Final Report on Project titled "*In vivo* Mammalian Tissue Response to Low Dose Ionizing Radiation: The Role of Endogenous Oxidative Metabolism and Intercellular Communication"

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The objective of the project was to elucidate the mechanisms underlying the biological effects of low dose/low dose rate ionizing radiation in organs/tissues of irradiated mice that differ in their susceptibility to ionizing radiation, and in human cells grown under conditions that mimic the natural *in vivo* environment. The focus was on the effects of sparsely ionizing cesium-137 γ rays and the role of oxidative metabolism and intercellular communication in these effects. Four Specific Aims were proposed. The integrated outcome of the experiments performed to investigate these aims has been significant towards developing a scientific basis to more accurately estimate human health risks from exposures to low doses ionizing radiation. By understanding the biochemical and molecular changes induced by low dose radiation, several novel markers associated with mitochondrial functions were identified, which has opened new avenues to investigate metabolic processes that may be affected by such exposure. In particular, a sensitive biomarker that is differentially modulated by low and high dose γ rays was discovered.

Major highlights:

1- Intercellular communication among irradiated cells exposed to low dose γ rays (1-10 cGy) delivered at very low dose rate (0.2 cGy/h) contributes, in the hours after irradiation, to protective responses against DNA damage induced by the radiation and from normal oxidative metabolism. Up-regulation of gap junction communication among cells exposed to a chronic dose of 10 cGy resulted in the cells harboring lower levels of chromosomal damage than what occurred spontaneously at the basal level. The effect was associated with modulation of cell cycle progression.

2- The Translationally Controlled Tumor Protein (TCTP) is up-regulated in normal human cells and tissues of mice exposed to low dose γ rays, but down-regulated in cells and tissues exposed to high dose γ rays (4 Gy). Upon exposure to doses as low as 1 cGy (a dose received in many diagnostic procedures), the TCTP level was greatly increased in human cells, with a significant enrichment in the nuclei. TCTP upregulation was dependent on the early sensors of DNA damage, specifically the protein ATM and the enzyme DNA-dependent protein kinase (DNA-PK). Importantly, this upregulation was associated with protective effects against DNA damage. As shown in the case of cells treated with DNA repair inhibitors in previous experiments, repair of γ-ray-induced chromosomal damage was compromised in TCTP-deficient cells. In the chromatin of irradiated cells, TCTP was found to exist in complex with ATM and γH2A.X, a protein that marks the sites of DNA damage. This finding is in agreement with TCTP's distinct localization with the foci of the DNA damage marker proteins γH2A.X, 53BP1, and P-ATM. Furthermore, TCTP was shown to interact with the DNA-binding subunits Ku70

and Ku80 of DNA-PK, a protein with a major role in repair of DNA double-strand breaks, a particularly harmful form of DNA damage.

In normal cells, TCTP did not affect such cell cycle progression towards division under normal, homeostatic conditions. However, TCTP had a prominent effect on stress-induced cell cycle checkpoints, which ensure that the cell cycle progresses without any DNA damage. We found that TCTP interacted with p53, a critical protein component of such checkpoints that maintains genomic integrity.

3- The expression patterns of many miRNA differed after exposure to either chronic or acute 10 cGy. The expression of miRNA *let-7e*, a negative regulator of *RAS* oncogene, and the *c-MYC* miRNA cluster were upregulated after 10 cGy chronic dose but were downregulated after 3 hours of acute 10 cGy. The *miR-21* was upregulated in chronic or acute low dose and moderate dose treated cells, and its target genes *hPDCD4*, *hPTEN*, *hSPRY2*, and *hTPM1* were found to be downregulated. These findings highlight the importance of dose rate in modulating the cellular response to ionizing radiation.

4- Exposure to a chronic dose of 10 cGy results in up-regulation of the cellular content of the antioxidant glutathione. Further, the DNA damage that occurs by exposure to acute-10 cGy is protected against by two ways: 1) Up-regulation of cellular antioxidant enzyme activity (overexpression of Mn-superoxide dismutase, catalase or glutathione peroxidase), and 2) inhibition of superoxide anion generation by flavin-containing oxidases. Together, the results support a significant role for oxidative metabolism in mediating low-dose radiation effects.

5- Aconitase activity and mitochondrial protein import are sensitive markers of exposure to ionizing radiation. The effects of γ rays on these endpoints vary in organs that differ in their sensitivity to ionizing radiation. Further, differential effects occur in mice that differ in their genetic susceptibility to radiation.

6- Oxidation/reduction reactions induced by low dose/low linear energy transfer ionizing radiation (γ rays) are similar to those caused by endogenous metabolism. Using cell cycle progression as an endpoint, the results of comprehensive studies provide direct evidence for such similarity.

7- In contrast to results in progeny of cells exposed to low mean doses of densely ionizing radiation (e.g. α particles, high charge and high energy particles), the micronucleus frequency (a surrogate form of DNA damage) in progeny of cells exposed to 10 cGy of γ rays delivered acutely or chronically was similar to that observed in the progeny of control cells. On the other hand, exposure of the progeny of 10 cGy-exposed cells to a 4 Gy acute challenge from γ rays results in significantly increased delay in the transition from G_1 to S phase of the cell cycle than what occurs in progeny of respective control cells.

8- To gain insight into the biochemical changes induced by low dose ionizing radiation, we determined global S-nitrosylation by 'Biotin Switch' assay in different organs of C57BL/6J mice exposed to acute 10 cGy of ^{137}Cs γ rays in the presence or absence of Iopamidol, a contrast agent used during computed tomography scans. To examine whether similar or distinct nitrosylation events are induced following high dose irradiation, mice were also exposed in parallel to 4 Gy. Significant dose and contrast agent-dependent changes were detected in organs that differ in their radiation sensitivity. Mass spectrometry analyses of modulated SNO-proteins suggested an effect on numerous pathways, including oxidative metabolism, DNA repair, Ras signaling and E3 ligase

function.

9- Together, the studies supported by this award highlighted the critical importance of radiation dose and quality in modulating targeted and non-targeted effects of low doses/low fluences of ionizing radiation. They are pertinent to developing a scientific basis for radiation protection standards, and may have translational applications in therapeutic regimens.

Publications that resulted with the support of award DE-FG02-07ER64344:

Peer-reviewed:

1. Jie Zhang, Sonia M. de Toledo, Badri N. Pandey, Guozheng Guo, Debkumar Pain, Hong Li and Edouard I. Azzam (2012) Role of the Translationally Controlled Tumor Protein in DNA Damage Sensing and Repair. *Proc. Natl. Acad. Sci. (Plus) USA*, 109: E926-33, 2012. PMID:22451927
2. M. Ahmad Chaudhry, Romaica A. Omaruddin, Bridget Kreger, Sonia M. de Toledo, and Edouard I. Azzam (2012) Micro RNA Responses to Chronic or Acute Exposures to Low Doses of Ionizing Radiation. *Molecular Biology Reports*, 39(7): 7549-58. PMID:22367372
3. Edouard I. Azzam, Jean-Paul Jay-Gerin and Debkumar Pain (2012) Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. *Cancer Letters*, 327 (1-2): 48-60. PMID:22182453
4. Edouard I. Azzam (2011) Exposure to low level environmental agents: The induction of hormesis. *Mutation Research*, 726(2), 89-90. PMID: 21807115
5. de Toledo SM, Buonanno M, Li M, Asaad N, Qin Y, Gonon G, Shim G, Galdass M, Boateng Y, Zhang J, Azzam EI (2011) The Impact of Adaptive and Non-Targeted Effects in the Biological Responses To Low Dose/Low Fluence Ionizing Radiation: the Modulating Effect of Linear Energy Transfer. *Health Physics* 100(3):290-292. PMID:21512606
6. Norman E. Ende and Edouard Azzam (2011) Planning for the Treatment of Mass Casualties Based on Pathology of the Fatalities of Hiroshima and Nagasaki. *International Journal of Radiation Biology* 87(4): 443-4. PMID: 21204615
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9. Marianne B. Sowa, Wilfried Goetz, Janet E. Baulch, Dinah N. Pyles, Jaroslaw Dziegielewski, Susannah Yovino, Andrew R. Snyder, Sonia M. de Toledo, Edouard I. Azzam and William F. Morgan (2010) Lack of evidence for low-LET radiation induced stressful bystander responses in normal human fibroblasts and colon carcinoma cells. *International Journal of Radiation Biology* **86**, 102-113. PMID: 20148696
10. Perumal Venkatachalam, Sonia M. de Toledo, Badri N. Pandey, Linda A. Tephly, A.

Brent Carter, John B. Little, Douglas R. Spitz, and Edouard I. Azzam (2008) Regulation of Normal Cell Cycle Progression by Flavin-Containing Oxidases. *Oncogene,* **27** (1), 20-31. PMID: 17637756

11. Edouard I. Azzam, Sonia M. de Toledo, Badri N. Pandey, and Perumal Venkatachalam (2007) Mechanisms underlying the expression and propagation of low dose/low fluence ionizing radiation effects. *International Journal of Low Radiation*, **4**, No.1, 61-68.
12. Sonia M. de Toledo and Edouard I. Azzam (2006) Adaptive and Bystander Responses in Human and Rodent Cell Cultures Exposed to Low Level Ionizing Radiation: The Impact of Linear Energy Transfer. *Dose-Response*, **4**, 291-301, 2006. PMID: 18648584
13. Sonia M. de Toledo, Nesrin Asaad, Venkatachalam Perumal, Ling Li, Badri N. Pandey, Roger W. Howell, Douglas R. Spitz and Edouard I. Azzam (2006) Adaptive responses to low dose/low dose-rate γ-rays in normal human fibroblasts cultured in three-dimensional architecture: The role of oxidative metabolism. *Radiat. Res.*, **166**, 849-857, 2006. PMID: 17149977
14. Badri N. Pandey, Donna M. Gordon, Sonia M. de Toledo, Debkumar Pain and Edouard I. Azzam (2006) Normal human fibroblasts exposed to high or low dose ionizing radiation: Differential effects on mitochondrial protein import and membrane potential. *Antioxidants and Redox Signaling (Forum Issue on "Redox Signaling & Tumor Biology)*, **8**, 1253-1261. PMID: 16910773

Manuscripts in Conference Proceedings:

1. Jie Zhang, Manuela Buonanno, Geraldine Gonon, Min Li, Mariann Galdass, Grace Shim, Sonia M. de Toledo and Edouard I. Azzam (2011) Bystander Effects and Adaptive Responses Modulate *In Vitro* and *In Vivo* Biological Responses to Low Dose Ionizing Radiation. Proceedings of the NATO Advanced Research Workshop on Radiological Issues Pertaining to Environmental Security and Ecoterrorism, Alushta, Ukraina, 9–14 October 2010. (Carmel E. Mothersill, Victoria Korogodina and Colin B. Seymour, Editors). Springer, Dordrecht, The Netherlands. Chapter 8, pp 86-101.

2. Edouard Azzam and John B. Little (2008) The Impact of Bystander Effects and Adaptive Responses in the Health Risks of Low Dose Ionizing-Radiation: the Modulating Effect of Linear Energy Transfer. Proceedings of the 12th International Congress on Radiation Protection, Buenos Aires, Argentina, 19-24 October 2008. Keynote Lecture I2.1

3. Edouard I. Azzam (2006) The roles and mechanisms of oxidative metabolism and intercellular communication in the propagation of low level radiation effects. *World Journal of Nuclear Medicine*, **5**, Supplement 1, S255-S257.

Manuscripts in preparation:

1. Min Li, Sonia M. de Toledo, Debkumar Pain and Edouard I. Azzam (2013) The Role of Genetic Susceptibility on *In Vivo* Effects of Low Dose γ Rays on Mitochondrial functions. *Free Radicals in Biology and Medicine* (in preparation)
2. Marianne Galdass, Min Li, Zhi Yang, Sonia M. de Toledo, Debkumar Pain, Douglas R.

Spitz and Edouard I. Azzam (2013) Succinate Dehydrogenase Regulates Mitochondrial Responses in γ-Irradiated Cells. *Radiation Research* (in preparation)

3. Salwa Bukhari, Changgong Wu, Sonia de Toledo ,Hong Li and Edouard I. Azzam (2013) *In vivo* Effect of Low Dose γ Rays on S-Nitrosylation in Presence and Absence of Iodine Contrast Agent. *Radiation Research* (in preparation)

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