

Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: A unifying concept in stress response biology

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Summary

Exposure of eukaryotic cells to ionizing radiation (IR) results in the immediate formation of free radicals that last a matter of milliseconds. It has been assumed that the subsequent alterations in multiple intracellular processes following irradiation is due to the initial oxidative damage caused by these free radicals. However, it is becoming increasingly clear that intracellular metabolic oxidation/reduction (redox) reactions can be affected by this initial IR-induced free radical insult and may remain perturbed for minutes, hours, or days. It would seem logical that these cellular redox reactions might contribute to the activation of protective or damaging processes that could impact upon the damaging effects of IR. These processes include redox sensitive signaling pathways, transcription factor activation, gene expression, and metabolic activities that govern the formation of intracellular oxidants and reductants. The physiological manifestations of these radiation-induced alterations in redox sensitive processes have been suggested to contribute to adaptive responses, bystander effects, cell cycle perturbations, cytotoxicity, heat-induced radiosensitization, genomic instability, inflammation, and fibrosis. While a great deal is known about the molecular changes associated with the initial production of free radicals at the time of irradiation, the contribution of perturbations in redox sensitive metabolic processes to biological outcomes following exposure to IR is only recently becoming established. This review will focus on evidence supporting the concept that perturbations in intracellular metabolic oxidation/reduction reactions contribute to the biological effects of radiation exposure as well as new concepts emerging from the field of free radical biology that may be relevant to future studies in radiobiology.

I. Historical perspective

It has been known for many years that exposure to ionizing radiation (IR) results in the formation of free radicals in living systems that are believed to persist for milliseconds and result in oxidative damage to biomolecules such as DNA, proteins,

and lipids that contribute to the biological effects of radiation [1–5]. In the presence of O₂, the radiation-induced free radicals that are formed initially are thought to include hydroxyl radical (OH[•]), superoxide (O₂^{•-}), and organic radicals (R[•]) [1–3]. Immediately upon irradiation in the presence of O₂, reactions of these free radicals gives

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rise to other reactive oxygen species including hydrogen peroxide (H_2O_2) and organic hydroperoxides (ROOH). These hydroperoxide species together with redox active metal ions (such as Fe and Cu), commonly found in biological matrices, are also believed to contribute to radiation-induced oxidative damage via Fenton type reactions [3,4,6]. It has been hypothesized that the free radical-mediated covalent modifications resulting from oxidative damage to critical biomolecules during and immediately (within 5 milliseconds) following irradiation result in most, if not all, of the biological effects of ionizing radiation.

The most compelling evidence in favor of this hypothesis has come from observations that manipulations of antioxidants (i.e. thiols, superoxide dismutases, hydroxyl radical scavengers, and hydroperoxide metabolizing enzyme systems) at the time of irradiation appear to alter the reactions of free radicals (and reactive oxygen species) leading to alterations in oxidative damage as well as alterations in the biological effects of IR [3,4,6–15]. In addition, following irradiation, cells and tissues appear to respond by increasing the expression of cellular antioxidant defenses and this increased antioxidant capacity has been hypothesized to be at least partially responsible for radiation-induced adaptive responses [16–20]. Taken together, this growing body of evidence clearly points to a causal relationship between radiation-induced free radical production at the time of irradiation and the long-term biological responses seen following exposure.

II. Metabolic oxidative stress

For many years it has been known that metabolism in mammalian cells primarily derives energy from the tightly controlled biochemical oxidation of substrates (i.e. carbohydrates, fats, and amino acids) to obtain reducing equivalents (electrons) necessary for mitochondrial electron transport chain mediated oxidative phosphorylation to produce ATP, with O_2 acting as the terminal electron acceptor [21,22]. In addition, reducing equivalents in the form of NADH or NADPH are essential for many cellular biosynthetic processes involved in replication, cell division, and macromolecular synthesis [22]. Therefore, the ability to

extract, store, and move electrons through complex biological structures via a series of biochemical oxidation/reduction (redox) reactions involving protein catalysts has been hypothesized to provide the essential 'life force' for maintaining metabolic homeostasis in mammalian cells [23].

This metabolic strategy, while extremely efficient, does lead to the formation of reactive oxygen species (ROS) as byproducts. Ideally the four-electron reduction of O_2 to form H_2O in Complex IV of the mitochondrial electron transport chain allows for the efficient removal of electrons from the system. The flow of electrons, through Complexes I–IV, pumps protons across the inner mitochondrial membrane creating a proton gradient that is utilized to drive ATP production by Complex V. However, there is the probability that 1-electron reductions of O_2 to form $\text{O}_2^{\cdot-}$ will occur proximal to Complex IV, probably at Complex I (NADH-dehydrogenase) or Complex III (ubiquinone-cytochrome b) of the electron transport chain [24]. Superoxide then rapidly dismutates to form H_2O_2 either spontaneously or through the catalytic function of superoxide dismutases [24,25]. It is estimated during normal respiration in mammalian mitochondria that as much as 1% of O_2 consumption could result in $\text{O}_2^{\cdot-}$ and H_2O_2 formation [24]. In addition to mitochondrial production of ROS, there are many cellular enzymes involved in wide variety of oxidative metabolic processes that also have the potential to generate ROS including NADPH oxidase enzymes, myeloperoxidase, xanthine oxidase, amino acid oxidases, cytochrome P450 enzymes, and several peroxisomal enzymes including flavoprotein dehydrogenase enzymes involved in β -oxidation of fatty acids and glycolate oxidase [25].

In complex living systems, metabolic production of $\text{O}_2^{\cdot-}$ and H_2O_2 would be expected to result in OH^{\cdot} formation via the transition metal catalyzed Haber–Weiss reaction [25]. In the presence of O_2 , OH^{\cdot} would be expected to abstract hydrogen atoms from critical biomolecules leading to the formation of R^{\cdot} and ROOH, resulting in oxidative damage. In the presence of the aforementioned highly reactive species, lipid peroxidation chain reactions would also be expected to increase [25–27]. Since lipid peroxidation is a self propagating process [25–27] that results in the formation of

diffusible cytotoxic byproducts (i.e. lipid hydroperoxides, epoxides, endoperoxides, and lipid aldehydes), it could dramatically amplify the initial number of reactive species produced by metabolic oxidative stress as well as the diffusion distance of these species [25–27]. In this fashion, the metabolic production of O_2^- and H_2O_2 in living systems could result in a spectrum of free radical reactions and oxidative damage similar (although at a much lower rate and in different cellular compartments) to that postulated for ionizing radiation. This concept has been invoked in the development of the Free Radical Theory of Oxygen Toxicity [28,29], the Free Radical Theory of Aging [30,31], and the Free Radical Theory of Cancer [32,33]. If free radical damage derived from metabolism were responsible for the pathological changes seen during aging, this theoretical construct might also explain why many of the same biological phenomena seen to increase as a function of aging (i.e. carcinogenesis, atherosclerosis, inflammatory diseases, fibrotic changes in normal tissues) are also induced by exposure to ionizing radiation.

The production of potentially deleterious prooxidants by oxidative metabolism is held in check by a complex and interdependent system of antioxidants in order to maintain the intracellular environment in a highly reduced steady state that is believed to fluctuate between redox potentials about -200 mV to -240 mV [34]. These antioxidants include lipid- and water-soluble small molecular weight dietary antioxidants (i.e. vitamin E, vitamin C, lipoic acid, β -carotene, etc.), small molecular weight peptides and cofactors, (i.e. glutathione, NADPH, pyruvate, thioredoxin, glutaredoxin, etc.) and antioxidant enzymes (i.e. catalase, superoxide dismutases, glutathione peroxidases, peroxiredoxins, glutathione transferases, etc.) [25,35]. When prooxidant production begins to exceed the ability of the cellular antioxidant capacity to maintain the normal steady state redox potential, a condition of oxidative stress exists [35]. If oxidative stress persists for prolonged periods, oxidative damage will accumulate in biomolecules. If the repair of oxidative damage cannot compensate for the increased production of oxidatively damaged biomolecules then injury, mutagenesis, carcinogenesis, accelerated senescence, and cell death can occur.

Metabolic oxidative stress and the subsequent deleterious consequences are amenable to intervention and this fact has generated a great deal of interest in the biomedical research community. When a cell 'senses' (see section below for details of redox sensitive signaling) shifts in metabolic oxidation-reduction reactions, it can respond by: (1) shifting metabolism away from pathways resulting in reactive species formation, (2) increasing metabolic antioxidant capacity, and (3) increasing pathways that repair oxidative damage to critical biomolecules. This can be accomplished immediately by transient activation or inactivation of redox sensitive proteins regulating these pathways and by the induction of gene expression pathways responsible for synthesizing new proteins. These pathways might also be manipulated via exogenous addition of: (1) small molecular weight dietary antioxidants and/or synthetic antioxidant enzymes, (2) substrates for the regeneration of reducing equivalents necessary for antioxidant pathways, and (3) genetic manipulations to enforce over expression of antioxidant enzymes can be accomplished to limit the effects of metabolic oxidative stress. In this regard, manipulations of pathways governing metabolic oxidative stress might also be utilized to decrease antioxidant capacity and/or increase prooxidant production to improve the cytotoxic response of cancer cells to therapeutic intervention. These various strategies are currently under investigation for a wide variety of degenerative diseases associated with aging, cancer, and inflammatory diseases thought to involve metabolic oxidative stress [36–42].

III. Metabolic oxidation/reduction reactions and cellular communication

Reactive species formed via metabolic activity can also act as second messengers in cell signaling cascades leading to shifts in gene expression patterns [43–49]. It is thought that this type of redox regulated signaling could function to coordinately regulate the availability of metabolic substrates with the orderly transition of cellular functions [33,50–56]. In general protein kinases are activated by oxidation reactions, while protein phosphatases and zinc finger proteins are inacti-

vated by oxidation reactions and transcription factor binding is increased by reduction reactions [43–60]. Redox sensitive changes in signaling proteins are for the most part reversible (in keeping with their proposed regulatory role). This property allows for the binary type ‘on-off’ switching believed to be necessary for the constant readjustment of metabolic activity to match changes in cellular function necessary to maintain a viable non-equilibrium steady state in living systems.

Typically the changes in the steady state intracellular redox environment that occur during normal fluctuations in metabolism or environmental stress are sensed by the movement of electrons to and from redox sensitive moieties (i.e. redox sensitive thiol residues and metal ions) on proteins involved with the initiation of signal transduction. These redox changes on specific proteins can result in altered conformation and/or activity that can initiate signaling cascades resulting in altered binding affinity, functional activity, and/or gene expression. The concept that subtle changes in redox sensitive moieties alters protein function seems logical given that most of the energy and reducing equivalents necessary for maintenance of homeostasis and responses to stress are derived from metabolic oxidation/reduction reactions. In this way subtle alterations in electron flow through redox sensitive signaling circuitry, within and between proteins, could provide a rapidly responsive, freely reversible, and direct mechanism to coordinately couple metabolic activity and gene expression processes during transitions between different non-equilibrium steady states necessary to maintain living systems.

ROS or reactive nitrogen species (RNS) derived from nitric oxide (NO), another biologically relevant free radical produced enzymatically by NO-synthases [61,62], could be essential mediators of this type of redox regulation. Similar to O_2^- , and H_2O_2 , NO is diffusible and levels of its production are intimately linked to the levels of metabolic activity. The diffusible nature of these species coupled with their reactivity make them logical candidates to initiate redox regulatory cascades allowing for communication between different cellular compartments. In this way, changes in electron flux through metabolic pathways in one cellular compartment could lead to

changes in the local flux of reactive species that could be sensed by redox sensitive signaling molecules, leading to alterations in gene expression pathways that provide the newly synthesized proteins necessary to transition between different non-equilibrium steady states.

IV. Metabolic oxidation/reduction reactions and cellular radiation response

Clearly, radiation-induced production of free radicals/ROS during the time of exposure contributes to oxidative damage as well as radiation response. Scavenging the reactive species produced at the time of irradiation with antioxidants can mitigate some of the effects of IR-induced injury; but this is clearly limited by the accessibility of the antioxidants to the site of IR-induced free radical production as well as the rate constants of the reactive species interacting with critical biomolecules. However, it is also becoming clear that if radiation damage includes disruption of critical biomolecules governing the metabolic production of prooxidant species, metabolic oxidative stress can also contribute to the biological effects of IR long after the time of exposure. As a result of this new concept and the historical understanding of how free radical production contributes to IR-induced damage, a new model of how metabolic oxidation/reduction reactions contribute to IR effects following exposure can now be considered.

Two pioneering studies implicating oxidative stress in IR response were done by Oberley's and Petkau's groups. Oberley's group [3] found that modifying intracellular or extracellular SOD activity in bacteria reduced the oxygen enhancement ratio implying that superoxide was at least partially responsible for the mechanisms leading to the well-known effects of oxygen on radiosensitization. In addition, Petkau et al. [7] were the first to show that IV injection of 35 $\mu\text{g/g}$ body weight CuZn SOD, 2 and 4 h following 6 or 8 Gy whole body x-irradiation, significantly protected Swiss mice from lethality at 30 days. These studies clearly showed that alterations in a metabolic enzyme with superoxide scavenging capability following radiation could result in radioprotection and implied that superoxide production (from some source) following IR was participating in

radiation-induced injury. These types of studies have continued with more recent observations that active SOD enzymes, SOD mimetic compounds and in some instances catalase can lead to inhibition of the deleterious effects induced by IR in a wide variety of *in vitro* and *in vivo* studies ranging from transformation assays, bystander effects, normal tissue damage associated with inflammatory responses, and fibrosis [12–14,18–20,63–69]. Furthermore recent studies using measurements of ROS and RNS derived from NO have also discovered that radiation exposure can induce increases in the metabolic production of these species for several minutes and hours post irradiation [70–72]. These same authors provided evidence that the source of this increase in radiation-induced prooxidant production could involve mitochondria and may be related to radiation-induced alterations in MAP kinase activation [70–72]. Finally, targeted irradiation of the cytoplasm, where many metabolic redox reactions occur, has been shown to induce mutations in nuclear DNA and manipulations of free radical scavenging capability will modify radiation-induced nuclear DNA damage under these conditions [73]. These results support the hypothesis that radiation-induced damage to cytoplasmic metabolic pathways that result in free radical production can contribute to heritable changes in the nuclear genome. The growing number of these types of findings seems to provide strong support for the hypothesis that metabolic sources of O_2^- and H_2O_2 (and possibly NO) contribute to radiation effects following exposure.

The specific metabolic reactions responsible for alterations in ROS (or RNS) production following radiation exposure is an area of considerably more controversy and appears to vary between model systems indicating that multiple different pathways are probably involved. In general, the proposed metabolic sources of prooxidant production fall into two categories that include components of mitochondrial electron transport chains [24,74] and oxidoreductase enzymes [25]. The activity of different metabolic pathways contributing to formation of these reactive species could be impacted upon by hundreds and perhaps thousands of different radiation-induced perturbations in structure, function, and gene expression pathways (i.e. mutations, direct damage, effects on

substrate availability, activity changes, etc.). Therefore, the target size for causing radiation-induced metabolic oxidative stress is very large which fulfills one requirement for explaining the self-perpetuating process of radiation-induced genomic instability [75].

V. Genomic instability and metabolic oxidative stress

It is well established that chronic metabolic oxidative stress can induce genomic instability and it has been suggested that mitochondria may participate in this process [33]. The major mitochondrial ETC complexes proposed to be involved with prooxidant production that are currently under investigation include Complex I (or NADH dehydrogenase), Complex II (succinate dehydrogenase), and Complex III (ubiquinone-cytochrome c oxidoreductase) [24,74]. The probability that univalent reductions of O_2 to form O_2^- will occur at any of these complexes during mitochondrial ETC activity would be expected to be governed by the residence time of the electrons on subunits in the electron transport chains (ETCs) that are accessible to O_2 , as well as the rate constants for the forward and reverse reactions of the reduced electron transport chain components with O_2 [76]. Mitochondrial ETC Complexes I–V are made up of 89 discrete protein subunits, 13 of which are coded for in mitochondrial DNA (mtDNA) and 76 of which are coded for in genomic DNA in the nucleus (nuDNA) [77]. A complex series of gene expression and protein biosynthetic processes must be coordinately regulated to result in the proper stoichiometric assembly of these complexes in the inner mitochondrial membrane [77]. It is possible that mtDNA and/or nuDNA coding for mitochondrial ETC subunits as well as the biochemical machinery necessary for the proper expression and assembly of ETC proteins is a primary target of oxidative damage during radiation exposure. In fact this possibility has already been suggested for oxidative damage during the aging process [78–80]. If radiation exposure induces disruptions in the proper assembly and/or function of mitochondrial ETCs (either by causing mutations or directly altering the protein structure/activity), an increase in residence time and/or accessibility of reduced

components of the ETCs to O_2 could result in an increase in the probability of one-electron reductions of O_2 to form $O_2^{\cdot-}$ and H_2O_2 . The resulting increased fluxes of $O_2^{\cdot-}$ and H_2O_2 could then lead to a condition of metabolic oxidative stress that could continue to cause further oxidative damage to critical biological structures long after radiation exposure. If radiation-induced damage (and/or damage caused by metabolic oxidative stress), resulted in mutations to either mtDNA and/or nuDNA coding for genes required for the proper assembly of ETCs (or other prooxidant producing enzyme systems), this aberrant condition of metabolic oxidative stress could also become a heritable trait. Therefore, this mechanism could potentially contribute to radiation-induced genomic instability that persists for many cell generations as well as in the progeny from irradiated animals.

In support of this hypothesis, persistent radiation-induced genomic instability, alterations in growth, and signaling have already been reported in cultured cells, bone marrow cells from irradiated animals, and the offspring of irradiated animals (reviewed in Morgan [75]). Also chronic exposure to either exogenously applied H_2O_2 or endogenous generation of ROS induced by hyperoxia has been shown to induce genomic instability and gene amplification [81]. Furthermore genomically unstable cells in culture (as well as bone marrow cells from irradiated animals) have also been shown to demonstrate alterations in parameters indicative of oxidative stress as well as mitochondrial dysfunction [82,83]. Future studies using these model systems should be able to rigorously address the involvement of metabolic oxidative stress resulting from damage to mitochondrial (or enzymatic) oxidative metabolism in radiation-induced genomic instability.

VI. Radiation-induced alterations in metabolic oxidation/reduction reactions and signal transduction

One of the most well characterized metabolic alterations observed immediately following IR exposure (and other oxidants) is increased Pentose Cycle activity leading to the regeneration of NADPH from $NADP^+$ coupled to the oxidation

of glucose-6-phosphate catalyzed by glucose-6-phosphate dehydrogenase (G6PD) [11,84]. Pentose Cycle induction following oxidative stress and/or IR is thought to provide reducing equivalents, in the form of NADPH, necessary for biosynthetic and repair processes. NADPH may also play a role as a cofactor in the reduction of hydroperoxides via the action of glutathione peroxidases and peroxiredoxins [11,45]. In this regard radiation and oxidative stress induced increases in glucose metabolism through the Pentose Cycle are thought to represent an immediate metabolic response to stress in an attempt to offset increases in oxidative damage with increases in protective and reparative processes.

Immediately following IR exposure, several signal transduction pathways (i.e. ERK1/2, JNK, p38, ATM, etc.) as well as transcription factors (i.e. AP1, NF- κ B, GADD153, p53, etc.) are activated resulting in the transcription of downstream genes thought to be involved in the radiation response [20,69–72,85–90]. The exact mechanisms responsible for sensing radiation induced free radical production leading to the activation of these signaling and gene expression pathways are not clearly understood. However, given that many of the aforementioned signaling and gene expression pathways have been reported to be sensitive to changes in intracellular oxidation/reduction reactions [20,57,69–72,85–90], it is tempting to hypothesize that radiation-induced changes in intracellular metabolic redox reactions could be initiating events leading to activation of signal transduction, transcription factors, and gene expression.

One of the potential links between IR-induced changes in oxidative metabolism and activation of signaling leading to alterations in transcription factor activation, is the Pentose Cycle. This would be expected, since NADPH is the source of electrons for the reduction of thioredoxin, which in the reduced form, is known to be involved with transcription factor activation [43–45,87,89]. In an elegant series of experiments, it was shown that thioredoxin reductase appears to pass electrons to thioredoxin, resulting in thioredoxin nuclear translocation and a subsequent interaction with redox factor-1 leading to the activation of the AP1 transcription factor [87,89]. It is tempting to speculate that radiation-induced

increases in Pentose Cycle activity may alter the availability of NADPH to donate electrons to thioredoxin reductase leading to the initiation of this signaling cascade. However, no data directly supporting this hypothesis is currently available. Since thioredoxin has been implicated in many different transcription factor activation pathways, this mechanism may also contribute to other radiation induced effects in addition to AP1 activation.

Another possible way that radiation-induced changes in Pentose Cycle activity may be linked to radiation-induced signal transduction and bystander effects is via the activity of NADPH oxidase enzymes. NADPH oxidases are flavin containing enzymes that have several different subunits and are known to be broadly expressed in both phagocytic cells associated with inflammatory responses, as well as non-phagocytic normal cells including fibroblasts and smooth muscle cells [91–96]. NADPH oxidases are also thought to be involved with signaling pathways leading to proliferation as well as fibrogenic responses [91–96]. NADPH oxidase enzymes take electrons from NADPH and produce O_2^- and H_2O_2 . Since the NADPH oxidase enzymes are dependent on NADPH to form ROS, changes in Pentose Cycle activity could potentially impact upon the levels of ROS produced by these enzymes by affecting substrate availability. Exposure of smooth muscle cells and fibroblasts to H_2O_2 -mediated oxidative stress is known to lead to increases in non-phagocytic NADPH oxidase activity [92] that contributes to H_2O_2 -induced cell injury [92]. In addition, several studies have suggested that inhibitors of flavin containing enzymes such as NADPH oxidase are capable of inhibiting radiation-induced signal transduction and injury in cells adjacent to irradiated cells ([69], reviewed in Azzam et al. [97]) suggesting that these enzymes may be involved in radiation-induced bystander effects. NADPH oxidase enzymes are also believed to be mediators of inflammatory injury, mutagenesis, growth disturbances, and transformation in a wide variety of biologically relevant pathologies including tumor promotion and vascular injury as well as normal cell signaling [91–97]. As such, they represent excellent candidates for studies of radiation-induced effects attributable to metabolic oxidative stress [91–97].

VII. Metabolic oxidative stress and radiation-induced clastogenic factors

Clastogenic factors are secreted by injured cells and are capable of diffusing significant distances causing genotoxic damage in cells that were never exposed to the original stressing agent [98]. It has been known for many years that radiation exposure is one of the agents capable of causing the release of clastogenic factors (reviewed in Morgan [75]). While the precise identity of radiation-induced clastogenic factors are not known, active agents found in preparations of clastogenic factors isolated following exposure to other insults are thought to include inosine triphosphate, lipid peroxidation products, and cytokines such as tumor necrosis factor ([98], TNF). The cell injury mediated by clastogenic factors is also inhibited by SOD, implicating superoxide in the genotoxic effects [98]. Furthermore, it has been shown that TNF is capable of inducing metabolic oxidative stress in target cells as well as inducing a superoxide-scavenging enzyme located in mitochondria (MnSOD, [99]). In addition over expression of MnSOD renders cells resistant to TNF [99], again implicating mitochondrial superoxide production in the biological effects of this cytokine.

Since radiation exposure and metabolic oxidative stress are capable of initiating lipid peroxidation reactions as well as cytokine release [100–102], it is tempting to hypothesize that these agents may be involved in radiation-induced clastogenesis as well as bystander effects. Furthermore, if radiation induces persistent metabolic oxidative stress by one of the mechanisms discussed earlier resulting in the formation of clastogenic factors via a redox regulated process, these clastogens might be significant contributors to persistent genomic instability. From this theoretical construct, it seems clear that several phenomena involved in delayed radiation effects including inflammation, fibrosis, carcinogenesis, bystander effects, and genomic instability could involve metabolic oxidative stress and the subsequent formation of clastogenic factors. This would seem to be a fertile area for future studies, since the biological effects of clastogens have been reported to be amenable to manipulation by exogenous administration of antioxidants ([98], reviewed in Morgan [75]).

VIII. Metabolic oxidative stress and radiation-induced adaptive responses

For many years it has been observed that sub-lethal or slightly toxic doses of radiation or oxidative stress induce adaptive responses that render cells resistant to further treatment by a second more cytotoxic challenge with the same or similar damaging agent [103–108]. During the intervening time between the pretreatment and challenge doses, signal transduction is induced leading to alterations in gene expression of proteins governing metabolic processes that are believed to protect the biological system from the second challenge dose. In this regard, several enzymes thought to perform antioxidant functions, such as MnSOD, proteins involved in glutathione metabolism, and epoxide hydrolase [16–20,109,110] are induced following radiation, suggesting a role in radiation inducible adaptive responses [18–20,109]. Furthermore, as noted earlier, several studies have suggested that in some systems, SOD administration provides some measure of radiation protection against metabolic oxidative stress even after radiation exposure [7]. Recently it was confirmed that MnSOD is over expressed in radioresistant variants isolated from MCF-7 human carcinoma cells following exposure to fractionated ionizing, again supporting the concept that this mitochondrial superoxide scavenging enzyme might play a role in radiation-inducible adaptive responses [20].

The causal relationship between MnSOD and resistance was confirmed when radioresistance in cells exposed to fractionated IR was reduced following expression of antisense MnSOD [20]. These cells also over expressed a group of stress responsive signaling proteins including p21, Myc, 14-3-3 zeta, Cyclin A, Cyclin B1, and GADD153. Radiation-induced expression of these six genes was suppressed in fibroblasts from MnSOD knock-out mice (–/–) as well as in radioresistant cells that expressed antisense MnSOD. The essential role of NF- κ B in the adaptive response was demonstrated by inhibiting NF- κ B nuclear translocation using mutant I κ B and this manipulation also inhibited radioresistance as well as reducing steady state levels of MnSOD, 14-3-3 zeta, GADD153, Cyclin A and Cyclin B1 mRNA [20]. In contrast, mutant I κ B was unable to inhibit radioresistance or

reduce 14-3-3 zeta, GADD153, Cyclin A and Cyclin B1 mRNAs in cells where MnSOD over expression was independent of NF- κ B [20]. This study was the first to provide clear evidence that a redox sensitive transcription factor induced by radiation (NF- κ B) was capable of regulating the expression of MnSOD, that in turn was capable of increasing the expression of genes that participate in radiation-induced adaptive responses. These results also support the speculation that redox sensitive signal transduction and a specific gene expression profile following radiation may play a role in radiation-induced adaptive responses by rendering cells resistant to metabolic oxidative stress derived from mitochondrial metabolism.

IX. Concluding remarks

This review has presented some of the evidence supporting the concept that metabolic oxidative stress derived from mitochondrial and enzymatic sources of prooxidant production can contribute to the deleterious effects of IR as well as IR-induced signaling and adaptive responses. It would appear from the number and persistence of these reports that in the new millennium many discoveries will likely arise from the pursuit of studies to more clearly define (1) the precise sources of this radiation-induced metabolic oxidative stress, (2) the oxidants and reactive byproducts that are produced, (3) the relative contribution of these damaging agents to deleterious effects of radiation in animals and humans, and (4) the factors that govern the mitigation of these effects that are amenable to manipulation in clinical settings. It is also likely that much can be learned from the experiences of the free radical biology community that could potentially be applied to the study of radiation-induced metabolic oxidative stress and in turn, the information gained from the study of radiation effects on metabolic oxidative stress may also be relevant to the basic biology of aging.

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