Final technical report

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Name of recipient:
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Effects of Low Dose Irradiation on NFkB Signaling Networks and Mitochondria

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Abstract:
Low dose ionizing radiation effects are difficult to study in human population because of the numerous confounding factors such as genetic and lifestyle differences. Research in mammalian model systems and in vitro is generally used in order to overcome this difficulty. In this program project three projects have joined together to investigate effects of low doses of ionizing radiation. These are doses at and below 10 cGy of low linear energy transfer ionizing radiation such as X-ray and gamma rays. This project was focused on cellular signaling associated with nuclear factor kappa B (NFkB) and mitochondria - subcellular organelles critical for cell aging and aging-like changes induced by ionizing radiation. In addition to cells in culture this project utilized animal tissues accumulated in a radiation biology tissue archive housed at Northwestern University (http://janus.northwestern.edu/janus2/index.php). Major trust of Project 1 was to gather all of the DoE sponsored irradiated animal (mouse, rat and dog) data and tissues under one roof and investigate mitochondrial DNA changes and micro RNA changes in these samples. Through comparison of different samples we were trying to delineate mitochondrial DNA quantity alterations and micro RNA expression differences associated with different doses and dose rates of radiation. Historic animal irradiation experiments sponsored by DoE were done in several national laboratories and universities between 1950's and 1990's; while these experiments were closed data and tissues were released to Project 1. Project 2 used cells in culture to investigate effects that low doses or radiation have on NFκB and its target genes manganese superoxide dismutase (MnSOD) and genes involved in cell cycle: Cyclins (B1 and D1) and cyclin dependent kinases (CDKs). Project 3 used cells in culture such as "normal" human cells (breast epithelial cell line MCF10A cells and skin keratinocyte cells HK18) and mouse embryo fibroblast (mef) cells to focus on role of NFkB protein and several other proteins such as survivin (BIRC5) in radiation dependent regulation of tumor necrosis factor alpha (TNFα) and its downstream signaling.

Summarized project activities

Project #1

Primary orientation of Project 1 was investigation of archival tissues and development of technologies suitable to extract information relevant for understanding effects of low dose and low dose rate radiation. Of all the nucleic acid molecules in archival samples, the greatest stability have DNA and micro RNAs (miRs). Because miRs are a novel class of highly conserved, small, non-coding RNAs, functioning most often as negative regulators of gene expression during proliferation, differentiation and apoptosis, we have decided to investigate their expression in our mouse archival samples. We executed an extensive investigation of archival spleens extracted from control and test animals exposed to more than 20 different exposure regimens. These samples were screened against a custom made miR microarray and miR expression patterns associated with development of spleen and lymphoid cancers were detected as well as miRs associated with absence of disease in animals that were exposed to radiation.

A select set of miRs was tested with mouse lymphoid cell lines in vitro as well. These cell lines have a similar genetic background but they also have a pronounced difference in their ability to withstand radiation injury. In terms of mRNA expression cell lines LYS and LYR differ by expression for several RNAs for inhibitors of cdks and several mRNAs involved in apoptosis. Similarly, in response to radiation, expression of miRs 665, 690, 1195 and 511, with target sequences in TNF-alpha, MnSOD and CDKs is increased more in the radioresistant cell line LYS than in the radiosensitive counterpart LYR. Because TNF-alpha, MnSOD and CDKs are within primary focus of Projects 2 and 3 we focused at these miRs. A closer inspection of conditioned cell media has shown that it contains exosomes with the same miRs, 665, 690, 1195 and 511, that can be detected in cells. Exosomes are secretory vesicles 30- 100nm in size, derived from fusion of multivesicular bodies to plasma membranes. They have a role in cell-to-cell communication and affect recipient cells as ligands or by transferring molecules between the cells. In the case of cell lines LYR and LYS, exosomes contain different (cell specific) amounts of the several miRs investigated; moreover, miR expression levels in exosomes are also modulated in response to radiation. Therefore, while projects 2 and 3 continue to focus on proteomic aspects of response to radiation that is dependent on MnSOD and Nf-KB, project 1 was oriented toward nucleic acid exploration that allows us to combine findings from cells and new animal research with investigation of archival tissues.

Project #2

This project tested the hypothesis that low dose radiation induces adaptive response and that this response is regulated by NFκB signaling. Among the genes involved in this process is MnSOD, one of the NFkB target genes. MnSOD plays a key role in LDR-induced adaptive radioprotection. Although mitochondria play an essential role in radiation response, the mechanism underlying mitochondrial pro- or anti- apoptotic pathways in low dose radiation-induced adaptive response remain elusive. This study tested the hypothesis that Cyclin D1/CDK4 is the key factor that delivers the adaptive signaling to mitochondria to induce an anti-apoptotic response. We found that Cyclin D1/CDK4 was indeed present in the mitochondrion and its mitochondrial translocation was significantly enhanced following exposure to low dose ionizing radiation (10 cGy X-rays) in human keratinocytes and an adaptive radioresistance and similar reaction was induced in irradiated mouse skin tissues in vivo. Cyclin D1/CDK4 was able to directly interact with mitochondrial antioxidase MnSOD, which may phosphorylate and activate MnSOD contributing to the adaptive radioresistance with reduced reactive oxygen species after irradiation and maintenan mitochondrial membrane potential. Our results demonstrated that cell cycle-regulated protein Cyclin D1/CDK4 is translocated to mitochondria to regulate mitochondrial function via direct interaction with mitochondrial antioxidase MnSOD in low dose

radiation-induced adaptive resistance. To detect and confirm mitochondrial functions in low dose rate induced adaptive response, human breast epithelial MCF10A cells and human skin keratinocytes HK18 cells were treated with 10cGy X-ray followed by 5Gy γ-ray. Adaptive radioprotection (clonogenic survival, mitochondrial membrane potential, apoptosis, etc.) were tested both in cultured cells and in vivo in irradiated mouse skin. Dynamic alterations of NFκB target genes including MnSOD, Cyclins (B1 and D1) and CDKs were associated with cell radiosensitivity. We also identified an MnSOD phosphorylation site that can be phosphorylated to enhance MnSOD enzymatic activity under LDR induced adaptive radioprotection. Finally we found a novel proteomics profile of MnSOD protein-protein interactions with an array of mitochondrial and cytoplasmic proteins under low dose rate induced adaptive radioprotection.

Project #3

This project was focused on developing molecularly modified cell lines and animal models with which to study the molecular mechanisms underlying the adaptive responses initiated by thiols and low dose radiation exposures. Mouse embryo fibroblast (mef) cells have been transfected with myc and ras oncogenes to allow for immortalization and long term growth both in vitro and in vivo. These cells were also modified to be defective in the tumor necrosis factor (TNF) signaling beginning with the cell surface receptors for TNF: TNFR1 and TNFR2. Under in vitro conditions wild type mef cells exhibit robust adaptive responses after exposure to 10 cGy; these led to resistance to high doses of radiation up to a 2 Gy dose. Endpoints included the reduction of micronuclei formation and the elevation of cell survival. Moreover, we found that the effects of low dose 10 cGy exposure equaled radiation protection obtained with 40 μM concentration of the free thiol form of amifostine. The maximum adaptive effect in both instances was observed at the time at which MnSOD (or SOD2) activity was found to be maximally elevated. In contrast, the mef cells knocked out for TNFR1, and 2 did not exhibit an adaptive response to either treatment. This suggested that the defect in these cells involves the ability to activate NFκB. The traditional adaptive response is measured by first exposing cells to a low dose of ionizing radiation (<10 cGy) or thiol followed by a high challenge dose of 2 Gy or more with this response being mediated via NFkB signaling and elevated MnSOD enzymatic activity. However, if the sequence of radiation exposures is reversed with a high 2 Gy dose followed by a small 10 cGy dose, an adaptive response is still observed but it is independent of MnSOD enzyme levels. In contrast to the standard adaptive response model, this novel exposure schema results in elevated levels of the survival related protein survivin which will inhibit the process of apoptosis and result in elevated survival as measured using colony forming assay methods. This survivin mediated-adaptive response occurs independently of TNFα signaling and is believed to be under the control of Akt/P13k/NFκB/survivin signaling. Survivin is known to be inducible in hematopoietic and endothelial stem cells and thus may be an important factor in the anti-radiation response induced by exposure to very low doses of ionizing radiation.

Products of this work included publications (see below) as well as irradiated animal tissue archive website

http://janus.northwestern.edu/janus2/index.php

Publications:

1. Murley JS, Kataoka Y, Miller RC, Li JJ, Woloschak G, Grdina DJ. SOD2-mediated

effects induced by WR1065 and low-dose ionizing radiation on micronucleus formation in RKO human colon carcinoma cells. Radiat Res. 2011 Jan;175(1):57-65. PMC3071890.
Significance of Research: A potential pathway of MnSOD to protect normal tissue under radiation treatment.

2. Liu W, Haley BM, Kwasny MJ, Li JJ, Grdina DJ, Paunesku T, Woloschak GE. The effects of radiation and dose-fractionation on cancer and non-tumor disease development. Int J Environ Res Public Health. 2012 Dec 18;9(12):4688-703. PMC3546784
Significance of Research: Cancer associated life shortening in response to radiation in Mus musculus is associated with higher doses and less fractionated radiation.

3. William Liu, Benjamin Haley, Mary J. Kwasny, Jian Jian Li, David J. Grdina, Tatjana Paunesku, Gayle E. Woloschak; Comparing radiation toxicities across species: an examination of radiation effects in Mus musculus and Peromyscus leucopus. Int J Radiat Biol. 2013 Jun;89(6):391-400.
Significance of Research: Life shortening in response to radiation was more significant in Peromyscus leucopus compared to Mus musculus and response to radiation appeared highly species specific.

4. Li, J. J. Phosphorylation of mitochondrial antioxidant MnSOD in the adaptive protection of cells against environmental oxidative stress. ACTA Biophysica Sinica, 28:19, 2012
Significance of Research: MnSOD plays a key role in regulation of mitochondrial function in low dose radiation induced adaptive response.

5. Grdina, D. J.,Murley, J. S., Miller, R. C., Mauceri, H. J., Sutton, H.J., Thirman, M. J., Li, J. J. Woloschak, G.E., and Weichselbaum, R. R. A manganese superoxide dismutase (SOD2) mediated adaptive response. Radiat Res. 2013 Feb;179(2):115-24. PMC3594688
Significance of Research: Discussion of the role of MnSOD for low dose radiation induced adaptive radioprotection.

6. Candas, D., Fan, M., Nantajit, D., Vaughan, A. T. M., Murley, J. S., Woloschak, G.E., Grdina, D. J., and Li, J.J. CyclinB1/CDK1-mediated radioprotection through Phosphorylation of Manganese Superoxide Dismutase. J Mol Cell Biol. 2013 Jun;5(3):166-75. PMCID: PMC3656610.
Significance of Research: Identification of a novel MnSOD enzymatic enhancement by CDK-mediated phosphorylation under LDR induced adaptive radioprotection.

7. Eldridge, A., Fan, M., Woloschak, G.E., Grdina, D.J., Chromy, B. A., and Li, J. J. Manganese superoxide dismutase interacts with a large scale of cellular and mitochondrial proteins in low dose radiation-induced adaptive radioprotection. Free Radic Biol Med. 2012 Nov 15;53(10):1838-47. PMCID: PMC3494792.
Significance of Research: Describing a novel pathway of mitochondrial antioxidant MnSOD can be activated and interact with other proteins in radiation adaptive response.

8. Jin C, Qin L, Shi Y, Candas D, Fan M, Lu CL, Vaughan AT, Shen R, Wu LS, Liu R, Li RF, Murley JS, Woloschak G, Grdina DJ, Li JJ. CDK4-mediated MnSOD activation and

mitochondrial homeostasis in radioadaptive protection. Free Radic Biol Med. 2015 Apr;81:77-87. PMC4359946.
Significance of Research: Describing roles of CDK4 and MnSOD in mitochondrial radiation adaptive response.

9. Grdina DJ, Murley JS, Miller RC, Mauceri HJ, Sutton HG, Li JJ, Woloschak GE, Weichselbaum RR. A survivin-associated adaptive response in radiation therapy. Cancer Res. 2013 Jul 15;73(14):4418-28. PMC3721325.
Significance of Research: Describing role of survivin in radiation adaptive response.

10. Grdina DJ, Murley JS, Miller RC, Woloschak GE, Li JJ. NFκB and Survivin-Mediated Radio-Adaptive Response. Radiat Res. 2015 Apr;183(4):391-7.
Significance of Research: Describing interaction between NFkB and survivin in radiation adaptive response.

11. 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 Dec 9;10(12):e0140989. doi: 10.1371/journal.pone.0140989. eCollection 2015. PMC4674094.
Significance of Research: Describing approaches suitable for modeling low dose radiation responses

12. Qin L, Fan M, Candas D, Jiang G, Papadopoulos S, Tian L, Woloschak G, Grdina DJ, Li JJ. CDK1 Enhances Mitochondrial Bioenergetics for Radiation-Induced DNA Repair. Cell Rep. 2015 Dec 15;13(10):2056-63. PMC4684969.
Significance of Research: Describing a novel pathway of CDK1 role in mitochondrial radiation adaptive response.

13. Miller RC, Murley JS, Rademaker AW, Woloschak GE, Li JJ, Weichselbaum RR, Grdina DJ. Very low doses of ionizing radiation and redox associated modifiers affect survivin-associated changes in radiation sensitivity. Free Radic Biol Med. 2016 Oct;99:110-119.
Significance of Research: Describing a novel role of survivin in radiation adaptive response.