FINAL TECHNICAL REPORT – DOE AWARD NO. DE-FG03-02ER63309

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Project Title: Mechanisms Underlying then Adaptive Response Against Spontaneous Neoplastic Transformation Induced by Low Doses of Low LET Radiation.

The goal of this project was to investigate mechanisms underlying the adaptive response seen following exposure of HeLa x skin fibroblast human hybrid cells to low doses of low LET radiation. It was proposed to investigate the contributions of three possible mechanisms. These were:

1. Upregulation of cellular antioxidant status.
2. Upregulation of DNA repair.
3. Upregulation of gap junction intracellular communication.

We have completed the study of the role of upregulation of reduced glutathione (GSH) as a possible mechanism underlying our observed suppression of transformation frequency at low radiation doses. First, we found no increase in intracellular GSH following low dose irradiation. Second, we found no effect of suppression of GSH by buthionine sulfoxime (BSO) on the transformation frequencies at low doses (0.1 and 4.0 cGy) of 60 kVp x-irradiation. We concluded that GSH levels do NOT play a significant role in modulating transformation frequency following low dose irradiation in our experimental system.

We have also completed our study of the possible role of upregulation of DNA repair in the observed adaptive response against neoplastic transformation by examining the effect of the poly-ADP-ribose polymerase (PARP) inhibitor, 3-aminobenzamide (3-AB), on the transformation frequency seen at low doses of Cs-137 gamma radiation. We examined doses of 0.5 and 5.0 cGy. No effect of 3-AB was seen at a dose of 0.5 cGy, but a significant increase in transformation frequency was seen at 5.0 cGy in the presence of 3-AB. We concluded that upregulation of DNA repair may be more important in modulating transformation at the higher dose. This conclusion is at least quantitatively similar to that reach by Rothkamm and Lobrich (*PNAS* 100: 5057-5062, 2003) in their study of the kinetics of removal of γ -H2AX nuclear foci (a putative marker for DNA dsb) from cells irradiated with very low doses. A manuscript describing the above studies has been submitted published in *Carcinogenesis* **24**:1961-1965, 2003.

In the context of possible mechanisms that could result in a suppression of transformation at low doses, we carried out a study in collaboration with Peter Johnston and Susan Short at the Gray Cancer Institute on the possible role of low dose cellular hyper-radiosensitivity of a transformation-sensitive subpopulation. We concluded that G2 cells could be such a population. The results were published in *Radiation Research* **159**:433-436, 2003.

Finally, we have completed two studies of the possible role of upregulation of gap junction intercellular communication (GJIC) in modulating transformation frequency at low doses of low LET radiation. The first approach used was to examine the effect of lindane, an inhibitor of connexin 43 function, on transformation frequency since we know that connexin 43 is the critical gap junction protein operative in our cells. While not optimal for GJIC effects, we used subconfluent cultures for these experiments, as that is what we had used for all studies to date. We first demonstrated, using scrape loading, that GJIC is inhibited in our cells following lindane treatment. We also demonstrated that phosphorylation of connexin 43 occurred following exposure

to a dose of 5.4 cGy of mammography energy x-rays (28 kVp), and that this phosphorylation was inhibited by lindane. The data indicated no effect of lindane on radiation-induced transformation frequencies at low radiation doses. We concluded that GJIC-mediated bystander effects do not play a role in modulating transformation frequency following low doses of low LET radiation when subconfluent cultures are irradiated. This research was published in *Radiation Research* **162**:646-654, 2004.

In order to optimize the opportunity for GJIC, we then carried out a study where confluent cultures were irradiated. The results indicated, that while the degree of low dose suppression was somewhat reduced compared to that seen for subconfluent cultures, it was not completely absent. Our conclusion is that any transformation-inducing, GJIC-mediated, bystander effects are insufficient to overcome the transformation-suppressing action of low doses of low LET radiation, whether they be by classic adaptive-response mechanisms (e.g. upregulation of DNA repair) or by bystander effects that result in suppression of transformation (selective killing of transformation prone subpopulations). This research has been submitted for publication.

Our research program was of sufficient interest to generate two invited reviews, and five invited presentations. For details see below.

Research supported by this grant has resulted in the following publications, conference proceedings and abstracts:

Peer-reviewed publications.

1. MC Pant, XY Lao, Q Lu, S Molloy, E Elmore and JL Redpath. Mechanisms of suppression of neoplastic transformation in vitro by low doses of low LET radiation. *Carcinogenesis* 24 : 1961-1965, 2003.
2. JL Redpath, SC Short, M Woodcock and PJ Johnston. Low dose reduction in transformation frequency compared to unirradiated controls: The role of hyper-radiosensitivity to cell death. *Radiat. Res.* 159 : 433-436, 2003.
3. JL Redpath. Radiation-induced neoplastic transformation in vitro : Evidence for a protective effect at low doses of low LET radiation. *Cancer and Metastasis Reviews* 23 : 333-339, 2004.
4. SJ Ko, X-Y Lao, S Molloy, E Elmore and JL Redpath. Neoplastic transformation in vitro following exposure to low doses of mammographic energy x-rays: Quantitative and mechanistic aspects. *Radiat Res* 162:646-654, 2004
5. JL Redpath. Nonlinear response for neoplastic transformation following low doses of low LET radiation. *Nonlinearity in Biology, Toxicology and Medicine* 3: 113-124, 2005
6. M Ko, X-Y Lao, R Kapadia, E Elmore and JL Redpath. Neoplastic transformation in vitro by low doses of ionizing radiation: role of adaptive response and bystander effects. *Mutation Res.* 2006, in press

Invited presentations/Conference proceedings.

1. JL Redpath, M Pant, XY Lao, Q Lu and E Elmore. Mechanisms of suppression of neoplastic transformation in vitro by low doses of low LET radiation. 12th International Congress of Radiation Research, Brisbane, Australia, Aug 17-22, 2003.
2. JL Redpath. Suppression of neoplastic transformation in vitro by low doses of low LET

radiation, in *Proceedings of the 14th Pacific Basin Nuclear Conference*, Honolulu, Hawaii, March 21-25, 2004.

3. JL Redpath, S Rightnar, G. Nelson and E Elmore, Low dose suppression of neoplastic transformation in vitro: Preliminary results with high energy protons. 3rd International Workshop on Space Radiation Research, Port Jefferson, NY, May 16-20, 2004.
4. JL Redpath. Nonlinear response for neoplastic transformation following low doses of low LET radiation. 3rd International Conference on Non-Linear Dose-Response Relationships in Biology, Toxicology and Medicine, Amherst, Mass, June 8-10, 2004.
5. JL Redpath, Adaptive responses and low dose radiation, International Workshop on Radiation-Induced Low Dose Effects, McMaster University, Hamilton, Ontario, Canada, Oct. 28- Nov. 9, 2004.

Abstracts.

6. JL Redpath, M Pant, XY Lao, Q Lu and E Elmore. Mechanisms of suppression of neoplastic transformation in vitro by low doses of low LET radiation. 12th International Congress of Radiation Research, Brisbane, Australia, Aug 17-22, 2003.
7. JL Redpath, X-Y Lao, M. Pant, S. Ko, C. Christie, T. Taylor and E. Elmore. Neoplastic transformation in vitro following exposure to low doses of low LET radiation. DOE Low Dose Radiation Research Program Workshop IV, Washington DC, Oct. 27-29, 2003.
8. JL Redpath. Suppression of neoplastic transformation in vitro by low doses of low LET radiation, in *Proceedings of the 14th Pacific Basin Nuclear Conference*, Honolulu, Hawaii, March 21-25, 2004.
9. SJ Ko, X Lao, S Molloy, E Elmore and JL Redpath. Neoplastic transformation in vitro following exposure to low doses of mammographic energy x-rays: Quantitative and mechanistic aspects. 51st Annual Meeting of the Radiation Research Society, St. Louis, MO, April 24-27, 2004.
10. JL Redpath, S Rightnar, G. Nelson and E Elmore, Low dose suppression of neoplastic transformation in vitro: Preliminary results with high energy protons. 3rd International Workshop on Space Radiation Research, Port Jefferson, NY, May 16-20, 2004.
11. JL Redpath. Nonlinear response for neoplastic transformation following low doses of low LET radiation. 3rd International Conference on Non-Linear Dose-Response Relationships in Biology, Toxicology and Medicine, Amherst, Mass, June 8-10, 2004.
12. JL Redpath, Adaptive responses and low dose radiation, International Workshop on Radiation-Induced Low Dose Effects, McMaster University, Hamilton, Ontario, Canada, Oct. 28- Nov. 9, 2004.
13. JL Redpath, X-Y Lao, R. Kapadia and E Elmore, Low dose studies of radiation-induced neoplastic transformation in vitro by low LET radiation. DOE Low Dose Radiation Research Program Workshop V, Bethesda, Maryland, April 25-27, 2005.
14. JL Redpath, X-Y Lao, M Ko, R. Kapadia. S. Rightnar, G. Nelson, and JL Redpath.

Neoplastic transformation in vitro induced by low doses of 232 MeV protons. 16th Annual NASA Space Radiation Investigator's Workshop, Port Jefferson, New York, May 15-18, 2005.