Mechanisms of Enhanced Cell Killing at Low Doses: Implications for Radiation Risk

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Research Objective

Our overall aim is to gather understanding of the mechanisms underlying low-dose hyper-radiosensitivity (HRS) and induced radioresistance (IRR). There is now some direct evidence that this dose-dependent radiosensitivity phenomenon reflects changes in the amount, rate or type of DNA repair, rather than indirect mechanisms such as modulation of cell-cycle progression, growth characteristics or apoptosis. There is also indirect evidence that cell survival-related HRS/IRR in response to single doses might be a manifestation of the same underlying mechanism that determines the well-known adaptive response in the two-dose case, thus HRS can be removed by prior irradiation with both high- and low-LET radiations as well as a variety of other stress-inducing agents such as hydrogen peroxide and chemotherapeutic agents.

Our goals in this project are therefore:

1. Identify which aspects of DNA repair (amount, rate and type) determine HRS/IRR,
2. Investigate the known link we have discovered between the extent of HRS/IRR and position in the cell cycle, focusing on changes in DNA structure and conformation which may modulate DNA repair,
3. Use the results from studies in (1) and (2) to distinguish, if necessary, between HRS/IRR and the adaptive response. The aim is to finally determine if these are separate or interlinked phenomena.

Use the results from studies in (1), (2) and (3) to propose a mechanism to explain HRS/IRR.

Research Progress and Implications, and Planned Activities

This report summarizes progress as of March 2000, which is 4 months into a three-year programme activated in November 1999.

1) Construction is underway of a high-speed automated epifluorescent cytometer that will be employed to i) measure low levels of DNA damage by use of the single-cell gel electrophoresis (“comet”) assay; ii) perform quantitative analysis of the distribution of chromatin and DNA-repair associated proteins.

2) In collaboration with J. Bourhis (Institut Gustave Roussy, Paris) we have identified DNA dependent protein kinase (DNA-PK) as a potential mediator of the HRS/IRR response. Analyses of the activity of this key DNA repair enzyme before and after low dose (0.2 Gy) irradiation in a panel of 10 cell lines were made. A significant correlation between the relative change in DNA-PK activity in response to irradiation and the extent of IRR was observed. We are currently extending this analysis to a larger panel of cell lines over the time course of IRR development. This includes cell lines both deficient, and subsequently complemented for PRKDC (with C. Kirchgesner, Stanford University).
Companion studies of PRKDC (and associated proteins such as the Ku70 and Ku80 proteins) distribution and expression are also underway.

3) A panel of GFP-chimera DNA constructs have been obtained that encode for chromatin associated and cytoskeletal proteins. Stable transfects of cells from the HRS/IRR panel are currently under selection. These will enable i) “real time” analysis of nuclear and chromatin conformation in response to low dose irradiation; ii) influence of over-expression of specific chromatin-associated proteins on the HRS/IRR response.

4) We have recently obtained a clone of a novel gene that has been shown to be involved in low dose radiation responses (DIR1; with T. Robson, University of Ulster, N. Ireland). This has been subcloned in sense and antisense orientations into plasmid vectors, under the control of both strong, constitutive and radiation-activatable gene promoters. Other genes involved in DNA damage sensing/repair processes (e.g. ATM, Ku70/80, PARP) are being cloned likewise. We have also designed ribozymes targeting these genes and oligonucleotides encoding these have been ordered. These will be cloned into our pREV1 ribozyme expression vector, which we have used previously to successfully target both the ATM and c-myc genes.

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