Project Summary

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Project Title: Mechanisms of enhanced cell killing at low doses: Implications for radiation risk

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Specific DOE problems that are being addressed

We have shown that cell lethality actually measured after exposure to low-doses of low-LET radiation, is markedly enhanced relative to the cell lethality previously expected by extrapolation of the high-dose cell-killing response. Net cancer risk is a balance between cell transformation and cell kill and such enhanced lethality may more than compensate for transformation at low radiation doses over at least the first 10 cGy of low-LET exposure. This would lead to a non-linear, threshold, dose-risk relationship. Therefore our data imply the possibility that the adverse effects of small radiation doses (<10 cGy) could be overestimated in specific cases. It is now important to research the mechanisms underlying the phenomenon of low-dose hypersensitivity to cell killing, in order to determine whether this can be generalized to safely allow an increase in radiation exposure limits. This would have major cost-reduction implications for the whole EM program.

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 July 2000, which is 8 months into a three-year programme activated in November 1999.

1) We have already established cell-plating techniques that improve the accuracy of clonogenic assays, as is required for examining the effects of low doses on cellular survival. However, low-dose clonogenic assays require large cell samples to maintain statistical accuracy. Manually counting the resulting colonies is a laborious task in which consistent objectivity is hard to achieve. This is true especially with some mammalian cell lines which form poorly defined or 'fuzzy' colonies typified by glioma or fibroblast cell lines. In collaboration with Paul Barber and Boris Vojnovic of the Advanced Technology Group at the GLCRT, a computer-vision-based automated colony counter has been developed. This system utilises novel imaging and image-processing methods involving a modified form of the Hough transform. The automated counter is able to identify less-discrete cell colonies. The results from the automated counts fall well within the distribution of the manual counts with respect to surviving fraction (SF) versus dose curves, SF values at 2 Gy (SF2) and total area under the SF curve (Dbar). This system also permits quantitative assessment of colony size, another potential indicator of cellular effects of low dose irradiation.

2) A novel low dose-rate irradiation system that utilises a 60-Co gamma source and an attenuating water tank has been developed to carry out simultaneous irradiations over a wide range of dose rates (1 to 100 cGy/h). We have now obtained definitive data indicating a greater reduction in cell survival per unit dose of irradiation at continuous low dose rate exposures of 2, 5 or 10 cGy h^{-1} compared with 20 and 60 cGy h^{-1}. We predicted this effect from our acute-dose HRS experiments. Previous explanations of such inverse dose-rate effects have invoked putative accumulation of cells in the G2 phase of the cell cycle as a G1 block is lost with decreasing dose rate. However, we have shown that i) G2 accumulation becomes less as the dose rate is reduced, ii) HRS/IRR is observed in p53 mutant cells and iii) HRS/IRR is observed in cells arrested by confluence during irradiation. Cells that do not exhibit HRS also fail to exhibit an inverse dose rate effect.

3) To examine the hypothesis that the HRS/IRR response of cells involves alterations in the repair capacity of cells we are currently developing non-clonogenic assays. Micronuclei are readily quantifiable lesions that correlate with the induction and repair of DSBs, chromosomal breaks and lethal lesions. Preliminary results with cytochalasin-B block micronucleus assay indicate that sensitivity at doses comparable to those employed in clonogenic assays is achievable. Current DSB assays do not provide the sensitivity to examine the repair of DSBs at low doses. 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. Evidence for an HRS/IRR response with these assays would support the hypothesis that DNA double strand break induction and/or repair are key events in the HRS/IRR response of cells.

4) In collaboration with J. Bourhis (Institut Gustave Roussy, Paris) we have identified DNA dependent protein kinase (PRKDC) 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 PRKDC 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. Kirchgessner, Stanford University). Companion studies of PRKDC (and associated proteins such as the Ku70 and Ku80 proteins) distribution and expression are also underway. At present, the change in extractable activity does not appear to be related to changes in overall amounts PRKDC

5) 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 nonsense 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.

Information Access: key recent publications

1. **Johnston PJ,** MacPhail SH, Stamato TD, Kirchgessner CU, Olive PL, 1998, Higher-order chromatin structure-dependent repair of DNA double-strand breaks: involvement of the V(D)J recombination double-strand break repair pathway. *Radiation Research*, **149,** 455-462.
2. **Joiner MC,** Lambin P and **Marples B,** 1999, Adaptive response and induced resistance. *Comptes Rendus de l'Académie des Sciences Série III*, **322,** 167-175.
3. Robson T, **Joiner MC,** Wilson GD, McCullough W, Price ME, Logan I, Jones H, McKeown, SR, and Hirst DG, 1999, A novel human stress response-related gene with a potential role in induced radioresistance. *Radiation Research*, **152,** 451-461.
4. Vaganay-Juéry S, Muller C, Abdulkarim B, Deutsch E, Marangoni E, Lambin P, Calsou P, Salles B, **Joiner M,** and Bourhis J, 2000, Decreased DNA-PK activity in human cancer cells exhibiting hypersensitivity to low-dose irradiation. *British Journal of Cancer*, in press.
5. Barber, P.R., Vojnovic, B., **Kelly, J., Boulton, P., Mayes, C., Woodcock, M.** and **Joiner, M.C.,** 2000, Automated counting of mammalian cell colonies. *Physics in Medicine and Biology*, in press
6. Short, S.C., **Kelly, J., Mayes, C.R., Woodcock, M.** and **Joiner, M.C.,** 2000, Low-dose hypersensitivity after fractionated low-dose irradiation *in vitro*. *International Journal of Radiation Oncology Biology Physics*, in press