

# Manipulations of Redox Metabolism for Enhancing Radiation Therapy Responses: A Historical Perspective and Novel Hypothesis



Since the discovery that free radical scavengers could protect against radiation induced injury in animals,<sup>1</sup> scientists studying radiation biology have been at the forefront of the field of redox biology and medicine. This seminal observation also led to the free radical theories of oxygen toxicity,<sup>2</sup> aging,<sup>3</sup> and cancer.<sup>4,5</sup> In addition, for the past 60 years, these theoretical constructs and resulting experimental data have also driven the development of several modern approaches for enhancing cancer therapy responses, while protecting normal tissues from radiochemotherapy. This work has also led to a paradigm shifting theoretical construct for understanding the relationship between oxidative metabolism and gene expression pathways that could be very useful moving forward for continuing to refine metabolic manipulations directed at improving radiochemotherapy.

## Radiation Biology and Redox Metabolism

For many years, it has been well recognized that the deposition of energy from ionizing radiation leads to the formation of free radicals directly on critical target molecules and well as indirectly from the radiolysis of H<sub>2</sub>O to form hydroxyl radical.<sup>6</sup> These free radicals are known to lead to covalent changes in critical biomolecules that significantly contribute to radiation-induced cell killing as well as transformation and normal tissue injury.<sup>6</sup> In the latter half of the 20th century, it has also become well-recognized that the initial free radical damage caused by ionizing radiation is accompanied by the induction of oxidative metabolic dysfunction that persists for many months and years following exposure, leading to normal tissue injury and genomic instability.<sup>6,7</sup> The recognition that these radiation-induced late normal tissue damage effects are indistinguishable from similar pathologies

induced during aging, has led to the proposition that the random stochastic free radical damage caused by ionizing radiation during cancer therapy may accelerate the onset of the persistent metabolic oxidative stress that has been associated with aging.<sup>6-8</sup>

## The “Horse and Cart” Model for Selectively Targeting Metabolism, Signal Transduction, and Gene Expression in Cancer Cells, While Protecting Normal Tissues

Albert Szent-Györgyi was one of the first scientists to propose that the life force necessary to maintain living systems was derived from easily oxidizable substrates providing for the movement of electrons from higher energy orbitals to lower energy orbitals releasing energy that could be harnessed by biological systems.<sup>9</sup> This theoretical construct together with the chemosmotic hypothesis proposed by Peter Mitchell<sup>10</sup> coupled to the discovery that mitochondria could produce superoxide (O<sub>2</sub><sup>•-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)<sup>11,12</sup> can now be unified with the free radical theories of cancer and aging<sup>6-8</sup> for the purpose of improving cancer therapy.

The “Horse (oxidative metabolism) and Cart (gene expression)” model provides a novel unified theoretical approach for understanding the fundamental regulation of mitochondrial oxidative metabolism, signal transduction, and gene expression in aging, cancer, and normal tissues injury (Fig. 1).<sup>8,13,14</sup> The essential oxidation/reduction (redox) metabolism which extracts, stores, and moves the electrons necessary for maintaining living cells occurs in the mitochondria/cytosol and can be considered the “horse” that is connected by redox sensitive signaling pathways to the “cart” representing the nuclear gene expression pathways (Fig. 1A). Given this model, it would be predicted that the mechanistic relationship between gene

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expression and glucose/O<sub>2</sub> metabolism would be based on the ability to extract electrons from food sources via metabolic oxidation/reduction reactions to generate ATP and reducing equivalents for biosynthesis (ie, NADH/NADPH), while consuming O<sub>2</sub> by 4 electron reductions to make H<sub>2</sub>O. The cellular proteins necessary to carry out these metabolic processes are regulated by the flow of ATP, reducing equivalents (in the form of NADH and NADPH), and reactive by-products of oxidative metabolism (ie, O<sub>2</sub><sup>•-</sup>, H<sub>2</sub>O<sub>2</sub>, organic hydroperoxides, aldehydes etc.) through redox sensitive signaling pathways to coordinately regulate the gene expression program required for maintaining cellular structure needed for normal function.

In normal cells, redox metabolism (horse) and gene expression (cart) are tightly coupled via nonequilibrium steady-state fluxes of reactive metabolic by-products and electron carriers through redox sensitive signal transduction pathways (Fig. 1A). In this way, the flow of electrons from reactions governing energy production and biosynthesis directly regulate gene expression pathways governing assembly of the protein and lipid structures necessary to maintain normal cellular functions and differentiation (Fig. 1A). When this nonequilibrium steady-state is disrupted, the reactive by-products of oxidative metabolism, such as O<sub>2</sub><sup>•-</sup>/H<sub>2</sub>O<sub>2</sub>, as well as reactive oxidative damage byproducts (ie, aldehydes and organic hydroperoxides), produced by the “horse,” can cause covalent damage in the genome, that if unrepaired or mis-repaired will lead to the gradual deterioration of the “cart” (Fig. 1B). The gradual deterioration of the “horse and cart” relationship can lead to dysfunction of cellular processes on multiple levels, due to stoichiometric mismatches between protein expression and the assembly of the mitochondrial/cytosolic metabolic machinery needed to maintain the efficiency of electron flows, leading to steadily increasing levels of reactive species produced as by-products of oxidative metabolism as a function of time. This increasing inefficiency in oxidative metabolism and increasing steady-state levels of reactive species can lead to the exponential deterioration of the genome during aging resulting in senescence and death (Fig. 1B). The increasing levels of reactive species and damage products that occur during aging are also accelerated by radiochemotherapy-induced damage and can be targeted (Fig. 1B, red X's) to inhibit normal tissue injury/degeneration during cancer therapy.

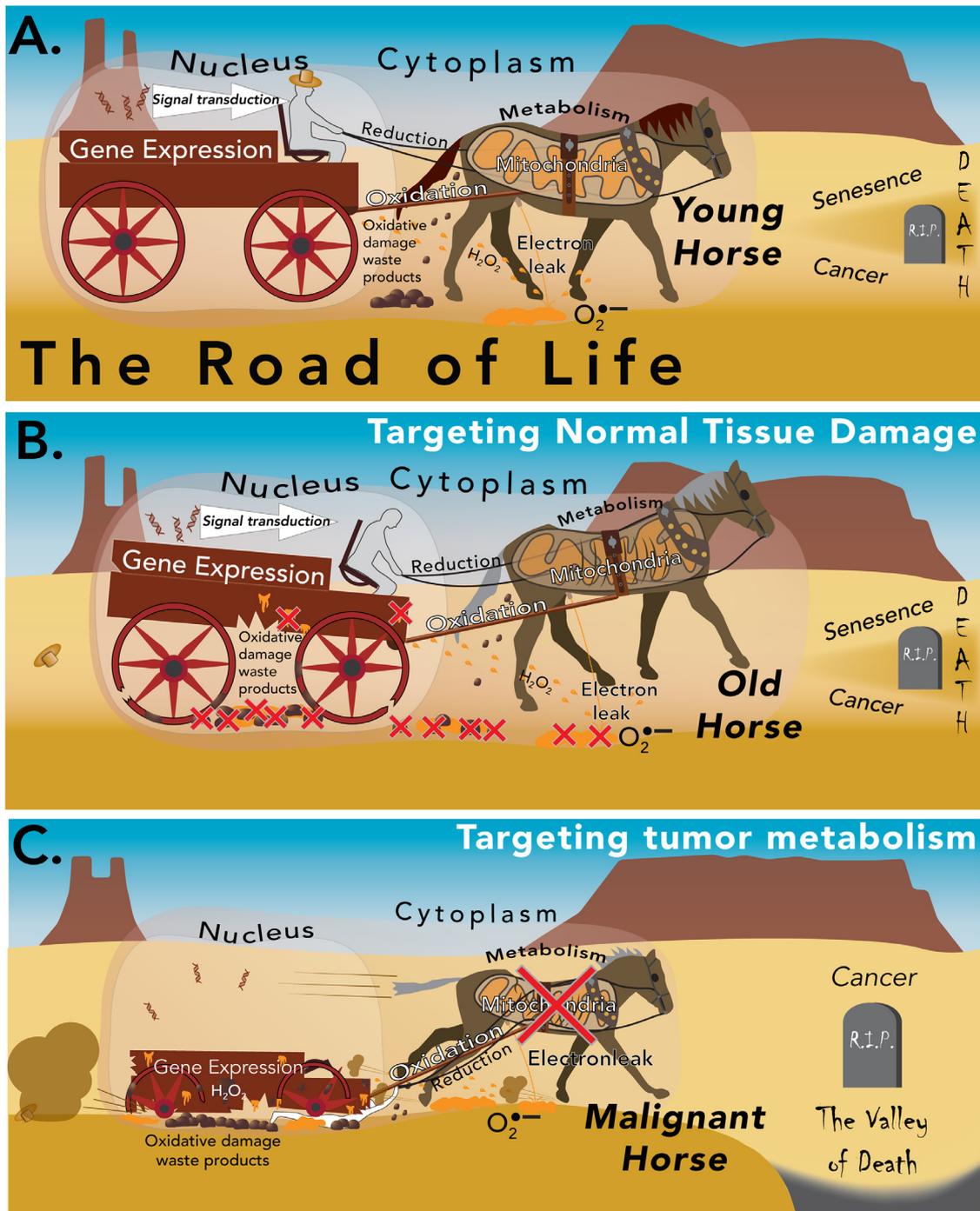
In this context 5 papers in this issue of seminars in radiation oncology address strategies being explored for targeting metabolism for the purpose of protecting against normal tissue responses, while enhancing tumor responses. The first of these papers by Schoenfeld et al. discusses the proposal to exploit pharmacological doses of ascorbate (given IV to blood levels of ~20 mM) to enhance metabolic oxidative stress in cancer tissues, while protecting normal tissues from radiation-induced injury.<sup>15</sup> The underlying mechanism for this approach is proposed to be the increased presence of redox active metal ions in cancer tissues making high levels of ascorbate selectively act as a pro-oxidant generating H<sub>2</sub>O<sub>2</sub> leading to cancer cell radiosensitization, while retaining antioxidant function in normal tissues protecting against

radiation injury. In the paper by Aykin-Burns et al. a similar approach to selectively protect normal tissues, while enhancing cancer cell therapeutic responses is proposed using vitamin E analogs where the differential redox biology of tocopherols is being exploited.<sup>16</sup> In a similar line of investigation the paper by Hillman explores the redox biology of soy isoflavones for inducing radiosensitization, while mitigating inflammatory responses leading to radiation-induced normal tissue injury.<sup>17</sup> In a final investigation along these same lines of inquiry, Mapuskar et al. discuss the utilization of small molecule superoxide dismutase mimics to inhibit radiation-induced normal tissue injury, while preserving cancer cell responses based on fundamental differences in oxidative metabolism leading to the formation of superoxide.<sup>18</sup>

## Cancer Biology and Cancer Therapy Responses

The proposed “horse and cart” model predicts that degenerative diseases associated with aging, including cancer development, arise from the disruption of metabolic homeostasis, where metabolic oxidation/reduction reactions are losing efficiency as a function of time and producing increasing levels of reactive by-products formed from 1-electron reductions of O<sub>2</sub> (electron leak) in mitochondrial electron transport chains. One electron reductions of O<sub>2</sub> lead to the formation of O<sub>2</sub><sup>•-</sup> and H<sub>2</sub>O<sub>2</sub>, which together with redox active labile metal ions (Fe<sup>+2</sup> and Cu<sup>+1</sup>) catalyze oxidation reactions on critical biomolecules leading to the formation of organic hydroperoxides, alkoxy/peroxy radicals, and aldehydes. This gradual dysregulation of metabolic oxidation reactions also disrupts the coordinated production of energy and reducing equivalents necessary for cell survival, proliferation, biosynthesis, and differentiated function that results in the acceleration of aging related pathologies including carcinogenesis. This model allows for the proposal that the flow of electrons during metabolism are both necessary for living systems as redox signaling molecules and detrimental in mediating damage to the genome causing increased metabolic inefficiency accelerating the processes of senescence, as well as onset of degenerative diseases associated with aging (Fig. 1B and C).

In the process of carcinogenesis, this model predicts that the cellular and metabolic dysregulation leading to malignant transformation is due to the age-related increases in steady-state levels of reactive oxygen species (ie, O<sub>2</sub><sup>•-</sup> and H<sub>2</sub>O<sub>2</sub>) and toxic products arising from free radical reactions that lead to immortalization of cells capable of cell division (possibly involving the induction of telomerase) as an adaptive response to the stress caused by the accumulation of oxidative damage to the genome. Once immortalization has occurred the transformed cell has adapted to the dysfunctional relationship between oxidative metabolism, signal transduction, and gene expression; but also exhibits significant genomic instability, loses the ability to perform differentiated function, loses control of cell proliferation, and



**Figure 1** The “horse and cart” model for understanding the fundamental relationship between mitochondrial oxidative metabolism, redox-regulated signal transduction, and gene expression in degenerative diseases associated with aging and cancer. (A) The essential redox metabolic processes occurring in the mitochondria and cytosol can be considered the “horse” and gene expression can be considered as the “cart”. In normal cells, oxidative metabolism (horse) and gene expression (cart) are tightly coupled via the nonequilibrium steady-state fluxes of reactive metabolic by-products and electron carriers through signal transduction pathways. (B) When this nonequilibrium steady-state is disrupted,  $O_2^{\bullet-}$  and  $H_2O_2$  as well as highly reactive oxidative damage products from oxidative metabolism (the horse) can cause the deterioration of the genome (the cart). If this disrupted oxidative metabolism continues to progress leading to the assembly of inefficient metabolic structures, the persistent exponential deterioration of the genome will inevitably lead to cancer induction and/or senescent death. (C) The dysregulated oxidative metabolism in cancer cells, which are immortalized and incapable of normal differentiated function will eventually drag the living system off the cliff into the valley of end stage malignancy and organismal death.

continues to progress to malignancy. In addition, the natural extension of this hypothesis is that continued targeting of only the malignant cell genomic dysregulation without targeting the malignant cell's metabolic dysfunction (Fig. 1C) will significantly limit the potential success of stopping cancer cells from overwhelming the organism (Fig. 1C). This theoretical construct predicts that the most efficient way to target metabolic frailties in cancer cells is to selectively increase the production of reactive species, while compromising the protective pathways. This model would also predict that exploiting the biochemical hubs of cancer cell redox metabolism including: (1) glycolysis, (2) NAD(H)/NADP(H) levels, (3) electron transport chain activity, (4) glutathione- and thioredoxin-dependent thiol metabolism, (5) folate metabolism, and (6) redox-active metal ion metabolism would be most efficacious for the development of combined modality cancer therapies.<sup>8</sup>

In the context of this metabolic targeting strategy, the paper by Lewis et al. provides a rigorous overview of NAD<sup>+</sup> metabolism in the context of the cellular responses to ionizing radiation as well as presenting novel combined modality therapy approaches that target NAD<sup>+</sup> metabolism to enhance radiation therapy responses.<sup>19</sup> In the paper by Ashcraft et al. the authors explore the novel preclinical as well as clinical evidence supporting exercise modulation of cancer therapy responses as well as the potential biological mechanisms involving alterations in oxidative metabolism that may account for these effects.<sup>20</sup> Another exciting line of investigation is reviewed in the paper by Floberg and Schwarz, where a strategy targeting glucose- and thiol- mediated hydroperoxide metabolism is central to the induction of selective metabolic oxidative stress in cancer vs normal cells with rigorous biochemical and preclinical support for potential early phase clinical trials.<sup>21</sup> In a similar exciting line of investigation Luo and Wicha review recent evidence focusing on targeting different redox states of cancer stem cells in triple negative breast cancer.<sup>22</sup> They discuss data showing that the plasticity of cancer stem cells can be manipulated through redox signaling pathways between mesenchymal to epithelial phenotypes targeting glucose and hydroperoxide metabolism for enhancing therapeutic responses.<sup>22</sup> In the final paper from Naz et al. novel strategies for imaging metabolic redox changes using electron paramagnetic resonance imaging of pO<sub>2</sub> and <sup>13</sup>C magnetic resonance imaging with hyperpolarized <sup>13</sup>C labeled pyruvate are evaluated as having significant potential for noninvasively evaluating redox changes in the tumor microenvironment for predicting therapy outcomes.<sup>23</sup>

## Conclusion

Overall, this issue of seminars in radiation oncology was assembled to emphasize exciting new avenues and recent advances in exploiting redox metabolism for improving radiochemotherapy both from a basic and translational science point of view. The historical perspective underlying these areas of research as well as a unifying hypothesis for

further advancing this research into clinical trials is also presented. We thank all the authors for their thoughtful contributions to this issue and hope that the reader can utilize the information presented to push forward with translational research efforts needed to realize the full potential of these approaches for improving the practice of medicine in radiation oncology.

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## References

1. Patt HM, Tyree EB, Straube RL, et al: Cysteine protection against X irradiation. *Science* 110:213-214, 1949
2. Gerschman R, Gilbert DL, Nye SW, et al: Oxygen poisoning and x-irradiation: a mechanism in common. *Science* 119:623-626, 1954
3. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 11:298-300, 1956
4. Oberley LW, Buettner GR. Role of superoxide dismutase in cancer: a review. *Cancer Res* 39:1141-1149, 1979
5. Oberley LW, Oberley TD, Buettner GR. Cell differentiation, aging and cancer: the possible roles of superoxide and superoxide dismutases. *Med Hypotheses* 6:249-268, 1980
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* 23:311-322, 2004
7. Spitz DR, Hauer-Jensen M. Ionizing radiation-induced responses: where free radical chemistry meets redox biology and medicine. *Antioxid Redox Signal* 20:1407-1409, 2014
8. Zhu Y, Dean AE, Horikoshi N, et al: Emerging evidence for targeting mitochondrial metabolic dysfunction in cancer therapy. *J Clin Invest* 128:3682-3691, 2018
9. Szent-Györgyi A. *Electronic biology and cancer*. New York, NY: Marcel Dekker Inc.; 1976. p. 34-35
10. Mitchell P. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. 1966. *Biochim Biophys Acta* 1807:1507-1538, 2011
11. Forman HJ, Kennedy J. Superoxide production and electron transport in mitochondrial oxidation of dihydroorotic acid. *J Biol Chem* 250:4322-4326, 1975
12. Boveris A, Cadenas E. Mitochondrial production of superoxide anions and its relationship to the antimycin insensitive respiration. *FEBS Lett* 54:311-314, 1975
13. Allen BG, Spitz DR. Physiologic and pathologic functions of mitochondrial ROS (Hockenbery, editor). *Mitochondria and Cell Death, Cell Death in Biology and Diseases*. New York, NY: Humana PressSpringer Science +Business Media; 2016. <https://doi.org/10.1007/978-1-4939-3612-0>

14. Allen BG, Spitz DR. Metabolic production of H<sub>2</sub>O<sub>2</sub> in carcinogenesis and cancer treatment. Redox-Active Therapeutics, Oxidative Stress in Applied Basic Research and Clinical Practice. (Batinic-Haberle, Reboucas, Spasojevic, St. Clair, and Warner, editors), Springer International Publishing Switzerland. 2016. <https://doi.org/10.1007/978-3-319-30705-3>
15. Schoenfeld JD, Alexander MS, Waldron TJ, et al. Pharmacological ascorbate as a means of sensitizing cancer cells to radio-chemotherapy while protecting normal tissue. (this issue **Seminars in Radiation Oncology**).
16. Aykin-Burns N, Pathak R, Boerma M, et al. Utilization of vitamin E analogs to protect normal tissues while enhancing anti-tumor effects. (this issue **Seminars in Radiation Oncology**).
17. Hillman GG: Soy isoflavones protect normal tissues while enhancing radiation responses. (this issue **Seminars in Radiation Oncology**).
18. Mapuskar KA, Anderson CM, Spitz DR, et al. Utilizing superoxide dismutase mimetics to enhance radiation therapy response while protecting normal tissues. (this issue **Seminars in Radiation Oncology**).
19. Lewis JE, Singh N, Holmila RJ, et al. Targeting NAD metabolism for enhancing radiation therapy responses. (this issue **Seminars in Radiation Oncology**).
20. Ashcraft KA, Warner AB, Jones LW, et al. Exercise as adjunct therapy in cancer. (this issue **Seminars in Radiation Oncology**).
21. Floberg JM, Schwarz JK Manipulation of glucose and hydroperoxide metabolism to improve radiation responses. (this issue **Seminars in Radiation Oncology**).
22. Luo M and Wicha MS Targeting cancer stem cell redox metabolism to enhance therapy responses. (this issue **Seminars in Radiation Oncology**).
23. Naz S, Kishimoto S, Mitchell JB, et al. Imaging metabolic processes to predict radiation responses. (this issue **Seminars in Radiation Oncology**).