear dose responses. Each bar represents a mean of four animals; error bars are standard errors of the mean (SEMs). (*Data from* Mizumatsu S, Monje ML, Morhardt DR, et al. Extreme sensitivity of adult neurogenesis to low doses of x-irradiation. Can Res 2003;63:4023; with permission; and Tada E, Parent JM, Lowenstein DH, et al. X-irradiation causes a prolonged reduction in cell proliferation in the dentate gyrus of adult rats. Neuroscience 2000;99:38.)

doses of irradiation than the production of glia. One interesting finding in rats [56] and mice [29,32] is that after irradiation, there is a striking increase in the number of proliferating microglia within the dentate SGZ; that effect was clearly dose dependent [29] and persistent [32].

Based on these in vivo data, it is clear that neural precursor cells and the overall process of neurogenesis are sensitive to irradiation. To determine if the radiation-induced failure of neurogenesis was attributable to an intrinsic cell defect after irradiation, precursor cells obtained from

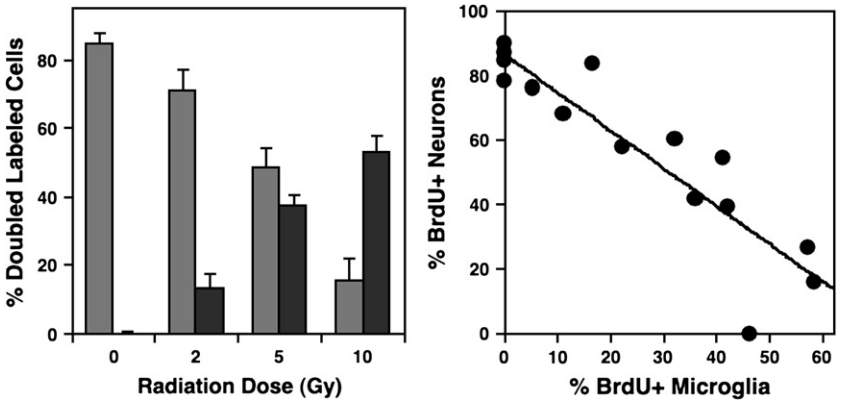

Fig. 5. Radiation-induced changes in neurogenesis are associated with indications of neuroinflammation. Two months after local brain irradiation of mice, in the dentate SGZ, there is a significant dose-related decrease in the number of newly born cells (BrdU-positive) that coexpress NeuN, a marker for mature neurons (*light bars, left panel*). At the same time, there is a significant increase in the number of activated microglia in the same area (*dark bars, left panel*). On the basis of individual animals, when neurogenesis (percentage of BrdU-positive neurons) is plotted against the percentage of BrdU-positive activated microglia, an extremely strong correlation ($r = 0.83$) is seen (*right panel*), suggesting a relation between neuroinflammation and reduced neurogenesis. In the left panel, each bar represents the mean value for four mice; error bars are standard errors of the mean (SEM). In the right panel, each circle represents one mouse; each mouse received a single x-ray dose ranging 2 to 10 Gy. (*Data from* Mizumatsu S, Monje ML, Morhardt DR, et al. Extreme sensitivity of adult neurogenesis to low doses of x-irradiation. Can Res 2003;63:4024,4025.)

whole hippocampal formations [38] from nonirradiated or irradiated rats were allowed to differentiate in culture [56]. Results from this study showed that some fraction of the precursor cells from normal and irradiated brains survived and was able to differentiate into immature neurons and glia. It also seemed that the basic differentiation machinery might have been aberrantly regulated after irradiation. These results were important because they suggested that in vivo, there are likely other factors that have an impact on the ability of surviving precursor cells to produce fully differentiated neurons. This was shown by Monje and colleagues [56], who stereotaxically transplanted highly enriched nonirradiated neural precursor cells into the dentate gyrus of rats irradiated 1 month previously with x-rays at a dose of 10 Gy. Tissues were collected 3 to 4 weeks later, and the fates of the transplanted cells were assessed using immunohistochemistry. Results from this study showed that although transplanted cells survived in nonirradiated and irradiated brains, the differentiation of cells into neurons was reduced by greater than 80% in the irradiated brain. This was interpreted to mean that local signaling within the dentate gyrus was defective, which likely represented a chronic disruption of the neurogenic microenvironment.

Radiation-induced inflammatory changes, including increased numbers of activated microglia, were reported in rats and mice after irradiation [29,32,56], and these changes were associated with altered neurogenesis (see Fig. 5). Furthermore, the dose-dependent increase in numbers of activated microglia was highly correlated with the dose-related decrease in neurogenesis (see Fig. 5) [29], suggesting that inflammation may play a critical role in the decreased neurogenesis seen after irradiation. Given the strong microglia presence after irradiation, along with studies reporting the role of monocyte chemoattractant protein (MCP-1) and its receptor, CCR2, in neuroinflammation [67], Rola and coworkers [32] made a qualitative appraisal of CCR2 immunoreactivity and showed a marked difference within the dentate gyrus between irradiated and nonirradiated mice (Fig. 6). Furthermore, the apparent upregulation of CCR2 was long lasting and was observed up to 9 months after irradiation with heavy ions [68]. Given these striking inflammatory changes after cranial irradiation, Monje and colleagues [57] showed that inflammatory blockage with the nonsteroidal anti-inflammatory agent indomethacin partially restored neurogenesis after a single 10-Gy dose of x-rays and suggested that this effect might be partially mediated by interleukin-6.

Anatomic and signaling relations between neural precursor cells and the microvasculature of neurogenic regions constitute another microenvironmental factor that plays a significant role in neurogenesis [36,38,69]. Proliferating precursor cells tend to be clustered around small vessels, and that association was lost after irradiation; in rats, the average distance from the midpoint of a BrdU-labeled precursor cell to the nearest vessel increased by approximate twofold after a dose of 10 Gy [56]. This led the authors of that study to suggest that the loss of this unique neurogenic anatomy indicated a fundamental disruption of the cellular and molecular interactions that are central to normal precursor cell biology in the dentate gyrus. Given this concept, Otsuka and coworkers [70] used the neutron capture reaction in boron to determine if the radiation sensitivity of neural precursor cells was dominated by direct radiation effects to the cells themselves or was mediated through changes in the microvasculature. In short, two boron-containing compounds were administered to groups of rats: one compound was restricted to the brain vasculature, whereas the other freely crossed the blood-brain barrier and entered cells. Rats were exposed to graded doses of thermal neutrons, which were absorbed by ^{10}B nuclei, resulting in the release of high-energy ionizing particles with ranges of 5 to 9 μm. Thus, in one case, the radiation was restricted to the blood and endothelial cells, whereas in the other case, the radiation was distributed throughout the brain. The results from this study showed that the selective irradiation of the microvasculature resulted in less loss of neural precursor cells than when the radiation dose was delivered uniformly to the parenchyma, indicating that the radiation response of precursor cells was attributable to changes not necessarily mediated through the vasculature.

Finally, oxidative stress is an intra- and extracellular factor that also may have a significant effect on neurogenesis, and there are in vitro data showing that such stress may constitute a biochemical mechanism regulating the fate of neural precursor cells [71]. Recent studies showed that the radiation response of hippocampal neural precursor cells in culture was characterized by specific cell cycle blocks, functional cell cycle checkpoints that were coupled with increases in the expression of p53 and other proteins, and elevated levels of reactive oxygen species (ROS) that were

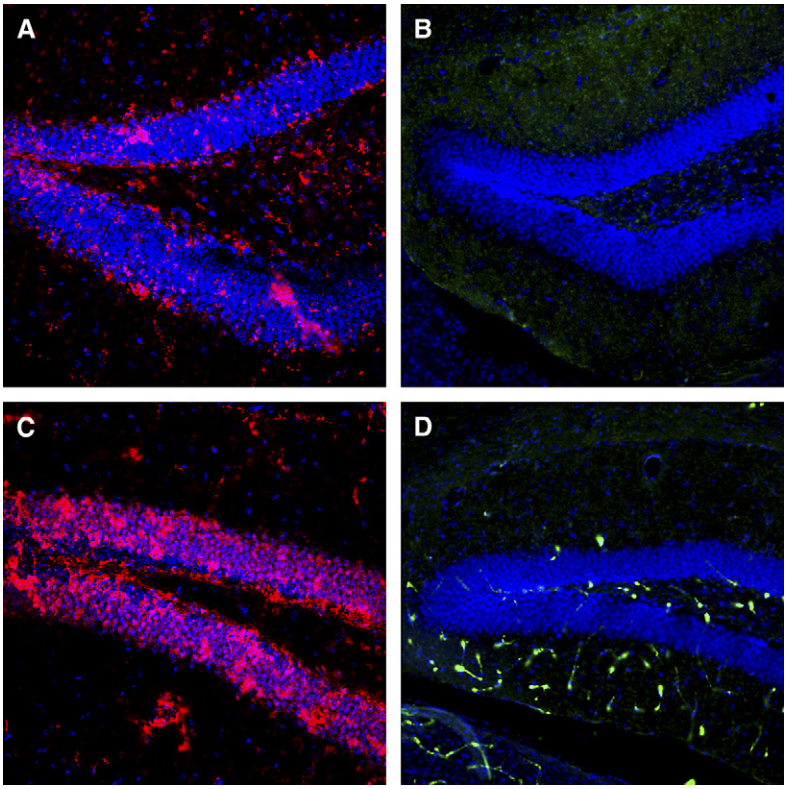

Fig. 6. Two months after x-irradiation, microenvironmental changes in the dentate gyrus include increased expression of the chemokine receptor CCR2 (*A, C*) and of the lipid peroxidation marker 4-hydroxynonenal (*B, D*). In both cases, there are qualitative increases in the extent of immunohistochemical labeling in the irradiated tissues. All micrographs are at original magnification ×20.

associated with early apoptosis. Furthermore, increased ROS levels in surviving precursor cells in culture persisted for at least 3 to 4 weeks after irradiation (Fig. 7) [72]. In that same study, immunohistochemistry and tissue extraction techniques were used to show that indications of oxidative stress could also be demonstrated after irradiation in the mouse hippocampal formation in vivo. Similarly, indications of ROS have been shown in rat hippocampus after whole-body irradiation with 10 Gy [62]. Given the importance of redox state in regulating multiple damage-responsive pathways in the CNS, it has been hypothesized that oxidative stress plays a major role in affecting neurogenesis and subsequent cognitive function after cell injury or depletion [73]. Cell culture studies and in vivo measurements suggest that neural precursor cells may be uniquely predisposed to redox regulation and that ROS may constitute critical environmental cues to control precursor cell division or differentiation [73]. ROS can be scavenged in vitro and in vivo by the antioxidant α-lipoic acid [73], and it is of interest that although treatment with α-lipoic acid can rescue specific indicators of neurogenesis, that rescue is reversed after drug withdrawal (Fig. 8). Recent work has also shown that treatment with the essential fatty acid precursor eicosapentaenoic acid before whole-body irradiation reduced ROS levels and resulted in less radiation-induced changes in long-term potentiation [62]. Taken together, these data suggest that antioxidant treatment strategies focused on reducing radiation-induced ROS could conceivably be used to restore neurogenesis and perhaps to ameliorate specific adverse sequelae of irradiation. Limiting oxidative stress after irradiation may also have the benefit of attenuating neuroinflammation, which could also help to restore precursor cell function.

There are compelling data using animal models that show the modulation of hippocampal neurogenesis is manifest as changes in cognitive

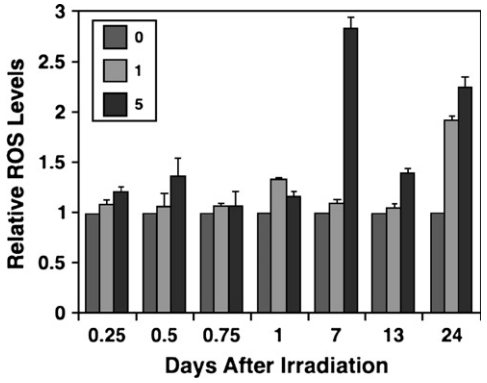

Fig. 7. ROS are generated in neural precursor cells from the hippocampus after low doses of x-rays. Exponentially growing cultures of rat neural precursor cells were irradiated, incubated with the ROS-sensitive fluorogenic dye CM-H2DCFDA, and assayed by fluorescence-activated cell sorting (FACS) analysis. In terms of acute response, ROS levels peak 12 hours after irradiation, which is the time of peak apoptosis in these cells [72]. There is also a persistent increase in ROS that is sustained for at least 1 month after irradiation. Bars represent the average of two independent measurements; error bars are standard deviations. All values were normalized to controls (set to unity) run the same day. (*Data from* Limoli CL, Giedzinski E, Rola R, et al. Radiation response of neural precursor cells: linking cellular sensitivity to cell cycle checkpoints, apoptosis and oxidative stress. Radiat Res 2004;161:23,24.)

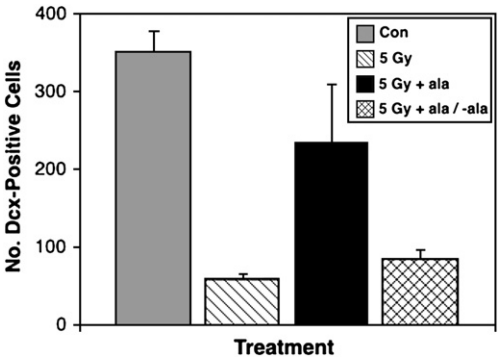

Fig. 8. Radiation-induced reductions in immature neurons in the mouse SGZ are partially rescued by antioxidant treatment. Immature neurons, the progeny of SGZ precursor cells, were detected using an antibody against doublecortin (Dcx), a protein associated with neuronal or neuroblast migration [84,86]. One week after a single dose of 5 Gy, the numbers of Dcx-positive cells in the dentate SGZ were reduced by approximately 85%. In mice given daily doses of the antioxidant α-lipoic acid (ala, 100 mg/kg) starting 1 day before irradiation and continuing for 1 week, there was substantial recovery of Dcx-positive cells relative to that seen after irradiation alone. In mice treated exactly the same way as described previously, except that ala treatment was stopped at 1 week and the mice were followed for another week, the apparent rescue of Dcx-positive cells was lost. This suggests that to be an effective strategy for reducing radiation-induced changes in neurogenesis, antioxidant treatment needs to be protracted.

performance. Treatments that decrease neurogenesis, such as drugs [74] or steroid exposure [75,76], are associated with impaired performance, whereas factors that increase neurogenesis, such as exercise or enriched environment, improve performance [26,77–79]. Recently, investigators have addressed the relation between neurogenesis and cognitive function after irradiation, and although cause and effect are difficult to prove, there clearly is an association between reduced neurogenesis in the dentate gyrus and impairments in hippocampal-dependent cognitive tasks [28,31,32,66,80]. Given the potentially devastating consequences of cranial irradiation on cognitive performance in human beings [9–12,81,82], experimental studies on neurogenesis and cognitive function are essential for the development of strategies and approaches to ameliorate or treat this serious sequela to cranial irradiation.

Summary and future considerations

Considerable data are now available regarding the radiation responsiveness of neural precursor cells that exist in the neurogenic regions of the mammalian forebrain. These cells and their progeny are extremely sensitive to irradiation, undergoing apoptosis after clinically relevant doses that do not result in overt tissue injury. In addition, there is compelling evidence that radiation significantly affects the whole process of neurogenesis and that the sensitivity depends, at least in part, on alterations in the microenvironment within which the precursor cells exist. Although provocative data exist suggesting that inflammation, oxidative stress, or morphologic relations influence neurogenesis, the precise mechanisms involved remain obscure and need to be investigated. Additionally, it is important to try to understand what these findings may mean in the context of radiation paradigms associated with the treatment of intracranial disease. This type of information should obviously have an impact in terms of radiation therapy, and it also should be useful in other situations that could affect neurogenesis, such as trauma, ischemia, and exposure to toxins

or chemotherapeutic agents. Understanding how neural precursor cells respond to noxious stimuli is likely to lead to new therapeutic approaches that should restore neurogenesis and perhaps improve cognitive performance.

Given the findings and uncertainties discussed previously, there are, in theory at least, ways that radiation-induced damage can be addressed within the context of stem/precursor cell biology. One of these would involve the ''recruitment'' or stimulation of endogenous neural stem/precursor cells to effect tissue repair. There is accumulating evidence that endogenous neurogenesis and gliogenesis may be integral components of an intrinsic self-repair process under certain conditions [83]. This makes the stimulation of endogenous stem/precursor cells for repair a particularly attractive approach, although, to date, there are few data addressing why such cells are not already able to effect repair under pathologic conditions. Critical and yet unresolved questions include the following:

1. Are these cells unable to repair cell/tissue damage?
2. Do they not receive an appropriate signal(s) for repair?
3. Are there inhibitory signals affecting proliferation migration, differentiation, integration, and, ultimately, appropriate functioning?

Perhaps by overcoming inhibiting signals it may be possible to enhance the ability of surviving stem/precursor cells to function in a reparative capacity. It may be as simple as using anti-inflammatory [57] or antioxidant approaches (see Fig. 8), although it seems much more likely that successful interventions would be far more complex.

Another possible way to address radiation injury could be the use of exogenous or transplanted neural stem/precursor cells, and there is one report showing that such an approach was used to ameliorate the expression of radiation-induced myelopathy in the rat spinal cord [84]. Considerable recent data are now being reported regarding the use of transplanted stem cells as vehicles of repair in the CNS after various types of injury or disease (reviewed by Kee and colleagues [85]). Some of those data are provocative, but there still is considerable uncertainty regarding the wide variety of factors and issues that can influence the ability of transplanted cells to move to a site of injury properly, proliferate or differentiate, and actually restore morphologic and functional normalcy. As summarized by Martino and Pluchino [83], there are numerous unsolved and challenging issues regarding how transplanted stem/precursor cells can be controlled and regulated; yet, if possible, the use of such cells could have a significant positive effect on the management of CNS injury and damage, including that induced by ionizing irradiation.

References

[1] Sheline GE, Wara WM, Smith V. Therapeutic irradiation and brain injury. Int J Radiat Oncol Biol Phys 1980;6:1215–28.
[2] Hopewell JW. Radiation injury to the central nervous system. Med Pediatr Oncol 1998;(Suppl 1):1–9.
[3] Tofilon PJ, Fike JR. The radioresponse of the central nervous system: a dynamic process. Radiat Res 2000;153:357–70.
[4] Burger PC, Mahaley MSJ, Dudka L, et al. The morphologic effects of radiation administered therapeutically for intracranial gliomas. A postmortem study of 25 cases. Cancer 1979;44:1256–72.
[5] Calvo W, Hopewell JW, Reinhold HS, et al. Time- and dose-related changes in the white matter of the rat brain after single doses of x rays. Br J Radiol 1988;61:1043–52.
[6] Fike JR, Gobbel GT. Central nervous system radiation injury in large animal models. In: Gutin PH, Leibel SA, Sheline GE, editors. Radiation injury to the nervous system. New York: Raven Press; 1991. p. 113–35.
[7] Hopewell JW. Late radiation damage to the central nervous system: a radiobiological interpretation. Neuropathol Appl Neurobiol 1979;5:329–43.
[8] van der Kogel AJ. Central nervous system radiation injury in small animal models. In: Gutin PH, Leibel SA, Sheline GE,editors. Radiation injury to the nervous system. New York: Raven Press; 1991. p. 91–111.
[9] Abayomi OK. Pathogenesis of irradiation-induced cognitive dysfunction. Acta Oncol 1996;35:659–63.
[10] Crossen JR, Garwood D, Glatstein E, et al. Neurobehavioral sequelae of cranial irradiation in adults: a review of radiation-induced encephalopathy. J Clin Oncol 1994;12:627–42.
[11] Roman DD, Sperduto PW. Neuropsychological effects of cranial radiation: current knowledge and future directions. Int J Radiat Oncol Biol Phys 1995; 31:983–98.
[12] Surma-aho O, Niemela M, Vilkki J, et al. Adverse long-term effects of brain radiotherapy in adult low-grade glioma patients. Neurology 2001;56: 1285–90.
[13] van der Kogel AJ. Radiation-induced damage in the central nervous system: an interpretation of target cell responses. Br J Cancer 1986;53:207–17.

[14] Rubin P, Johnston CJ, Williams JP, et al. A perpetual cascade of cytokines postirradiation leads to pulmonary fibrosis. Int J Radiat Oncol Biol Phys 1995; 33:99–109.
[15] Gage FH. Mammalian neural stem cells. Science 2000;287:1433–8.
[16] Gage FH, Ray J, Fisher LJ. Isolation, characterization, and use of stem cells from the CNS. Annu Rev Neurosci 1995;18:159–92.
[17] Temple S, Alvarez-Buylla A. Stem cells in the adult mammalian central nervous system. Curr Opin Neurobiol 1999;9:135–41.
[18] Doetsch F, Caille I, Lim DA, et al. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 1999;97:703–16.
[19] Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. Proc Natl Acad Sci USA 1993;90:2074–7.
[20] Seri B, Garcia-Verdugo JM, McEwen BS, et al. Astrocytes give rise to new neurons in the adult mammalian hippocampus. J Neurosci 2001;21: 7153–60.
[21] Doetsch F, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 1997;17: 5046–61.
[22] Luskin MB, McDermott K. Divergent lineages for oligodendrocytes and astrocytes originating in the neonatal forebrain subventricular zone. Glia 1994; 11:211–26.
[23] Morshead CM, Reynolds BA, Craig CG, et al. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron 1994;13:1071–82.
[24] Markakis EA, Gage FH. Adult-generated neurons in the dentate gyrus send axonal projections to field CA3 and are surrounded by synaptic vesicles. J Comp Neurol 1999;406:449–60.
[25] Zhao C, Teng EM, Summers RG Jr, et al. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. J Neurosci 2006;26:3–11.
[26] van Praag H, Christie BR, Sejnowski TJ, et al. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 1999; 96:13427–31.
[27] Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature 1997;386:493–5.
[28] Madsen TM, Kristjansen PE, Bolwig TG, et al. Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. Neuroscience 2003;119:635–42.
[29] Mizumatsu S, Monje ML, Morhardt DR, et al. Extreme sensitivity of adult neurogenesis to low doses of x-irradiation. Can Res 2003;63:4021–7.
[30] Monje ML, Palmer T. Radiation injury and neurogenesis. Curr Opin Neurol 2003;16:129–34.
[31] Raber J, Rola R, LeFevour A, et al. Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. Radiat Res 2004;162:39–47.
[32] Rola R, Raber J, Rizk A, et al. Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice. Exp Neurol 2004;188:316–30.
[33] Tada E, Parent JM, Lowenstein DH, et al. X-irradiation causes a prolonged reduction in cell proliferation in the dentate gyrus of adult rats. Neuroscience 2000;99:33–41.
[34] Bull ND, Bartlett PF. The adult mouse hippocampal progenitor is neurogenic but not a stem cell. J Neurosci 2005;25:10815–21.
[35] Morshead CM, van der Kooy D. A new 'spin' on neural stem cells? Curr Opin Neurobiol 2001;11: 59–65.
[36] Shen Q, Goderie SK, Jin L, et al. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. Science 2004;304: 1338–40.
[37] Lim DA, Tramontin AD, Trevejo JM, et al. Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. Neuron 2000;28:713–26.
[38] Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 2000;425:479–94.
[39] Cavanagh JB, Hopewell JW. Mitotic activity in the subependymal plate of rats and the long-term consequences of x-irradiation. J Neurol Sci 1972;15: 471–82.
[40] Hopewell JW, Cavanagh JB. Effects of x irradiation on the mitotic activity of the subependymal plate of rats. Br J Radiol 1972;45:461–5.
[41] Hubbard BM, Hopewell JW. Quantitative changes in cellularity of the rat subependymal plate after x-irradiation. Cell Tissue Kinet 1980;13:403–13.
[42] Bellinzona M, Gobbel GT, Shinohara C, et al. Apoptosis is induced in the subependyma of young adult rats by ionizing irradiation. Neurosci Lett 1996;208: 163–6.
[43] Shinohara C, Gobbel GT, Lamborn KR, et al. Apoptosis in the subependyma of young adult rats after single and fractionated doses of x-rays. Cancer Res 1997;57:2694–702.
[44] Tada E, Yang C, Gobbel GT, et al. Long term impairment of subependymal repopulation following damage by ionizing irradiation. Exp Neurol 1999; 160:66–77.
[45] Doetsch F, Garcia-Verdugo JM, Alverez-Buylla A. Identification of the in vivo stem cells in the adult mammalian brain. In: Proceedings of the 24th Society of Neuroscientists Meeting. 1998. p. 1275.
[46] Morshead CM, Craig CG, van der Kooy D. In vivo clonal analyses reveal the properties of endogenous

neural stem cell proliferation in the adult mammalian forebrain. Development 1998;125:2251–61.
[47] Paulus U, Potten CS, Loeffler M. A model of the control of cellular regeneration in the intestinal crypt after perturbation based solely on local stem cell regulation. Cell Prolif 1992;25:559–78.
[48] Dorr W. Three A's of repopulation during fractionated irradiation of squamous epithelia: asymmetry loss, acceleration of stem-cell divisions and abortive divisions. Int J Radiat Biol 1997;72:635–43.
[49] Gritti A, Parati EA, Cova L, et al. Multipotential stem cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. J Neurosci 1996;16:1091–100.
[50] Reynolds BA, Weiss S. Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. Dev Biol 1996;175:1–13.
[51] Craig CG, Tropepe V, Morshead CM, et al. In vivo growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. J Neurosci 1996;16:2649–58.
[52] Cameron HA, Hazel TG, McKay RDG. Regulation of neurogenesis by growth factors and neurotransmitters. J Neurobiol 1998;36:287–306.
[53] Decker L, Picard-Riera N, Lachapelle F, et al. Growth factor treatment promotes mobilization of young but not aged adult subventricular zone precursors in response to demyelination. J Neurosci Res 2002;69:763–71.
[54] Jin K, Sun Y, Xie L, et al. Neurogenesis and aging: FGF-2 and HB-EGF restore neurogenesis in hippocampus and subventricular zone of aged mice. Aging Cell 2003;2:175–83.
[55] Smith MT, Pencea V, Wang Z, et al. Increased number of BrdU-labeled neurons in the rostral migratory stream of the estrous prairie vole. Horm Behav 2001; 39:11–21.
[56] Monje ML, Mizumatsu S, Fike JR, et al. Irradiation induces neural precursor-cell dysfunction. Nat Med 2002;8:955–62.
[57] Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. Science 2003;302:1760–5.
[58] Nagai R, Tsunoda S, Hori Y, et al. Selective vulnerability to radiation in the hippocampal dentate granule cells. Surg Neurol 2000;53:503–6 [discussion: 506–7].
[59] Peissner W, Kocher M, Treuer H, et al. Ionizing radiation-induced apoptosis of proliferating stem cells in the dentate gyrus of the adult rat hippocampus. Brain Res Mol Brain Res 1999;71:61–8.
[60] Sasaki R, Matsumoto A, Itoh K, et al. Target cells of apoptosis in the adult murine dentate gyrus and O4 immunoreactivity after ionizing radiation. Neurosci Lett 2000;279:57–60.
[61] Uberti D, Piccioni L, Cadei M, et al. P53 is dispensable for apoptosis but controls neurogenesis of mouse dentate gyrus cells following gamma-irradiation. Brain Res Mol Brain Res 2001;93:81–9.
[62] Lonergan PE, Martin DS, Horrobin DF, et al. Neuroprotective effect of eicosapentaenoic acid in hippocampus of rats exposed to gamma-irradiation. J Biol Chem 2002;277:20804–11.
[63] Rola R, Otsuka S, Obenaus A, et al. Indicators of hippocampal neurogenesis are altered by ^{56}Fe irradiation in a dose dependent manner. Radiat Res 2004; 162:442–6.
[64] Parent JM, Tada E, Fike JR, et al. Inhibition of dentate granule cell neurogenesis with brain irradiation does not prevent seizure-induced mossy fiber synaptic reorganization in the rat. J Neurosci 1999;19: 4508–19.
[65] Casadesus G, Shukitt-Hale B, Stellwagen HM, et al. Hippocampal neurogenesis and PSA-NCAM expression following exposure to 56Fe particles mimics that seen during aging in rats. Exp Gerontol 2005;40: 249–54.
[66] Raber J, Fan Y, Matsumori Y, et al. Irradiation attenuates neurogenesis and exacerbates ischemia-induced deficits. Ann Neurol 2004;55:381–9.
[67] Banisadr G, Queraud-Lesaux F, Boutterin MC, et al. Distribution, cellular localization and functional role of CCR2 chemokine receptors in adult rat brain. J Neurochem 2002;81:257–69.
[68] Rola R, Sarkissan V, Obenaus A, et al. High-LET irradiation induced inflammation and persistent changes in markers of hippocampal neurogenesis. Radiat Res 2005;164:556–60.
[69] Louissaint A Jr, Rao S, Leventhal C, et al. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. Neuron 2002;34: 945–60.
[70] Otsuka S, Coderre JA, Micca PL, et al. Depletion of neural precursor cells following local brain irradiation is due to radiation dose to the parenchyma not the vasculature. Radiat Res 2006;165:582–91.
[71] Smith J, Ladi E, Mayer-Proschel M, et al. Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. Proc Natl Acad Sci USA 2000;97: 10032–7.
[72] Limoli CL, Giedzinski E, Rola R, et al. Radiation response of neural precursor cells: linking cellular sensitivity to cell cycle checkpoints, apoptosis and oxidative stress. Radiat Res 2004;161:17–27.
[73] Limoli CL, Rola R, Giedzinski E, et al. Cell density dependent regulation of neural precursor cell function. Proc Natl Acad Sci USA 2004;101: 16052–7.
[74] Shors TJ, Miesegaes G, Beylin A, et al. Neurogenesis in the adult is involved in the formation of trace memories. Nature 2001;410:372–6.
[75] Cameron HA, Gould E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. Neuroscience 1994;61:203–9.

[76] Lemaire V, Koehl M, Le Moal M, et al. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc Natl Acad Sci USA 2000;97:11032–7.
[77] van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 1999;2: 266–70.
[78] van Praag H, Shubert T, Zhao C, et al. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 2005;25:8680–5.
[79] van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci 2000;1:191–8.
[80] Snyder JS, Hong NS, McDonald RJ, et al. A role for adult neurogenesis in spatial long-term memory. Neuroscience 2005;130:843–52.
[81] Grill J, Renaux VK, Bulteau C, et al. Long-term intellectual outcome in children with posterior fossa tumors according to radiation doses and volumes. Int J Radiat Oncol Biol Phys 1999;45:137–45.
[82] Meyers CA, Geara F, Wong PF, et al. Neurocognitive effects of therapeutic irradiation for base of skull tumors. Int J Radiat Oncol Biol Phys 2000; 46:51–5.
[83] Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci 2006;7:395–406.
[84] Rezvani M, Birds DA, Hodges H, et al. Modification of radiation myelopathy by the transplantation of neural stem cells in the rat. Radiat Res 2001;156: 408–12.
[85] Kee N, Sivalingam S, Boonstra R, et al. The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. J Neurosci Methods 2002;115: 97–105.
[86] Nacher J, Crespo C, McEwen BS. Doublecortin expression in the adult rat telencephalon. Eur J Neurosci 2001;14:629–44.