

# Brain Damage and Patterns of Neurovascular Disorder after Ionizing Irradiation. Complications in Radiotherapy and Radiation Combined Injury

Authors: Gorbunov, Nikolai V., and Kiang, Juliann G.

Source: Radiation Research, 196(1): 1-16

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RADE-20-00147.1

The BioOne Digital Library (<u>https://bioone.org/</u>) provides worldwide distribution for more than 580 journals and eBooks from BioOne's community of over 150 nonprofit societies, research institutions, and university presses in the biological, ecological, and environmental sciences. The BioOne Digital Library encompasses the flagship aggregation BioOne Complete (<u>https://bioone.org/subscribe</u>), the BioOne Complete Archive (<u>https://bioone.org/archive</u>), and the BioOne eBooks program offerings ESA eBook Collection (<u>https://bioone.org/esa-ebooks</u>) and CSIRO Publishing BioSelect Collection (<u>https://bioone.org/csiro-ebooks</u>).

Your use of this PDF, the BioOne Digital Library, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at <u>www.bioone.org/terms-of-use</u>.

Usage of BioOne Digital Library content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne is an innovative nonprofit that sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

## REVIEW

# Brain Damage and Patterns of Neurovascular Disorder after Ionizing Irradiation. Complications in Radiotherapy and Radiation Combined Injury

Nikolai V. Gorbunov<sup>*a*,1</sup> and Juliann G. Kiang<sup>*b*,*c*,*d*,2</sup>

<sup>a</sup> MCG, Rockville, Maryland; and <sup>b</sup> Armed Forces Radiobiology Research Institute, Departments of <sup>c</sup> Pharmacology and Molecular Therapeutics and <sup>d</sup> Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland

Gorbunov, N. V. and Kiang, J. G. Brain Damage and Patterns of Neurovascular Disorder after Ionizing Irradiation. Complications in Radiotherapy and Radiation Combined Injury. *Radiat. Res.* 196, 1–16 (2021).

Exposure to ionizing radiation, mechanical trauma, toxic chemicals or infections, or combinations thereof (i.e., combined injury) can induce organic injury to brain tissues, the structural disarrangement of interactive networks of neurovascular and glial cells, as well as on arrays of the paracrine and systemic destruction. This leads to subsequent decline in cognitive capacity and decompensation of mental health. There is an ongoing need for improvement in mitigating and treating radiation- or combined injury-induced brain injury. Cranial irradiation per se can cause a multifactorial encephalopathy that occurs in a radiation dose- and time-dependent manner due to differences in radiosensitivity among the various constituents of brain parenchyma and vasculature. Of particular concern are the radiosensitivity and inflammation susceptibility of: 1. the neurogenic and oligodendrogenic niches in the subependymal and hippocampal domains; and 2. the microvascular endothelium. Thus, cranial or total-body irradiation can cause a plethora of biochemical and cellular disorders in brain tissues, including: 1. decline in neurogenesis and oligodendrogenesis; 2. impairment of the blood-brain barrier; and 3. ablation of vascular capillary. These changes, along with cerebrovascular inflammation, underlie different stages of encephalopathy, from the early protracted stage to the late delayed stage. It is evident that ionizing radiation combined with other traumatic insults such as penetrating wound, burn, blast, systemic infection and chemotherapy, among others, can exacerbate the radiation sequelae (and vice versa) with increasing severity of neurogenic and microvascular patterns of radiation brain damage. © 2021 by Radiation Research Society

#### INTRODUCTION

There is growing concern regarding the long-term adverse effects of ionizing radiation on nervous tissues are associated with crucial aspects of brain constituents related to mental activity, when the radiation-induced organic brain injury severely affects mental health (1-3). The consistent sequelae of high-dose radiation to the brain include alteration of neuronal architecture, suppression of adult neurogenesis, and induction of neuroinflammation, vascular impairment, autoimmune response, radiation myelopathy and neurological disorders, which can ultimately lead to declining in cognitive capacity (1-16). Extensive research has been done to explore the response of brain tissues to X rays and gamma rays, resulting in a dramatic improvement in radiation treatment modalities (4-26). Nevertheless, even Gamma Knife and CyberKnife® procedures, with the stereotactic precision, produce scattered radiation to the normal parenchymal and vascular tissues outside the target areas, presenting an ongoing challenge to radiation oncologists (27-28).

Notably, the knowledge and perceptions of radiobiological responses/effects has been constantly evolving over the last century, undergoing revisions and encompassing new data from modern molecular and cell biology, molecular histopathology, micro-irradiation techniques for single-cell target, human and animal genetics, functional genomics, and systems biology (3 Omics), along with implementation of computational predictive models, big data research including clinical data on radiation, occupational and military medicine (1-48). Ultimately, this effort has led to a paradigm shift towards precision radiation oncology as well as radiation protection and radiation countermeasures based on integrative radiation systems biology (49-51).

Referring to radiation oncology, implementation of the systems biology methodology for optimization of ionizing radiation combined with chemotherapy or biological response modifier (BRM) therapy could revolutionize the future therapeutic modalities for cancer treatment. Indeed, while the main objective of combined-modality therapy is to

<sup>&</sup>lt;sup>1</sup> Previously employed at Armed Forces Radiobiology Research Institute, Uniformed Services University, Bethesda, MD.

<sup>&</sup>lt;sup>2</sup> Address for correspondence: Armed Forces Radiobiology Research Institute, Scientific Research, 4555 South Palmer Road, BLDG 42, Bethesda, MD 20889-5648; email: juliann.kiang@usuhs.edu.

maximize the ratio of normal tissue preservation against tumor cell reduction, clinical observations have often shown worsening long-term adverse radiation effects on the brain during the period after the "double-impacts" produced due to radiation treatment combined with chemo- or BRM therapy (12, 51).

Importantly, a crucial feature in the induction of adverse effects of radiation combined injury is the synergy of different etiological factors, i.e., radiation combined with concurrent or subsequent secondary trauma (such as penetrating wound, blunt, blast, thermal, hemorrhage, etc.) or insults from chemical toxins and infections. For example, this has been described in detail in a variety of models employing total-body irradiation combined with a secondary insult occurring from radiation-induced aseptic inflammation, immune suppression, coagulopathy and a high susceptibility to bacterial translocation from wound and *Enterobacteriaceae* bacteremia (45). A decrease in the LD<sub>50/30</sub> dose for gamma rays has been reported, in which animals received radiation combined with either skin wound, burn or laceration (45).

The available clinical data on radiation combined injury are limited and mostly originate from the cohorts of atomic bomb survivors and victims of the Chernobyl nuclear accident. Even more limited are clinical observations from these groups on radiation combined brain injury; thus, clarification of its pathogenesis based on these cohorts may be challenging. Nonetheless, presumably, the effects of high-dose radiation can include a decreased ability to recover from mechanical (e.g., wound, blunt or blast) trauma due to suppression of normal neurogenesis, oligodendrogenesis and vasculogenesis, and induction of parenchymal and neurovascular inflammation. Moreover, these conditions can increase the risk of a tertiary effect, i.e., intracranial infections. Overall, importantly, brain trauma, infection or chemotherapy/chemotoxins can exacerbate the radiation encephalopathy sequelae (and vice versa). Predictive models of these outcomes should be included in the integrative radiation systems biology methodology and employ data from translation research and combined radiation therapy and clinical data from nuclear and radiological accidents and incidents (12, 49, 51).

This review addresses the molecular and cellular mechanisms mediating differential effects induced by high-dose (>2 Gy) exposure of X rays and gamma-photons in the cerebral parenchymal and brain stroma that are associated with brain dysfunction. Specifically, this review focuses on the countermeasures against cerebrovascular impairment caused by ionizing radiation and/or radiation combined injury.

### X-ray and Gamma-Photon Tissue Irradiation: Molecular Effects Induced by Ionization and the Mechanisms of Cell Injury

The energy absorbed by tissues and body fluids upon ionizing irradiation is dissipated within radiolysis of water and organic and inorganic biomolecules (52-55).

The generated "primary reactive products" of radiolysis are subjected to different types of recombination yielding either the "secondary cytotoxic" products or adducts with intact "bystander" constituents (54-56). Thus, the impact of a pulse radiation to a single cell can per se produce an instant effect on vital pathways that control transcriptional, translational and post-translational events, cell metabolic homeostasis, redox balance, cell-to-cell communication, growth, differentiation or aberrations (56-63). Moreover, it appears that living cells are equipped with numerous molecular mechanisms [e.g., redox sensing transcriptional factors, nuclear factor (erythroid-derived 2)-like 2 (Nrf2), NF-kB, activator protein 1 (AP1) and mitogen-activated protein kinase (MAPK)], to respond to different types of cytotoxic stress and/or impacts of the damage-associated molecular patterns (DAMPs), reactive oxygen species (ROS) or the reactive electrophilic species, as a part of the intrinsic defense control. Noticeably, radiation-induced damage to the cell constituents occurs in a dose-dependent manner. Thus, the extent of the subsequent cell remodeling ultimately leads to either cell restoration or a complete cell loss (e.g., cell senescence or death) (64–67). In this respect, considering the high energy of the penetrating radiation, the molecular damage to the cell and tissue structures from the direct radiation impact is not specific, although the consequent reactive responses to the radiation-produced injury are. In general, this specificity defines levels of radiosensitivity of cells in different tissues and systems.

As the ionization energy is delivered to the targeted molecules, the electrons are subsequently ejected and transferred to the oxidized molecules, yielding in the biological environment, including the water ion-radicals  $(H_2O^{\bullet+})$ , an array of carbon-, oxygen-, sulfur-, and nitrogencentered ion-radicals and the excited water molecule  $(H_2O^*)$ and then, the discharged water-caged electrons  $(e_{ag})$ , hydroxyl radical (HO<sup>•</sup>), nucleophilic hydril (H<sup>•</sup>) and other free radical species. There are numerous transient products vielded in irradiated cells which disappear within a millisecond via numerous redox reactions; namely the water ion-radical  $(H_2O^+)$ , the hydrated ejected electrons (e<sup>-</sup><sub>ad</sub>), hydroxyl radical (HO<sup>•</sup>), nucleophilic hydril (H), and an array of carbon-, oxygen-, sulfur-, and nitrogen-centered ion-radicals in different biomolecules (52-54, 56). Moreover, these transient oxidized species disappear within a millisecond via numerous redox reactions with antioxidants or other redox-susceptible biomolecules, thus propagating oxidative stress (52, 53, 68, 69). In particular, this is referred to as the strong electrophilic hydroxyl radical (HO<sup>•</sup>), which has a capacity to oxidize organic molecules with diffusiondependent constant rates (69, 70). Biomolecule-centered radicals and the caged e- can either remain due to intramolecular recombination or react with the solvated oxygen to form numerous peroxides and superoxide anion radicals  $(O_2^{\bullet})$  (52, 53, 56). These secondary ROS can be either promptly utilized in the Haber-Weiss/Fenton-type reactions and by these means, activate the free-radical and radical-free metabolic pathways, or proceed to quenching by antiradical/ antioxidant redox mechanisms (54-57, 70-73). As an end point of these redox reactions, a rise in non-radical electrophiles and DAMPs in the irradiated tissues and biofluids can exacerbate cytotoxicity due to the primary radiation impact (74-79).

It would appear that the "secondary" ROS could potentially amplify the "primary" oxidative stress; however, theoretical calculations indicate that the yields of products, generated as a consequence of a primary ionization event and essential for "secondary" oxidative hit, are lower by orders of magnitude than those produced by normal cellular metabolism (80). Therefore, the concept of amplification of the radiation-induced "primary" oxidative stress has been further refocused on the shift of metabolic and pro-inflammatory redox pathways in irradiated cells (62, 63, 66, 77, 81-85). Indeed, numerous observations indicate that the radiation-induced radiolysis can affect crucial cellular constituents and tilt calcium homeostasis, and thus can activate a cascade of metabolic responses leading to a prolonged oxidative stress which propagates systemically (64, 80-87). Moreover, there are several redox mechanisms, which have been proposed to drive the metabolic oxidative stress. Among these are activation of: 1. Constitutive and inducible nitric oxide synthases (62, 81); 2. NADPH oxidase (76, 83); 3. Monoamine oxidase (88); 4. Radiation-inducible micro-RNA miR-193a-3p (89); 5. Transient receptor potential (TRP) proteins (90); and 6. ER-mitochondrial axis (83, 91-93).

In this particular role, the radiation-induced imbalance of mitochondrial redox machinery is the major consumer of oxygen in aerobes as well as the major source of metabolically produced ROS in most cells (83, 91-94). This is especially important, considering that the mitochondrial volume represents a substantial radiation target, i.e., 4-30% cell volume depending on the cell type (95). Recent extensive investigations of the radiation effects on mitochondria have shown that these organelles have a superior role in response to radiation hit by triggering: 1. Short- and long-term metabolic responses (e.g., a decrease in oxidative phosphorylation); 2. Metabolic amplification of the "primary" ROS yield from radiolysis; and 3. The cytochrome C-dependent mechanism of programmed cell death (84, 91-94). Numerous in vitro and in vivo observations indicate that radiation effects on mitochondria have multifactorial characteristics. First, mitochondrial DNA is very susceptible to radiation damage. In response to the formation of lesions in irradiated mitochondria, expression of specific mitochondrial genes that are related to cell survival can be upregulated. They can also induce a compensatory increase in the mitochondrial DNA copy number, i.e., "mitochondrial polyploidization" (84). The reactive products of radiolysis such as ROS, RNS, RCS, RLS can produce post-translational modifications of mitochondrial proteins followed by functional alterations, such as the following: 1. A prolonged dysregulation of the respiratory electron transport chain (affecting complex I, complex II, complex III and succinate/pyruvate-mediated respiratory capacity) with subsequent increases in ROS production; 2. Alterations in oxidative phosphorylation; 3. Remodeling of the mitochondrial-ER network, activation of mitophagy, and changes in the mitochondrial mass; 4. Increases in intraorganelle communications and [Ca<sup>2+</sup>]-mediated propagation of oxidative stress; and 5. Increases in permeability and swelling (83, 84, 91-96). Altogether, these reactions may constitute the "mitochondrial ROS-induced ROS" mechanism (97), and thus sustain the effect of prolonged oxidative stress in irradiated tissues (83, 84, 87, 93, 94). Thus, the radiation-associated "metabolic" activation of ROS can further sustain and propagate the systemic electrophilic stress to form a variety of non-targeted detrimental effects on tissues and organ systems.

Oxidation of biomolecules due to radiolysis and the Fenton mechanism has been extensively investigated over the past decades. For a long time, the radiobiological effects were associated with radiation-induced clustered DNA lesions, i.e., two or more individual lesions within one or two helical turns of the DNA that occur after passage of a single radiation track through a nucleus. Mitochondrial DNAs (mtDNAs) are equally susceptible to radiation injury (98). Radiation can induce clustered DNA cleavage through the direct impact of the ionizing photons on the DNA as well as through the indirect action of reactive chemical species formed near the DNA due to radiolysis and oxygenation. Indirect effects are attributed to oxidative damage by ROS, primarily by hydroxyl radicals generated in radiolysis and the Fenton reactions (70, 85, 98, 99). However, the "direct" damage of DNA induced by photons occurs randomly in sugar and base moieties leading to strand breakage, release of free (unaltered) DNA bases, phosphates, and the formation of intermediate DNA free radicals and the TBA, i.e., 2-thiobarbituric acid, reactive products. The hydroxyl radicals react with the bases of DNA rather than the sugars (70). In these events, the main reaction is the addition of the hydroxyl radical to the  $\pi$ bonds of the bases. In the presence of oxygen, the resulting pyrimidine carbon-based radicals can be converted to the corresponding peroxyl radicals, as are sugar-based radicals. Hydroxyl radical adducts of purines can be further subjected to cleavage or undergo recombination. The ultimate intramolecular recombination of base-centered radicals can lead to oxidation of the base and sugar moieties and the oxidative DNA cleavage (55, 70, 71, 95-100).

While the targeted DNA damage and epigenetic alterations are considered to be crucial mechanisms of the radiation-induced cell death, mutagenesis and genomic instability, the emerging role of other types of biomolecules modified due to radiation is of growing interest. Thus, like nucleic acids and nucleotides, deleterious effects of radiation on proteins (or peptides and amino acids), carbohydrates and lipid polyunsaturated fatty acids (PUFA) can occur either due to their radiolysis followed by formation of RC<sup>•</sup>, ROO<sup>•</sup>, RO<sup>•</sup>, RS<sup>•</sup> and RN<sup>•</sup>, or due to oxidation by ROS, primarily by hydroxyl radicals, resulting from radiation exposure and the Fenton reactions. This free radical oxidation results in an array of reactive electrophiles, which can cause further post-translational modification proteins, activation of cell defense mechanisms, or detrimental effect on homeostatic responses (76, 85, 100, 101), Recently defined important electrophiles produced due to radiation exposure and peroxidation are the protein-, amino acid- and lipid-derived carbonyls (55, 67, 73, 104, 105). Direct protein carbonylation, i.e., post-translational modification resulting in the addition of reactive carbonyl groups to proteins, occurs as a consequence of oxidation of lysine, arginine, histidine, proline, glutamate, and threonine residues, and fragmentation products of peptide bond cleavage reactions. Protein carbonyls are reported to be detectable for a while after radiation exposure (55, 85, 102, 103).

The radiation-induced oxidation of lipids generates a large number of reactive intermediates, i.e., reactive lipid species (RLS). Thus, radiation-induced cleavage of PUFA or abstraction of a proton from PUFA by hydroxyl radical leads to addition of molecular oxygen to form PUFAperoxides followed by their chemical degradation (Hock cleavage). These peroxidation reactions yield a plethora of PUFA-derived electrophilic ("soft") carbonyls, which includes  $\alpha,\beta$ -unsaturated aldehydes and ketones, such as 2-alkenals, 4-hydroxy-2-alkenals (4-HNE), 4-oxo-2-alkenals (4-ONE), acrolein, and malondialdehyde (MDA) (85, 102). The molecular construction containing  $\alpha$ ,  $\beta$ -unsaturated carbonyl conjugated with the diene displays efficient electron-withdrawing properties when reacting with nucleophiles, such as cysteine thiol, lysine, or histidine residues, via the Michael mechanism, producing a variety of intraand intermolecular covalent adducts. These covalent modifications result in a free carbonyl attached to the protein that appears as "secondary" carbonylation (75, 85, 102). In addition to carbonylation, the PUFA-derived aldehydes can react with the amine moiety of lysine residues to form the Schiff base adducts, which can further undergo intra-protein recombinations (102). Importantly, RLS such as MDA or 4-HNE can react with DNA and RNA (nuclear and mitochondrial) as well, in particular, with the guanine and adenine bases yielding etheno adducts (i.e., ethenobases). The produced aberrations in the nucleic acids can lead to mis-transcriptions and thus to altered transcriptional products. Moreover, by building Schiff bases with histones and other transcription regulatory proteins in the nucleus, these RLS can provoke clastogenic effects and promote epigenetic alterations. Notably, PUFA derivatives with conjugated dienes are particularly susceptible to in vivo nitration with endogenously produced RNS yielding nitroalkenes, another group of electrophilic RLS (75). Biological effects of  $\alpha,\beta$ -unsaturated aldehydes and ketones have been well addressed in the literature recently and the multidiversified interference of these products has been associated with the cell protein machineries situated in cytosol and crucial organelles (e.g., mitochondria, ER and nuclei), and formation of immunogens, inflammagens, and DAMPs in the biofluids recognizable by PRRs on non-targeted cells (76, 77, 98, 100, 102, 106, 107). Based on these observations, post-translational modification of proteins with PUFA-derived carbonyls, i.e., RLS, has also been proposed as a novel signaling mechanism that modulates the cell redox-stress responses, including those mediated by Nrf2, NF- $\kappa$ B, heat-shock proteins 70 and 90, heat-shock factor 1 (HSF-1) and APE/EpRE (75, 100, 102, 105, 106).

Notably, excessive generation of RLS implicated in the Michael addition/reaction can spread carbonyl (electrophilic) stress from the targeted cells to bystander cells via the gap junction network or the extracellular vesicle mechanisms, thus producing non-targeted effects including mitochondrial dysfunction, ER stress, disturbance of calcium homeostasis, and epigenetic and clastogenic dysregulations (86, 100, 102). Overall, while the "radiolysis phase" is short lived, it causes devastating systemic effects, i.e., radiation-induced damage to proteins and lipids, posttranslational modification of proteins, impairment of DNA, epigenetic alterations and formation of aberrant organelles generates an array of mediators of stress, danger and inflammation that interfere with cell communication systems and homeostatic control (55, 64, 87, 108, 109). In conjunction with these events, activation of free radical reactions, formation of ROS, RNS, RCS, RLS, the Schiff and etheno adducts, the products of protein sulfhydryl oxidation as well as depletion of antioxidants are major features of radiolysis in tissues and fluids, and therefore, are considered to be biomarkers of oxidative stress after radiation exposure.

### Differential Susceptibility of Brain Structures and Systems to Gamma-Photon Irradiation and Combined Injury

Impairment of the brain neuroregulatory activity and functions as well as cognitive capacity due to radiation or radiation combined injury is the result of induced organic injury to the brain tissues, the structural disarrangement of interactive networks of neurovascular and glial cells, as well as on arrays of the paracrine and systemic destructive cues originated from reactive responses to the impacts. The response and structural and functional changes in brain cells to radiation vary among different animal species (and humans), cell phenotype, age and brain morphological area and zones (e.g., the cerebral cortex vs. the hippocampus; the lateral ventricles vs. the third ventricle) and are largely suggested to fall in a linear dose-response pattern (4, 7, 18, 29, 30, 46-48, 110-118). However, the scope of injury and the consequences of the impacts are determined by cell susceptibility and their spatial-temporal status in the cell interactive networks of the tissues, whether they are neuronal, glial or neurovascular (7, 23, 24, 26, 117, 119*123*). Notably, the differentiation stage and radiation dose/ dose rate can predispose the fate of affected cells. Indeed, there are some particular phenotypes in the brain that are able to proliferate, e.g., the cells of the subependymal zone, glial and vascular endothelial progenitors, which are more sensitive to radiation damage and undergo apoptosis at lower doses compared to non-proliferating (e.g., terminally differentiated and  $G_0$  stage) parenchymal and vascular cells. Proliferating cells are also prone to senescence (*117*, *119–122*, *124–135*).

Evidently, the pathogenesis of the radiation-induced brain disease has a multifactorial character and depends on a latency of cell responses, the dynamic of the radiation-induced structural alterations and severity of the organic injury (*117*, *122*, *127*, *128*, *136*, *137*).

Moreover, a growing body of data from clinical and translational research indicate that radiation effects on the parenchyma cells and brain physiology are aggravated by the associated damage to the microvascular endothelium leading to cerebrovascular inflammation, breach of the brain blood barrier, and impairment of metabolic homeostasis (6, 14, 26, 41, 117, 118, 136). Therefore, structural and functional integrity, and the homeostatic and mental functions may decline gradually within weeks through years postirradiation (117, 118, 121, 138).

Overall, gamma photons per se, with their high ability to penetrate the cranial cavity, can, to a certain degree, directly affect all brain cell phenotypes (parenchymal and stromal), as well as interstitial fluids in all parts and areas of the brain regardless of radiation dose(s) given. However, it is widely accepted that radiation exposure below some certain single dose (as threshold) may not produce either radiation sickness or long-term adverse health effects in humans and animals, despite a presence of transient lesions in photon-targeted cells (3, 4, 29, 48, 117, 118, 125). Thus, the risk of brain cancer is considerable at radiation doses >0.2 Gy (110, 111, 118, 119, 139–141), while a single dose to human <0.02 Sv (or <0.02 Gy gamma photons) or cumulative lifetime exposures to <0.1Sv count as "no evidence for irradiation-related diseases" (142 - 145).

The estimation of limiting doses for direct radiation effects in the CNS is based on morphological and functional deficits. Thus, in humans, radiation doses between 0.35–0.50 Gy cause transient radiation sickness, with nausea and vomiting, that can develop a few days postirradiation (4, 46, 47, 145). In addition, delayed neurological disorder (>30 days) induced by 0.1–1.0 Gy radiation can originate from morphological and functional changes within the neuronal and vascular networks (14, 23, 26, 117, 118). A severe form of delayed neuropathy, myelopathy, neuroinflammation, vascular impairment, and decline in cognitive capacity occurs after exposure to doses ranging from 1–5 Gy (i.e., LD<sub>50/60</sub> for human total-body irradiation) followed by supportive care (14, 23, 26, 117, 118, 141). Furthermore, single doses of radiation over 20 Gy produces the acute

cerebrovascular syndrome characterized by very short prodromal and latent phases followed by neurological problems, including headache, seizures, cerebral edema, abnormal cognition, neurological deficits and loss of consciousness, as well as death (4, 26, 46, 47, 146, 147). Given that radiation oncology treatments of the head and neck employ high doses (i.e., cumulative dose ~60 Gy or more with 2–6 Gy per fraction), the brain tissue is regarded as a dose-limiting organ, with considerable concerns about radiation-induced vascular injury (1, 2, 25, 26, 28, 112, 118–122, 148–151). Most importantly, the brain microvasculature represents the largest vascular network in humans and animals, and up to 20% of the total oxygen consumed in the body is provided to the brain (152–154).

Based on the clinical sequelae, radiation-induced brain injury can be characterized as protracted acute, early delayed and late delayed injury (25, 26, 118, 155–157). Protracted acute cerebrovascular injury develops in hours to days depending on radiation dose to the cranium (147, 157). Early delayed brain injury occurs 1–4 months postirradiation and can involve alterations in the architecture of neuronal network, cerebral edema, transient micro-symptoms of organic damage to the nervous system and demyelination followed by perturbations in the functional activity of the nervous system (118, 121, 157). Although both of these early injuries can result in severe reactions, they are considered to be resolvable.

In contrast, late delayed brain injury is characterized histopathologically by gray matter organic damage, ataxia, vascular abnormalities, demyelination and, ultimately, white matter necrosis, which usually begins to occur 4-6 months postirradiation and can develop over years thereafter (118). The late delayed injuries have been considered irreversible and progressive (13, 14, 16, 22, 25, 26, 112, 114, 116, 118, 120, 122, 155-158). Thus, radiation-induced volumetric changes related to cortical atrophy (i.e., temporal and limbic cortex) have been reported in these patients a year after radiotherapy. This volumetric effect occurs in a dosedependent manner at a dose range of 1-60 Gy with greater effects at higher doses (159). It has also been found that doses above 28.6 Gy resulted in a greater than 20% probability of cortical atrophy, and the estimated decrease in cortical thickness is -0.0033 mm (P < 0.001) for every 1 Gy increase in the dose (159).

The explanation for this pathology has been exclusively based on alterations in mitotic activity, impairment of the proliferating capacity and apoptotic responses of either glial cells (e.g., astrocytes and oligodendrocytes in the forebrain subependyma) or vascular endothelial cells (6, 22, 112, 114, 122, 129, 141–143, 158, 160, 161). Evidently, irradiation of the subependymal zone can also dramatically affect a population of the adult neural stem cells that sustain brain tissue remodeling and cognitive functions (7, 23, 39, 116, 117, 127, 149, 160). Moreover, the radiation parenchymal effects can be exacerbated by the radiation-induced profound loss of capillary density within the hippocampus



FIG. 1. Diagram of the mid-sagittal plane of the human brain with indication of distinct radiosensitivity of brain structures/zones and radiation-response centers. Clinical data. • Radiation-induced nausea and vomiting occur after irradiation at doses >0.35 Gy; the effect develops with a dose-dependent latency and is due to responses of the chemoreceptor trigger zone of the medulla oblongata to the radiation-induced pro-inflammatory stimuli. • Cortical atrophy occurs in a dose-dependent manner (1-60 Gy irradiation; 1 Gy increments) with greater effects at higher doses, where the temporal and limbic cortex exhibits the greatest change in cortical thickness per Gy, compared to other regions (159). Radiation therapy at doses above 28.6 Gy results in a >20% probability to manifest cortical atrophy (170). • Brain exposure to a single 5–15 Gy radiation dose can cause structural/functional alterations in glial cells and ultimately leads to the fiber demyelination (16, 112, 114, 118, 171, 172). • Late delayed effects of cranial radiotherapy (35-40 Gy fractionated, 1.8 Gy per day) include reduction in the white matter volume (e.g., the corpus callosum, olfactory bulbs, the anterior commissure) (173). • A long-term effect of radiation at moderate-to-high-doses includes a risk for development of brain tumors (174). Translational research. • Brain irradiation, single fractions ranging from 0.5–15 Gy, can trigger structural/functional alterations in hippocampal cells and induce apoptosis of mitotically active brain cells in SEZ and dentate gyrus, thus detrimentally suppressing normal neurogenesis and oligodendrogenesis, affecting myelination and white matter development, particularly in the corpus callosum, parietal cortex and subventricular zone. Impairment of neurogenesis includes a significant shortening in dendritic length, reductions in the number of dendritic branches and branch points, and declines in the dendritic spine density that can occur after a month postexposure. Moreover, the above effects are frequently accompanied by a chronic microglia activation and can upregulate neuroinflammation and leads to decline in the cognitive function (8, 14, 16–18, 23, 39, 65, 115, 117, 118, 122, 123, 130–132, 175, 176). • Microvascular degeneration can occur after 0.1 Gy single dose exposure (rarefaction of capillary density in mouse brain) (117, 144). A dramatic increase in endothelial aberration appears after irradiation at doses > 4 Gy (16). These effects are associated with a decline in vasculogenesis and a development of neurovascular inflammation in hippocampus, the fimbria, and the ventricle choroid plexus that exacerbate cognitive impairment (112, 114, 116-119, 121, 160, 177).

region leading to perfusion impairment (121, 161). Overall, the extensive damage to the parenchymal and/or to the stromal cells as well as a progressive loss of their progenitors can lead to deterioration by neuroinflammation along with breach of the blood-brain barrier (BBB) that can ultimately lead to white matter lesions (5, 7, 25, 26, 115, 116, 128, 144, 158, 162). With the above considerations, there are two conceptually different hypotheses proposed to explain the mechanisms underlying the radiation-induced temporal and structural alterations of brain tissues and the radiation brain disease. One of these is based on parenchyma-associated pathogenic mechanisms, and the other is focused on the vascular etiology of the disease (5, 6, 39, 116, 118, 120–122, 138, 162, 163).

The parenchymal hypothesis of radiation-induced brain injury is focused on the neural progenitor cells (NPCs) and oligodendrocyte precursors (OP) that are required for adult neurogenesis and oligodendrogenesis that occur in the specific microenvironments, i.e., the angiogenic and astroglial niches of the subventricular zone and the subependymal zone at the lateral ventricles as well as in the dentate gyrus at the hippocampus (Fig. 1). Thus, endothelial cells and astrocytes situated in the niches control, promote and mediate NPC renewal and/or further differentiation to neurons and oligodendrocytes (5–7, 9, 13–16, 39, 114–117, 122, 124, 144, 158, 160, 162–164). Cranial irradiation is shown to induce: 1. Apoptosis in the subependymal zone and the dentate gyrus; and 2. A dose-dependent loss of the neuronal

stem cells as well as suppression of proliferation and differentiation of the surviving NPCs. Moreover, radiation toxicity affects reproductive capacity of oligodendrocyte type-2 astrocyte (O-2A) progenitor cells essential for the oligodendrocyte maturation (*10*, *13*, *14*, *15*, *21*, *22*, *39*, *122*, *130*, *156*, *162–164*).

The vascular hypothesis is supported by a large body of data that has described the radiation-induced vascular structural changes, including vessel dilation, vasculopathy and depletion of endothelial progenitors, appearance of senescent and aberrant endothelial cell, as well as activation of endothelial apoptosis and vascular inflammation (7, 120, 121, 128, 136, 154). Quantitative studies of irradiated animal brains have also demonstrated time- and dose-dependent reductions in the number of endothelial cell nuclei and blood vessel lengths (112, 119, 121, 161).

The vascular tissue displays a biphasic mode in response to radiation. The first acute phase can occur within 24 h postirradiation where the underlying mechanisms are associated with the radiation-induced apoptosis of endothelial cells (133). The second late phase of radiation effects on vasculature beds requires month(s) for development and features capillary collapse, thickening of basement membrane and a loss of the endothelial clonogenic activity (133). Notably, the radiosensitivity of vascular tissues can vary based on the vessel caliber, i.e., macrovessels >300 microns, microvessels <300 microns, arterioles and venules >10 microns and capillaries <10 microns (137). Evidently, capillaries are the most radiosensitive components of the vasculature (165). Thus, Dimitrievich et al. (166, 167) have demonstrated that sensitivity of the capillaries to single Xray doses ranging from 200 to 2,000 rad has been significantly greater than that of larger vessels. They have also shown that the major features of radiation injury have been represented by capillary disruption, and extravasation of blood components as well as proinflammatory alterations (166). There is reported evidence that the high vulnerability of capillary beds to radiation is due specifically to high radiation sensitivity of their endothelium, which is the major structural constituent of capillary walls (136, 168, 169). Referring to the radiation-induced brain disease, the microvasculature represents the principal histo-hematic interface in the CNS that sustains and controls the nervous tissue homeostasis (10). Therefore, the radiation-induced damage to the normal capillary endothelium can result in a breach of the brain-blood barrier leading to severe health outcomes.

Overall, while it is widely accepted that radiation does not produce pathognomonic morphologic features, that is, radiation-induced alterations may occur as a result of injuries by other factors, nonetheless, it is evident that microvascular injury may drive some unique self-sustaining mechanisms of chronic radiation diseases (6, 7, 119, 137). Neurological disorders resulting from brain irradiation are listed in Table 1. Endothelial Cell Response to Radiation Exposure and the Vascular Patterns of CNS Effects after Radiotherapy and Radiation Combined Injury

Microvascular Endothelium in Integrity of Brain Vascular Barrier. The endothelium of the microvascular beds comprises specific cell phenotypes and represents a part of (BBB) that tightly controls the brain immunochemical homeostasis (10). The BBB is a highly organized multicellular gate-keeping structure, which sustains the brain tissue immunochemical homeostasis by regulating the molecular trans-endothelial transport between the parenchyma and the systemic circulation and by restricting translocation of the peripheral immune cells. Importantly, there is a lack or low level of constitutive expression of major histocompatibility I molecules on neurons and oligodendrocytes of the adult brain. The limited permeability of the CNS microvascular endothelium is mostly attributed to its intrinsic low pinocytic activity and a high level of efflux transporters. This BBB-specific cell layer is tightly bound by the tight junction (TJ) and the adherent junction (AJ) molecules that are situated in the intercellular space between the adjacent endothelial cells.

There is a large body of evidence to indicate that the vascular cellular components along with the parenchymal components constitute structural/functional modules, named as the multicellular neurovascular units (NVUs). NVUs can be defined as complex functional and anatomical structures composed of endothelial cells with their TJs/AJs, a basal lamina covered with pericytes and smooth muscular and parenchymal cells, including astrocytes, neurons, interneurons and adjacent perivascular microglia (144, 178, 179). Moreover, the vascular endothelial monolayer is embedded in a complex meshwork of interacting proteins, glycoproteins, proteoglycans, glycolipids and extracellular vesicles that constitute glycocalyx and extracellular matrix. Thus, the NVU architecture depicts the influence of parenchymal, mural, extracellular components and paracrine factors in the unique function of the brain microvascular endothelium (10, 24, 178). Moreover, NVUs can represent functional platforms for integrating responses of pro- and antiinflammatory pathways under normal and pathological conditions (41).

Radiation Induces Endothelial Remodeling and Changes Endothelial Function. Evidently, the vascular endothelial cells in the NVU construct are vulnerable to direct radiation impacts, indirect bystander effects and the secondary inflammatory factors (6, 9, 16, 79, 121, 133, 144, 151). This makes the microvascular tissue extremely susceptible to radiation injury. Indeed, radiation-induced biochemical alterations and stressogenic stimuli can drive morphological and functional alterations in endothelial cells, namely expression of pro-inflammatory phenotypes, remodeling of tight junctions and NVU interactions, increases in permeability of endothelial lining of microvasculature, cell death and detachment from the basement membrane (136, 144,

	6			6	
Туре	Dose to brain	Latency	Duration	Prodromes, morphological and/or functional pathology	
Acute	>20 Gy single	Minutes-hour	2 Days	Fatigue, headache, fever, nausea, vomiting, hypotension, encephalopathy, major impairment of cognitive function, cerebellar ataxia, cerebral edema, increase in intracranial pressure, respiratory distress, cardiovascular shock, cerebral anoxia, death. (145–147)	
	4–16 Gy single WBI, supportive care	Hours–days	Days-weeks	Fatigue, headache, fever, nausea, vomiting, hypotension, encephalopathy, impairment of cognitive function, acute psychosis, cerebellar ataxia, cerebral edema, subarachnoid-parenchymal hemorrhage. Chance of death after >8 Gy irradiation (4, 46, 47, 145–147).	
	20–60 Gy fractionated radiotherapy	Days	Weeks	Fatigue, headache, fever, hypotension, nausea, vomiting, temporal encephalopathy with impairment of cognitive function, neuroinflammation, respiratory distress, cerebral edema ( <i>14</i> , <i>16</i> , <i>144</i> , <i>157</i> ).	
Early delayed	3–60 Gy, single or fractionated (radiotherapy or translational research)	Weeks	4–6 months	<ul> <li>Encephalopathy, temporal cognitive dysfunction, transient myelopathy, endocrine dysfunction, vasculopathy.</li> <li>Reversible suppression of the brain stem cells (2, 14, 121, 123, 144, 157, 162, 175). [Note, suppression of neurogenesis and vasculogenesis can occur due to &lt;2.0 Gy radiation (122)].</li> <li>In a range of 5 Gy– 60 Gy, radiation at 20–50 Gy induced white matter injury which became significant</li> </ul>	
Late delayed	3–60 Gy, single or fractionated (radiotherapy or translational research)	4–6 Months to 1 year	Years–lifetime	<ul> <li>~3 months posturradiation (112).</li> <li>Cranial neuropathy, myelopathy, vasculopathy, loss of mitotically-active cells (precursors of neurones and oligodendrocytes), demyelination, white matter necrosis, gliosis, neuroinflammation, cerebral atrophy, progressive and irreversible cognitive dysfunction. High risk for progressive dementia and endocrine dysfunction. High risk for development of malignant neoplasm (2, 14, 17, 22, 26, 117, 121, 144, 156, 159–162, 168–176).</li> <li>Radiotherapy at 20–50 Gy causes long-term mental impairment. Mental decline in children after 30–35 Gy can be discernible 4–6 months thereafter and can become pronounced 2–3 years later (112).</li> <li>50–60 Gy irradiation induced dry granular or fibrinoid necrosis, with calcification, perivascular fibrosis, collagenization and vessel telangiectasia, with all changes occurring within 6 months of treatment (112).</li> </ul>	

 TABLE 1

 Neurological Disorders Resulted from Brain Ionizing Irradiation

180, 181). These vascular effects are often exacerbated by neuroinflammatory responses associated with radiation, such as decreases in expression of protein kinase B (Akt) and anti-inflammatory cytokines, and increases in expression of pro-inflammatory mitogen-activated protein kinases (MAPK) (182). Ultimately, this microvascular remodeling can result in a BBB breach and an intraparenchymal hemorrhage.

There is some evidence that high-dose (>2 Gy) radiation is associated with development of aberrant endothelial cells, whereby the affected cells can either transfer from the quiescent state to a pro-inflammatory phenotype or proceed to mitotic or apoptotic cell death, depending on the delivered radiation dose (11, 76, 120, 121, 128, 151, 181, 183, 184).

The endothelial pro-inflammatory phenotypes are characterized by the expression of cytokines, chemokines, and adhesion molecules that facilitate the recruitment and homing immune cells to sites of infection or tissue injury (9, 11, 118, 121, 142, 144). Naturally, endothelial cell activation is a normal part of the vascular defense mechanisms. In the event of radiation exposure, there are several pathways, including DAMP-activating pathways, which can trigger pro-inflammatory endothelial response (185, 186). Evidently, DAMP implicates signaling cascades activated via toll-like receptors, purinergic receptors and inflammasomes. Thus, exposure of endothelial cells to the radiation-produced DAMP, dual-function alarmins, such as HMGB1, and exosomes, can upregulate pro-inflammatory responses mediated by NF-kB, MAPK and interferon regulatory factor 3 (IRF3) (187, 188). Ultimately, these reactions can result in expression of several adhesion molecules such as intercellular adhesion molecule (ICAM)- 1, vascular cell adhesion molecule (VCAM)-1, and E-selectin (184).

Apoptosis of microvascular endothelial cells can occur within 24 h postirradiation to >5 Gy of gamma photons or X rays in a dose-dependent manner (123, 181, 183, 184, 189). The data accumulated on this topic over the past 30 vears indicate that endothelial apoptosis drives the early microvascular radiation toxicity (190). These acute vascular effects in different tissues, including brain, can appear within days to weeks postirradiation. Chronic effects are associated with the endothelial cell senescence in cerebral vascular beds and these transformations may occur within months to years (137). Thus, the applied cumulative radiation dose, implemented fraction size, intrinsic nature of the vascular bed and specificity of brain areas subjected to irradiation, together can determine radiation outcomes. Moreover, another factor that defines the endothelial response to ionizing radiation is the degree of differentiation of endothelial cells. Notably, endothelial progenitor cells (EPCs) are not only attributed to embryonic tissues, but the pool of peripheral blood EPCs originates from bone marrow and resident sources of many other tissues. It appears that this reservoir of EPCs can actively contribute to vascular remodeling, whereas the radiation-induced suppression of proliferation and ablation of EPCs causes vascular dysfunction (10, 191).

In addition to the above, there is a large body of published research with a focus on elucidating the molecular pathways leading to endothelial apoptosis. In this respect, of particular interest are the intrinsic mechanisms activated by radiation-induced damage to DNA, mitochondria and plasma membrane. The apoptosis mechanism activated by DNA damage implicates the p53-dependent pathway in which p53 activates transcription of the pro-apoptotic BH3-only proteins PUMA, NOXA or Bax, while the "mitochondrial damage associated" mechanism is mediated by the subsequently released cytochrome C (62, 132, 134).

Moreover, much of the literature based on *in vitro* and *in vivo* research have also suggested that the radiation-induced endothelial cell apoptosis is largely mediated by the lipid second messenger ceramide upon activation of acid/neutral sphingomyelinases (ASMase/NSMase) hydrolyzing sphingomyelin and releasing ceramide (*133, 136, 192*). One crucial target of ceramide is the RAC1/MEKK1 pathway, which interacts with the protein kinase MAPK8 regulating apoptosis through effector caspases, i.e., caspase-1, caspase-3 and caspase-6, as well as the autocrine stimulation of the death receptor pathway. Interestingly, this protein kinase is also implicated in the extrinsic apoptotic mechanism activated by external cues such as TNF- $\alpha$  (*193*).

The Radiation-Induced Endothelial Damage and Microvascular Impairment. As discussed above, the recent concepts of cellular mechanisms of the radiation-induced impairment of brain microvasculature regard the vascular endothelium as one of the main targets in radiation exposure. Thus, a high-dose radiation exposure or repeated fractional radiotherapy can exceed the adaptive physiological response and intrinsic resilience of the endothelium, thereby leading to endothelial dysfunction. This pathological condition can first appear in the form of insufficient responses to paracrine/endocrine/physiological stimuli and a failure of the endothelium to perform its normal, physiologic functions. Then, the radiation-induced massive formation of aberrant endothelial cells would lead to reduction of the cell density in the microvasculature that culminates in deterioration of the vascular tone, and vascular inflammation and declining integrity of BBB. This string of events, along with evident development of coagulopathy associated with declining the levels of platelets in the peripheral blood, often culminates in the parenchymal hemorrhage as a part of the sequelae of the acute disease (47, 194).

It is widely accepted that upon nuclear/radiological accidents or acts of nuclear detonation, the ionizing radiation would, with high-percentage estimates, be confounded by physical trauma (including burns) or exposure to toxic chemicals; this could also occur with infection with endemic, environmental or weaponized pathogens. A combination of these factors results in combined injury, which is more severe compared to due exposure to the same radiation dose/dose rate alone (45). Although the mechanisms of this synergistic interaction are not clearly understood, evidently a combination of factors can, while damaging/deranging biological barriers, also synergistically induce immune suppression and upregulate cascades of the systemic and local reactive responses in the injured parenchymal and vascular tissues. Ultimately, these alterations increase bacterial translocation, susceptibility to sepsis and the multiple organ failure outcomes (45).

Since Lawrence *et al.* reported *Enterobacteriaceae* bacteremia and subsequent sepsis as major factors of animal mortality after irradiation, the crucial role of bacterial breach, translocation and sepsis in radiation sequelae has been broadly documented in clinical observations and a variety of animal models of radiation combined injury (45, 195).

It is worth noting that the development of immunosuppression in irradiated animals can increase susceptibility to bacterial inflammagens and septicemia in order of magnitude compared to nonirradiated controls (45, 195). This phenomenon also suggests implication of the "secondary" septic responses in vasculature and parenchymal tissues sensitized by "the primary" radiation exposure. Moreover, these secondary responses can align with coagulopathy and the generalized hemorrhagic Shwartzman-like reaction, which leads to brain hemorrhage documented in animal models in the early delayed phase postirradiation (194).

Taken together, based on the information above, it is reasonable to suggest that acute and/or delayed encephalopathy due to nuclear accidents (196) can be aggravated by the infection-induced impairment of endothelium in the cerebral vasculature. Implication of neurovascular pattern in the encephalopathy due to combined impacts would be even more evident when such combination constitutes radiation and acute or sub-acute traumatic brain injuries characterized by mechanical damages to vasculature and intracranial hemorrhage.

Overall, a decline in brain function due to radiation combined injury is a challenge to civilian and military physicians. Development of new therapeutic modalities for managing the associated neurovascular injury could be a crucial step to address this challenge.

#### Perspectives on Mitigation of Microvascular Injury due to Radiation Therapy and Radiation Combined Impacts

Over time, several approaches and strategies have been considered for reducing radiation effects in the endothelium and brain cells, which are based on recruiting intrinsic resources for repair/resistance capacity and on the reduction/modulation of responses that drive pro-apoptotic pathways or senescence (12, 65, 133, 136, 197). These strategies include: Increase in capacities of the EPC-clonogen-generating tissues and the growth factor-producing cells; 2. Development of recombinant growth factors (e.g., VEGF); and 3. Molecular interference approaches using small molecules such as antioxidants, transcriptional modulators, inhibitors of the central renin-angiotensin system, inhibitors/modulators of the protein kinase pathways, among others. There is a large body of recently-published literature on these topics (12, 197–199).

In this respect, remarkable advances have been made based on investigations into the radioprotective effects of the basic fibroblast growth factor (bFGF) on the endothelium (133, 200). Here, it was suggested that the radioprotection resulted from suppression of the ceramide-related apoptosis (discussed above) where the PKC system operates as a bFGF effector (200). Moreover, implementation of the emerging cell therapy techniques for reconstitution of damaged microvasculature using donor EPCs and mesenchymal stromal cells presents a new opportunity for managing cerebrovascular impairment caused by radiation, trauma, sepsis or combination thereof (201-204).

A growing body of translational data suggests that the ghrelin peptide, an endogenous ligand of the growth hormone secretagogue receptor, can ameliorate vascular impairment under different pathological conditions (205–207). Ghrelin originally was reported to induce growth hormone release through pituitary GHSR-1a stimulation. However, recently published studies have indicated multiple paracrine, autocrine and endocrine roles of ghrelin, reflecting the ubiquitous expression of GHSR-1 $\alpha$  in a variety of tissues and organs. Thus, in addition to the established effects on food intake and growth hormone release, ghrelin has emerged as a potent immunoregulatory and anti-inflammatory agent. Moreover, ghrelin can also ameliorate neuronal apoptotic transformations in models of

brain injury, making it an effective intrinsic neuroprotector (206, 208, 209). There is evidence to suggest that ghrelin can inhibit neuronal and endothelial apoptosis by activating the extracellular-signaling-regulated-kinase (ERK)1/2, mitogen-activated protein kinase (MAPK) (180, 210), protein kinase A (PKA) and protein kinase C pathways (PKC). The activation of these pathways is associated with reduced activation of BAX, an improved Bcl2/BAX ratio and suppression of apoptosis/improved cell survival (210, 211).

There are promising published studies that have shown the pro-survival effects of ghrelin in rodent models of radiation injury and the combined radiation injury (radiation accompanied by sepsis or trauma) (180, 192). Using a mouse model of acute radiation injury and combined radiation injury (skin trauma), Kiang *et al.* has previously reported on the mitigating effects of ghrelin in hematopoietic tissue (180) and cerebral vasculature (180).

#### CONCLUSIONS

Radiation and radiation combined injury induce severe brain injury in a dose- and trauma score-dependent manner and, in part, are associated with neurovascular impairment. Whether the combined injury was the result of nuclear industrial accidents, detonation of nuclear/radiological devices or radiation therapy, it can nevertheless lead to devastating human health outcomes including severe brain injury. Research and development of new therapeutic modalities for radiation- and radiation combined brain injury should include the integrative radiation systems biology methodology, and should employ analyses of big data obtained from translational research, combined radiotherapy (*169*) and clinical data from nuclear and radiological accidents and incidents.

#### ACKNOWLEDGMENTS

The writing of this review was supported by AFRRI RBB34363, RAB 33529, RAB310934, NIH/NIAID IAA YI-AI-5045-04 and JPC-7 VP000276-01 to JGK. This review has been cleared and approved by AFRRI and USUHS leadership management. The views, opinions and findings contained in this report are those of the authors and do not reflect official policy or positions of the Armed Forces Radiobiology Research Institute, the Uniformed Services University of the Health Sciences, the Department of Defense, or the U.S. government. The commercial products identified in this article do not necessarily imply recommendation or endorsement by the federal government and do not imply that the products identified are necessarily the best available for the purpose.

Received: June 17, 2020; accepted: April 2, 2021; published online: May 12, 2021

#### REFERENCES

- Crossen JR, Garwood D, Glatstein E, Neuwelt EA. Neurobehavioral sequelae of cranial irradiation in adults: a review of radiation-induced encephalopathy. J Clin Oncol 1994; 12:627– 42.
- Roman DD, Sperduto PW. Neuropsychological effects of cranial radiation: current knowledge and future directions. Int J Radiat Oncol Biol Phys 1995; 31:983–98.

- 3. Kovalchuk A, Kolb B. Low dose radiation effects on the brain from mechanisms and behavioral outcomes to mitigation strategies. Cell Cycle 2017; 16:1266–70.
- Dainiak N, Waselenko JK, Armitage JO, MacVittie TJ, Farese AM. The hematologist and radiation casualties. Hematology Am Soc Hematol Educ Program 2003; 473–96.
- Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. Science 2003; 302:1760–65.
- Coderre JA, Morris GM, Micca PL, Hopewell JW, Verhagen I, Kleiboer BJ, et al. Late effects of radiation on the central nervous system: role of vascular endothelial damage and glial stem cell survival. Radiat Res 2006; 166:495–503.
- Lee WH, Sonntag WE, Mitschelen M, Yan H, Lee YW. Irradiation induces regionally specific alterations in pro-inflammatory environments in rat brain. Int J Radiat Biol 2010; 86:132– 44.
- Mizumatsu S, Monje ML, Morhardt DR, Rola R, Palmer TD, Fike JR. Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. Cancer Res 2003; 63:4021–7.
- Moravan MJ, Olschowka JA, Williams JP, O'Banion MK. Cranial irradiation leads to acute and persistent neuroinflammation with delayed increases in T-cell infiltration and CD11c expression in C57BL/6 mouse brain. Radiat Res 2011; 176:459– 73.
- Monje ML, Mizumatsu S, Fike JR, Palmer TD. Irradiation induces neural precursor-cell dysfunction. Nat Med 2002; 8:955– 62.
- Yuan H, Goetz DJ, Gaber MW, Issekutz AC, Merchant TE, Kiani MF. Radiation-induced up-regulation of adhesion molecules in brain microvasculature and their modulation by dexamethasone. Radiat Res 2005; 163:544–51.
- Robbins ME, Zhao W, Garcia-Espinosa M.A, Diz DI. Reninangiotensin system blockers and modulation of radiation-induced brain injury. Curr Drug Targets 2010; 11:1413–22.
- Okada S, Okeda R. Pathology of radiation myelopathy. Neuropathology 2001; 21:247–65.
- 14. Greene-Schloesser D, Moore E, Robbins ME. Molecular pathways: radiation-induced cognitive impairment. Clin Cancer Res 2013; 19:2294–300.
- 15. Tome WA, Gokhan S, Gulinello ME, Brodin NP, Heard J, Mehler MF, et al. Hippocampal-dependent neurocognitive impairment following cranial irradiation observed in pre-clinical models: current knowledge and possible future directions. Br J Radiol 2016; 89:20150762.
- Makale MT, McDonald CR, Hattangadi-Gluth JA, Kesari S. Brain irradiation and long-term cognitive disability: Current concepts. Nat Rev Neurol 2017; 13:52–64.
- 17. Vogel FS. Effects of high dose gamma radiation on the brain and individual neurons. In: Haley TJ, Snider RS, editors. Response of the nervous system to ionizing radiation. New York; London: Academic Press: 1962. p. 249–60.
- Balentov S, Hnilicova P, Kalenska D, Murin P, Hajtmanova E, Lehotsky J, et al. Effect of whole-brain irradiation on the specific brain regions in a rat model: Metabolic and histopathological changes. Neurotoxicology 2017; 60:70–81.
- Taylor ML, Kron T. Consideration of the radiation dose delivered away from the treatment field to patients in radiotherapy. J Med Phys 2011; 36:59–71.
- Lawrence YR, Li XA, el Naqa I, Hahn CA, Marks LB, Merchant TE, et al. Radiation dose-volume effects in the brain. Int J Radiat Oncol Biol Phys 2010; 76:S20–7.
- Yang T, Wu SL, Liang JC, Rao ZR, Ju G. Time-dependent astroglial changes after gamma knife radiosurgery in the rat forebrain. Neurosurgery 2000; 47:407–15, discussion 415–16.
- 22. Panagiotakos G, Alshamy G, Chan B, Abrams R, Greenberg E, Saxena A, et al. Long-term impact of radiation on the stem cell

and oligodendrocyte precursors in the brain. PLoS One 2007; 2:e588.

- Parihar VK, Limoli CL. Cranial irradiation compromises neuronal architecture in the hippocampus. Proc Natl Acad Sci USA 2013; 110:12822–7.
- Bylicky MA, Mueller GP, Day RM. Radiation resistance of normal human astrocytes: the role of non-homologous end joining DNA repair activity. J Radiat Res 2019; 60:37–50.
- Schultheiss TE, Kun LE, Ang KK, Stephens LC. Radiation response of the central nervous system. Int J Radiat Oncol Biol Phys 1995; 31:1093–112.
- Tofilon PJ, Fike JR. The radioresponse of the central nervous system: a dynamic process. Radiat Res 2000; 153:357–70.
- 27. Petti PL, Chuang CF, Smith V, Larson DA. Peripheral doses in CyberKnife radiosurgery. Med Phys 2006; 33:1770–9.
- Shinde A, Akhavan D, Sedrak M, Glaser S, Amini A. Shifting paradigms: whole brain radiation therapy versus stereotactic radiosurgery for brain metastases. CNS Oncol 2019; 8:CNS27.
- 29. Kovalev EE, Smirnova OA. Estimation of radiation risk based on the concept of individual variability of radiosensitivity. In: Reeves GI, Ainsworth EJ, editors. AFRRI Contract Report 96-1. Bethesda, MD: Armed Forces Radiobiology Research Institute; 1996:1–202.
- 30. Ruhm W1, Azizova T, Bouffler S, Cullings HM, Grosche B, Little MP, et al. Typical doses and dose rates in studies pertinent to radiation risk inference at low doses and low dose rates. J Radiat Res 2018; 59:ii1–10.
- De-Coursey E. Human pathological anatomy of ionizing radiation effects of the atomic bomb explosions. Mil Surg 1948; 102:427–32.
- 32. Cronkite EP. The hemorrhagic syndrome of acute ionizing radiation illness produced in goats and swine by exposure to the atomic bomb at Bikini, 1946. Blood 1950; 5:32–45.
- 33. Pan HY, Mazur LM, Martin NE, Mayo CS, Santanam L, Pawlicki T, et al. Radiation oncology health information technology: is it working for or against us? Int J Radiat Oncol Biol Phys 2017; 98:259–62.
- 34. Lee WJ, Choi Y, Ko S, Cha ES, Kim J, Kim YM, et al. Projected lifetime cancer risks from occupational radiation exposure among diagnostic medical radiation workers in South Korea. BMC Cancer 2018; 18;1206.
- Davidson ST. Any dose is too high. Environ Health Perspect 2005; 113:A735.
- Vaiserman A, Koliada A, Zabuga O, Socol Y. Health impacts of low-dose ionizing radiation: Current scientific debates and regulatory issues. Dose Response 2018; 16:1559325818796331.
- 37. Kerns SL, Ostrer H, Rosenstein BS. Radiogenomics: using genetics to identify cancer patients at risk for development of adverse effects following radiotherapy. Cancer Discov 2014; 4:155–65.
- Shouse SS, Warren SL, Whipple GH. II. Aplasia of marrow and fatal intoxication in dogs produced by roentgen radiation of all bones. J Exp Med 1931; 53:421–35.
- Pellmar TC, Lepinski DL. Gamma radiation (5–10 Gy) impairs neuronal function in the guinea pig hippocampus. Radiat Res 1993; 136:255–61.
- 40. Ghita M, Fernandez-Palomo C, Fukunaga H, Fredericia PM, Schettino G, Brauer-Krisch E, et al. Microbeam evolution: from single cell irradiation to pre-clinical studies. Int J Radiat Biol 2018; 94:708–18.
- 41. Yin E, Nelson DO, Coleman MA, Peterson LE, Wyrobek AJ. Gene expression changes in mouse brain after exposure to lowdose ionizing radiation. Int; J Radiat Biol 2003; 79:759–75.
- 42. Otsuka K, Koana T, Tauchi H, Sakai K. Activation of antioxidative enzymes induced by low-dose-rate whole-body gamma irradiation: Adaptive response in terms of initial DNA damage. Radiat Res 2006; 166:474–8.

- Amundson SA. Functional genomics and a new era in radiation biology and oncology. Bioscience 2008; 58:491–500.
- 44. Brothwell MRS, West CM, Dunning AM, Burnet NG, Barnett GC. Radiogenomics in the era of advanced radiotherapy. Clin Oncol (R Coll Radiol) 2019; 31:319–25.
- 45. Kiang JG, Olabisi A. Radiation: A poly-traumatic hit leading to multi-organ death. Cell Biosci 2019; 9:25.
- Diagnosis and treatment of radiation injuries. Safety reports series
   Vienna: International Atomic Energy Agency; 1998.
- Follow-up of delayed health consequences of acute accidental radiation exposure. IAEA-TECDOC-1300. Vienna: International Atomic Energy Agency; 2002.
- 48. Health risks from exposure to low levels of ionizing radiation. BEIR (Biological Effects of Ionizing Radiation) VII Phase 2 Report, National Research Council. Washington, DC: National Academy Press; 2006
- 49. Brahme A, Lind BK. A systems biology approach to radiation therapy optimization. Radiat Environ Biophys 2010; 49:111–24.
- 50. Mayo CS, Phillips M, McNutt TR, Palta J, Dekker A, Miller RC, et al. Treatment data and technical process challenges for practical big data efforts in radiation oncology. Med Phys 2018; 45:e793–e810.
- Unger K. Integrative radiation systems biology. Radiat Oncol 2014; 9:21.
- 52. Coderre J. Radiation chemistry. Chapter 7. In: 22.55J Principles of Radiation Interactions. Fall 2004. MIT OpenCourseWare. License: Creative Commons BY-NC-SA. Cambridge, MA: Massachusetts Institute of Technology. (https://ocw.mit.edu)
- LaVerne JA. OH radicals and oxidizing products in the gamma radiolysis of water. Radiat Res 2000; 153:196–200.
- 54. Singh A, Singh H. Time-scale and nature of radiation-biological damage: approaches to radiation protection and post-irradiation therapy. Prog Biophys Mol Biol 1982; 39:69–107.
- 55. O'Neill P, Wardman P. Radiation chemistry comes before radiation biology. Int J Radiat Biol. 2009; 85:9–25.
- 56. Azimzadeh O, Scherthan H, Sarioglu H, Barjaktarovic Z, Conrad M, Vogt A, et al. Rapid proteomic remodeling of cardiac tissue caused by total body ionizing radiation. Proteomics 2011; 11:3299–311.
- 57. Benderitter M, Vincent-Genod L, Pouget JP, Voisin P. The cell membrane as a biosensor of oxidative stress induced by radiation exposure: a multiparameter investigation. Radiat Res 2003; 159:471–83.
- 58. Corre I, Niaudet C, Paris F. Plasma membrane signaling induced by ionizing radiation. Mutat Res 2010; 704:1–7.
- 59. An JH, Kim J, Seong J. Redox signaling by ionizing radiation in mouse liver. Ann N Y Acad Sci 2004; 1030:86–94.
- 60. Gorbunov NV, Pogue-Geile KL, Epperly MW, Bigbee WL, Draviam R, Day BW, et al. Activation of the nitric oxide synthase 2 pathway in the response of bone marrow stromal cells to high doses of ionizing radiation. Radiat Res 2000; 154:73–86.
- 61. Choi KM, Kang CM, Cho ES, Kang SM, Lee SB, Um HD. Ionizing radiation-induced micronucleus formation is mediated by reactive oxygen species that are produced in a manner dependent on mitochondria, Nox1, and JNK. Oncol Rep 2007; 17:1183–8.
- Shinomiya N. New concepts in radiation-induced apoptosis: 'Premitotic apoptosis' and 'postmitotic apoptosis'. J Cell Mol Med 2001; 5:240–53.
- Gajdusek C, Onoda K, London S, Johnson M, Morrison R, Mayberg M. Early molecular changes in irradiated aortic endothelium. J Cell Physiol 2001; 188:8–23
- 64. Vo NTK, Shahid M, Seymour CB, Mothersill CE. Effects of dose rate on the reproductive cell death and early mitochondrial membrane potential in different human epithelium-derived cells

exposed to gamma rays. Dose Response 2019; 17:1559325819852508.

- 65. Kiang JG, Fukumoto R, Gorbunov NV. Lipid peroxidation after ionizing irradiation leads to apoptosis and autophagy. In: Angel C, editor. Lipid Peroxidation. Rijeka, Croatia: InTech Open Access Publisher; 2012. p. 261–78.
- 66. Davies MJ, Forni, LG, Willson RL. Vitamin E analogue Trolox CEsr and pulse-radiolysis studies of free-radical reactions. Biochem J 1988; 255:513–22.
- 67. Mezyk SP. Rate constant determination for the reaction of hydroxyl and glutathione thiyl radicals with glutathione in aqueous solution. J Phys Chem 1996; 100: 8861–66.
- Breen AP, Murphy JA. Reactions of oxyl radicals with DNA. Free Radic Biol Med 1995; 18:1033–77.
- Ostdal H, Davies MJ, Andersen HJ. Reaction between protein radicals and other biomolecules. Free Radic Biol Med 2002; 33:201–9.
- Jones DP. Radical-free biology of oxidative stress. Am J Physiol Cell Physiol 2008; 295:C849–68.
- Bild W, Ciobica A, Padurariu M, Bild V. The interdependence of the reactive species of oxygen, nitrogen, and carbon. J Physiol Biochem 2013; 69:147–54.
- Azzam EI, de Toledo SM, Little JB. Stress signaling from irradiated to non-irradiated cells. Curr Cancer Drug Targets 2004; 4:53–64.
- Schopfer FJ, Cipollina C, Freeman BA. Formation and signaling actions of electrophilic lipids. Chem Rev 2011; 111:5997–6021.
- Uchida K. Redox-derived damage-associated molecular patterns: Ligand function of lipid peroxidation adducts. Redox Biol 2013; 1:94–96.
- 75. Ilnytskyy Y, Kovalchuk O. Non-targeted radiation effects an epigenetic connection. Mutat Res 2011; 714:113–25.
- Kwon JE, Kim BY, Kwak SY, Bae IH, Han YH. Ionizing radiation-inducible microRNA miR-193a-3p induces apoptosis by directly targeting Mcl-1. Apoptosis 2013; 18:896–909.
- Rodel F, Frey B, Multhoff G, Gaipl U. Contribution of the immune system to bystander and non-targeted effects of ionizing radiation. Cancer Lett 2015; 356:105–13.
- Leach JK, Van Tuyle G, Lin PS, Schmidt-Ullrich R, Mikkelsen RB. Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. Cancer Res 2001; 61:3894–901.
- Leach JK, Black SM, Schmidt-Ullrich RK, Mikkelsen RB. Activation of constitutive nitric-oxide synthase activity is an early signaling event induced by ionizing radiation. J Biol Chem 2002; 277:15400–6.
- Spitz DR, Azzam EI, Li JJ, Gius D. Metabolic oxidation/ reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology. Cancer Metastasis Rev 2004; 23:311–22.
- 81. Wang Y, Liu L, Pazhanisamy SK, Li H, Meng A, Zhou D. Total body irradiation causes residual bone marrow injury by induction of persistent oxidative stress in murine hematopoietic stem cells. Free Radic Biol Med 2010; 48:348–56.
- 82. Kam WW, Banati RB. Effects of ionizing radiation on mitochondria. Free Radic Biol Med 2013; 65:607–19.
- 83. Reisz JA, Bansal N, Qian J, Zhao W, Furdui CM. Effects of ionizing radiation on biological molecules-mechanisms of damage and emerging methods of detection. Antioxid Redox Signal 2014; 21:260–92.
- Zhao W, Robbins ME. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. Curr Med Chem 2009; 16:130–43.
- Tulard A, Hoffschir F, de Boisferon FH, Luccioni C, Bravard A. Persistent oxidative stress after ionizing radiation is involved in inherited radiosensitivity. Free Radic Biol Med 2003; 35:68–77.
- 86. Collins-Underwood JR, Zhao W, Sharpe JG, Robbins ME.

NADPH oxidase mediates radiation-induced oxidative stress in rat brain microvascular endothelial cells. Free Radic Biol Med 2008; 45:929–38.

- 87. Mancuso M, Pasquali E, Leonardi S, Rebessi S, Tanori M, Giardullo P, et al. Role of connexin43 and ATP in long-range bystander radiation damage and oncogenesis in vivo. Oncogene 2011; 30:4601–8.
- Klumpp D, Misovic M, Szteyn K, Shumilina E, Rudner J, Huber SM. Targeting TRPM2 channels impairs radiation-induced cell cycle arrest and fosters cell death of T cell leukemia cells in a Bcl-2-dependent manner. Oxid Med Cell Longev 2016; 2016:8026702.
- Zhang B, Wang Y, Pang X, Su Y, Ai G, Wang T. ER stress induced by ionising radiation in IEC-6 cells. Int J Radiat Biol 2010; 86:429–35.
- Zhang B, Davidson MM, Zhou H, Wang C, Walker WF, Hei TK. Cytoplasmic irradiation results in mitochondrial dysfunction and DRP1-dependent mitochondrial fission. Cancer Res 2013; 73:6700–10.
- 91. Kobashigawa S, Suzuki K, Yamashita S. Ionizing radiation accelerates Drp1-dependent mitochondrial fission, which involves delayed mitochondrial reactive oxygen species production in normal human fibroblast-like cells. Biochem Biophys Res Commun 2011; 414:795–800.
- Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. J Exp Med 2011; 208:417–20.
- 93. Anastacio MM, Kanter EM, Makepeace CM, Keith AD, Zhang H, Schuessler RB, et al. Relationship between mitochondrial matrix volume and cellular volume in response to stress and the role of ATP-sensitive potassium channel. Circulation 2013; 128:S130–5.
- 94. Brady NR, Elmore SP, van Beek JJ, Krab K, Courtoy PJ, Hue L, Westerhoff HV. Coordinated behavior of mitochondria in both space and time: a reactive oxygen species-activated wave of mitochondrial depolarization. Biophys J 2004; 87:2022–34.
- Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial ROS-induced ROS release: an update and review. Biochim Biophys Acta 2006; 1757:509–17.
- 96. Georgakilas AG, O'Neill P, Stewart RD. Induction and repair of clustered DNA lesions: what do we know so far? Radiat Res 2013; 180:100–9.
- Cadet J, Douki T, Gasparutto D, Ravanat JL. Oxidative damage to DNA: formation, measurement and biochemical features. Mutat Res 2003; 531:5–23.
- Higdon A, Diers AR, Oh JY, Landar A, Darley-Usmar VM. Cell signalling by reactive lipid species: new concepts and molecular mechanisms. Biochem J 2012; 442:453–64.
- 99. Anuranjani, Bala M. Concerted action of Nrf2-ARE pathway, MRN complex, HMGB1 and inflammatory cytokines – implication in modification of radiation damage. Redox Biol 2014; 2:832–46.
- 100. Grimsrud PA, Xie H, Griffin TJ, Bernlohr DA. Oxidative stress and covalent modification of protein with bioactive aldehydes. J Biol Chem 2008; 283:21837–41.
- 101. Kim YC, Barshishat-Kupper M, McCart EA, Mueller GP, Day RM. Bone marrow protein oxidation in response to ionizing radiation in C57BL/6J mice. Proteomes 2014; 2:291–302.
- 102. Duran L, Tappel AL. Production of carbonyl compounds and sulfur compounds on irradiation of amino acids. Radiat Res 1958; 9:498–501.
- 103. Wong CM, Marcocci L, Liu L, Suzuki YJ. Cell signaling by protein carbonylation and decarbonylation. Antioxid Redox Signal 2010; 12:393–404.
- 104. Haberzettl P, Hill BG. Oxidized lipids activate autophagy in a JNK-dependent manner by stimulating the endoplasmic reticulum stress response. Redox Biol 2013; 1:56–64.

- 105. Picklo MJ, Azenkeng A, Hoffmann MR. Trans-4-oxo-2-nonenal potently alters mitochondrial function. Free Radic Biol Med 2011; 50:400–7.
- 106. Hei TK, Zhou H, Chai Y, Ponnaiya B, Ivanov VN. Radiation induced non-targeted response: mechanism and potential clinical implications. Curr Mol Pharmacol 2011; 4:96–105.
- 107. Berbee M, Fu Q, Boerma M, Wang J, Kumar KS, Hauer-Jensen M. Gamma-tocotrienol ameliorates intestinal radiation injury and reduces vascular oxidative stress after total-body irradiation by an HMG-CoA reductase-dependent mechanism. Radiat Res 2009; 171:596–605.
- *108.* Valentin J. Low-dose extrapolation of radiation-related cancer risk. Ann ICRP 2005; 35:1–140.
- 109. Tubiana M, Aurengo A. Dose-effect relationship and estimation of the carcinogenic effects of low doses of ionising radiation: The Joint Report of the Academie des Sciences (Paris) and of the Academie Nationale de Medecine. Int J Low Radiat 2006; 2:135– 53.
- 110. Calvo W, Hopewell JW, Reinhold HS, Yeung TK. 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.
- 111. The 2007 Recommendations of the International Commission on Radiological Protection. Oxford: ICRP publication 103. Ann ICRP 2007; 37:1–332.
- 112. Steen RG, Koury M, Granja CI, Xiong X, Wu S, Glass JO, et al. Effect of ionizing radiation on the human brain: White matter and gray matter T1 in pediatric brain tumor patients treated with conformal radiation therapy. J Radiat Oncol BioI Phys 2001; 49:79–91.
- 113. Jenrow KA, Brown SL, Lapanowski K, Naei H, Kolozsvary A, Kim JH. Selective inhibition of microglia-mediated neuroinflammation mitigates radiation-induced cognitive impairment. Radiat Res 2013; 179:549–56.
- 114. Tome WA, Gokhan S, Brodin NP, Gulinello ME, Heard J, Mehler MF, et al. A mouse model replicating hippocampal sparing cranial irradiation in humans: a tool for identifying new strategies to limit neurocognitive decline. Sci Rep 2015; 5:14384.
- 115. Casciati A, Dobos K, Antonelli F, Benedek A, Kempf SJ, Belles M, et al. Age-related effects of X-ray irradiation on mouse hippocampus. Oncotarget 2016; 7:28040–58.
- 116. Makale MT, McDonald CR, Hattangadi-Gluth JA, Kesari S. Mechanisms of radiotherapy-associated cognitive disability in patients with brain tumours. Nat Rev Neurol 2017; 13:52–64.
- 117. Reinhold HS, Calvo W, Hopewell JW, van der Berg AP. Development of blood vessel-related radiation damage in the fimbria of the central nervous system. Int J Radiat Oncol Biol Phys 1990; 18:37–42.
- 118. Hopewell JW, Campling D, Calvo W, Reinhold HS, Wilkinson JH, Yeung TK. Vascular irradiation damage: its cellular basis and likely consequences. Br J Cancer Suppl 1986; 7:181–91.
- 119. Warrington JP, Ashpole N, Csiszar A, Lee YW, Ungvari Z, Sonntag WE. Whole brain radiation-induced vascular cognitive impairment: mechanisms and implications. J Vasc Res 2013; 50:445–57.
- 120. Shinohara C, Gobbel GT, Lamborn KR, Tada E, Fike JR. Apoptosis in the subependyma of young adult rats after single and fractionated doses of X-rays. Cancer Res 1997; 57:2694–702.
- 121. Chakraborti A, Allen A, Allen B, Rosi S, Fike JR. Cranial irradiation alters dendritic spine density and morphology in the hippocampus. PLoS One 2012; 7:e40844.
- 122. Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 2000; 425:479–94.
- 123. Lowe XR, Bhattacharya S, Marchetti F, Wyrobek AJ. Early brain response to low-dose radiation exposure involves molecular networks and pathways associated with cognitive functions, advanced aging and Alzheimer's disease. Radiat Res 2009; 171:53–65.

- 124. Goldberg JS, Hirschi KK. Diverse roles of the vasculature within the neural stem cell niche. Regen Med 2009; 4:879–97.
- 125. Chopra H, Hung M.K, Kwong DL, Zhang CF, Pow EHN. Insights into endothelial progenitor cells: Origin, classification, potentials, and prospects. Stem Cells Int 2018; 2018:9847015.
- 126. Nordal RA, Wong CS. Molecular targets in radiation-induced blood-brain barrier disruption. Int J Radiat Oncol Biol Phys 2005; 62:279.
- 127. Brush J, Lipnick SL, Phillips T, Sitko J, McDonald JT, McBride WH. Molecular mechanisms of late normal tissue injury. Semin Radiat Oncol 2007; 17:121–30.
- 128. van der Maazen RWM, Kleiboer BJ, Verhagen I, van der Kogel AJ. Irradiation in vitro discriminates between different O-2A progenitor cell subpopulations in the perinatal central nervous system of rats. Radiat Res 1991; 128:64–72.
- 129. Gobbel GT, Bellinzona M, Vogt AR, Gupta N, Fike JR, Chan PH. Response of postmitotic neurons to X-irradiation: implications for the role of DNA damage in neuronal apoptosis. J Neurosci 1998; 18:147–55.
- 130. Chow BM, Li YQ, Wong CS. Radiation-induced apoptosis in the adult central nervous system is p53-dependent. Cell Death Differ 2000; 7:712–20.
- 131. Li YQ, Cheng Z, Wong S. Differential apoptosis radiosensitivity of neural progenitors in adult mouse hippocampus. Int J Mol Sci 2016; 17:pii:E970.
- 132. Pena LA, Fuks Z, Kolesnick RN. Radiation-induced apoptosis of endothelial cells in the murine central nervous system: protection by fibroblast growth factor and sphingomyelinase deficiency. Cancer Res 2000; 60:321–7.
- 133. Ungvari Z, Podlutsky A, Sosnowska D, Tucsek Z, Toth P, Deak F, et al. Ionizing radiation promotes the acquisition of a senescence-associated secretory phenotype and impairs angiogenic capacity in cerebromicrovascular endothelial cells: Role of increased DNA damage and decreased DNA repair capacity in microvascular radiosensitivity. J Gerontol A Biol Sci Med Sci 2013; 68:1443–57.
- 134. Li YQ, Chen P, Haimovitz-Friedman A, Reilly RM, Wong CS. Endothelial apoptosis initiates acute blood-brain barrier disruption after ionizing radiation. Cancer Res 2003; 63:5950–6.
- *135.* Fajardo LF. The pathology of ionizing radiation as defined by morphologic patterns. Acta Oncol 2005; 44:13–22.
- 136. Naveed M, Zhou QG, Han F. Cerebrovascular inflammation: A critical trigger for neurovascular injury? Neurochem Int 2019; 126:165–77.
- 137. Wilke C, Grosshans D, Duman J, Brown P, Li J. Radiationinduced cognitive toxicity: pathophysiology and interventions to reduce toxicity in adults. Neuro Oncol 2018; 20:597–607.
- *138.* Chien L, Chen WK, Liu ST, Chang CR, Kao MC, Chen KW, et al. Low-dose ionizing radiation induces mitochondrial fusion and increases expression of mitochondrial complexes I and III in hippocampal neurons. Oncotarget 2015; 6:30628–39.
- 139. Prise KM. New advances in radiation biology. Occup Med (Lond) 2006; 56:156–61.
- 140. Pierce DA, Preston DL. Radiation-related cancer risks at low doses among atomic bomb survivors. Radiat Res 2000; 154:178– 86.
- 141. Haley BM, Paunesku T, Grdina DJ, Woloschak GE. The increase in animal mortality risk following exposure to sparsely ionizing radiation is not linear quadratic with dose. PLoS One 2015; 10:e0140989.
- 142. Lumniczky K, Szatmari T, Safrany G. Ionizing radiation-induced immune and inflammatory reactions in the brain. Front Immunol 2017; 8:517.
- *143.* Anno GH, Baum SJ, Withers HR. Symptomatology of acute radiation effects in humans after exposure to doses of 0.5 to 30 Gy. Health Phys 1989; 56:821–38.

- 144. Mettler FA, Upton AC. Medical effects of ionizing radiation. Philadelphia: Saunders; 1995.
- 145. Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, et al. Strategic National Stockpile Radiation Working Group. Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. Ann Intern Med 2004; 140:1037–51.
- 146. West CM, Barnett GC. Genetics and genomics of radiotherapy toxicity: towards prediction. Genome Med 2011; 3:52.
- 147. Marsh JC, Gielda BT, Herskovic AM, Abrams RA. Cognitive sparing during the administration of whole brain radiotherapy and prophylactic cranial irradiation: Current concepts and approaches. J Oncol 2010; 2010:198208.
- 148. Hansasuta A, Choi CY, Gibbs IC, Soltys SG, Tse VC, Lieberson RE, et al. Multi-session stereotactic radiosurgery for vestibular schwannomas: single institution experience with 383 cases. Neurosurgery 2011; 69:1200–9.
- 149. Baker DG, Krochak RJ. The response of the microvascular system to radiation. Cancer Invest 1989; 7:287–94.
- 150. Walker EJ, Shen F, Young WL, Su H. Cerebrovascular casting of the adult mouse for 3D imaging and morphological analysis. J Vis Exp 2011; 57:e2958.
- 151. Marin-Padilla M. The human brain intracerebral microvascular system: development and structure. Front Neuroanat 2012; 6:38.
- 152. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev 1997; 77:731–58.
- 153. Sheline GE. Radiation therapy of brain tumors. Cancer 1977; 39:873–81.
- 154. Greene-Schloesser D, Robbins ME. Radiation-induced cognitive impairment–from bench to bedside. Neuro Oncol 2012; 14:iv37– 44.
- 155. Walker AJ, Ruzevick J, Malayeri AA, Rigamonti D, Lim M, Redmond KJ, et al. Postradiation imaging changes in the CNS: how can we differentiate between treatment effect and disease progression? Future Oncol 2014; 10:1277–97.
- 156. Shah R, Vattoth S, Jacob R, Manzil FF, O'Malley JP, Borghei P, et al. Radiation necrosis in the brain: imaging features and differentiation from tumor recurrence. Radiographics 2012; 32:1343–59.
- 157. Karunamuni R, Bartsch H, White NS, Moiseenko V, Carmona R, Marshall DC, et al. Dose-dependent cortical thinning after partial brain irradiation in high-grade glioma. Int J Radiat Oncol Biol Phys 2016; 94:297–304.
- 158. van den Maazen RWM, Kleiboer BJ, Berhagen I, van der Kogel AJ. Repair capacity of adult rat glial progenitor cells determined by an in vitro clonogenic assay after in vitro or in vivo fractionated irradiation. Int Radiat Biol 1993; 63:661–6.
- 159. Brown WR, Blair RM, Moody DM, Thore CR, Ahmed S, Robbins ME, et al. Capillary loss precedes the cognitive impairment induced by fractionated whole-brain irradiation: a potential rat model of vascular dmentia. J Neurol Sci 2007; 257:67–71.
- 160. Sundgren PC, Cao Y. Brain irradiation: effects on normal brain parenchyma and radiation injury. Neuroimaging Clin N Am 2009; 19:657–68.
- 161. Burns TC, Awad AJ, Li MD, Grant GA. Radiation-induced brain injury: low-hanging fruit for neuroregeneration. Neurosurg Focus 2016; 40:E3.
- 162. Song H, Stevens CF, Gage FH. Astroglia induce neurogenesis from adult neural stem cells. Nature 2002; 417:39–44.
- 163. Fajardo LF, Berthrong M. Vascular lesions following radiation. Pathol Annu 1988; 23 Pt 1:297–330.
- 164. Dimitrievich GS, Fischer-Dzoga K, Griem ML. Radiosensitivity of vascular tissue. I. Differential radiosensitivity of capillaries: a quantitative in vivo study. Radiat Res 1984; 99:511–35.

- 165. Fischer-Dzoga K, Dimitrievich GS, Griem ML. Radiosensitivity of vascular tissue. II. Differential radiosensitivity of aortic cells in vitro. Radiat Res 1984; 99:536–46.
- 166. Ljubimova NV, Levitman MK, Plotnikova ED, Eidus LK. Endothelial cell population dynamics in rat brain after local irradiation. Br J Radiol 1991; 64:934–40.
- 167. Mendonca MS, Chin-Sinex H, Dhaemers R, Mead LE, Yoder MC, Ingram DA. Differential mechanisms of x-ray-induced cell death in human endothelial progenitor cells isolated from cord blood and adults. Radiat Res 2011; 176:208–16.
- 168. Karunamuni RA, Moore KL, Seibert TM, Li N, White NS, Bartsch H, et al. Radiation sparing of cerebral cortex in brain tumor patients using quantitative neuroimaging. Radiother Oncol 2016; 118(1):29–34.
- 169. Peper M, Steinvorth S, Schraube P, Fruehauf S, Haas R, Kimmig BN et al. Neurobehavioral toxicity of total body irradiation: a follow-up in long-term survivors. Int J Radiat Oncol Biol Phys 2000; 46:303–11.
- 170. Makola M, Douglas Ris M, Mahone EM, Yeates KO, Cecil KM. Long-term effects of radiation therapy on white matter of the corpus callosum: a diffusion tensor imaging study in children. Pediatr Radiol 2017; 47:1809–16.
- 171. Reddick WE, Glass JO, Palmer SL, Wu S, Gajjar A, Langston JW, et al. Atypical white matter volume development in children following craniospinal irradiation. Neuro Oncol 2005; 7:12–9.
- 172. Braganza MZ, Kitahara CM, Berrington de Gonzalez A, Inskip PD, Johnson KJ, Rajaraman P. Ionizing radiation and the risk of brain and central nervous system tumors: a systematic review. Neuro Oncol 2012; 14:1316–24.
- 173. Betlazar C, Middleton RJ, Banati RB, Liu GJ. The impact of high and low dose ionising radiation on the central nervous system. Redox Biol 2016; 9:144–56.
- 174. Beera KG, Li YQ, Dazai J, Stewart J, Egan S, Ahmed M, et al. Altered brain morphology after focal radiation reveals impact of off-target effects: implications for white matter development and neurogenesis. Neuro Oncol 2018; 20:788–98.
- 175. Calvo W, Hopewell JW, Reinhold HS, van den Berg AP, Yeung TK. Dose-dependent and time-dependent changes in the choroid plexus of the irradiated rat brain. Br J Radiol 1987; 60:1109–17.
- 176. Maki T, Hayakawa K, Pham LDD, Xing C, Lo EH, Arai K. Biphasic mechanisms of neurovascular unit injury and protection in CNS diseases. CNS Neurol Disord Drug Targets 2013; 12:302–15.
- 177. McConnell HL, Kersch CN, Woltjer RL, Neuwelt EA. The translational significance of the neurovascular unit. J Biol Chem 2017; 292:762–70.
- 178. Paemeleire K. The cellular basis of neurovascular metabolic coupling. Acta Neurol Belg 2002; 102:153–7.
- 179. Heckmann M, Douwes K, Peter R, Degitz K. Vascular activation of adhesion molecule mRNA and cell surface expression by ionizing radiation. Exp Cell Res 1998; 238:148–54.
- 180. Kiang JG, Smith JT, Anderson MN, Umali MV, Ho C, Zhai M, et al. A novel therapy, using Ghrelin with pegylated G-CSF, inhibits brain hemorrhage from ionizing radiation or combined radiation injury. Pharm Pharmacol Int J 2019; 7:133–45.
- 181. Ahmad M, Khurana NR, Jaberi JE. Ionizing radiation decreases capillary-like structure formation by endothelial cells in vitro. Microvascular Res 2007; 73:14–19.
- 182. Hildebrandt G, Maggiorella L, Rodel F, Rodel V, Willis D, Trott KR. Mononuclear cell adhesion and cell adhesion molecule liberation after X-irradiation of activated endothelial cells in vitro. Int J Radiat Biol 2002; 78:315–25.
- *183.* Hellweg CE. The nuclear factor kappaB pathway: a link to the immune system in the radiation response. Cancer Lett 2015; 368:275–89.
- 184. Dong X, Tong F, Qian C, Zhang R, Dong J, Wu G, et al. NEMO modulates radiation-induced endothelial senescence of human

umbilical veins through NF-kappaB signal pathway. Radiat Res 2015; 183:82–93.

- 185. Fiuza C, Bustin M, Talwar S, Tropea M, Gerstenberger E, Shelhamer JH, et al. Inflammation-promoting activity of HMGB1 on human microvascular endothelial cells. Blood 2003; 101:2652–60.
- 186. Dent P, Yacoub A, Fisher PB, Hagan MP, Grant S. MAPK pathways in radiation responses. Oncogene 2003; 22:5885–96.
- 187. Hu S, Gao Y, Zhou H, Kong F, Xiao F, Zhou P, et al. New insight into mitochondrial changes in vascular endothelial cells irradiated by gamma ray. Int J Radiat Biol 2017; 93:470–6.
- 188. Meyn RE, Stephens LC, Milas L. Programmed cell death and radioresistance. Cancer Metastasis Rev 1996; 15:119–31.
- 189. Malinovskaya NA, Komleva YK, Salmin VV, Morgun AV, Shuvaev AN, Panina YA, et al. Endothelial progenitor cells physiology and metabolic plasticity in brain angiogenesis and blood-brain barrier modeling. Front Physiol 2016; 7:599.
- 190. Lozano J, Menendez S, Morales A, Ehleiter D, Liao WC, Wagman R, et al. Cell autonomous apoptosis defects in acid sphingomyelinase knockout fibroblasts. J Biol Chem 2001; 276:442–8
- 191. Verheij M, Bose R, Lin XH, Yao B, Jarvis WD, Grant S, et al. Requirement for ceramide-initiated SAPK/JNK signaling in stress-induced apoptosis. Nature 1996; 380:75–9.
- 192. Gorbunov NV, Kiang JG. Ghrelin therapy decreases incidents of intracranial hemorrhage in mice after whole-body ionizing irradiation combined with burn trauma. Int J Mol Sci 2017; 18:1693.
- 193. Brook I, Elliott TB, Ledney GD. Chapter 17. Infection after ionizing radiation. In: Zak O, Merle A, Sande MA, editors. Handbook of animal models of infection. San Diego, CA; London, UK: Academic Press; 1999. p. 151–61
- 194. Kehoe AD, Nikiforov AM, Alexanin SS, Neronov EG, Tikhomirova OV, Shun'kov VB, et al. Angiotensin-converting enzyme genotype and encephalopathy in Chernobyl cleanup workers. Eur J Neurol 2009; 16:95–100.
- 195. Uckun FM, Tuel-Ahlgre L, Song CW, Waddick K, Myers DE, Kirihara J, et al. Ionizing radiation stimulates unidentified tyrosine-specific protein kinases in human B-lymphocyte precursors, triggering apoptosis and clonogenic cell death. Proc Natl Acad Sci U S A 1992; 89:9005–9.
- 196. Li M, Ping G, Plathow C, Trinh T, Lipson KE, Hauser K, et al. Small molecule receptor tyrosine kinase inhibitor of plateletderived growth factor signaling (SU9518) modifies radiation response in fibroblasts and endothelial cells. BMC Cancer 2006; 24:6–79.
- *197.* Jain KK. The handbook of neuroprotection. Germany; New York: Springer; 2019. p. 1–287.
- 198. Haimovitz-Friedman A, Balaban N, McLoughlin M, Ehleiter D, Michaeli J, Vlodavsky I, et al. Protein kinase C mediates basic fibroblast growth factor protection of endothelial cells against radiation-induced apoptosis. Cancer Res 1994; 54:2591–7.
- 199. Yu Q J, Tao H, Wang X, Li MC. Targeting brain microvascular endothelial cells: a therapeutic approach to neuroprotection against stroke. Neural Regen Res 2015; 10:1882–91.
- 200. Qiu J, Hirschi KK. Endothelial cell development and its application to regenerative medicine. Circ Res 2019; 125:489– 501.
- 201. Zhou Y, Shao A, Xu W, Wu H, Deng Y. Advance of stem cell treatment for traumatic brain injury. Frontiers Cell Neurosci 2019; 13:301–10.
- 202. Bonsack B, Heyck M, Kingsbury C, Cozene B, Sadanandan N, Lee JY, et al. Fast-tracking regenerative medicine for traumatic brain injury. Neural Regen Res 2020; 15:1179–90.
- 203. Tesauro M, Schinzari F, Caramanti M, Lauro R, Cardillo C. Metabolic and cardiovascular effects of ghrelin. Int J Pept 2010; 2010:864342.

- 204. Lopez NE, Gaston L, Lopez KR, Coimbra RC, Hageny A, Putnam J, et al. Early ghrelin treatment attenuates disruption of the blood brain barrier and apoptosis after traumatic brain injury through a UCP-2 mechanism. Brain Res 2012; 1489:140–8.
- 205. Katare R, Rawal S, Munasinghe P, Tsuchimochi H, Inagaki T, Fujii Y, et al. Ghrelin promotes functional angiogenesis in a mouse model of critical limb ischemia through activation of proangiogenic microRNAs. Endocrinology 2016; 157:432–45.
- 206. Spencer SJ, Miller AA, Andrews ZB. The role of ghrelin in neuroprotection after ischemic brain injury. Brain Sci 2013; 3:344–59.
- 207. Chung H, Seo S, Moon M, Park S. Phosphatidylinositol-3-kinase/ Akt/glycogen synthase kinase-3 beta and ERK1/2 pathways mediate protective effects of acylated and unacylated ghrelin against oxygen-glucose deprivation-induced apoptosis in primary rat cortical neuronal cells. J Endocrinol 2008; 198:511–21.
- 208. Baldanzi G, Filigheddu N, Cutrupi S, Catapano F, Bonissoni S,

Fubini A, et al. Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. J Cell Biol 2002; 159:1029–37.

- 209. Shah KG, Wu R, Jacob A, Blau SA, Ji Y, Dong W, et al. Human ghrelin ameliorates organ injury and improves survival after radiation injury combined with severe sepsis. Mol Med 2009; 15:407–14.
- 210. Kiang JG, Smith JT, Cannon G, Anderson MN, Ho C, Zhai M, et al. Ghrelin, a novel therapy, corrects cytokine and NF-kB-AKT-MAPK network and mitigates intestinal injury induced by combined radiation and skin-wound trauma. Cell Biosci 2020; 10:63.
- 211. Kiang JG, Zhai M, Lin B, Smith JT, Anderson MN, Jiang S. Cotherapy of pegylated-G-CSF and ghrelin for enhancing survival after exposure to lethal radiation. Front Pharmacol 2021; 2021:628018.