RADIATION RESEARCH 138, S32–S36 (1994)

Multiphasic Survival Curves for Cells of Human Tumor Cell Lines: Induced Repair or Hypersensitive Subpopulation?

P. Lambin,*,†,§ B. Fertil,‡ E. P. Malaise† and M. C. Joiner*,[1]

*CRC Gray Laboratory, P.O. Box 100, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, United Kingdom;
†Laboratoire de Radiobiologie Cellulaire (Unité Inserm 247), Institut Gustave-Roussy, 94805 Villejuif, France;
‡Laboratoire de Biostatistiques (Unité Inserm 194), Hôpital la Pitié-Salpétrière, 75014 Paris, France;
and §Department of Radiation-Oncology, University Hospital St. Rafaël, Leuven, Belgium

Lambin, P., Fertil, B., Malaise, E. P. and Joiner, M. C. Multiphasic Survival Curves for Cells of Human Tumor Cell Lines: Induced Repair or Hypersensitive Subpopulation? *Radiat. Res.* 138, S32–S36 (1994).

Survival of the cells of three human tumor cell lines of differing radiosensitivity was measured after irradiation with single doses of X rays (0.05–5 Gy). At doses below 1 Gy, cells were more radiosensitive than predicted by back-extrapolating the high-dose response. This difference was more marked for cells of the *radioresistant* cell lines than the radiosensitive cell line so that the "true" initial slopes of the survival curves, at very low doses, were similar for the cells of the three cell lines. This phenomenon could reflect an induced radioresistance so that low doses of X rays are more effective *per gray* than higher doses, because only at higher doses is there sufficient damage to trigger repair systems or other radioprotective mechanisms which can then act during the time course for repair of DNA injury.

INTRODUCTION

The linear-quadratic (LQ) model is now used widely to model the shapes of radiation cell survival curves *in vitro*. As dose is reduced, the LQ equation predicts a decreasing slope to the relationship between surviving fraction (SF) and dose (d), which asymptotically approaches an "initial" slope termed the α value. Specifically, $-\ln(SF) = \alpha d + \beta d^2$.

We demonstrated previously that the LQ model substantially *underestimated* the effect of *single* X-ray doses <1 Gy in asynchronous V79 hamster cells (*1*) and HT29 human tumor cells (*2*) *in vitro*: as the dose was reduced to very low values, the slope of the cell survival curve asymptotically approached a value several times steeper than the α value derived from the LQ model fitted to the data at doses >1 Gy and extrapolated into the low-dose region. We have argued that this increased low-dose effectiveness is unlikely to be due to subpopulations of cells with different radiosensitivity (e.g. in different phases of the cell cycle) and could be a manifestation of "induced" radioprotection. We have proposed that low doses may be more effective per gray because only at higher doses is there sufficient initial damage to trigger repair systems or other radioprotective mechanisms (*1*, *2*).

In this paper, we report a comparison of the low-dose survival response of cells of three human tumor cell lines of differing radiosensitivity: relatively radioresistant HT29 and Be11 cells with surviving fractions at 2 Gy (SF2) of 74 and 67%, respectively, and highly radiosensitive SW48 cells with SF2 = 16%.

MATERIALS AND METHODS

The HT29 and SW48 cell lines were derived originally from colorectal tumors and the Be11 line from a melanoma. All cells were maintained in monolayer culture *in vitro*, in Eagle's Minimum Essential Medium (MEM) supplemented with 20% fetal calf serum, penicillin (80 units ml^{-1}) and streptomycin (0.25 mg ml^{-1}), and were passaged routinely once a week using a calcium-free salt solution with 0.1% trypsin and 0.04% EDTA.

Cells were irradiated with X rays generated by a Pantak unit operating at 240 kVp with filtration of 0.25 mm Cu + 1 mm Al giving an HVL of 1.3 mm Cu. A dose rate of 0.18 Gy min^{-1} was chosen to ensure exposure times of at least 17 s and hence accurate dosimetry in the low-dose range. Cell survival was determined after single doses of up to 5 Gy but focusing on survival at doses less than 1 Gy. Flasks were irradiated 6 h after plating.

Cell survival was measured using a Dynamic Microscopic Image Processing Scanner (DMIPS) cell analyzer (*3*, *4*) according to the method of Marples and Joiner (*1*), with the usual criterion of 50 cells or more per colony as confirmed by manual microscopic examination of all originally recorded cell locations in each flask after 6–7 days' incubation at 37°C. Surviving fraction (SF) was calculated by dividing the plating efficiency of irradiated cells by the plating efficiency of sham-irradiated cells. In all three cell lines, at the high survival levels investigated (SF > 20%), a low incidence of slow-growing colonies allowed reliable scoring at this time; surviving colony sizes were almost universally well in excess of 50 cells and were easily distinguished from abortive colonies with fewer than 50 cells. Additionally, we have found that for HT29 cells, surviving fraction determined by this method is independent of the time of colony scoring at 6, 7 or 8 days after plating (P. Lambin *et al.*, unpublished), and this agrees with a recent finding by Spadinger *et al.* (*3*), where survival was similar at 4 and 6 days after plating V79 hamster cells.

RESULTS

Figure 1 and the left panel of Fig. 1 in the accompanying paper (*4*) show the survival measured in the two radioresistant cell lines, HT29 and Be11. Increased low-dose sensitivity, relative to an extrapolated high-dose LQ prediction (shown as a dotted line), can be seen clearly as a substantial downward "kink" in the survival curve as the dose is reduced below 1 Gy. In radiosensitive SW48 cells (Fig. 2), there was apparently no increased low-dose sensitivity.

[1]To whom correspondence should be addressed.

033-7587/94 $5.00

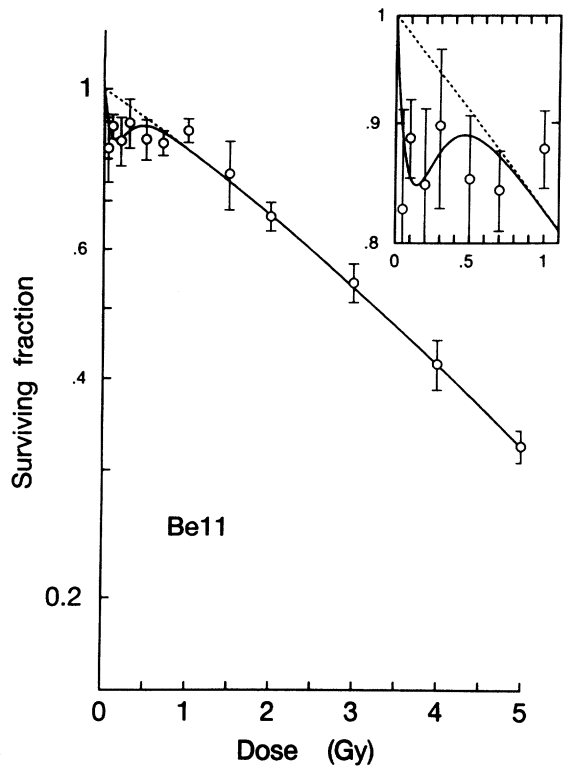

FIG. 1. Survival of radioresistant Be11 cells irradiated with 240 kVp X rays. Data points show the mean ± SEM of four experiments with multiple repeats at some doses (generally between five and seven determinations of survival per dose). The dotted line shows the fit of the linear-quadratic model to the X-ray data at ≥2 Gy. The solid line shows the fit of the induced repair model to all the X-ray data. At X-ray doses <1 Gy, the LQ model substantially underpredicts the effect of X rays, which is better fitted by the induced repair model. The inset shows the region of the survival curve between 0 and 1 Gy magnified.

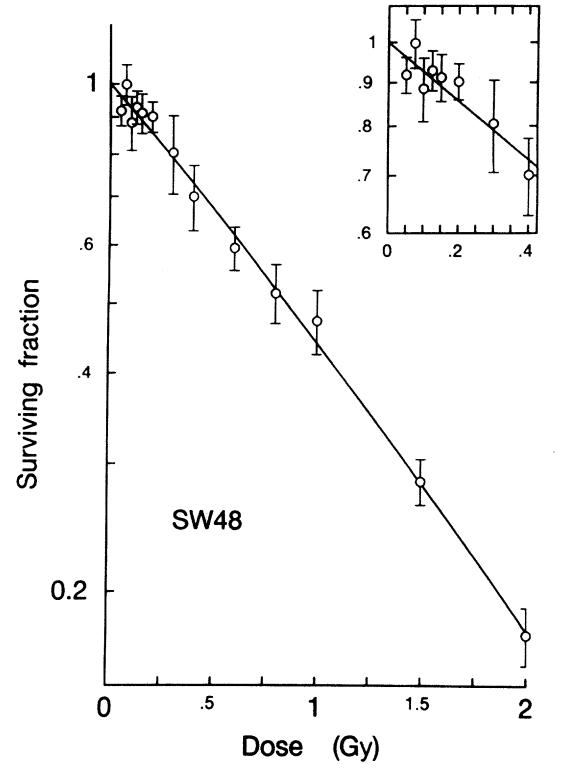

FIG. 2. Survival of radiosensitive SW48 cells irradiated with 240 kVp X rays. Data points show the mean ± SEM of seven experiments with multiple repeats at some doses (generally between five and ten determinations of survival per dose). The response of SW48 cells is well described by the LQ model (solid line) with no evidence of low-dose hypersensitivity. The inset shows the region of the survival curve between 0 and 0.4 Gy magnified.

To describe these data, we have developed a modification of the LQ equation in which the α term is dependent on dose: at very low doses α is large, and it decreases with increasing dose in an exponential fashion with a "rate" determined by a dose constant, d_c, of about 0.2 Gy. This is a simple model for a process of induced radioresistance, where the degree of induction depends on dose. Specifically,

$$SF = \exp\{-\alpha_r[1 + (\alpha_s/\alpha_r - 1)e^{-d/d_c}]d - \beta d^2\}. \quad (1)$$

The parameter α represents the low-dose slope of the survival curve extrapolated from high doses; this is α from the simple LQ model fitted to the data at higher doses only, shown by the dotted lines. The parameter α_s is the actual α value measured and is the slope of the solid lines at very low doses ($d \ll d_c$). The ratio α_s/α_r therefore indicates the magnitude of induced radioprotection as the dose increases above 1 Gy ($d \gg d_c$). We have previously termed this equation the "induced repair" model (*1*, *2*, *5*, *6*), and it is shown as solid lines in Fig. 1 and the left panel of Fig. 1 in the accompanying paper (*4*). The fit of this model to all the data in the two figures is statistically better than the fit of the LQ model, with a lower minimum residual variance. However, for Fig. 2, no statistical advantage could be obtained by fitting the data with the induced repair model and α_s and d_c could not be determined. This suggests the absence of induced radioresistance in this cell line, although the existence of such a phenomenon cannot be ruled out at lower doses than tested in these experiments. We are now accumulating data on human HX142 neuroblastoma cells and a line of human AT fibroblasts (AT5BIVA), which are also indicating a lack of low-dose substructure in these very radiosensitive cells.

DISCUSSION

The DMIPS has allowed us to study the surviving fraction of human cell lines irradiated with doses of 0.05 to 5 Gy, corresponding to less than the first decade of cell killing. At doses less than 1 Gy, the DMIPS method has considerably greater resolution than conventional cell plating assays and is accurate enough to show surviving fractions significantly less than 100% for most of the doses tested. This extra precision with the DMIPS procedure is obtained because in each flask, a precisely *known* number of cells are located and their positions recorded for subsequent revisiting to assess colony formation (*3*, *5*). The distribution of

surviving cells is therefore binomial rather than Poisson.

The low-dose shapes of the survival curves of HT29 and Be11 cells are similar to the shapes of the survival curves for TN-368 insect cells (*8–10*), budding yeast (*11*) and algae (*12*). That this type of "multiphasic" curve cannot represent the sum of two genetically distinct moieties, has been proven mathematically (*11*). We have therefore considered three possible explanations for the increased effectiveness of low doses, which are fundamentally different.

First, could some unidentified experimental artefact have produced these results? Based on the considerable evidence of such radiation responses in the literature (see ref. *2* and Joiner, this issue), we consider this unlikely. However, in each experiment, we were careful to randomize fully the order of plating followed by irradiation and scanning among the different dose groups. The scanning of the flasks and the analysis of cell survival were both performed blindly. In addition, for the HT29 cell line, we found an increased sensitivity to low doses of X rays but *not* to low neutron doses (*2*); Boreham and Mitchel (*13*) also found that high-LET radiations were less able to induce repair in yeast. From Fig. 2, apparently there is *no* low-dose hypersensitivity in the more radiosensitive cell line SW48. Certain yeast mutants (e.g. *RAD52)* also do not show induced repair (*14*), but the specific mutation (if any) conferring radiosensitivity on SW48 has not been identified. Considering all this evidence does suggest, on balance, that the pattern of X-ray survival found in these cell lines has *not* been influenced by some unknown artefact of the DMIPS procedure and is therefore a genuine phenomenon.

Second, a seemingly obvious explanation for these observations is that the low-dose substructure in the survival curve is determined by a small subpopulation of sensitive cells, in a sensitive phase of the cell cycle, for example. Indeed, such biphasic survival curves are often seen in *in vivo–in vitro* assays of survival of cells from irradiated tumors containing a significant amount of hypoxic (radioresistant) cells, and it is well known that particular phases of the cell cycle are more radiosensitive than the average. However, the most compelling argument against this explanation is that the notional small subpopulation that determines the shape of the cell survival curve at low doses would have to be unreasonably radiosensitive. For example, with HT29 cells, 7% of the total cell population would need to have a $\overline{D}$ of 0.042 Gy (*2*). The AT cell lines, traditionally considered as the most radiosensitive, have a mean $\overline{D}$ of about 0.55 Gy (*15*), and even if we take into account possible cyclic variations in radiosensitivity, it is hard to imagine that 7% of any cell population could have a $\overline{D}$ 13 times lower than AT cells and 81 times lower than the majority of the HT29 population. Only an approximate 10-fold maximum variation in the radiosensitivity of mammalian cells has been reported *in vitro* between the different phases of the cell cycle (*16*). In the HT29 cell line (*4*) there is a distinct region of the survival curve from about 0.3–0.7 Gy where survival does not change with dose. If this is to be explained by proposing that this net response is the sum of the responses of cell subpopulations in different phases of the cycle, then it can be shown mathematically that at least one of those subpopulations must respond to radiation by *increasing* cell survival—in other words, a case of radiation creating life. This seems unreasonable. An increase in the absolute plating efficiency of the cells as a result of radiation could also explain this effect, but it is very difficult to distinguish this from a genuine increase in survival. Furthermore, Marples and Joiner (*1*) have also demonstrated low-dose substructure in V79 cells, where it is more difficult to argue for any increase in absolute plating efficiency since this is already close to 100% in this cell line. Two further arguments against the involvement of cell cycle phase-specific radiosensitivity in these single-dose experiments are that first, the hypersensitivity at low doses was not seen with the sensitive cell line SW48 despite a cell cycle time (28 h) on the same order as that of Be11 (30 h) and HT29 (25 h) cells, and second, Marples and Joiner (*1*) have also demonstrated that hypersensitivity of V79 hamster cells to low X-ray doses is similar in asynchronous and partially synchronized cell populations.

A third possibility to explain the low-dose substructure in the response of the resistant cell lines is that the radiosensitivity of the *whole* cell population decreases with increasing dose over the range 0–1 Gy, an idea that has been termed induced repair by several authors (*17–20*, for example) and is embodied in Eq. (1). If induced radioresistance is the explanation for the low-dose substructure in the response of HT29 and Be11 cells to single doses, then it should be possible, in split-dose experiments, for a first dose to initiate an adaptive response that provides protection against a subsequent exposure as has been found in studies on human lymphocytes (*20*). We have done such experiments with HT29 cells, using an interdose interval of 4 h as in the lymphocyte studies (*20*). Cells irradiated twice (2×0.25 Gy) had a significantly higher overall SF than cells irradiated once with 0.5 Gy. In other words, there was split-dose recovery as expected. However, the response to 2×0.25 Gy was composed of unequal responses to each of the two doses, with significantly less response to the second dose than the first dose. This is consistent with the existence of an adaptive response in these cells which in turn supports the substructure seen in the single-dose survival curve at doses <1 Gy (see left panel of Fig. 1 in ref. *4*).

Hypersensitivity to single doses was not seen with the sensitive cell line SW48, and this suggests a link to radiosensitivity; cells would therefore be intrinsically radiosensitive to clinically sized doses because they

have a diminished inducible response. This hypothesis is supported by preliminary results indicating a "nonmultiphasic" survival curve also obtained with another very radiosensitive cell line, HX142 (P. Lambin *et al.*, unpublished) and with the AT5BIVA line of human AT fibroblasts (B. Singh *et al.*, unpublished). Although there is no evidence for specific mutations conferring radiosensitivity in either SW48 or HX142 cells, the preliminary data for AT fibroblasts certainly agree with the lack of inducible repair seen in sensitive mutants of lower eukaryotes (ref. *14*, for example).

Therefore, although it is still possible that the differing radiosensitivity of two or more cell populations, for example in different stages of the cycle, does play some role in determining the low-dose shape of the X-ray survival curve, we feel we have ruled this out as a *sole* explanation for the effects seen. Rather, it is likely that there is a dose-dependent inducible radioprotective process that is necessary to explain the phenomenon.

Comparison with Other Studies

As a necessary part of an induced-radioresistance or induced-repair hypothesis, it is now clear that ionizing radiation can indeed induce expression of genes (*21*), production of *m*RNA (*22*) and synthesis of proteins (*23*), particularly specific DNA-binding proteins, in nuclei (*24*). Induced protection has been reported many times in mammalian cells exposed to DNA-damaging agents (see refs. *19*, *25*, *26*, for example). Induced systems that protect specifically against radiation damage have also been documented in yeast (*14*, *17*), *E. coli* (*27*), algae (*28–30*), protozoan cells (*31*), plant cells *in vitro* (*32*), insect cells (*33*), human lymphocytes (*20*) and human fibroblasts (*25*). It is therefore not unreasonable to hypothesize that the repair of DNA is, at least partly, inducible by X rays and that this may be responsible for the shape of the cell survival curve over the first gray.

Inducible repair systems have been proposed to explain the observations from a large series of studies (*20*) in which human lymphocytes irradiated with a small dose of X rays or by tritiated thymidine become less susceptible to the effects of subsequent irradiation given 4–6 h later, as assessed by the number of chromosome aberrations. While this "adaptive response" supports the present data, as discussed above, the "inducing" dose in the lymphocyte studies was very low (about 0.01 Gy, ref. *19*), compared with the higher value (~0.2–0.6 Gy) determined for end points for killing of mammalian cells *in vitro* (refs. *1*, *2*, this study) and *in vivo* (*6*, *7*) and the considerably higher value (~100 Gy) determined in the studies of insect cells (*8–10*). The lower value of inducing dose in lymphocytes may reflect the split-dose experimental design: it is possible that if several hours are available after a priming dose before the challenge dose, then this may be sufficient time for up-regulation of radioresistance even if the priming dose is very small. In single-dose studies, however (Fig. 1 for example), significant radioresistance has to be induced within probably the first hour of irradiation to have an impact on repair of DNA injury which is probably complete within a few hours. This shorter induction time may require the larger doses suggested by the inflexion points around 0.5 Gy in Fig. 1 and the left panel of Fig. 1 in ref. (*4*). We are now determining whether much smaller doses than 0.25 Gy can be used to induce radioresistance in further split-dose studies in HT29 cells.

For normal tissues irradiated *in vivo*, the LQ model reasonably predicts changes in the effect of radiation with fractionation for doses greater than 1 Gy per fraction. If an induced protection mechanism comparable to that proposed *in vitro* existed in normal tissues, it would be detected in fractionation experiments only with doses per fraction less than 0.5 Gy. In fact, three studies *have* indicated hypersensitivity to low doses per fraction *in vivo* in mouse skin and kidney and a rat tumor (*6*, *7*, *34*). In the kidney study, any influence of the cell cycle could probably be discounted because the labeling index of cells extracted from the organ is less than 0.3%. These data *in vivo* are entirely consistent with our observations in human tumor cells *in vitro*. It might therefore be important to take account of a higher than expected biological effect of very low doses per fraction delivered in the penumbrae of fields treated by conventional radiotherapy. However, one must bear in mind that, although the effect per unit total dose could be much higher, the *total* doses in these regions are still very small so the final damage is unlikely to exceed tolerance. However, it may be important to consider the possible extra effect of low doses outside the target volume if regions nearby are retreated or a possible increased incidence of mutation, and therefore of secondary cancers outside the target volume (*35*).

In conclusion, with lethality as the end point, we have shown that cells of two human tumor cell lines, HT29 and Be11, considered as radioresistant at the usual X-ray doses, are hypersensitive at doses less than 0.5 Gy. This phenomenon is not apparent with a sensitive cell line (SW48), and at very low doses the radiosensitivity of all the cell lines is more similar. A possible explanation for the different response patterns of these three cell lines at very low doses is that the magnitude of cellular radioresistance measured above 1 Gy may correlate with the apparent inducible response over the first gray.

ACKNOWLEDGMENTS

This work was supported by the Cancer Research Campaign UK, grant no. B17-913001 of the European Community, the Ligue Nationale Française Contre le Cancer (Comité des Hauts-de-Seine) and le Comite de radioprotection de l' Electricité de France. We would also like to acknowledge the assistance of Helen Johns, Karin Haustermans and Monique Ramaekers in the preparation of these data for publication.

REFERENCES

1. B. Marples and M. C. Joiner, The response of Chinese hamster V79 cells to low radiation doses: Evidence of enhanced sensitivity of the whole cell population. *Radiat. Res.* **133**, 41–51 (1993).

2. P. Lambin, B. Marples, E. P. Malaise, B. Fertil and M. C. Joiner, Hypersensitivity of a human tumour cell line to very low radiation doses. *Int. J. Radiat. Biol.* **63**, 639–650 (1993).
3. I. Spadinger and B. Palcic, Cell survival measurements at low doses using an automated image cytometry device. *Int. J. Radiat. Biol.* **63**, 183–189 (1993).
4. P. Lambin, J. Coco-Martin, J. D. Legal, A. C Begg, C. Parmentier, M. C. Joiner and E. P. Malaise, Intrinsic radiosensitivity and chromosome aberration analysis using fluorescence *in situ* hybridization in cells of two human tumor cell lines. *Radiat. Res.* **138**, S40–S43 (1994).
5. B. Palcic and B. Jaggi, The use of solid-state image sensor technology to detect and characterize live mammalian cells growing in tissue culture. *Int. J. Radiat. Biol.* **50**, 345–352 (1986).
6. M. C. Joiner, J. Denekamp and R. L. Maughan, The use of 'top-up' experiments to investigate the effect of very small doses per fraction in mouse skin. *Int. J. Radiat. Biol.* **49**, 565–580 (1986).
7. M. C. Joiner and H. Johns, Renal damage in the mouse: The response to very small doses per fraction. *Radiat. Res.* **114**, 385–398 (1988).
8. T. M. Koval, Multiphasic survival response of a radioresistant lepidopteran insect cell line. *Radiat. Res.* **98**, 642–648 (1984).
9. T. M. Koval, Inducible repair of ionising radiation damage in higher eukaryotic cells. *Mutat. Res.* **173**, 291–293 (1986).
10. T. M. Koval, Enhanced recovery from ionizing radiation damage in a lepidopteran insect cell line. *Radiat. Res.* **115**, 413–420 (1988).
11. C. A. Beam, R. K. Mortimer, R. G. Wolfe and C. A. Tobias, The relation of radioresistance to budding in *Saccharomyces cerevisiae*. *Arch. Biochem. Biophys.* **49**, 110–122 (1954).
12. R. J. Horsley and C. M. Pujara, Study of the inflexion of X-radiation survival curves for synchronized cell populations of the green alga (*Oedogonium cardiacum*). *Radiat. Res.* **40**, 440–449 (1969).
13. D. R. Boreham and R. E. J. Mitchel, DNA lesions that signal the induction of radioresistance and DNA repair in yeast. *Radiat. Res.* **128**, 19–28 (1991).
14. R. E. J. Mitchel and D. P. Morrison, Inducible DNA-repair systems in yeast: Competition for lesions. *Mutat. Res.* **183**, 149–159 (1987).
15. P. J. Deschavanne, D. Debieu, B. Fertil and E. P. Malaise, Re-evaluation of *in vitro* radiosensitivity of human fibroblasts of different genetic origins. *Int. J. Radiat. Biol.* **50**, 279–293 (1986).
16. W. K. Sinclair, Cell cycle dependence of the lethal radiation response in mammalian cells. *Curr. Top. Radiat. Res. Q.* **7**, 264–285 (1972).
17. J. Calkins and W. Todd, Evidence for a triggered or activated radiation repair system in *Saccharomyces cerevisiae*. *Int. J. Radiat. Biol.* **14**, 487–491 (1968).
18. M. C. Joiner and B. Marples, Does induced repair determine the lethal effects of very low radiation doses? In *Radiation Science: Of Molecules, Mice and Men* (J. Denekamp and D. G. Hirst, Eds.), pp. 74–78. Report 24, British Institute of Radiology, London, 1992.
19. S. Sengupta and S. B. Bhattacharjee, Induction of repair functions by hydrogen peroxide in Chinese hamster cells. *Int. J. Radiat. Biol.* **53**, 935–942 (1988).
20. S. Wolff, J. K. Wiencke, V. Afzal, J. Youngblom and F. Cortes, The adaptive response of human lymphocytes to very low doses of ionizing radiation: A case of induced chromosomal repair with the induction of specific proteins. In *Low Dose Radiation: Biological Bases of Risk Assessment* (K. F. Baverstock and J. W. Stather, Eds.), pp. 446–454. Taylor & Francis, London, 1989.
21. M. A. Papathanasiou, N. C. Kerr, J. H. Robbins, O. W. McBride, I. J. Alamo, S. F. Barrett, I. D. Hickson and A. J. Fornace, Induction by ionizing radiation of the *gadd45* gene in cultured human cells: Lack of mediation by protein kinase C. *Mol. Cell. Biol.* **11**, 1009–1016 (1991).
22. G. E. Woloschak, C-M. Chang-Liu and P. Shearin-Jones, Regulation of protein kinase C by ionizing radiation. *Cancer Res.* **50**, 3963–3967 (1990).
23. D. A. Boothman, I. Bouvard and E. N. Hughes, Identification and characterization of X-ray-induced proteins in human cells. *Cancer Res.* **49**, 2871–2878 (1989).
24. S. P. Singh and M. F. Lavin, DNA-binding protein activated by gamma radiation in human cells. *Mol. Cell. Biol.* **10**, 5279–5285 (1990).
25. L. Samson and J. L. Schwartz, Evidence for an adaptive DNA repair pathway in CHO and human skin fibroblast cell lines. *Nature* **287**, 861–863 (1980).
26. S. Lindquist, The heat-shock response. *Annu. Rev. Biochem.* **55**, 1151–1191 (1986).
27. E. C. Pollard, D. J. Fluke and D. Kazanis, Induced radioresistance: an aspect of induced repair. *Mol. Gen. Genet.* **184**, 421–429 (1981).
28. P. E. Bryant, Changes in sensitivity of cells during exposure to radiation at low dose-rate. *Int. J. Radiat. Biol.* **22**, 67–73 (1972).
29. A. Howard and F. G. Cowie, Induced resistance in *Closterium*: Indirect evidence for the induction of repair enzyme. *Radiat. Res.* **75**, 607–616 (1978).
30. S. Santier, R. Gilet and E. P. Malaise, Induced radiation resistance during low-dose-rate gamma irradiation in plateau-phase *Chlorella* cells. *Radiat. Res.* **104**, 224–233 (1985).
31. J. Calkins, An unusual form of response in X-irradiated protozoa and a hypothesis as to its origin. *Int. J. Radiat. Biol.* **12**, 297–301 (1967).
32. H. P. Leenhouts, M. J. Susma, M. Litwiniszin, C. Droerties and K. H. Chadwick, Radiation stimulated repair in *Saintpaulia* cells *in vivo*. In *Radiation Biology and Chemistry* (H. E. Edwards, S. Navaratnam, B. J. Parsons and G. O. Phillips, Eds.), pp. 227–236. Elsevier, Amsterdam, 1979.
33. T. M. Koval, Inducible repair of ionizing radiation damage in higher eukaryotic cells. *Mutat. Res.* **173**, 291–293 (1986).
34. H. P. Beck-Bornholdt, T. Maurer, S. Becker, M. Omniczynski, H. Vogler and F. Wurschmidt, Radiotherapy of the rhabdomyosarcoma R1H of the rat: Hyperfractionation—126 fractions applied within 6 weeks. *Int. J. Radiat. Oncol. Biol. Phys.* **16**, 701–705 (1989).
35. N. E. A. Crompton, B. Barth and J. Kiefer, Inverse dose-rate effect for the induction of 6-thioguanine-resistant mutants in Chinese hamster V79-S cells by ^{60}Co γ rays. *Radiat. Res.* **124**, 300–308 (1990).