Adaptive Response Against Spontaneous Neoplastic Transformation In Vitro Induced by Ionizing Radiation

Project ID: 69848

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Research Objective

The following paragraphs outline a project which addresses the area "Understanding biological responses to radiation and endogeneous damage". The proposed research addresses the following key questions: (a) "How much do low doses of radiation protect against subsequent low doses of ionizing radiation?". The experimental system to be employed to address these questions is the HeLa x skin fibroblast human hybrid cell system with which the investigators have over 12 years of experience and, importantly, with which they have made several observations which can be expended upon to address the question posed above. The endpoint to be employed is neoplastic transformation frequency. Two radiation sources will be used. These are Cs-137 gamma rays (0.66 MeV gamma energy) and 80 kVp X-rays used in fluoroscopy. While these two energies of photons are in what is normally considered the low LET range (0.4 to 5.0 keV/u), they do have a difference in biological effectiveness of perhaps a factor of 2. Notice 99-14 specifically notes the need to quantify the generality and extent of the apparent adaptive response in cells irradiated with small doses of radiation. This is the focus of the proposed effort. Total doses to be used, whether single or fractionated, will be no more than 10 cGy. Appropriate attention is given to the necessity for accurate dosimetry. The experimental protocol is designed such that the data obtained will be amenable to the rigorous statistical analysis so essential to evaluate low dose effects. The investigators have already demonstrated that irradiation with a single dose of 1cGy results in a neoplastic transformation frequency that is a factor of two LOWER than that of sham-irradiated cells (Radiation Research, 149, 517-520, 1998). It is proposed to expand on this observation by establishing a dose-response relationship (e.g. 0.1, 1.0 and 10.0 cGy) as well for repeated low dose exposures (e.g. 10 x 0.1 cGy and 10 x 1 cGy). These proposed experiments directly address the question posed in Notice 99-14. Transformation frequencies will be compared to those predicted by linear extrapolation of high dose data (2 and 4 Gy) through the spontaneous background frequency. This will provide an estimate of the factor by which such linear extrapolations can overestimate low dose data. It is also proposed to take advantage of another observation in the investigators laboratory to address, albeit indirectly, the question of the role of repair in this response. The investigators have previously shown that post-irradiation holding at room temperature prior to plating for focus formation results in enhanced cell kill and enhanced neoplastic transformation, most likely through the promotion of misrepair. It is now hypothesized that if the adaptive response involves the induction of a repair process then the reduction in transformation frequency following exposure to 1 cGy would be abrogated by post-irradiation holding at room temperature.

Research Progress and Implications (3/10/00)

The research design for this project described three phases (IA & IB, II and III). Phase IA was designed to be the first to be tackled and consisted of expanding our initial observation using a dose of 1 cGy to include studies with 0.1 and 10.0 cGy. It was estimated that this phase would take 6 to 9 months to complete. The initial step was to screen serum lots for use over the whole proposed

funding period. This was done and a serum lot was chosen (September and October). The experimental design for phase IA required six repeats of a protocol which included eight arms: 0, 0.1, 1.0, and 10.0 cGy with both immediate and delayed post-irradiation plating. This number of repeats were considered necessary for rigorous statistical analysis. Three of these repeats were accomplished over the period November through January. Unfortunately, the spontaneous (0 cGy) transformation frequencies were lower than had been obtained in the initial serum screen, and too low to hope to be able to obtain decent statistics for the experiment. After some trouble shooting regarding optimizing incubator pH it has been decided to screen further serum lots before proceeding any further. We are in the middle of that now.