Mechanisms of enhanced cell killing at low doses: implications for radiation risk

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The background

Over the past decade, we have discovered that:

- Radiation sensitivity can be dose-dependent: small acute exposures to low-LET radiation (and exposures at very low dose rates) are more lethal per unit dose than larger exposures above a threshold (typically 5–40 cGy) at which radioresistance increases.
- These dual phenomena are termed low-dose hypersensitivity (HRS) at very low doses and increased radioresistance (IRR) as the dose increases (see panel 3).
- HRS/IRR are widespread in both immortalized and non-immortalized human cells *in vitro*, and in animal normal-tissue models *in vivo*. HRS has also been measured in the epidermis of patients undergoing radiotherapy.
- HRS may be the constitutive response of cells systems to low-dose radiation exposures.
- Direct evidence suggests 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.
- Indirect evidence suggests that cell survival-related HRS/IRR in response to single doses might be related to the well-known *adaptive response* in the two-dose case.

Net cancer risk is a balance between cell transformation and cell kill. Our hypersensitive low-dose cell-survival responses suggest that cell lethality could more than compensate for transformation at low-LET radiation doses up to about 10 cGy. This would lead to a non-linear, threshold, dose-risk relationship implying that the cancer risk from small radiation doses (<10 cGy) could be *overestimated* in specific cases.

The goals

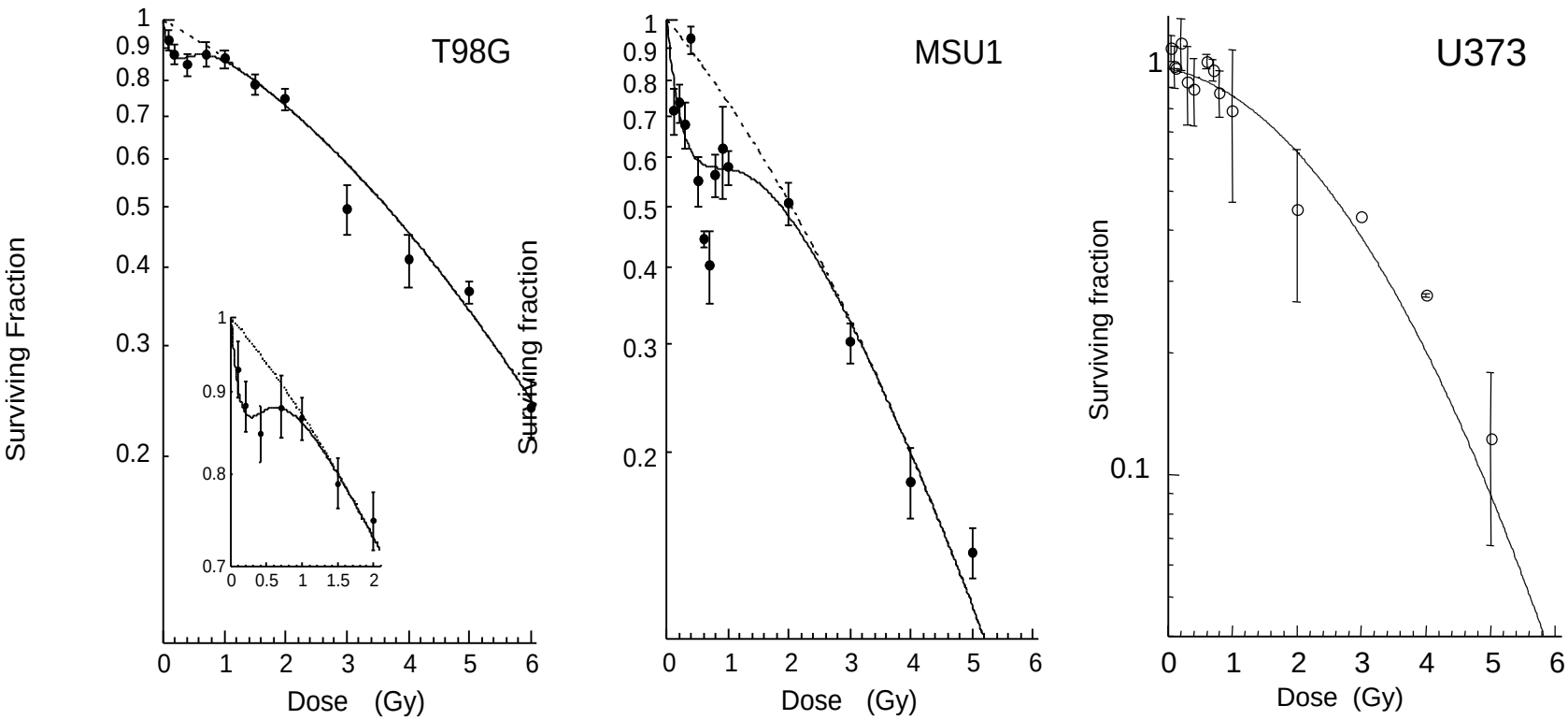
Our overall aim is to gain understanding of the mechanisms underlying HRS/IRR.

Specifically,

1. Identify which aspects of DNA repair (amount, rate, type, location and pathway) 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.
4. Use the results from studies in (1), (2) and (3) to propose a mechanism to explain HRS/IRR.

The survival response

Here are examples of HRS, in three established human cell lines *in vitro*.
Note the extremely radiosensitive response below 10 cGy:
for T98G glioma $\alpha = 1.56\ Gy^{-1}$; for MSU1.1 fibroblasts, $\alpha = 2.56\ Gy^{-1}$.
In U373, there is no HRS: $\alpha = 0.053\ Gy^{-1}$.



Modelling the survival response

The real lethality of very low doses compared with lethality predicted from measurements made at higher dose, is described by α_s/α_r in the following equations:

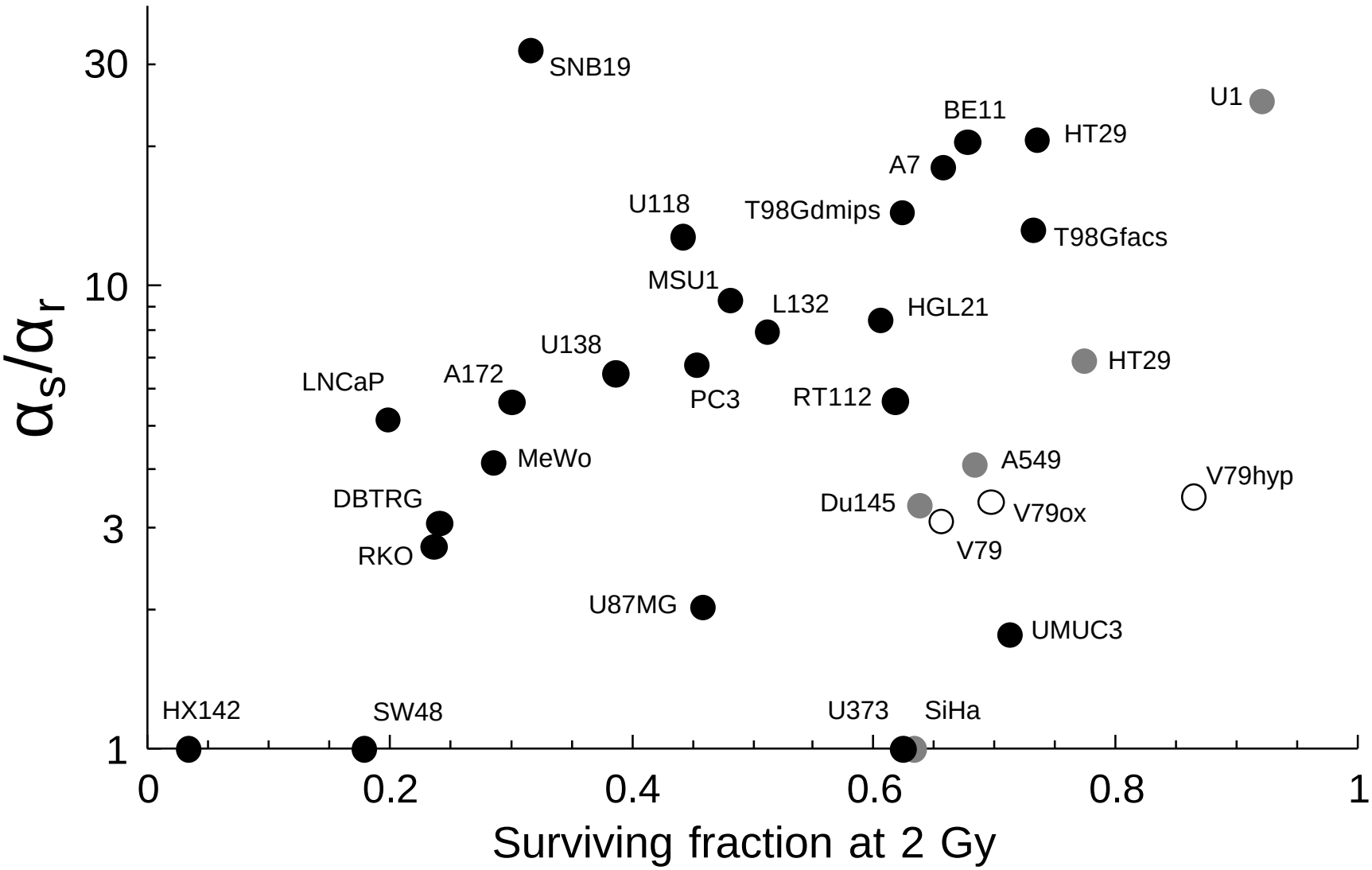
$$\text{Surviving fraction} = \exp(-\alpha d - \beta d^2)$$

where

$$\alpha = \alpha_r \left(1 + \left(\frac{\alpha_s}{\alpha_r} - 1\right) e^{-d/d_c}\right).$$

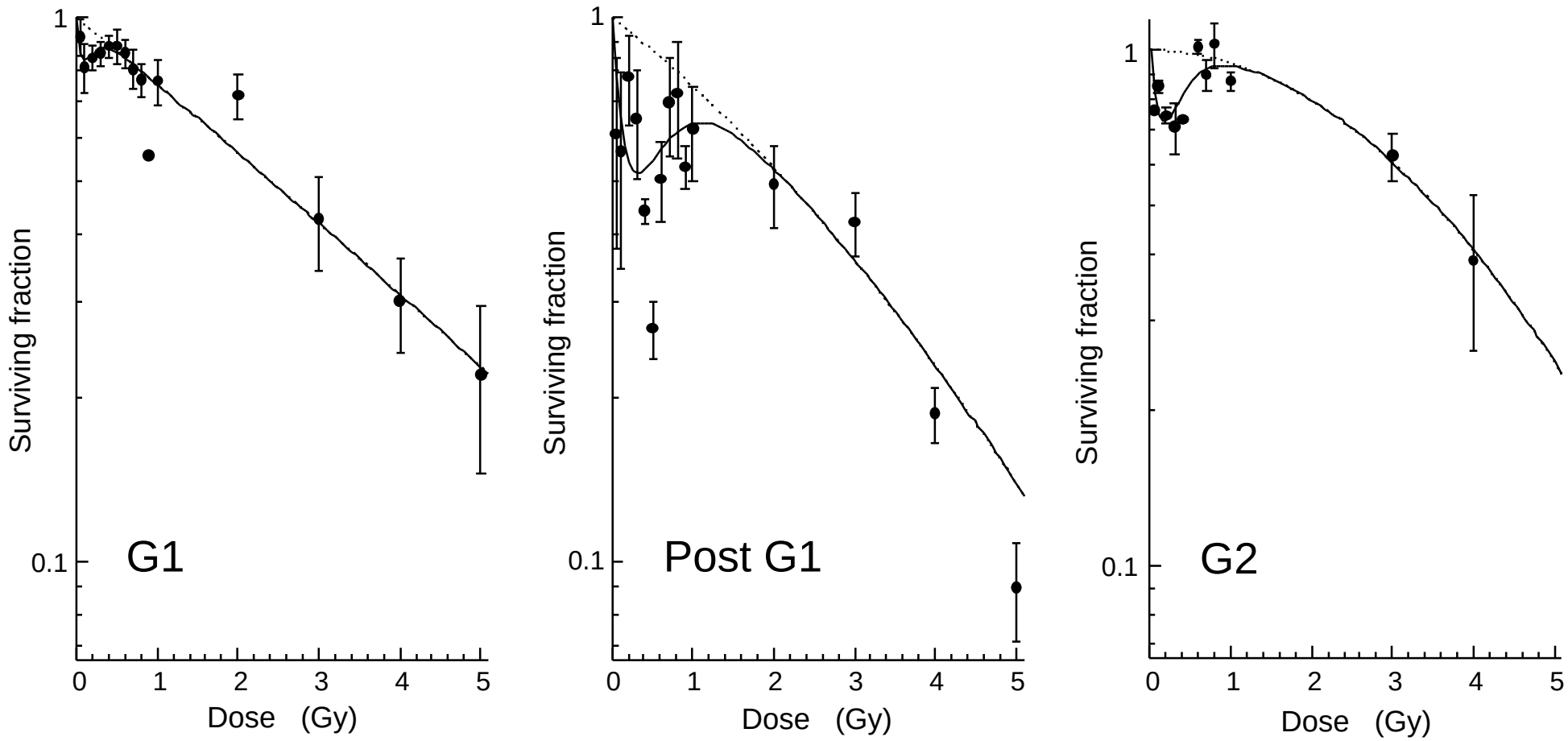
We developed this model to describe measured cell survival after exposure to the complete range of doses, right down to zero. For example, the solid lines in the cell survival plots (panel 3) show the fit of these equations to the data.
Cell lines which are more resistant to high doses (*e.g.* 2 Gy) usually demonstrate the biggest increase in sensitivity when exposed to very low doses.

HRS occurs in about 85% of cell lines tested



HRS is cell-cycle dependent

HRS is substantially more prominent in post-G1 (also in G2) compared with G1-phase cells. This observation should help point us in the direction of likely mechanisms. For example in T98G cells:



Unlikely mechanisms:

Apoptosis: HRS is seen in cell lines with no apoptotic response.

Sensitive subpopulations: HRS occurs in the presence and absence of cell-cycle delays at either the G1 or G2 checkpoints. HRS occurs in confluence- and low serum-arrested cells.

Possible mechanisms:

DNA repair:

- induction of IRR by prior treatment with DNA damaging agents;
- absence of IRR in some repair-deficient mutant cell lines;
- inhibition of IRR by modifers of DNA repair;
- apparent increase in DNA repair by prior treatment with small doses of DNA damaging agents;
- decrease in HRS/IRR with increasing LET;
- changes in DNA-PK activity correspond with changes in HRS/IRR response.

Our plan

DNA repair?

Compare the amount, rate and fidelity of repair with PFGE and comet assays with and without priming by small doses in the HRS region. Use established plasmid-based dsb-repair fidelity assays and develop a rapid DNA-break rejoining assay. Measure activites of DNA repair proteins such as DNA dependent protein kinase. Use ribozyme and antisense strategies to modulate individual repair pathways and determine effect on HRS/IRR.

The adaptive response?

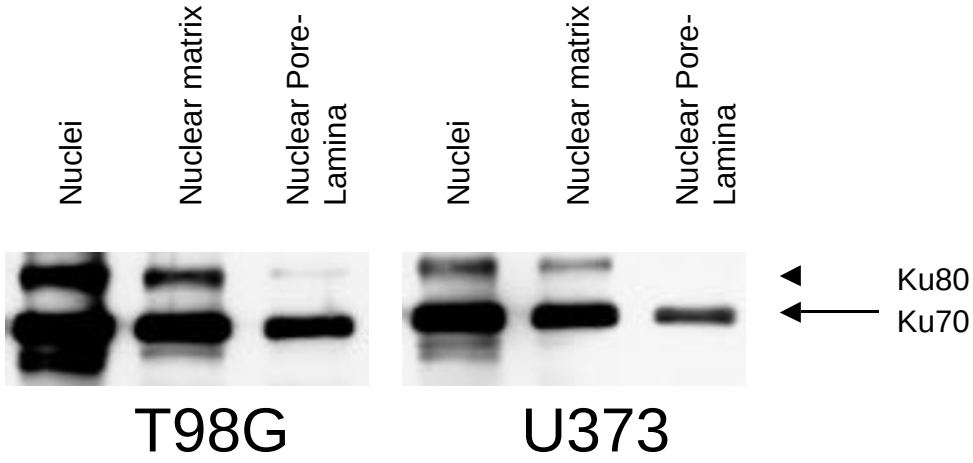
Using high-expressing HRS/IRR cell lines; measure response to successive small doses in the HRS domain as a function of interval between them. Short-term changes in radiosensitivity would indicate fundamental differences between HRS/IRR and the adaptive response.

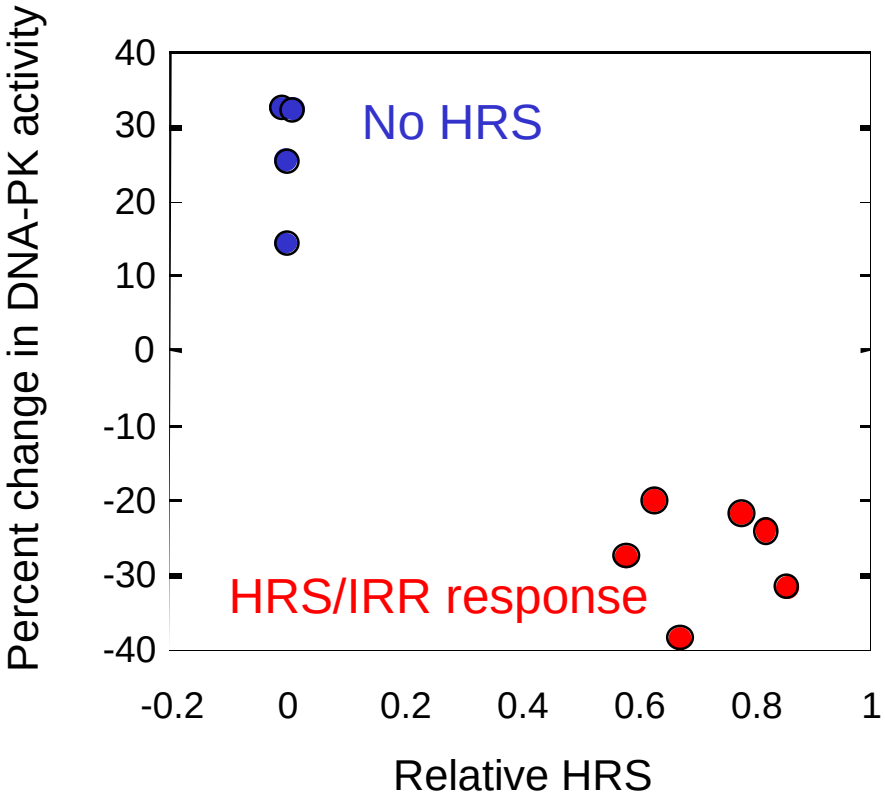
DNA organisation?

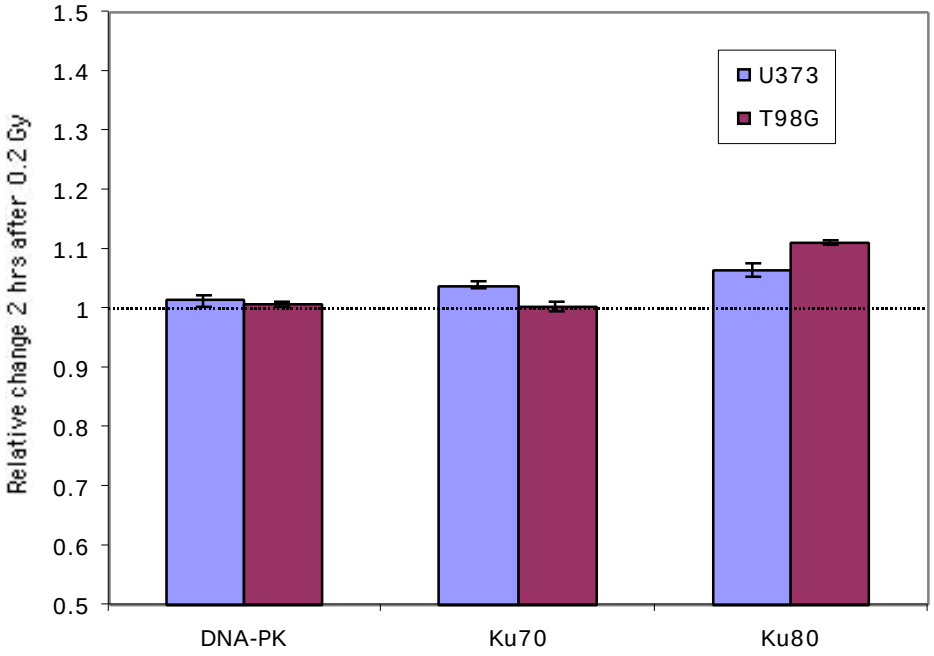
First, evaluate HRS/IRR as a function of cell-cycle position and compare with initial/residual DNA damage. Determine changes in DNA conformation and chromatin structure using special variations of comet/FISH assays and cytometric assays and correlate with cell cycle position and level of HRS/IRR.

HRS/IRR corresponds to changes in DNA-PK activity

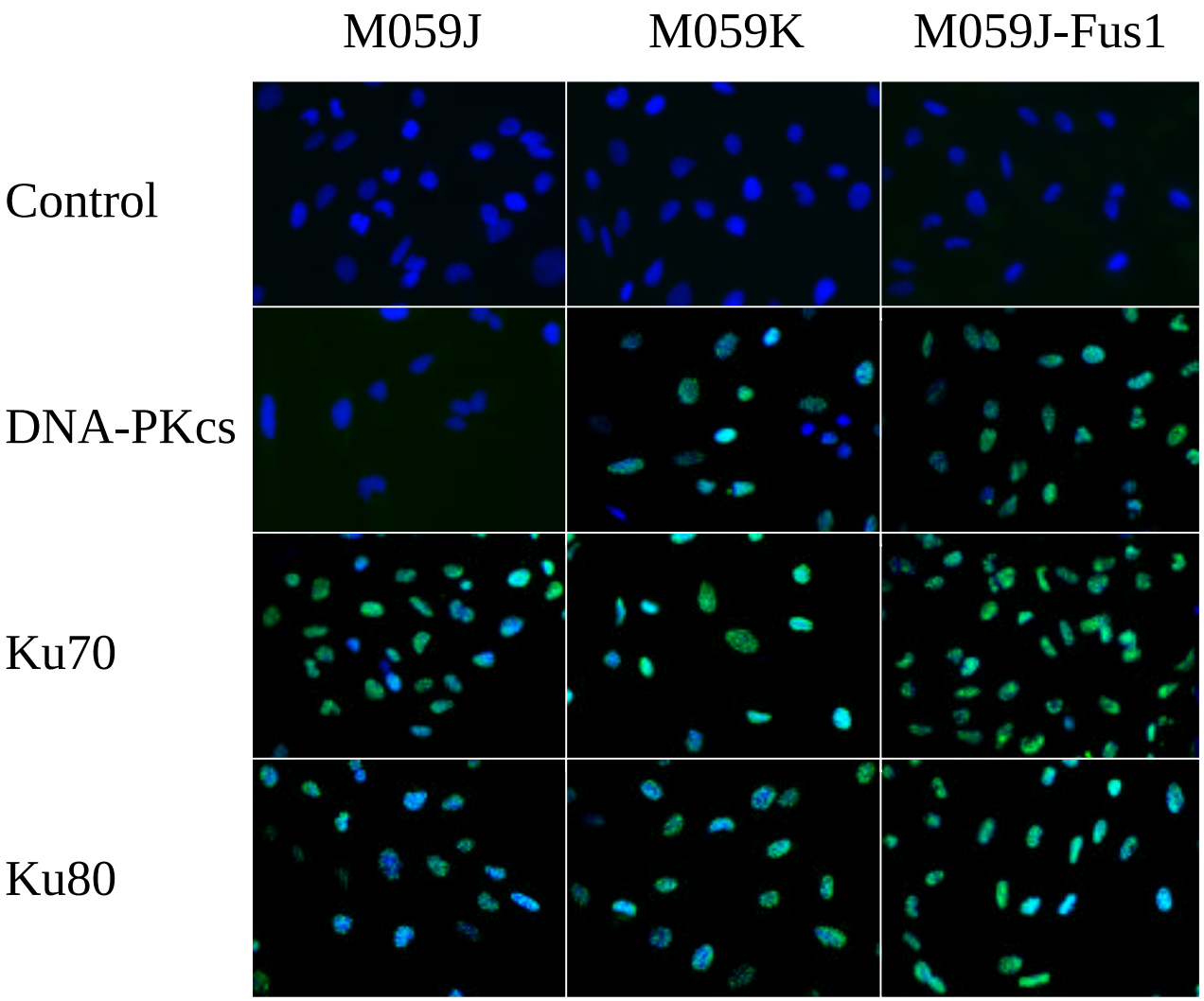
After a 0.2 Gy dose of X-rays, extractable DNA-PK activity (below) is reduced in cells exhibiting HRS/IRR (*e.g.* T98G) and increased in cells with no HRS (*e.g.* U373). Constitutive levels of Ku70 and Ku80 remain constant in cells with and without HRS/IRR responses as measured by Western blotting (right panel). Levels of DNA-PKcs, Ku70 and Ku80 do not change in response to 0.2 Gy as measured by fluorescent immuno-cytometry (lower-right panel).







Future work



We wish now to look at DNA-PK expression in more detail. These are 3 glioblastoma cell lines. MO59J and MO59K are derived from the same original tumour, but MO59J lacks DNA-PKcs whereas MO59K expresses this protein normally. MO59J-fus1 is a variant of MO59J which has been complemented with a fragment of human chromosome 8 that contains the DNA-PKcs gene (a gift from C. Kirchgessner, Stanford University). We shall use these cell lines to study the relationship between HRS and DNA-PK function in more detail.

The panel shows cells stained for DNA-PKcs, Ku70 and Ku80 (green), and counterstained with Hoechst 33342 (blue).

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