AN ASSOCIATION BETWEEN LOW-DOSE HYPER-RADIOSENSITIVITY AND THE EARLY G2-PHASE CHECKPOINT

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The phenomenon of low-dose hyper-radiosensitivity (HRS) is an effect in which cells die from excessive sensitivity to low doses (<0.3 Gy) of ionizing radiation but become more resistant (IRR) to larger doses. Current model to explain HRS suggests that the effect is a consequence of ineffective early G2-phase arrest of cells irradiated in G2 phase. In our previous study, using flow-cytometry-based clonogenic survival assay, the HRS response was demonstrated for the asynchronous and G2-phase fibroblasts of four of the 25 patients with cervix cancer. To determine an association between the occurrence of HRS and the activity of early G2-phase checkpoint, the number of mitotic cells (staining positive for phosphorylated histone H3) as a function of radiation dose was assessed for the asynchronous fibroblasts of four HRS+ positive and four HRS- negative patients.

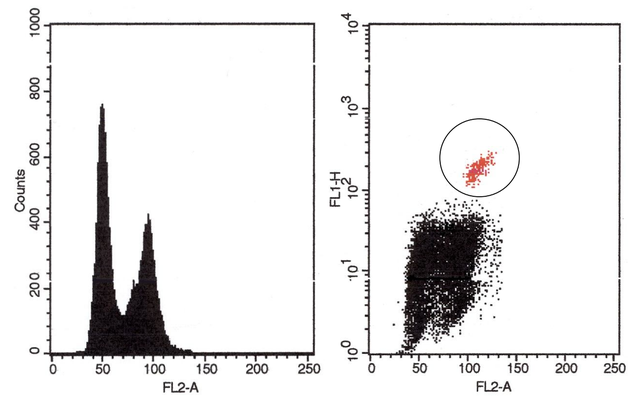
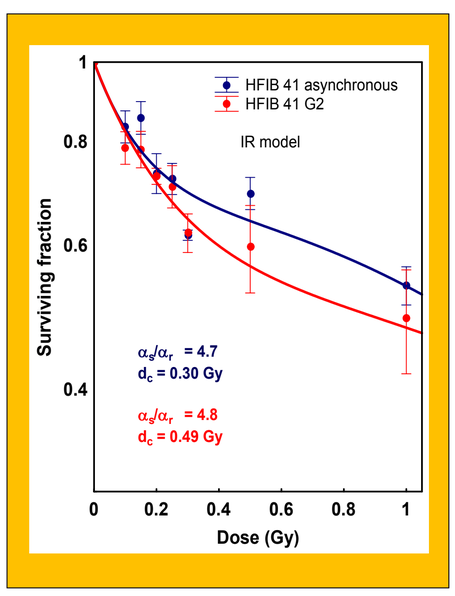
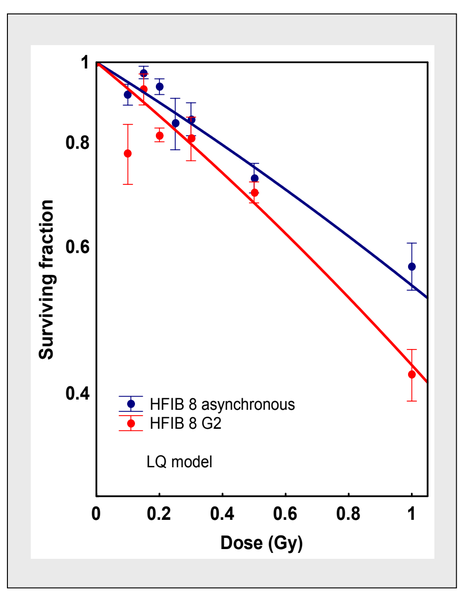
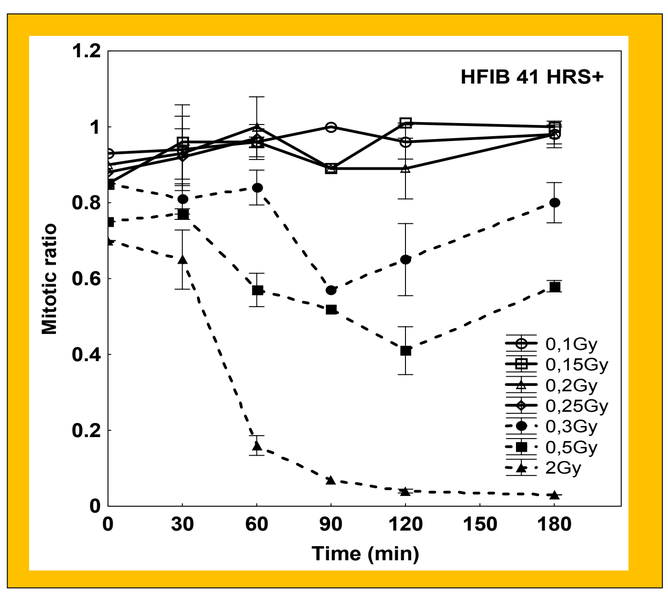


Fig 1. Flow cytometric results. DNA content and cell cycle distribution were analyzed after PI staining (left). Mitotic cells (stained positive with anti-phospho-histone H3) are encircled (right).







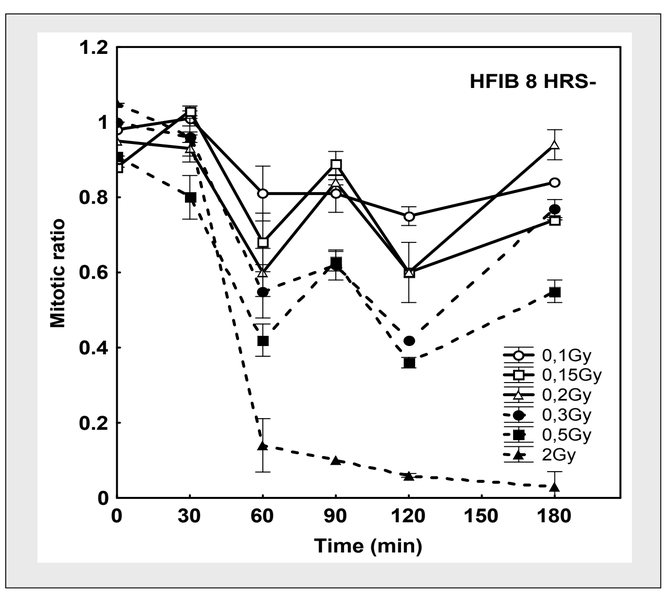


Fig 2. Survival of asynchronous (blue lines) and G2-phase (red lines) fibroblasts of HRS+ positive patient (left) and HRS- negative patient (right). Each point represents the mean ± SEM of 3-6 experiments. The lines show the fits of the induced-repair (IR) model (left) and linear-quadratic (LQ) model (right).

Fig 3. Measurement of asynchronous fibroblasts entering mitosis as a function of time after irradiation. The mitotic ratio is the ratio of mitotic cells for irradiated and non-irradiated cells. HRS+ positive fibroblasts (left) continue to enter mitosis at a constant rate after doses <0.3 Gy, indicating not active early G2-phase checkpoint. Conversely, HRS- negative fibroblasts (right) exhibit an active G2-phase arrest at all doses. In HRS+ positive cells (>0.3 Gy) and in HRS- negative cells (at all doses) the meaningful decrease in the mitotic ratio occurs as early as 1h after exposure. Data points represent mean ± SEM of 3 experiments.

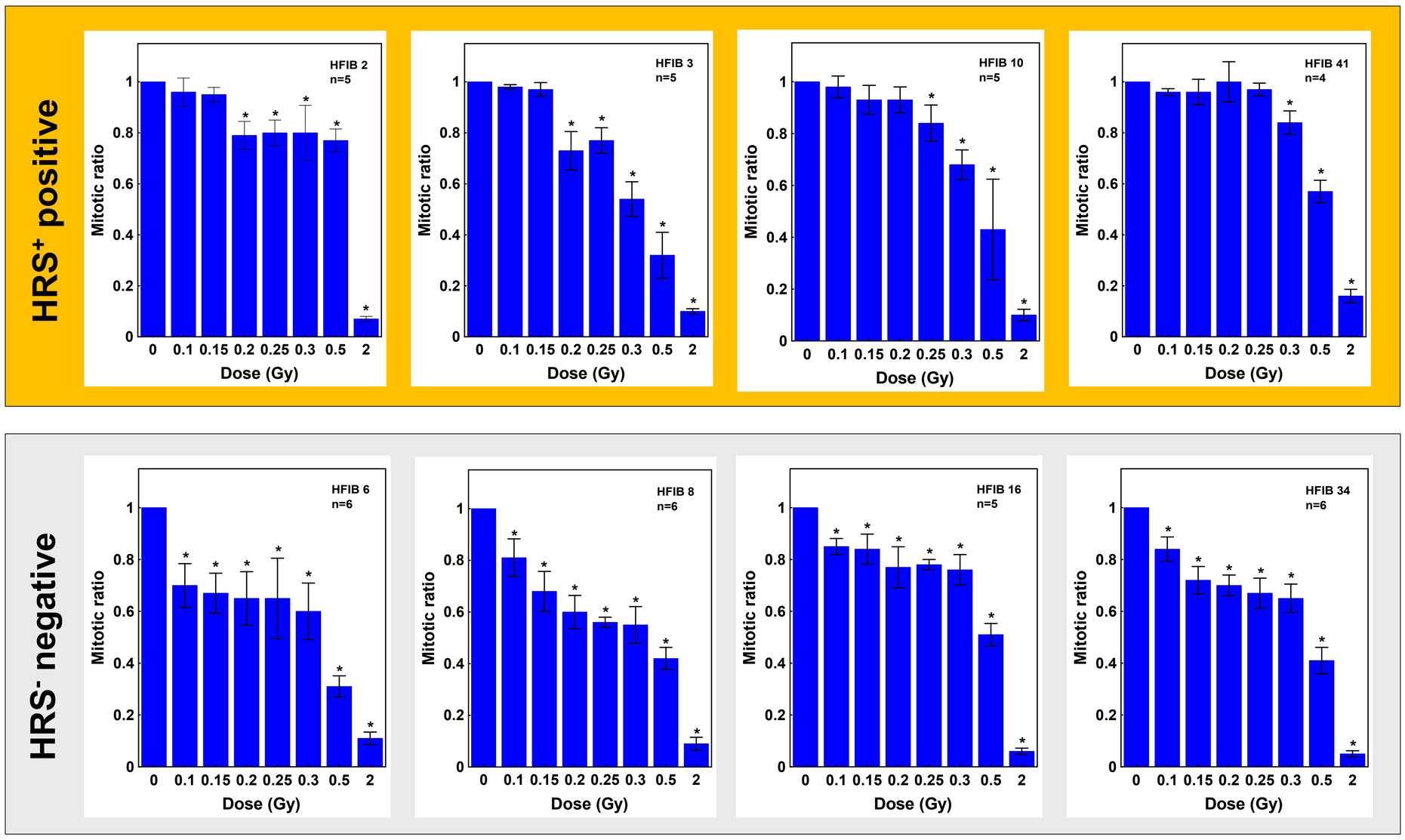


Fig 4. Measurement of asynchronous fibroblasts entering mitosis as a function of radiation dose 1h after irradiation. After low doses <0.3 Gy no reduction in the mitotic ratio was observed for HRS+ positive fibroblasts (upper panel). In contrast, HRS- negative fibroblasts (lower panel) exhibited an immediate post-irradiation decrease in the mitotic ratio even at the lowest doses. Data points represent mean ± SEM of 4-6 experiments.

Conclusion

No significant reduction in mitotic ratio, evident for HRS+ positive fibroblasts irradiated with doses lower than 0.3 Gy, provides support to the relationship between the HRS response and failure of the early G2-phase checkpoint.

Work supported by National Science Center grant no. N N401 102539.