INTRODUCTION

The use of high-energy beams to eliminate rapidly dividing tumor cells has undoubtedly cured a large and growing number of patients from their lethal cancer. However, collateral damage to the surrounding tissue is a treatment- and dose-limiting factor. After pelvic radiotherapy, damage to non-cancerous intestinal tissue results in five syndromes that can arise years to decades after treatment (Steineck et al., 2017): fecal-urgency syndrome, fecal-leakage syndrome, excessive-mucus discharge, excessive-gas discharge and blood discharge. Collectively, these syndromes are manifestations of pelvic-radiation disease (Andreyev et al., 2011). Pelvic-radiation disease causes profound distress, limits the ability to work, and restricts social activities in millions of cancer survivors worldwide (Andreyev 2007). Intriguingly, there are also long-lasting symptoms after pelvic-cancer treatment that are related to the central nervous system (CNS), such as reduced cognitive function, depression, and mental fatigue (Jakobsson et al., 2010).

The symptoms related to CNS functioning after cancer treatment are generally attributed to the impact of chemotherapy which, in contrast with pelvic radiotherapy, is delivered systemically. Since radiotherapy is usually combined with chemotherapy, the extent of the possible contribution of pelvic radiotherapy to late cognitive dysfunction is unknown. Only a small number of studies on cognitive symptoms has been made. Vistad et al. (2007) reported that one
third of cervical cancer survivors who had received radiotherapy, but not chemotherapy, suffered from chronic fatigue (assessed as both physical and mental fatigue) five years or more after treatment. This study found a correlation between chronic fatigue and intestinal function, and between chronic fatigue and depression (Vistad et al., 2007). In a study by Kurita et al., gynecological cancer survivors were found to be at greater risk of developing cognitive impairment than their cancer history-free twins and the authors were unable to attribute the increased risk to chemotherapy (Kurita et al., 2011). In inflammatory bowel disease (IBD; Crohn’s disease and Ulcerative Colitis), where the integrity of the intestinal wall is severely compromised, depression and cognitive dysfunction are common features (Attree et al., 2003).

The link between inflammation in the bowel and impact on cognitive functioning has been proposed to be found in cytokines secreted from the inflamed intestine that cross the blood–brain barrier (Tadin Hadjina et al., 2019). There are also studies relating intestinal inflammation to mental illness (Chen et al., 2019). Adult hippocampal neurogenesis, a neuroplasticity process where stem- and progenitor cells in the subgranular zone of the hippocampal dentate gyrus generate new neurons in the granule cell layer (GCL), is involved in memory, learning and mood regulation. Adult hippocampal neurogenesis is known to be particularly sensitive to changes in the microenvironment and inflammatory stimuli, and its disruption is believed to contribute to cognitive dysfunction (Chesnokova et al., 2016).

Consumption of foods rich in dietary fiber is a strong promotor of intestinal health that could possibly modulate the potential effects of pelvic radiotherapy on brain health (Wedlake et al., 2017). It has already been shown in animal models that the consumption of dietary fiber modulates the response of the intestine to irradiation (Sureban et al., 2015), and that it may also have a beneficial impact on pelvic-radiation disease in pelvic-cancer survivors (Wedlake et al., 2017). Importantly, a lack of fiber reduces the thickness of the protective inner mucus layer lining the intestine. With a weakened barrier between the mucosa and the pathogens in the lumen, antigens may penetrate the intestinal wall and trigger inflammatory activity (Desai et al., 2016) whereby, for an example, cytokines may reach the brain and modulate neurogenesis (Shi et al., 2020).

To test the hypothesis that irradiation-induced intestinal injury could have an impact on hippocampal neurogenesis we used an in-house developed murine model of pelvic radiotherapy (Bull et al., 2017), delivering four fractions of 8 Gray (Gy) to the mouse colorectum with a linear accelerator. To further probe whether the consumption of dietary fiber, or the lack thereof, could modify possible effects of irradiation on brain health, mice were fed with a diet rich in fiber (basal chow mix with bioprocessed oat bran as the fiber source), or a fiber-deprived diet (basal chow mixed with starch) two weeks before irradiation, and continuously throughout the experiment for up to 18 weeks after irradiation. Parameters of neurogenesis were then evaluated in the hippocampus using immunohistochemistry and stereology-based methods. In addition, serum cytokine levels were correlated to cell proliferation and immature neurons.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6J mice were purchased from Charles River Laboratories (Germany). Mice were kept in groups of five with constant room temperature at 24 °C and a 12-hour light cycle. The mice were provided food and water ad libitum. All experimental procedures were approved by the Gothenburg Committee of Swedish Animal Welfare Agency (ethical permit 1458-2018).

Irradiation procedure

For the irradiation procedure we used a linear accelerator (TrueBeam; Varian Medical Systems Inc., Charlottesville, Virginia, USA) with 6 MV nominal photon energy. Mice to be irradiated were anesthetized using isoflurane (MSD Animal Health, UK) and placed in a silicone mold to ensure identical positioning of the irradiation field; a 5 mm bolus over the mice ensured equal distribution of the radiation. The small irradiation field was placed so that approximately 1.5 cm of the distal colon was irradiated but not the spinal cord and testicles. For a more detailed description, see (Bull et al., 2017). Sham-irradiated mice were placed under the linear accelerator but received anesthesia only, the irradiated mice received 8 Gy in 4 fractions with a 5.9 Gy/min dose rate at 12-hour time intervals. The dose variation within the target volume was estimated to be ±5%. The irradiation procedure took at most five minutes for each animal. To determine how much scattered radiation the linear accelerators produced that could reach the brain, we measured scattered radiation adjacent to the head using a diode during the irradiation procedure. Scattered radiation close to the head during the irradiation procedure did not exceed 20 mGy. In another study, 20 mGy a day given for 300 consecutive days did not affect parameters of adult neurogenesis (Kempf et al., 2016).

Experimental design

The C57BL/6J mice were divided into four groups for each time point studied, each group consisting of 10 mice (n = 10). Two diets were provided, one with 15% fiber from bioprocessed oat bran (“High oat”; H-oat), and one fiber-free diet where the oat bran had been replaced by starch (0% fiber; No fiber), ensuring similar isocaloric content. The four groups were: irradiated mice fed with 15% fiber diet (H-oat irr), sham-irradiated mice fed with 15% fiber diet (H-oat sham-irr), irradiated mice fed a fiber-free diet (No fiber irr), sham-irradiated mice fed a fiber-free diet (No fiber sham-irr). Each group was placed on its diet two weeks before irradiation, and the diets were continued throughout the experiment (Fig. 1 A). The bioprocessed oat bran was provided by Glucanova AB (Lund, Sweden) and prepared according to European patent # 2996492. Bioprocessed oat bran and/or starch was added to a custom-made basal mixture in powdered form.
A  Experimental design

Radiation, 8 Gy in four fractions (12 hours inbetween) - 2 weeks  0 weeks  1 week  6 weeks  18 weeks

Post-irradiation time-points for follow-up

H-oat or No fiber dietary intervention

B  Hippocampal region analyzed

C  Granule cell layer volume

1 week post-irradiation  6 weeks post-irradiation  18 weeks post-irradiation

Two-way ANOVA significance for (§)=diet, (●)=irradiation, (#)=interaction
* = post-hoc significance
(TD.160816, Envigo Teklad Diets, Madison, Wisconsin, USA). Both diets were provided in a porridge-like consistency.

**Tissue sample collection**

Due to the collection of multiple organs for additional studies, perfusion was not performed but brains were immersion-fixed. The mice were deeply anesthetized with isoflurane (MSD Animal Health, UK) prior to blood collection through cardiac puncture followed by decapitation. The brains were removed, and the left and right hemispheres were separated. The left hemisphere was snap frozen in liquid nitrogen, while the right hemisphere was put in 6% formaldehyde solution (Histofix; HistoLab Products, AB) overnight and then developed using 3,3'-diaminobenzidine (DAB, Saveen Werner AB) diluted in TBS with H2O2 and NiCl2. Finally, the sections were rinsed in tap water to deactivate DAB, mounted on slides and then left overnight to dry. Once dried, the slides were coverslipped with X-Tra-Kitt (Medite GmbH).

**Immunohistochemistry**

For immunohistochemistry, sections were rinsed three times in TBS (tris-buffered saline) and blocked with 3% donkey serum (BD Biosciences) for 30 min to avoid unspecific binding. After blocking, the sections were incubated in primary antibodies with markers against ionized calcium binding adaptor molecule 1 (Iba1) for microglia (rabbit anti-Iba1, 1:2000, Wako Industries), Ki67 (rabbit anti-Ki67, 1:500, Abcam) for proliferating cells and NeuN (rabbit anti-NeuN, 1:1000, Merck Millipore) for mature neurons overnight at 4 °C in 3% donkey serum in TBS containing 0.1% TX100. The next day, the sections were rinsed in TBS for 3 × 10 min and incubated in fluorochrome-coupled secondary antibody (donkey anti-rabbit Alexa Fluor 555, 1:1000, Invitrogen/Molecular Probes) for 1 h at room temperature. After the incubation, sections were rinsed in TBS for 3 × 10 min, and then mounted onto slides and coverslipped with 4',6-diamidino-2-phenylindole (DAPI) ProLong Gold, (Invitrogen/Molecular Probes). To label immature neurons in the granule cell layer with immunohistochemistry, an antibody detecting doublecortin (DCX) (anti-rabbit DCX 1:500, Abcam) was used. The sections were rinsed in TBS for 3 × 10 min and treated with sodium citrate 10 mM at 80 °C in a water bath, rinsed in TBS again and then incubated for 30 min in 0.6% H2O2 followed by 30 min in a blocking solution (3% donkey serum in TBS with 0.1% TX100) to avoid unspecific binding. The sections were then incubated in block solution containing primary antibody against DCX, overnight at 4 °C. The next day, the primary antibody was washed off with TBS and then incubated in secondary antibody (biotinylated donkey anti-rabbit antibody, 1:1000, Jackson ImmunoResearch Laboratories Inc.) in block solution for 1 h and rinsed with TBS. After incubation in avidin–biotin-peroxidase (Vectastain Elite ABC Kit, Vector Laboratories) for 1 h, sections were rinsed and a dark brown or black stain resulted. After washing in TBS for 10 min, sections were rinsed in TBS for 5 min and mounted on slides and then left overnight to dry. Once dried, the slides were coverslipped with X-Tra-Kitt (Medite GmbH).

**Volume assessment and cell quantification**

For quantitative assessment of histological parameters, we used stereology-based methods and a Leica DM6000 microscope (Leica Microsystems, Wetzlar, Germany) equipped with a semi-automated stage and Stereo Investigator software (Microbrightfield, Williston, VT, USA). For volume of the GCL layer, the outline of the GCL was traced in 20× magnification, starting from where GCL was divided into a dorsal and ventral part until the dorsal blade disappeared (Fig. 1B). The resulting area was then multiplied with the section thickness (25 μm) and series (1:12). For the quantification of cells, every sixth or twelfth section was analyzed, but since some sections towards the midline were fragile and lost during processing, we chose to report total number of cells from an equal number of sections taken at similar locations. For Ki67, Iba1 and DCX, all labeled cells were counted exhaustively. NeuN+ neurons in the GCL were counted using stereology-based principles as follows: the GCL was traced using the Stereo Investigator software, whereafter a grid (80 μm grid size) with counting frames (20×20 μm) at each grid intersection was placed over the region of interest. NeuN+ cells falling within or touching the green line of the counting frame but not the red, were then counted at 40× resolution. To ensure a high precision of the estimate, a minimum of 200 cells and 20 counting frames were analyzed per section. The total number of NeuN+ cells per section was then estimated by the software, and this estimation was consistent with the manual count.

Fig. 1. Experimental design and granule cell volumes. (A) Male C57BL/6J mice (n = 10 per treatment and diet group) were fed either a fiber-free (“No fiber”) or a fiber-rich diet consisting of bioprocessed oat bran (High oat; “H-oat”) starting 2 weeks before the irradiation procedure (“−2 weeks”). Mice were then irradiated (“0 weeks”) with 32 Gy, delivered in four fractions of 8 Gy using a linear accelerator, and continued on their respective diet until euthanized at either 1 week, 6 weeks or 18 weeks after irradiation. (B) The right hemisphere was sagittally sectioned in a 1:12 series, starting where the hippocampus was clearly divided into a dorsal and ventral blade, and continuing towards the midline until the dorsal blade disappeared. (C) Average volumes of the granule cell layer at 1, 6 and 18 weeks after irradiation. A fiber-free diet induced a small and transient reduction of the GCL volume at 6 weeks. Neither irradiation, nor dietary fiber intake affected the size of the GCL at 1 and 18 week time points. P ≤ 0.05 significance is indicated by * = irradiation, § = diet, # = interaction.
multiplied with the series (12) to yield the total number of NeuN+ cells in the volume between the first and last section.

**Serum cytokine quantification**

Serum cytokine levels at all three time points after consumption of the No-fiber and H-oat diets have been published previously with a detailed protocol (Patel et al., 2020). In short, blood was drawn terminally during anesthesia through cardiac puncture. Serum was harvested after allowing whole blood to clot in room temperature followed by centrifugation. Serum cytokines were then quantified using the Bio-Plex Mouse Cytokine 23-Plex Assay (Bio-Rad Laboratories AB, Solna, Sweden). The 23 cytokines included in the panel were interleukin (IL)-1, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, eotaxin, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, keratinocyte-derived chemokine (KC), monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α and β, regulated upon activation normal T-cell expressed and secreted (RANTES), and tumor necrosis factor (TNF)-α. Serum cytokine levels were analyzed against DCX+ immature neurons for the 1, 6 and 18-weeks timepoints, and for Ki67+ proliferating cell numbers for the 6-week timepoint (where a statistically significant change had been identified).

**Statistics**

The software GraphPad Prism 8 (GraphPad software LLC, San Diego, CA, USA) was used to analyze the data. When comparing two factors (diet and irradiation) a two-way ANOVA was applied. For comparing between sham-irradiated and irradiated, a pairwise post-hoc test with Bonferroni correction for multiple testing (alpha 0.05) was applied. Grubb’s test was used to identify and remove outliers (three in total; one outlier was found for DCX in the three separate time points). Animals were not included if the sections were not complete or torn. After Pearson’s correlation analysis between hippocampal cell numbers and serum cytokines, linear regression was performed. Without correction for multiple testing, only the correlation slopes that were significantly deviating from zero with a P-value ≤ 0.05 and a goodness-of-fit $r^2 ≥ 0.7$ were reported. For the bar graphs, the results are presented as mean ± S. E.M. For all analyses, a difference with a $P$-value ≤ 0.05 was considered statistically significant. Descriptive statistics are shown in Supplementary Table 2 and 3.

**RESULTS**

**Volume of the GCL layer**

A fiber-free diet induced a small and transient but statistically significant reduction of GCL volume at 6 weeks (Fig. 1C; $^P = 0.028$ for diet, two-way ANOVA).

**Cell proliferation in the sub granular zone**

To determine whether colorectal irradiation or diet could affect the proliferative capacity of the stem- and progenitor cells in the hippocampus, we quantified the total number of Ki-67+ cells in the subgranular zone at three different time points: 1 week, 6 weeks, 18 weeks (Fig. 2). We found no significant difference at the acute 1-week time point (Fig. 2A), but at the intermediate time point (6 weeks), irradiation caused a reduction in the number of proliferating cells regardless of diet (Fig. 2B; $^*P = 0.016$ for irradiation, two-way ANOVA), with the greatest reduction in the No fiber group (15.8% loss of Ki-67+ proliferating cells) when compared to H-oat group (9.2% loss of Ki-67+ proliferating cells), although the difference was not statistically significant after correction for multiple testing ($P = 0.064$, Bonferroni post-hoc test). Four months after irradiation (18 weeks), the negative impact of irradiation on the number of Ki-67+ cells was no longer found (Fig. 2C).

**Neuronal maturation in the sub granular zone**

To evaluate whether changes in cell proliferation led to changes in the population of immature neurons, we quantified the number of immature neurons per millimeter length of the GCL using the marker DCX. At 1 week after irradiation there was no difference between any of the groups in the number of DCX+ cells per mm GCL (Fig. 3A). At 6 weeks, irradiation increased the number of immature neurons per mm GCL compared to sham-irradiation (Fig. 3D; $^*^*P = 0.002$ for irradiation, two-way ANOVA), with the largest increase (32%) in the high-oat group (Fig. 3D; $^*^*^*P = 0.002$ for H-oat sham-irr vs. H-oat irr, two-way ANOVA followed by Bonferroni post-hoc test). At 18 weeks, sham-irradiated mice in the No-fiber group had fewer DCX+ cells than sham-irradiated mice in the H-oat group, and irradiation induced a large increase in DCX+ cells in the No-fiber group but not in the H-oat group (Fig. 3G; $^*^*P = 0.022$ for interaction, two-way ANOVA and $^*^*^*P = 0.001$, Bonferroni post-hoc test).

An estimation of the maturational stage of the DCX+ cells in the subgranular zone can be made by quantifying the orientation of cell processes, where horizontal processes indicate a maturing young neuron. At 1 week, a two-way ANOVA revealed that there was an interaction between diet and irradiation ($^*^*^*P = 0.015$ for interaction, two-way ANOVA), so that after irradiation, there was a 16% reduction in early immature neurons in the high-oat group ($p = 0.026$, post-hoc Bonferroni test) (Fig. 3B). At 6 weeks and 18 weeks after irradiation, there were no differences in the percentage of cells in a specific maturational stage (Fig. 3E, F and 3H, I).

**Neuronal numbers**

To quantify the number of mature post-mitotic neurons in the granule cell layer, we used the marker NeuN and stereology-based methods. Since neurons in the mouse granule cell layer require a month or more to mature,
we did not quantify NeuN+ cells at the acute time point. There were no differences in the number of mature neurons between the groups 6 weeks or 18 weeks after irradiation (Fig. S1A-B).

**Inflammatory activity**

Since we found significant changes in both proliferative activity and the number of immature neurons at six weeks, we quantified microglia in the hippocampus at this time point as an indicator for ongoing inflammatory activity in the hippocampus. Each of the three hippocampal areas, the molecular layer, the granule cell layer and the hilus, were analyzed separately using the microglial marker Iba1. We did not find any differences in the density of Iba1+ microglia between the groups in any of the three areas (Fig. S2).

**Cytokine correlations**

No correlations between any of the serum cytokines and proliferating Ki67+ cells in the hippocampus were found at six weeks post-irradiation. For DCX+ cells, using a cut-off value at r^2 ≥ 0.7 without correction for multiple testing, we found a negative correlation at 1 week (Fig. 4A) for MIP-1α in both H-oat (r^2 = 0.71) and No-fiber (r^2 = 0.88) irradiated animals, and for eotaxin (r^2 = 0.72) in H-oat irradiated animals. No correlations with an r^2 ≥ 0.7 were found in sham-irradiated animals. For the 6-week timepoint no correlations with an r^2 ≥ 0.7 were found. For the 18-weeks timepoint (Fig. 4B), negative correlations were found in irradiated H-oat animals between DCX+ cells and IL-9 (r^2 = 0.78), IL-10 (r^2 = 0.70), MIP 1b (r^2 = 0.73) and in sham-irradiated H-oat animals (Fig. 4C) between DCX+ cells and MCP-1 (r^2 = 0.76). A positive correlation was found in irradiated H-oat animals between DCX+ cells and IL-12p40 (r^2 = 0.74, Fig. 4B).

**DISCUSSION**

The study was conceived on the basis of testimonials from pelvic-organ cancer survivors and clinical studies showing that pelvic-organ cancer survivors experience cognitive sequelae that can be chronic (Kurita et al., 2011). The impact of pelvic-organ cancer treatment on cognitive function is commonly attributed to chemotherapy. To our knowledge, this is the first study to report that irradiation confined to the distal bowels can affect parameters of hippocampal neurogenesis, a brain structure heavily involved in memory, learning and mood regulation. We found that colorectal irradiation resulted in a...
decrease in cell proliferation in the hippocampal subgranular zone six weeks after irradiation. The loss of proliferating cells, presumably neuronal precursors, was exacerbated in animals that were on a diet devoid of fiber. Moreover, irradiation had a lasting impact on the number of immature DCX+ neurons, an effect that could be modulated by the intake of bioprocessed oat bran, rich in dietary fiber. Despite these findings, we were not able to find any changes in the population size of mature granule cells, the main type of neurons in the dentate gyrus of the hippocampus, probably reflecting the brain’s ability to “buffer” for smaller changes in the early stages of neurogenesis.

Irradiation clearly caused a decrease in cell proliferation in the hippocampus six weeks after irradiation, an effect that appeared slightly stronger, albeit not statistically significant, in animals devoid of fiber. Thus, there is a possibility that a diet rich in fiber would be protective against irradiation-induced loss of cell proliferation, had we induced more severe damage to the distal bowels. In a study on diet and hippocampal neurogenesis, mice fed with a diet containing nuts, cocoa, vegetable oils rich in unsaponifiable fatty acids, and flours rich in soluble fibers had more newly generated cells in the subgranular zone (Valente et al., 2009). Another study showed that chemically induced colitis promoted the activation of the quiescent pool of the neural stem cell niche, along with an increase in fast proliferating progenitor cells (Sox2+ cells) and neuroblasts (DCX+ cells) (Gampierakis et al., 2020). In our study, the number of DCX+ cells increased at six weeks in a long-lasting response to irradiation. Increasing numbers of DCX+ cells as a reaction to injury has been reported before (Gampierakis et al., 2020), and perhaps the increase was a response to the loss of cell proliferation. At 18 weeks, the increase in DCX+ cells after irradiation was significantly larger in the animals

Fig. 3. Changes in DCX+ cells in the various immature stages. Immature neurons were quantified as DCX+ cells per mm GCL. The two stages of neuronal maturation were determined by the orientation of the cell processes: horizontal for early immature neurons and vertical for late immature neurons. The data for the maturational stages are presented as a percentage of total cells quantified. (A) At 1 week after irradiation, the total number of DCX+ cells per mm length of the GCL was unchanged. (B, C) There was a differential effect of irradiation on the percentage early and late immature neurons depending on the dietary intake. (D) At 6 weeks, there was an effect of irradiation, increasing the number of DCX+ cells per mm GCL, with a larger increase in the high-oat animals. (E, F) Irradiation induced a shift towards a lower percentage of early immature neurons. (G) At 18 weeks, there was an effect of irradiation, increasing the number of DCX+ cells per mm GCL, with a larger increase in the no-fiber animals. Moreover, dietary fiber intake modified the number of DCX+ cells where Sham-irradiated no-fiber animals had fewer DCX+ cells per mm GCL than sham-irradiated high-oat animals. In addition, there was an interaction between dietary fiber intake and irradiation. (H, I) Similar to the 6-week timepoint, irradiation had induced a shift towards a lower percentage of early immature neurons. Scale bar (J) 50 μm, scale bar (K) 25 μm. P ≤ 0.05 significance is indicated by * = irradiation, § = diet, ¦ = interaction.
devoid of fiber. We know from previous experiments that irradiation-induced crypt degeneration peaks at one week in mice fed a fiber-free diet (Malipatiolla et al., unpublished results), thus there is a breach in the intestinal integrity at this time point. However, we were unable to find any correlations between the number of degenerat-
ing crypts in the intestine, and DCX⁺ cells in the hippocampus (data not shown). Possibly, gross comparisons of the pathophysiological hallmarks in the irradiated bowel with markers of neurogenesis require much larger sampling sizes than used here.

We also evaluated whether irradiation or diet had an impact on the maturational progress of the DCX⁺ immature neurons at early, intermediate or late time points after irradiation. This was done by determining the orientation of the cell processes; the very immature points after irradiation. This was determined by determining neuronal maturation stages was previously described by Plumpe and coworkers (Plumpe et al., 2006). Overall, the ratios between the more mature young neurons and the immature neurons were constant, between 1:6 and 1:7. We found that there was a shift towards a higher percentage of DCX⁺ neurons that had transitioned into the more mature stages after irradiation if the animals were fed bioprocessed oat bran, but the findings concerning the DCX⁺ cell population were difficult to interpret. One possible explanation for the fluctuations in the data between the time points and groups could be that DCX⁺ cells are sensitive to the levels of cytokines circulating in the blood stream. We have previously found that the mice had quite different serum pro-inflammatory cytokine profiles between the time points and the four treatment groups, and this could play a role (Patel et al., 2020). Pro-inflammatory cytokines are known to have a deleterious impact on hippocampal neurogenesis, but in the current experiment, cytokine-induced inflammatory activity within the CNS would likely be mild due to the limited radiation field. In line with this, we did not find evidence of a relationship between any of the 23 serum cytokines quantified and the reduced cell proliferation in the GCL at six weeks after irradiation, nor did we find evidence of microglia activation. Of note, there was a negative correlation between MIP1α and number of DCX⁺ cells at the acute phase in both irradiated groups, with a trend towards higher cytokine levels and significantly fewer DCX⁺ cells in the irradiated animals on a fiber-free diet. MIP1α is an inflammatory chemokine and its receptors are expressed in the dentate gyrus (Tran et al., 2007), where MIP-1α has been shown to negatively impact neurogenesis and impair learning and memory functions (Marciniak et al., 2015). Others have shown a lasting cytokine response in the hippocampus after dextran sodium sulphate-induced colitis, together with increased expression of Iba1 and reduced levels of DCX (Zonis et al., 2015), but in that model the inflammatory component is stronger than in our model of pelvic radiotherapy. Fatigue has been linked to elevated levels of circulating blood cytokines such as IL-12 in IBD patients, that have a stronger inflammatory condition than pelvic cancer survivors (Vogelaar et al., 2017). Whether similar mechanisms, albeit more subtle, are in action after pelvic radiotherapy remains to be proven, especially since the inflammatory component is not considered a prominent feature in pelvic-radiation disease. Care should be taken when interpreting correlations, and the correlations found in the present study are merely suggestions of possible candidates for a link between intestinal inflammation and hippocampal health. Pathological changes in the gut can perturb brain homeostasis in many other ways, such as via the production of toxic metabolites leaking into the blood stream and vagal nerve stimulation (Bonaz et al., 2018 and Obrenovich, 2018). Both cytokines and toxins could increase the permeability of the blood brain barrier, thereby disrupting the tight control on what enters the brain (Obrenovich, 2018). In some studies, a blood–brain-barrier dysfunction has been proposed as a root cause for psychological and neurological disorders (Chen et al., 2019).

Using a well-established marker for mature neurons, we evaluated the number of neurons with a stereology-based method at 6 and 18 weeks after irradiation. Since neuronal maturation requires 4–6 weeks, we did not quantify mature neurons one week after treatment. Irradiation of the colorectum did not have an impact on the number of mature neurons, neither did diet. A large number of immature neurons are produced by an excess of rapidly proliferating progenitor cells, which then goes through a strict selection process with the result that only a fraction of the newly produced cells becomes fully matured and integrated neurons (Plumpe et al., 2006). We believe that the hippocampal stem cell niche would “buffer” against a decrease in cell proliferation, unless a very profound injury in the intestine is induced through which the indirect impact on hippocampal cell proliferation is so large that it cannot be compensated for. Likewise, when DCX-positive, the immature neurons can stay in a specific stage for a prolonged period or can, if needed, move quickly through each stage (Plumpe et al., 2006). Therefore, the number of DCX⁺ cells does not necessarily reflect the production rate of new neurons. Such mechanisms are well-known to protect the vulnerable hippocampal niche from environmental stressors. Thus, the findings presented here do not prove that colorectal irradiation has a negative impact on the end result of adult neurogenesis and the study was not designed to test the functionality of the mature neurons. However, the current study does show that colorectal irradiation has the capacity to modify the various stages of neurogenesis.

Recently, several reports have revealed the major impact that dietary fiber has on intestinal health. In a study conducted on 165 patients randomized to three groups with varying levels of fiber intake during pelvic radiotherapy, high-fiber intake was protective both acutely and at a one-year follow-up (Wedlake et al., 2017). In another study conducted by us, a high consumption of citrus fruit, rich in dietary fiber, reduced symptoms of pelvic-radiation disease (Hedelin et al., 2019). While the cause of the outcome in these studies were not investigated, it is known that a lack of fiber in the diet causes erosion of the protective inner mucus layer lining the colon, both by reducing the production of mucus by goblet cells as well as by increasing the degradation of mucus by bacteria (Desai et al., 2016). Bioprocessed oat bran, the fiber source we used in this study, is rich...
in readily available beta-glucan that produce an especially high yield of butyrate, a short-chain fatty acid produced during microbial fermentation. Butyrate act as an energy source for the colonocytes (Wong et al., 2006), and goblet cells will use butyrate to produce a protective mucus layer lining the epithelium (Hamer et al., 2008), preventing pathogens and inflammatory substances such as lipopolysaccharides (LPS) from penetrating the mucosa (Staka et al., 2015). Thus, a fiber-rich diet can prevent systemic inflammation by strengthening the mucosal barrier, which could have an indirect impact on brain health as well (Desai et al., 2016). Shi et al. evaluated the effects of oat β-glucan supplementation on the reduced cognitive function in mice fed a Western diet, which is a high-fat, low-fiber diet. The supplementation of beta-glucan ameliorated cognitive dysfunction, in part by protecting against engulfment of synapses by hippocampal microglia. The results from the study further suggested that LPS and pro-inflammatory serum cytokines from a leaky intestine triggered the neuroinflammation and cognitive dysfunc-
tion, and that the supplementation of beta-glucan pre-
vented this by protecting the intestinal barrier and the microbial community (Shi et al., 2020). Possibly, metabolites from the colon may also reach the brain to various extent depending on the state of the microbiota and the intestinal injury, and the short-chain fatty acid butyrate has been shown to be able to stimulate hippocampal cell proliferation (Silva et al., 2020). In contrast, a fiber-deficient Western diet appears to reduce hippocampal volume, as seen in humans (Jacka et al., 2015), which resonates with our own findings, where we found a small volume reduction of the GCL. The decline was however transient, likely masked over time as a larger decline in volume occurred with the ageing of the mice.

A weakness of our study is that we cannot rule out bioactivity from other compounds in the bioprocessed oat bran, since it also contains, in addition to a high fiber content, other compounds such as lipids, biologically active phytochemicals and vitamins. Nevertheless, to follow-up on our findings would be of importance from a clinical perspective, since many pelvic-organ cancer survivors are given the advice to reduce their fiber intake (Ahlin et al., 2018). Such advice appears to be based on the common notion that fiber consump-
tion will increase intestinal discomfort acutely, but it is not evidence-based and could be counterproductive in the long run.

The strength of our study is the use of linear accelerators, equipment that is employed by the clinic to treat cancer patients. The use of linear accelerators allows us to precisely delineate the radiation field and deliver fractionated radiation with a high dose certainty and with a dose rate identical to that given to patients. The effect that colorectal irradiation had on parameters of neurogenesis was perhaps subtle, but increasing the radiation field would have resulted in the damage of other organs, preventing the disentangling of the intestinal radiation effects per se on the hippocampal niche. Increasing the dose would likely also have prevented long-term follow up due to a reduced survival in our mice. Pelvic cancer patients typically receive one fraction a day (Erlandsson et al., 2017), however, the cell cycle of the mouse intestinal stem cell is faster and the time between fractions can therefore be reduced while still allowing for DNA damage repair. We have found the gross pathophysiological hallmarks in the mucosa of our mice to be similar to that found in the mucosa of irradiated pelvic-cancer survivors (Malipatlolla et al., 2019). Never-
theless, we postulate that for humans, the effects of pelvic radiotherapy on hippocampal parameters might be more profound than in mice, since mice are more radioresistant than humans. For an example, in our model of pelvic radiotherapy the mice do not suffer from diarrhea and/or constipation, two common symptoms in pelvic-cancer survivors.

Taken together, our findings show that that pelvic radiotherapy, in itself, may contribute to the lasting impact that pelvic-organ cancer treatment has on cognitive function, and that measures intended to prevent intestinal damage may also preserve brain health in pelvic-cancer survivors.

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DISCLOSURES

A. Rascon is a part-time employee of Glucanova, the provider of the bioprocessed oat bran. She is also the author of the patent for its preparation.

M. Kalm is an employee at AstraZeneca, however this contribution is only within her associate professorship at Gothenburg University.

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**APPENDIX A. SUPPLEMENTARY DATA**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroscience.2021.08.030.