FORUM EDITORIAL

Ionizing Radiation-Induced Responses: Where Free Radical Chemistry Meets Redox Biology and Medicine

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Abstract

The biological effects of ionizing radiation (IR) from environmental, medical, and man-made sources, as well as from space exploration are of broad health concern. During the last 40 years it has become evident that, in addition to short-lived free radical-mediated events initiated within microseconds of exposure and generally thought to dissipate within milliseconds, IR-induced production of reactive oxygen and nitrogen species as well as changes in redox signaling linked to disruption of metabolic processes persist long after radiation exposure. Furthermore, persistent IR-induced increases in the metabolic production of reactive oxygen and nitrogen species appear to significantly contribute to the delayed effects of IR exposure, including induction of adaptive responses at low doses as well as carcinogenesis, fibrosis, inflammation, genomic instability, and acceleration of the onset of degenerative tissue injury processes associated with aging. The ability to identify the specific metabolic mechanisms and dose–response relationships that contribute to adaptive responses as well as persistent IR-induced injury processes holds great promise for identifying novel strategies to mitigate the deleterious effects of IR exposure as well as for gathering mechanistic information critical for risk assessment. This Forum contains original and review articles authored by experts in the field of radiobiology focusing on novel mechanisms involving redox biology and metabolism that significantly contribute to the persistent biological effects seen following IR exposure. *Antioxid. Redox Signal.* 20, 1407–1409.

Introduction

DURING THE 50 YEARS following the discovery by Patt in 1949 that thiol-containing free radical scavengers were capable of providing significant protection from ionizing radiation (IR)-induced injury and cell killing (7), a great deal of research has focused on identifying the biomolecules that represent the critical targets of free radical damage initiated by IR exposure (1, 3). This work led to the predominant hypothesis of mammalian radiobiology in the last half of the 20th century, that IR-induced free radical-mediated DNA damage to cells that had the potential to divide and maintain tissue structure and function by self-renewal, represented the most critical target governing the ability of mammals to survive moderate (1–10 Gy) doses of radiation exposure (1, 3).

These studies found that postmitotic mammalian tissues were generally more radioresistant than tissues that required self-renewal, but again the critical targets were presumed to be the biomolecules necessary for maintaining structure and function. At very high IR doses (>40 Gy), the oxidation of lipids and membrane structure/function was thought to be the critical target for rapid onset death processes. At more moderate doses (10–40 Gy), the genetic material coding for the proper turnover of functional proteins in postmitotic cells as well as the ability of supporting cells in postmitotic tissues to rejuvenate stromal cells were thought to be the critical targets (3).

In addition, central to the study of radiobiology was the concept that IR-induced free radical-mediated events were primarily responsible for IR-induced injury (1, 3). This is thought to occur either directly by interaction of IR with critical organic biomolecules (designated RH) resulting in

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the ejection of electrons leading to the formation of an organic-free radical ($R^{\bullet}$) or indirectly by the ionization of H_2O molecules leading to the formation of hydroxy radicals ($\bullet OH$) in close enough proximity to abstract an $H^{\bullet}$ from a critical biomolecule again forming $R^{\bullet}$. In either circumstance, the free radicals formed following radiation were thought to be either chemically scavenged (or repaired) by thiol-free radical scavengers (such as GSH or cysteine) to be restored to their original structure (RH or H_2O), or if O_2 were present, $R^{\bullet}$ could react to form $ROO^{\bullet}$, which was thought to fix the damage in a relatively unrepairable condition. This free radical chemistry was believed to explain the dramatic effect of O_2 on the enhancement of IR-induced tissue injury that was universally noted (1, 3, 9).

In the last quarter of the 20th century following the discovery of the superoxide dismutase enzymes by McCord and Fridovich (4), the scientific community began to accept the fundamental principle that many of the same free radicals that were formed as a result of IR interacting with biological material were also formed as by-products of oxidative metabolism [reviewed in Spitz *et al.* (9)]. In 1976, Oberley *et al.*, Petkau *et al.*, and Misra and Fridovich independently reported that manipulation of superoxide dismutase activity both before and after IR exposure could protect from the biological effects of IR in bacterial and mammalian model systems (5, 6, 8). These discoveries led to the proposal that the cellular biochemical machinery responsible for the metabolic production of free radicals and other reactive oxygen and nitrogen species derived from superoxide and nitric oxide could remain perturbed for minutes, hours, days, and even years after exposure to IR [reviewed in Spitz *et al.* (9)]. Furthermore, it has also become clear that these persistent IR-induced increases in reactive oxygen and nitrogen species contribute significantly to the delayed effects of IR exposure, including carcinogenesis, fibrosis, inflammation, genomic instability, and the acceleration of degenerative tissue injury processes associated with aging [reviewed in Spitz *et al.* (9)]. In addition to these observations, the induction of adaptive responses following low doses of radiation has been shown to render cells and tissues resistant to subsequent exposures to higher doses of radiation by superoxide dismutase dependent mechanisms (2). Understanding the specific biochemical mechanisms and reactive species formed from metabolism following radiation as well as how these processes can be manipulated, holds great promise for identifying novel strategies to enhance therapeutic responses to radiation, mitigate the deleterious effects of radiation, as well as gather mechanistic information critical for radiation risk assessment. The current Forum includes three original scientific articles as well as four review articles focused on the development of tools and strategies for unraveling the specific mechanisms involved in manipulating radiation responses by targeting redox metabolism following exposure to IR.

In the original article from Pathak *et al.*, the authors report the construction and characterization of a novel, Cre-Lox-driven, transgenic mouse model that overexpresses the inhibitory GTP cyclohydrolase I feedback regulatory protein (GFRP). This inhibits the synthesis of the cofactor, 5,6,7,8-tetrahydrobiopterin (BH4), thought to be involved in several redox regulatory processes, including the NO synthase activity and NADPH oxidase activity (this Forum). These authors find that compared with control littermates, GFRP transgenic mice exhibited reduced BH4 levels as well as low levels of

glutathione and differential mitochondrial bioenergetic profiles. After whole body radiation, GFRP transgenic mice also showed decreased BH4/7,8-dihydrobiopterin ratios, increased vascular oxidative stress, and reduced white blood cell counts compared with controls. This novel mouse model demonstrates the mechanistic involvement of BH4-dependent metabolic processes in acute radiation responses and shows great promise for providing a powerful tool for studying the role of BH4 in both whole body and tissue-specific radiation responses.

In the original article from Coleman *et al.*, the authors report that acute radiation-induced liver injury and perturbations in mitochondrial oxidative metabolism seen in mice lacking the mitochondrial protein deacetylase, Sirtuin 3, are mediated by the superoxide anion (this Forum). The authors go on to show that parameters of radiation-induced injury were significantly attenuated by intraperitoneal injection of the highly specific superoxide dismutase mimetic, GC4401. This work provides strong evidence for the causal role of superoxide from mitochondrial metabolism in acute IR-induced liver injury and suggests that GC4401 could be used as a radioprotective compound *in vivo*.

In the original article from Tseng *et al.*, the authors report that exposure to low doses of charged particle irradiation relevant to space travel could induce oxidative stress in neural stem and precursor cells as well as significantly impair novel object recognition in mice 2 and 12 weeks following exposure (this Forum). These data provide evidence that acute exposure of neural stem cells and the intact central nervous system to mission relevant doses and influences of charged particle space radiations can elicit persistent metabolic oxidative stress lasting weeks to months that is associated with impaired cognition. These data support the hypothesis that astronauts subjected to radiations during space travel may be at heightened risk for experiencing performance decrements as well as long-term neurocognitive impairment.

In the Forum review from Alexandrou and Li, the authors explore the mechanisms involved with low dose ionizing radiation (LDIR)-induced adaptive responses and the cross talk between DNA damage and cellular metabolism (this Forum). They focus on the role of cell cycle regulators, including Cyclin D1/CDK4 and Cyclin B1/CDK1 complexes that are actively involved in the regulation of mitochondrial functions via phosphorylation of mitochondrial target proteins. They propose that the mechanistic regulation of LDIR-mediated mitochondrial activity via Cyclin B1/CDK1 is an important network that represents a target for therapeutic interventions aimed at reducing radiation injury and cancer risk.

In the Forum review from Shao *et al.*, the authors explore the mechanisms involved with understanding the redox regulatory mechanisms by which radiation causes hematopoietic stem cell (HSC) injury (this Forum). They focus on the induction of HSC apoptosis via the p53-Puma pathway; promotion of HSC differentiation via activation of the G-CSF/Stat3/BATF-dependent checkpoint; induction of HSC senescence via reactive oxygen species and p38; and mechanisms governing radiation-induced damage to the HSC microenvironment. The authors then use these mechanistic insights to propose novel strategies to prevent and/or mitigate IR-induced bone marrow suppression.

In the Forum review from Miao *et al.*, the authors explore the mechanisms involved with understanding the inter-

relationships between IR-induced oxidative stress and proinflammatory mediators in prostate cancer development and treatment (this Forum). The focus of the review is on the role of interleukin (IL)-6, IL-8, tumor necrosis factor- α , and transforming growth factor- β in different biological consequences ranging from cell death to the development of radioresistance. The authors propose that cancer cells, relative to normal cells, exist in condition of greater metabolic oxidative stress and secrete more proinflammatory mediators. They go on to propose that this biochemical imbalance in cancer cells, relative to normal cells, can be exploited for the purpose of developing new cancer therapies that selectively activate cell death pathways in cancer cells with minimal side effects on normal tissues.

In the final Forum review from Li *et al.*, the authors review the redox mechanisms involved in both targeted and non-targeted bystander effects that are relevant to exposure to high atomic number and high energy (HZE) particles encountered during space travel (this Forum). The significance of secondary radiation generated from interaction of the primary particles with biological material as well as the mitigating effects of antioxidants on various cellular injuries is discussed. The authors propose that a clear elucidation of the redox mechanisms underlying the cellular responses to HZE particles will be useful in reducing the uncertainty associated with current models for predicting long-term health risks of space radiation as well as understanding the inter-relationships between metabolic oxidative stress and proinflammatory mediators in cancer therapies using high linear energy transfer particle radiations.

Acknowledgments

This work was supported, in part, by grants from National Institutes of Health (CA182804 and CA133114) as well as the Department of Energy (DE-SC0000830) to D.S., and by National Institutes of Health (AI67798 and CA71382) and the Veterans Administration to M.H.-J.

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Date of first submission to ARS Central, December 16, 2013;
date of acceptance, December 19, 2013.

Abbreviations Used

BH4 = 5,6,7,8-tetrahydrobiopterin
GFRP = GTP cyclohydrolase I feedback regulatory protein
HSC = hematopoietic stem cell
IL = interleukin
IR = ionizing radiation
LDIR = low dose ionizing radiation