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Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of exposure to ionizing radiation

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The paradigm of genetic alterations being restricted to direct DNA damage after exposure to ionizing radiation has been challenged by observations in which cells that are not exposed to ionizing radiation exhibit responses typically associated with direct radiation exposure. These effects are demonstrated in cells that are the descendants of irradiated cells (radiation-induced genomic instability) or in cells that are in contact with irradiated cells or receive certain signals from irradiated cells (radiation-induced bystander effects). There is accumulating evidence that radiation-induced genomic instability may be a consequence of, and in some cell systems may also produce, bystander interactions involving intercellular signalling, production of cytokines and free-radical generation. These processes are also features of inflammatory responses that are known to have the potential for both bystander-mediated and persisting damage as well as for conferring a predisposition to malignancy. Thus, radiation-induced genomic instability and untargeted bystander effects may reflect inter-related aspects of inflammatory-type responses to radiation-induced stress and injury and contribute to the variety of pathological consequences of radiation exposures.
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Radiation-induced genomic instability

Significant biological consequences of exposure to ionizing radiation include cell death, gene mutations and chromosome aberrations. These effects are conventionally attributed to an irreversible change resulting from the deposition of energy in the DNA of an irradiated cell being fixed either during the processing and enzymatic repair of DNA damage or during DNA replication. Accordingly, it has been widely accepted that most of these changes take place immediately following exposure. Thus, if the damage were repaired, the progeny of an irradiated cell would appear normal (Figure 1a), but if misrepaired, the progeny would be expected to show any transmissible radiation-induced genetic change and all cells derived from such a cell would exhibit the same genetic change, that is, the effect would be clonal (Figure 1b). However, many laboratory studies have demonstrated nonclonal chromosome aberrations (Pampfer and Streffer, 1989; Kadhim *et al*., 1992, 1994; Marder and Morgan, 1993; Holmberg *et al*., 1995, 1998; Grosovsky *et al*., 1996; Watson *et al*., 2001) and mutations (Little *et al*., 1990, 1997; Chang and Little, 1992; Harper *et al*., 1997) in the clonal progeny of irradiated cells. In addition, it has been apparent for many years that radiation-induced cytotoxicity, defined as loss of reproductive potential, may be delayed for up to six generations of cell replication (Puck and Marcus, 1956; Elkind and Sutton, 1959; Trott and Hug, 1970) with death occurring randomly among the progeny cells (Thompson and Suit, 1969). More recently, the progeny of irradiated cells have been shown to exhibit an enhanced death rate and loss of reproductive potential that persists for many generations and possibly indefinitely in established cell lines (Seymour *et al*., 1986; Gorgojo and Little, 1989; Little *et al*., 1990; Seymour and Mothersill, 1992; Brown and Trott, 1994; Mothersill *et al*., 1998). The terms lethal mutations and delayed reproductive death are used interchangeably for this delayed death phenotype. All the various dysgenetic effects (Simons, 1995) in which delayed death, gene mutations and a variety of chromosomal abnormalities can be demonstrated in cells that are not themselves irradiated but are the progeny of cells exposed to ionizing radiation many cell divisions previously have been interpreted as manifestations of a radiation-induced genomic instability (Figure 1c). Induced instability is a genome-wide process (Li *et al*., 1992, 1994; Grosovsky *et al*., 1996) and the cellular phenotype is similar to that of the inherited chromosome instability syndromes, characterized by spontaneously high levels of chromosomal abnormalities and mutations (Meyn, 1997; Wright, 1999; Futaki and Liu, 2001). Despite the apparent similarities, radiation-induced genomic instability seems to reflect epigenetic processes rather than mutation of genome maintenance genes (Clutton *et al*., 1996a; Limoli *et al*., 1998b; Morgan *et al*., 2002; Nagar *et al*., 2003). However, the radiation-induced chromosomal instability phenotype in both haemopoietic tissue (Watson *et al*., 1997) and mammary epithelium

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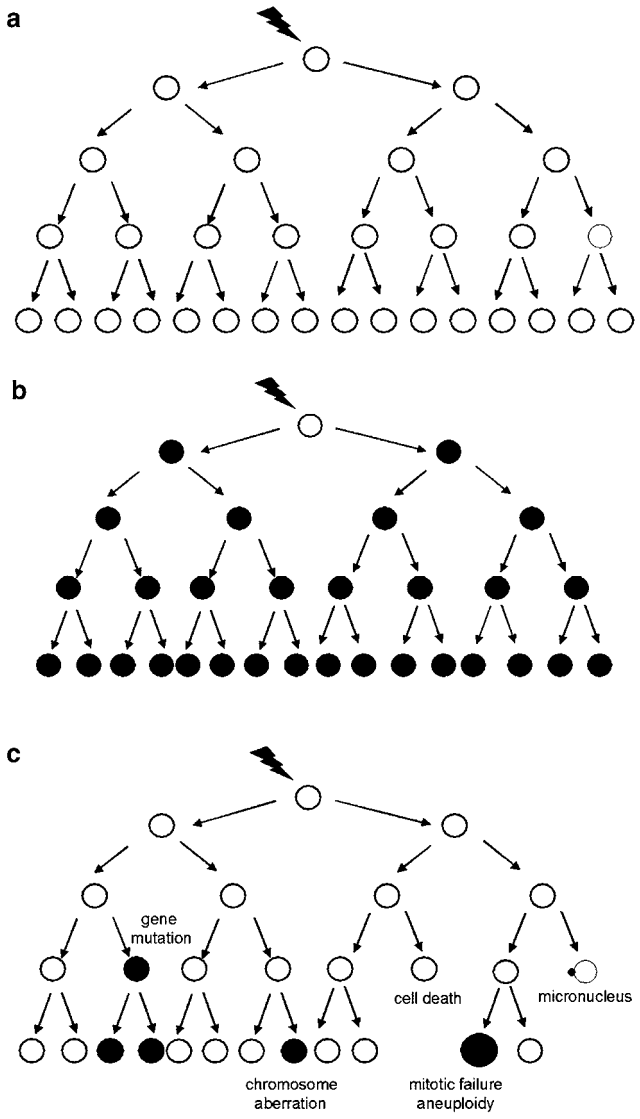

Figure 1 Models of the responses of clonogenic cells to ionizing radiation with mutations and/or chromosomal aberrations shown as filled circles and apparently normal cells as open circles. (**a**) If a cell faithfully repairs DNA damage then its clonal descendants will appear normal. (**b**) If a cell is directly mutated by radiation then all its descendants will express the same mutation. (**c**) Radiation-induced genomic instability is characterized by nonclonal effects in descendant cells

(Ponnaiya *et al*., 1997) is strongly influenced by genetic factors with some genotypes being susceptible and others relatively resistant.

The number of studies designed to assess radiation-induced genomic instability *in vivo* are limited, but the earliest report of the induction of chromosomal instability in normal cells is that of mouse zygote irradiation (Pampfer and Streffer, 1989). In this study, skin fibroblast cultures obtained from foetuses derived from X-irradiated zygotes were found to have a two- to three-fold increase in the types of abnormalities found as spontaneous aberrations in controls. After whole body irradiation with either X-rays or neutrons, chromosomal instability was demonstrated in the bone marrow for up to 24 months postirradiation, indicating that chromosomal instability can be initiated and maintained *in vivo* (Watson *et al*., 2000, 2001) as well as being initiated *in vitro* and perpetuated *in vivo* as demonstrated using a bone marrow transplantation protocol (Watson *et al*., 1996, 2000). In the whole body irradiation studies, there was no direct relationship between cells expressing stable and unstable aberrations, but there was significant interindividual variation in the expression of both stable and unstable aberrations. The mice used in these studies were inbred (and therefore genetically identical), irradiated at the same time and had concurrent age-matched controls. Thus, interindividual variation in the expression of chromosomal aberrations must reflect the biological variation that might be expected of a complex *in vivo* system. Furthermore, when compared to *in vitro* studies, the *in vivo* data showed less damage per cell and fewer cells demonstrating chromosomal instability. This difference can be attributed largely to the cellular defence mechanisms that have evolved to recognize and remove aberrant cells.

Mechanisms underlying radiation-induced genomic instability

At present, the mechanism of induction of instability by ionizing radiation is not fully understood nor is it clear whether all endpoints reflect a common mechanism. In all the various studies, the frequency of induced instability is orders of magnitude greater than that of conventional gene mutation frequencies and although in some studies using established cell lines a large number of postirradiation cell divisions before assay might have allowed for selection of a radiation-induced gene mutation that confers a mutator phenotype, overall the data indicate that the mechanism underlying induced instability is epigenetic. Typically, the spontaneous frequency of gene mutations in mammalian cells is of the order of 10^{-6} and this increases 10-fold to $\sim 10^{-5}$ (0.001% of surviving clonogenic cells) after exposure to 1 Gy X-rays. However, approximately 10% of surviving cells produce clones that exhibit delayed hypoxanthine phosphoribosyl-transferase (*hprt*) mutations (Harper *et al*., 1997; Little *et al*., 1997; Little, 1998) and a similar or much greater proportion (in some cases up to $\sim 50\%$) exhibit chromosomal instability (Kadhim *et al*., 1992, 1994; Marder and Morgan, 1993). In a comparative study of *hprt* mutations induced directly by irradiation or arising as a consequence of induced instability, 75% of those induced directly by X-rays ('conventional' mutations) involved partial or total gene deletions and 25% small-scale or point mutations, whereas only 28% of the delayed mutations were associated with large deletions and the majority were small-scale changes (Little *et al*., 1997). This observation of a mutation spectrum more like that of spontaneously arising mutations than conventional radiation-induced muta-

tions is similar to many of the cytogenetic investigations in which the aberrations associated with radiation-induced chromosomal instability are similar to those arising spontaneously in the cells (Pampfer and Streffer, 1989; Kadhim *et al*., 1994; Watson *et al*., 2001). Unstable aberrations characteristic of radiation-induced chromosomal instability may commonly result in apoptosis and this may account for a component of the delayed reproductive death/lethal mutation phenotype in some cell systems (Limoli *et al*., 1998b; Mothersill *et al*., 2000a).

It is well established that cultured cells acquire spontaneous mutational changes as a consequence of free-radical toxicity, attributable largely to the generation of hydrogen peroxide from polyamine substrates (Parchment and Natarajan, 1992) and *in vivo*, major causes of spontaneous DNA damage are oxidative damage associated with normal metabolism (Chance *et al*., 1979), reactive oxygen species (ROS) produced by phagocytic cells (Babior, 1984, 2000) and from lipid peroxidation (Tappel, 1973). Thus, the association of radiation-induced chromosomal instability with increased intracellular ROS, oxidative DNA base damage and vulnerability to free-radical-mediated membrane damage in haemopoietic cells (Clutton *et al*., 1996a) and an association of increased ROS with radiation-induced delayed death caused by ongoing apoptosis or necrosis in CHO cells (Limoli *et al*., 1998b, 2001) provided a potential epigenetic mechanism for radiation-induced genomic instability. However, some death may also result from signal antonymy generating an apoptotic response to conflicting simultaneous signals for proliferation and cell cycle arrest (Hibner and Coutinho, 1994).

The role of DNA strand breakage as the molecular lesion responsible for initiating genomic instability has been investigated in a comparative study of radiation and chemical agents and ionizing radiation, bleomycin and neocarzinostatin were found to be equally effective at eliciting delayed chromosomal instability (Limoli *et al*., 1997). Exponentially growing cells cultured in a medium containing bromodeoxyuridine, then exposed to nonionizing UVA light in the presence of the dye Hoechst 33258, showed significant levels of DNA strand breaks and base damage and clonal populations derived from single progenitor cells surviving such photolysis exhibited chromosomal instability (Limoli *et al*., 1998a). However, treatment with four different restriction endonucleases did not result in any delayed instability, indicating that DNA strand breakage *per se* does not necessarily produce instability (Limoli *et al*., 1997). That agents other than ionizing radiation induce an instability phenotype in cultured cells was also demonstrated by the production of a delayed reproductive death phenotype in the progeny of nontransformed human keratinocytes exposed to a range of environmental mutagens or cytotoxic compounds. The results clearly linked the delayed death phenotype with substances that induced DNA strand breaks (Mothersill *et al*., 1998). However, all the substances that were effective in inducing instability in this study (ionizing radiation, nonionizing ultraviolet radiation, nickel, cadmium, hydrogen peroxide and bleomycin) are known to induce oxidative stress. Subsequently, it was demonstrated that cadmium and nickel could produce chromosomal instability in human diploid fibroblasts *in vitro* and the effect could not be predicted from acute toxicity data as instability was demonstrated at levels where no acute toxic effects could be demonstrated (Coen *et al*., 2001). More recently, in a study of human skin keratinocytes (HaCaT cells) exposed to UV-A radiation, delayed reproductive death, elevated mutation frequency and an increase in micronucleus formation up to 21 days after initial exposure were all consistent with an instability phenotype (Phillipson *et al*., 2002). Reversal of these effects using catalase was consistent with a role for hydrogen peroxide in instability. Overall, the results of investigations of other agents capable of inducing an instability phenotype are consistent with the free-radical model proposed for radiation-induced instability (Clutton *et al*., 1996a; Limoli *et al*., 1998b, 2001).

Radiation-induced bystander effects

In addition to instability-generated effects, the paradigm of genetic alterations being restricted to direct DNA damage has also been challenged by a number of developments in which mutations have been induced by cytoplasmic irradiation or by a variety of effects, normally detected in irradiated cells, being demonstrated in cells that are not themselves irradiated, but in the neighbourhood of irradiated cells or exposed to factors produced by irradiated cells. Such effects are collectively regarded as radiation-induced bystander effects. One of the first radiation-induced bystander effects was reported in 1992 as an unexpectedly high frequency of sister chromatid exchanges in cultures of Chinese hamster ovary cells after exposures where less than 1% of cell nuclei were actually traversed by an α-particle (Nagasawa and Little, 1992). Similar results were reported in studies of cultured primary human lung fibroblasts (Deshpande *et al*., 1996) and subsequent studies (Nagasawa and Little, 1999) demonstrated a significantly higher frequency of mutations at the *hprt* locus than would be predicted by a back extrapolation from the data for higher doses. All these studies indicated that the target size for genetic damage was greater than the nucleus or indeed the whole cell.

A limitation of using low fluence α-particle irradiation is that it is not possible to define the precise location of the traversed cell and relate this to a response in a nontraversed cell. This is where microbeam technology (Brenner and Hall, 2002) that can irradiate with submicrometre targeting capabilities is ideally placed to advance the understanding of these processes. The initial reports of microbeam experiments that challenged the paradigm of nuclear irradiation being required to produce mutations were of the induction of mutations by cytoplasmic irradiation (Wu *et al*., 1999) and micronucleated or apoptotic human fibroblasts exceed-

ing the number of cells traversed by α-particles (Prise *et al*., 1998). These studies have been refined and extended (Belyakov *et al*., 1999, 2001) to demonstrate bystander-mediated damage comprehensively that often results in cell death.

In a comparison of cytoplasmic versus nuclear irradiations, it was demonstrated that mutations at the CD95 (S1) locus of human-hamster hybrid A_L cells could be induced by a single α-particle traversal of the cytoplasm, where minimal toxicity and a maximal plateau was reached after four to eight cytoplasmic traversals resulting in a mutant fraction of $\sim 3\times$ background (Wu *et al*., 1999). In contrast, nuclear irradiation-induced mutations increased linearly with dose over a wide range. The spectrum of recovered mutations also differed depending on whether irradiation was targeted to the nucleus or the cytoplasm. Nuclear irradiation mutants were predominately large deletions, whereas mutants induced by cytoplasmic irradiation consisted of localized changes, probably reflecting base damage by ROS. The effects of a free-radical scavenger and a thiol-depleting drug indicated that the mutagenicity of cytoplasmic irradiation depends on the generation of ROS, particularly hydroxyl radicals. An important implication of this study is that α-particle traversals of the cytoplasm may contribute a significant proportion of overall mutant yield in the environmentally relevant very low-dose region. The difference in mutation spectrum is similar to the difference between *hprt* mutations induced directly by X-rays (mainly deletions) and those arising as a consequence of radiation-induced genomic instability (mainly small-scale mutations) (Little *et al*., 1997); that is, mutations induced by cytoplasmic irradiation or as a consequence of instability are similar to those arising spontaneously. Subsequent studies demonstrated mutations in A_L cells not traversed by an α-particle by a mechanism that depended on cell–cell communication (Zhou *et al*., 2000) and irradiation of only 10% of a confluent population with a single α-particle per cell resulted in a mutant yield similar to that observed when all the cells in the population were irradiated. The effect was eliminated in cells pretreated with octanol or in cells carrying a dominant-negative connexin 43 vector to inhibit gap-junction-mediated intercellular communication (Zhou *et al*., 2001).

In more recent investigations, a primary explant technique has been used to model the *in vivo* micro-architecture of normal urothelium. A total of 10 individual cell nuclei were irradiated either on the periphery of the explant outgrowth, where proliferating cells were located, or in the centre, where the cells were terminally differentiated. The fraction of apoptotic and micronucleated cells was measured 3 days later and a significant bystander-induced damage was observed after irradiation of cells at the periphery, but not at the centre. Approximately 2000–6000 cells could be damaged by the irradiation of the 10 cells initially, suggesting a cascade mechanism of cell damage induction. However, the fraction of micronucleated and apoptotic cells did not exceed 1–2% of the total number of the cells within the explant outgrowth and there was evidence for premature terminal differentiation that was interpreted as a protective effect. The potential to produce bystander-induced damage in this system seemed to depend on the proliferative status of the irradiated cell (Belyakov *et al*., 2002, 2003).

Mechanisms underlying radiation-induced bystander effects

The bystander effect, operationally defined as the induction of radiation-induced effects in nonirradiated cells, may reflect the occurrence of at least two separate mechanisms for the transfer of a damaging signal from irradiated cells. One mechanism seems dependent on gap-junction intercellular communication stimulating a damage-signalling pathway mediated by the tumour suppressor gene product p53 (Azzam *et al*., 1998, 2001). Other studies implicate a second mechanism in which irradiated cells secrete cytokines such as TGF-β or IL-8 or other factors that act to increase intracellular levels of ROS in unirradiated cells (Lehnert and Goodwin, 1997a, b; Narayanan *et al*., 1997; Iyer and Lehnert, 2000).

The first evidence for a p53-mediated bystander effect was reported in a study of rat lung epithelial cells in culture exposed to low-dose α-irradiation (Hickman *et al*., 1994). Using flow cytometry, a greater proportion of cells expressed the p53 protein than were hit by an α-particle. A role for gap-junction-mediated communication in inducing this signalling pathway was then reported by Little and coworkers (Azzam *et al*., 1998), who investigated the response of confluent cultures of primary human diploid fibroblasts exposed to low fluences of α-particles. When 5% of nuclei were traversed by a particle, an overall three- to four-fold increase was observed in the protein levels of p53 and its downstream target CDKN1A (also commonly known as CDKN1A/$p21^{waf1/cip1}$, a protein involved in cell cycle checkpoint function). The increased level of expression was eliminated by pretreatment with the gap-junction intercellular communication inhibitor lindane. An *in situ* immunofluorescence technique was then used to observe the patterns of expression in cultures in which approximately 2% of cells were irradiated. Increased expression of CDKN1A/p21 was observed in a clustered pattern; some groups of cells displayed elevated levels, whereas other groups of cells in the same culture remained at background levels of expression. Using genetically engineered cells, connexin 43 gap junctions were directly implicated in mediating this bystander effect (Azzam *et al*., 2001).

The second type of mechanism mediating bystander effects by secretion of a factor or factors was first demonstrated by Lehnert and coworkers as an increase in sister chromatid exchanges in unirradiated human lung fibroblasts exposed to the medium in which cells had been irradiated with low fluences of α-particles (Lehnert and Goodwin, 1997a). This protocol can be

used to observe a bystander effect for periods of at least 24 h after the radiation exposure, suggesting a continual production/secretion of factors perhaps until the return of cellular DNA damage response to basal levels. The bystander signal also induces an elevation in intracellular levels of ROS, including superoxide and hydrogen peroxide. This effect is postulated to be critical in the transmission of damage (Lehnert and Goodwin, 1997a, b). Elimination of the bystander effect by heat treatment of the harvested medium or by treatment of irradiated cells with protein synthesis inhibitors indicates that the secreted factors are proteins (Lehnert and Goodwin, 1997b; Narayanan *et al*., 1997; Iyer and Lehnert, 2000) and there is evidence linking the NADPH oxidase/NF-κB pathway to this bystander effect (Iyer and Lehnert, 2000). The untargeted induction of sister chromatid exchanges in lung fibroblasts does not demonstrate a linear relationship to dose over the range of 1–13 cGy but is maximally induced by the lowest doses investigated ($\sim$1 cGy), suggesting a switch mechanism for the activation of a generalized cellular response after damage to a large non-nuclear cellular target. Interestingly, the gap-junction-dependent bystander effect for the induction of CDKN1A/p21 in skin fibroblasts exhibits a similar lack of linear response being maximally induced at $\sim$1 cGy (Azzam *et al*., 2001). In other studies of normal human lung fibroblasts exposed *in vitro* to a low dose of α-particles, proliferation was stimulated and this response also occurred when unirradiated cells were treated with supernatants from irradiated cells. The promitogenic response was attributed to increases in the concentrations of a cytokine (TGF-β1) in cell supernatants; a concentration that also induced intracellular ROS in unirradiated cells and, unlike the gap-junction-mediated mechanism in skin fibroblasts (Azzam *et al*., 2001), led to decreased cellular levels of CDKN1A/p21 and also p53 (Iyer and Lehnert, 2000). Potentially related to the mechanisms mediating cytogenetic damage not requiring gap-junctional communication is the finding that the medium in which certain cells have been irradiated contains an activity, probably a protein, that produces cytotoxic effects in nonirradiated cells (Mothersill and Seymour, 1998; Lyng *et al*., 2000, 2002; Mothersill *et al*., 2000b, 2001; Seymour and Mothersill, 2000). The first detectable effect of the medium containing the cytotoxic activity on recipient cells is a rapid (1–2 min) calcium pulse followed 30 min–2 h later by changes in mitochondrial membrane permeability and the induction of ROS (Lyng *et al*., 2000, 2002); a role for mitochondrial metabolism is suggested by the lack of signal production by cells that lacked functional glucose-6-phosphate dehydrogenase (Mothersill *et al*., 2000b).

Clastogenic factors: long-range acting nontargeted mechanisms

Prior to the recent studies of bystander effects, there are numerous reports that irradiated cells may produce factors *in vivo* that can affect the survival and function of unirradiated cells. Probably the first publication dates from 1954 when damage to the sternal bone marrow of children receiving splenic irradiation for chronic granulocytic leukaemia was reported (Parsons *et al*., 1954). Subsequently, it was reported that plasma from X-irradiated patients could cause chromosome damage in lymphocytes held in short-term culture (Hollowell and Littlefield, 1967) and a number of reports confirmed that a transferable factor capable of causing chromosome breaks in unirradiated lymphocytes was induced by total-body irradiation (Goh and Sumner, 1968; Scott, 1969; Lloyd and Moquet, 1985) with considerable interindividual variation in both production and response (Littlefield *et al*., 1969). A stable clastogenic activity in the plasma obtained from atomic bomb survivors 31 years after exposure (Pant and Kamada, 1977) and a similar activity in Chernobyl liquidators (Emerit *et al*., 1994, 1997) has been reported by Emerit and her colleagues who have also described clastogenic factors being produced by other cellular stresses and in patients with a variety of chromosome instability syndromes and inflammatory disorders (Emerit, 1990, 1994). These clastogenic factors are produced via superoxide and also induce the production of superoxide; this may be the explanation for their persistence over many years. The vicious circle of clastogenic factor formation and action shifts the pro-oxidant/antioxidant balance in cells towards the pro-oxidant state and clastogenic factors can be regarded as markers of oxidative stress. Their clastogenic activity may be related to the formation of lipid peroxidation products (Emerit *et al*., 1991), inosine nucleotides (Auclair *et al*., 1990) and cytotoxic cytokines (Emerit *et al*., 1995); all candidates for mediating radiation-induced bystander effects.

A link between radiation-induced bystander effects and genomic instability

A potential link between radiation-induced genomic instability and bystander effects was indicated by the persistent reduction in the cloning efficiency of non-irradiated normal and malignant epithelial cell lines exposed to a medium from irradiated cultures (Mothersill and Seymour, 1997). The medium irradiated in the absence of cells had no effect nor did irradiated medium from a fibroblast cell line, but irradiated medium from epithelial cells had an extremely toxic effect on nonirradiated fibroblasts. More recently, the medium harvested from an irradiated human keratinocyte cell line up to 35 population doublings postirradiation was shown to induce rapid calcium fluxes and subsequent loss of mitochondrial membrane potential and increases in ROS in nonirradiated cells (Lyng *et al*., 2002). The data are consistent with the induction of cell death in nonirradiated cells by a signal produced by irradiated cells and with the progeny of irradiated cells producing the signal for many generations in culture.

The first link between radiation-induced chromosomal instability and bystander effects was provided by the observation that chromosomal instability was expressed in the progeny of more clonogenic haemopoietic stem cells than were traversed by an α-particle (Kadhim *et al*., 1992). This discrepancy, indicating that cells exhibiting chromosome instability might be derived from nonirradiated cells, was subsequently confirmed by a direct experiment in which, by interposing a shielding grid between the source of α-particles and the cells, the ratio of irradiated to nonirradiated clonogenic cells was manipulated such that the majority of exposed clonogenic stem cells were killed (Lorimore *et al*., 1998). Although survival data confirmed the expected reduction in the number of clonogenic cells traversed and killed after irradiation by the introduction of the shielding grid, there was no expected reduction in the number of descendant clones exhibiting chromosomal instability. As the expression of instability was similar with or without shielding a bystander mechanism could be largely or, indeed, wholly responsible for chromosomal instability in haemopoietic cells after α-particle irradiation (Figure 2a). The original study demonstrating *in vitro* chromosomal instability after low-fluence irradiation (Kadhim *et al*., 1992) is compatible with such a model. Interestingly, using microbeam technology, the induction of genomic instability in human lymphocytes by targeted α-particles (Kadhim *et al*., 2001) has recently been shown to have a significant bystander-mediated component (Kadhim, 2002).

To investigate the potential for an indirect mechanism of instability *in vivo*, mixtures of irradiated and nonirradiated haemopoietic cells were transplanted using a sex mismatch congenic transplantation protocol such that cytogenetic scoring could distinguish not only host-derived cells from donor-derived cells but also cells derived from the irradiated or nonirradiated donor stem cells (Watson *et al*., 2000). Using this system in which relatively few stem cells were transplanted, chromosomal instability was observed in the progeny of both irradiated and nonirradiated stem cells up to 12 months post-transplantation. At the sampling times chosen, cells derived from the transplanted donor stem cells would have reconstituted the haemopoietic system and restored steady-state haemopoiesis in which, at any one time, all haemopoietic cells would be derived from a limited number of long-term repopulating stem cells. Thus, it is exceedingly unlikely that any cells examined were present in the original irradiated population and it is also unlikely that the cells studied were the direct progeny of the irradiated stem cells. Rather, they would be the descendants of stem cells that were themselves the progeny of the original transplanted long-term repopulating stem cells. Instability transmitted from stem cells to their descendants could explain the cytogenetic aberrations observed in the descendants of irradiated stem cells, but to explain chromosomal instability in the distant progeny of nonirradiated stem cells a bystander-type model has to be invoked (Watson *et al*., 2000; Lorimore and Wright, 2003) and such a mechanism might well be involved in what appears to be a direct

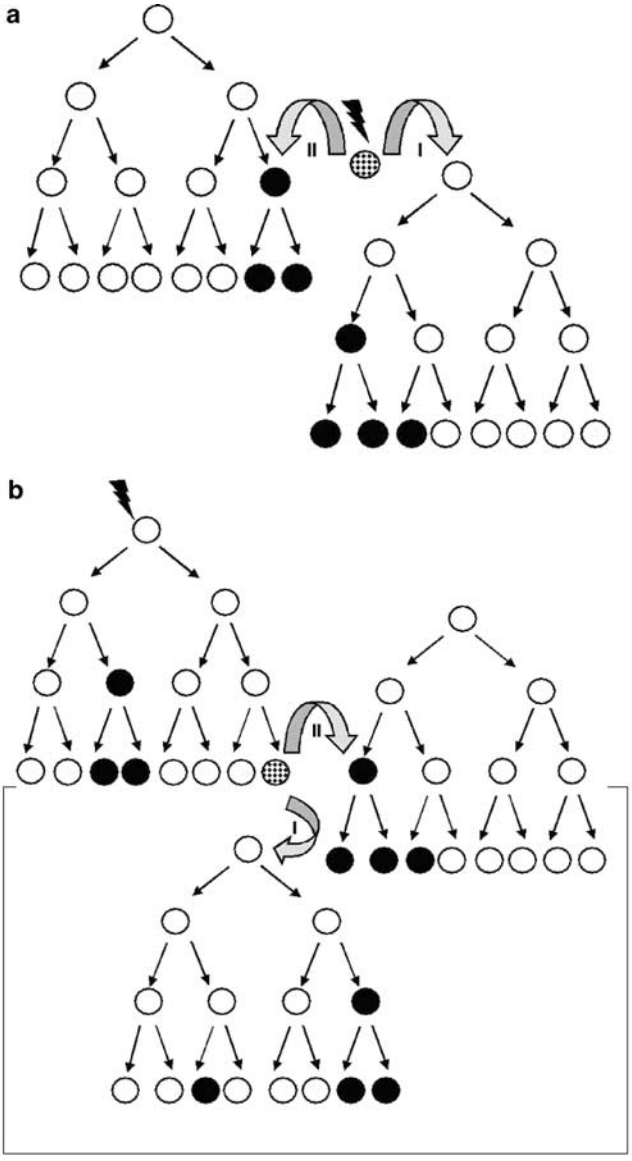

Figure 2 Models of the inter-relationship between radiation-induced genomic instability in haemopoietic tissues (Lorimore *et al*., 1998; Lorimore and Wright, 2003) with mutations and/or chromosomal aberrations shown as filled circles and apparently normal cells as open circles and an 'activated cell' capable of producing a bystander signal as a hatched circle. (**a**) A bystander signal may induce a transmissible genomic instability or a damage response in an unirradiated clonogenic cell (I) or in a cell that has descended from an unirradiated clonogenic cell (II). In this model, instability may be a consequence of bystander signalling from an 'activated cell' and bystander effects may initiate and be responsible for radiation-induced genomic instability. (**b**) Radiation-induced genomic instability transmitted from an irradiated clonogenic cells may result in an 'activated cell' capable of inducing a bystander-mediated transmissible genomic instability or a damage response in an unirradiated clonogenic cell (I) or in a cell that has descended from an unirradiated clonogenic cell (II). In this model, radiation-induced genomic instability produces cells that provide a bystander signal able to induce instability; that is, RIBE can be both a cause (**a**) and/or a consequence (**b**) of RIGI

transmission mechanism (Figure 2b). Important implications of the various experimental findings exploring instability and bystander effects in the haemopoietic system are that chromosomal instability can be induced by a bystander mechanism and that bystander effects may be produced as a consequence of instability, that is, bystander effects can be both a cause and a consequence of radiation-induced genomic instability (Lorimore and Wright, 2003). Evidence that these effects are not restricted to experimental models is provided by a recent report of a 35-year-old man accidentally exposed to acute high-dose total body neutron radiation who received a stem cell transplant from his HLA-identical sister. In monitoring this patient, chromosomal instability in donor female cells was demonstrated consistent with a bystander effect of the neutron exposure (Chiba *et al*., 2002).

An additional link between induced instability and bystander effects has been provided by investigations of both delayed apoptosis and radiation-induced chromosomal instability in the Chinese hamster-human hybrid GM10115 cell line (Marder and Morgan, 1993; Limoli *et al*., 1998b). In these cells a high level of recombination involving interstitial telomere repeat-like sequences characterizes the instability. Using the comet assay to investigate whether there was an elevated level of endogenous DNA breaks in chromosomally unstable clones, no significant difference between nonirradiated and radiation-induced chromosomally unstable clones was found. Since elevated levels of endogenous breaks were not detected in unstable clones, it was proposed that bystander effects lead to the activation of recombinational pathways that perpetuate the unstable phenotype. Specifically, it was suggested that radiation induces conditions and/or factors that stimulate the production of ROS and these reactive intermediates contribute to a chronic pro-oxidant environment that cycles over multiple generations, promoting chromosomal recombination and other phenotypes associated with genomic instability (Morgan *et al*., 2002). Subsequently, a 'death-inducing effect' of exposing GM10115 cells to a medium in which unstable GM10115 cells had been cultured was implicated in the delayed death associated with the chromosomally unstable clones (Nagar *et al*., 2003).

Inflammatory-type processes as sources of bystander signals *in vivo*

The cells responsible for bystander-mediated chromosomal instability in unirradiated haemopoietic cells *in vivo* (Watson *et al*., 2000; Lorimore and Wright, 2003) are likely to be of the mononuclear phagocytic (monocyte/macrophage) lineage with characteristics in common with the activated phagocytes found in inflammatory conditions as such cells are able to produce gene mutations (Weitzman and Stossel, 1981), DNA base modifications (Dizdaroglu *et al*., 1993), DNA strand breaks (Birnboim, 1982; Shacter *et al*., 1988) and cytogenetic damage (Weitberg *et al*., 1983) in neighbouring cells. *In vivo*, because of their migratory properties, it is possible that activated phagocytic cells generated as a consequence of induced instability may also contribute to genetic damage in nonhaemopoietic cells. That instability-derived activated phagocytes may produce genetic lesions in neighbouring cells has similar implications to the mechanisms proposed to explain the relationship between inflammation and carcinogenesis (Weitzman and Gordon, 1990; Rosin *et al*., 1994; Maeda and Akaike, 1998; Darveau, 1999).

Recent studies have revealed indirect nontargeted mechanisms that result in increased numbers of macrophages exhibiting the phenotype of activated phagocytes after whole body irradiation (Lorimore *et al*., 2001). Nitric oxide synthase expression, lysosomal enzyme activity and the capacity to produce superoxide were increased in these cells and time course investigations correlated enzyme induction with phagocytosis of apoptotic cells. That the macrophage activation was associated with the phagocytic clearance of radiation-induced apoptotic cells, rather than activation being a direct effect of radiation, was confirmed by a number of investigations using p53−/− mice that lack p53-dependent radiation-induced apoptosis. Further investigations of the tissue response to apoptosis revealed an unexpected accumulation of neutrophils at the margins of the splenic blood vessels and in splenic tissue. These are classical signs of an acute inflammatory response and the timing coincided with the increased macrophage activity. This response was also an indirect consequence of the irradiation and associated with radiation-induced apoptosis, as it was not observed in irradiated p53−/− mice. While it would be expected that the cell death resulting from irradiation requires rapid phagocytic clearance, the increase in enzyme activity after phagocytosis, the length of time that activated macrophages persist and the inflammatory nature of the process would not be expected as apoptosis is generally regarded as a noninflammatory process. However, the findings are remarkably similar to the neutrophil infiltration observed in the thymus after irradiation (Uchimura *et al*., 2000) and *in vitro* studies have shown that phagocytosis of apoptotic cells results in the production of both pro- and anti-inflammatory cytokines (Giles *et al*., 2000; Gregory, 2000). Additionally, nitric oxide can be either pro- or antiapoptotic, can either downregulate or upregulate p53 activity (Brune *et al*., 1996; Brockhaus and Brune, 1999) and may be pro- or anti-inflammatory (Nathan and Shiloh, 2000) depending on the context. Thus, it is possible that nonspecific inflammatory-type responses to radiation-induced stress and injury may contribute to the wide variety of bystander-mediated effects and to genomic instability. Studies *in vitro* of cells, other than haemopoietic cells, that implicate soluble factors and processes involving ROS in nontargeted effects would be consistent with free-radical/cytokine-mediated mechanisms comparable to an inflammatory reaction. It is of particular interest that a persistent subclinical inflammation among Japanese A-bomb

survivors has recently been reported and it is suggested that radiation-induced enhancement of inflammatory reactions might contribute as an epigenetic and/or bystander effect to the development of several radiation-induced disorders, including nonmalignant conditions (Neriishi *et al*., 2001; Hayashi *et al*., 2003).

The response to damaging signals

The mechanisms underlying the response to DNA damage can be regarded as three interlinked processes of recognition of injury, damage assessment and response implementation (Rich *et al*., 2000). The recognition and response processes are not activated in a simple linear manner because there are multiple responses to DNA damage that trigger both repair and apoptotic processes. Malfunctioning of these pathways may result in genetic instability and malignancy. The pathways that signal the response to radiation DNA damage involve the ATM protein, a member of the phosphatidylinositol 3-kinase-like family (Durocher and Jackson, 2001; Shiloh, 2001). ATM is the gene mutated in the chromosomal instability syndrome ataxia telangiectasia and the ATM protein can directly bind and phosphorylate p53, enhancing its ability to transactivate downstream responsive genes such as CDKN1A/p21. Cell cycle arrest allows for a period of damage assessment and recruitment of DNA repair proteins or, if the damage exceeds the repair capacity of the cell, the initiation of apoptotic processes (Zhou and Elledge, 2000). In the absence of DNA-PK, p53 phosphorylation attenuates Bax but not CDKN1A/p21 induction after irradiation. Conversely, the activation of p53 in the absence of ATM does not affect Bax, but abolishes CDKN1A/p21 induction (Barlow *et al*., 1997; Wang *et al*., 2000) and these are examples of how genetic modifiers of the damage response might influence outcome (Figure 3a). However, there are also multiple interactions between checkpoint and apoptotic programmes that contribute to the heterogeneity of downstream events in response to DNA damage and the complexity of these interactions may result in cell death even though DNA repair mechanisms have been initiated. Inherited mutations in many of these damage recognition and response genes confers a high relative risk of malignancy, although the overall incidence of inherited human cancers represents less than 5% of all cases (Mohrenweiser and Jones, 2000) and increasing evidence suggests that the majority of tumours induced by damaging environmental agents arise in people with low penetrant genetic variants. Although such genetic polymorphisms individually provide only a small increase in susceptibility, they are important risk factors due both to their higher incidences throughout the population and the potential for synergistic effects of mixtures of variants (Mohrenweiser and Jones, 2000; Nebert, 2000; Knudsen *et al*., 2001; Pharoah *et al*., 2002).

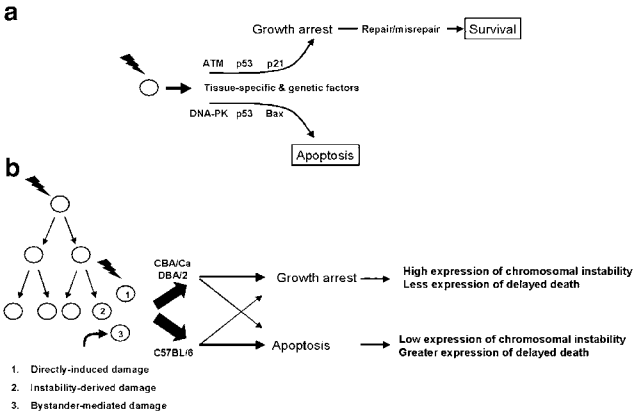

Figure 3 (**a**) A schematic representation of how genetic modifiers influencing the p53 response pathway would reduce or reinforce the apoptotic response in a genotype-dependent manner. (**b**) The implications of genetic modification of responses in the p53 pathway for the expression of radiation-induced genomic instability and bystander effects in haemopoietic cells are shown schematically as a differential sectoring of response in genetic strains, susceptible to radiation-induced chromosomal instability and expressing low levels of delayed death (CBA/Ca and DBA/2) and those relatively resistant to the expression of chromosomal instability expressing high levels of delayed death (C57BL/6)

As the very earliest events in radiation-induced malignancy are not known (Hall, 2001) it is not unreasonable to assume that there might be a link between radiation-induced genomic instability and malignancy (Little, 2000; Mothersill and Seymour, 2000) and perhaps a specific role as a 'critical early event associated with initiation of the carcinogenic process' (Ullrich and Ponnaiya, 1998). Although there is evidence of genetic instability in radiogenic tumours, it may not be possible to distinguish between instability that may be a consequence of malignant changes in cells and instability that might reflect the delayed effects of exposure to ionizing radiation, that is, is such instability a cause or a consequence of malignancy. However, it has been suggested that instability-generated p53 point mutations are important in the genesis of thorotrast-induced tumours (Iwamoto *et al*., 1999; Ishikawa *et al*., 2001) and that the high level of genome instability in secondary solid tumours developing after radiotherapy of bilateral retinoblastoma, including uncommon p53 mutations, is attributable to instability rather than a direct effect of ionizing radiation (Lefevre *et al*., 2001). Clearly, any process that increases the frequency with which genetic changes arise will increase the probability of relevant genetic changes in potential target cells and the correlation between the genotype-dependent expression of chromosomal instability in mouse mammary epithelial and susceptibility to mammary tumours supports a role for radiation-induced instability in the process of tumorigenesis (Ponnaiya *et al*., 1997; Ullrich and Ponnaiya, 1998).

The genotype dependency of the expression of radiation-induced chromosomal instability (Ponnaiya

et al., 1997; Watson *et al*., 1997) and apoptotic response (Clutton *et al*., 1996b; Mothersill *et al*., 1999; Wright, 2002), together with the many observations of genetic factors influencing the response to ionizing radiation, prompted an investigation to study the potential for genetic modification of cell-type-specific p53 and apoptotic responses that might relate to genetically determined differences in the longer-term outcomes of radiation exposure (Lorimore *et al*., 2001; Wallace *et al*., 2001; Coates *et al*., 2003). In these investigations, strain-dependent differences in the amount, timing, distribution and transcriptional activity of p53 in tissues taken from identically treated inbred strains of mice were demonstrated. Three strains, two susceptible to radiation-induced chromosomal instability (DBA/2 and CBA/Ca), the other relatively resistant to the expression of chromosomal instability (C57BL/6), showed distinct apoptotic responses after whole body ionizing radiation exposure consistent with a genotype-dependent inverse correlation between the potential to express chromosomal aberrations and cell death (Figure 3b).

An important factor that influences radiation responses is the differential activation of p53-response genes. Transcriptionally active p53 can represses or activate genes, and there is increasing evidence for differential activation of different p53 target genes in different cells and tissues (Gorospe *et al*., 1997; Yu *et al*., 1999; Bouvard *et al*., 2000; Burns *et al*., 2001; Fei *et al*., 2002). It is now clear that the differential induction of specific p53-target genes is a genetically modified process (Wallace *et al*., 2001; Coates *et al*., 2003). The data for haemopoietic cells are consistent with a greater p53-mediated transcriptional activation of the CDKN1A/p21 gene following irradiation tending to reduce the apoptotic response (CBA/Ca and DBA/2) and a greater upregulation of Bax reinforcing the apoptotic programme (C57BL/6). Irradiated murine urinary epithelium exhibits similar genotype-dependent apoptotic responses that reflect the range of responses seen in the human urothelium (Mothersill *et al*., 1999). In contrast to these findings, the relationship between delayed death and chromosomal instability exhibits a more complex relationship in irradiated human HPV-G keratinocytes (Mothersill *et al*., 2000a) and there is no evidence for differential CDKN1A/p21 expression in the mouse intestinal epithelium and no appreciable differences in Bax expression (Coates *et al*., 2003), clearly showing that the genetic modification of p53-transcriptional induction of CDKN1A/p21 and Bax is a tissue-specific process and that the inverse correlations between CDKN1A/p21, Bax and apoptosis do not hold for all cell types.

Conclusions

The paradigm of genetic alterations being restricted to direct DNA damage after exposure to ionizing radiation has been challenged by observations in which radiation induces conditions and/or factors that result in non-irradiated cells exhibiting a wide range of responses conventionally associated with direct DNA damage. These nontargeted effects are collectively referred to as radiation-induced genomic instability and radiation-induced bystander effects. Their expression is influenced by cell-type and genetic factors and in some cases by the type of radiation exposure. It is likely that either effect may be a cause or a consequence of the other, but at present it is not known to what extent these untargeted effects contribute to overall cellular radiation responses especially *in vivo*. Experimental studies of bystander effects have documented a wide range of responses involving gap-junction-mediated or cytokine-like activation of signalling processes that influence levels of ROS. Intercellular signalling, production of cytokines and free radicals are all features of inflammatory responses and such responses may be protective or damaging depending on the context. In addition to the potential for persisting and bystander-mediated damage, inflammatory processes may confer a predisposition to malignancy and other pathological consequences. Thus, radiation-induced genomic instability and bystander effects may reflect inter-related aspects of inflammatory-type responses to radiation-induced stress and injury and together with directly mediated effects may be involved in the variety of the pathological consequences of radiation exposures (Figure 4). Recent evidence suggests that for certain cell types, whether a damaging signal is a consequence of direct radiation or arises as a consequence of an untargeted process, there are genotype-dependent and cell-type specific modifiers of the responses that influence the efficiency with which a damaged cell initiates an apoptotic response or growth arrest. These genetically modified signalling processes may contribute to the underlying mechanisms for the probability of tumour development and the type of tumour induced by exposure to a given genotoxic agent being strongly dependent on genetic background. The genetic background that produces the more effective

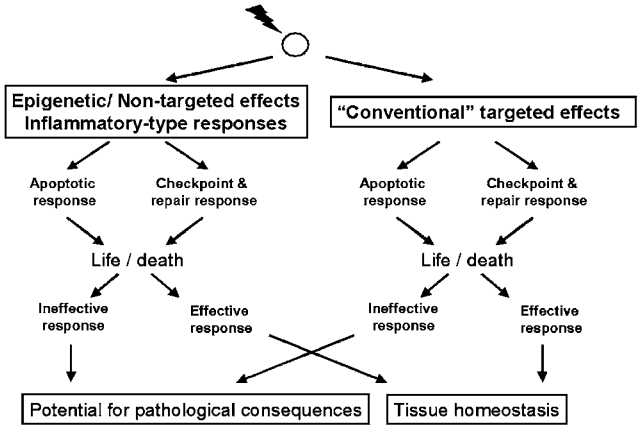

Figure 4 A schematic representation of the pathways by which a cell may respond to ionizing radiation resulting in the potential for pathological consequences from direct effects and/or indirect nontargeted effects

apoptotic response and phagocytic clearance would be less predisposed to adverse consequences of irradiation due to a more effective elimination of unstable and potentially malignant cells. Thus, both the degree of radiation-induced genomic instability and the potential consequences of this induced phenotype appear to represent a balance between the production of genotoxic/clastogenic factors and the response of the cell to such damaging agents. Both signal production and signal response may be significantly influenced by genetic and cell-type specific factors.

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References

Auclair C, Gouyette A, Levy A and Emerit I. (1990). *Arch. Biochem. Biophys.,* **278,** 238–244.
Azzam EI, de Toledo SM, Gooding T and Little JB. (1998). *Radiat. Res.,* **150,** 497–504.
Azzam EI, de Toledo SM and Little JB. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 473–478.
Babior BM. (1984). *Blood,* **64,** 959–966.
Babior BM. (2000). *Am. J. Med.,* **109,** 33–44.
Barlow C, Brown KD, Deng CX, Tagle DA and Wynshaw-Boris A. (1997). *Nat. Genet.,* **17,** 453–456.
Belyakov OV, Folkard M, Mothersill C, Prise KM and Michael BD. (2002). *Radiat. Prot. Dosimetry,* **99,** 249–251.
Belyakov OV, Folkard M, Mothersill C, Prise KM and Michael BD. (2003). *Br. J. Cancer,* **88,** 767–774.
Belyakov OV, Malcolmson AM, Folkard M, Prise KM and Michael BD. (2001). *Br. J. Cancer,* **84,** 674–679.
Belyakov OV, Prise KM, Trott KR and Michael BD. (1999). *Int. J. Radiat. Biol.,* **75,** 985–993.
Birnboim HC. (1982). *Science,* **215,** 1247–1249.
Bouvard V, Zaitchouk T, Vacher M, Duthu A, Canivet M, Choisy-Rossi C, Nieruchalski M and May E. (2000). *Oncogene,* **19,** 649–660.
Brenner DJ and Hall EJ. (2002). *Radiat. Prot. Dosimetry,* **99,** 283–286.
Brockhaus F and Brune B. (1999). *Oncogene,* **18,** 6403–6410.
Brown DC and Trott KR. (1994). *Int. J. Radiat. Biol.,* **66,** 151–155.
Brune B, Golkel C and von Knethen A. (1996). *Biochem. Biophys. Res. Commun.,* **229,** 396–401.
Burns TF, Bernhard EJ and El-Deiry WS. (2001). *Oncogene,* **20,** 4601–4612.
Chance B, Sies H and Boveris A. (1979). *Physiol. Rev.,* **59,** 527–605.
Chang WP and Little JB. (1992). *Mutat. Res.,* **270,** 191–199.
Chiba S, Saito A, Ogawa S, Takeuchi K, Kumano K, Seo S, Suzuki T, Tanaka Y, Saito T, Izutsu K, Yuji K, Masuda S, Futami S, Nishida M, Suzuki G, Gale RP, Fukayama M, Maekawa K and Hirai H. (2002). *Bone Marrow Transplantation,* **29,** 935–939.
Clutton SM, Townsend KM, Walker C, Ansell JD and Wright EG. (1996a). *Carcinogenesis,* **17,** 1633–1639.
Clutton SM, Townsend KMS, Goodhead DT, Ansell JA and Wright EG. (1996b). *Cell Death Differ.,* **3,** 141–148.
Coates PJ, Lorimore SA, Lindsay K and Wright EG. (2003). *J. Pathol.*. in press.
Coen N, Mothersill C, Kadhim M and Wright EG. (2001). *J. Pathol.,* **195,** 293–299.
Darveau R. (1999). *Nat. Biotechnol.,* **17,** 19.
Deshpande A, Goodwin EH, Bailey SM, Marrone BL and Lehnert BE. (1996). *Radiat. Res.,* **145,** 260–267.
Dizdaroglu M, Olinski R, Doroshow JH and Akman SA. (1993). *Cancer Res.,* **53,** 1269–1272.
Durocher D and Jackson SP. (2001). *Curr. Opin. Cell Biol.,* **13,** 225–231.
Elkind M and Sutton H. (1959). *Nature,* **184,** 1293–1295.
Emerit I. (1990). *Methods Enzymol.,* **186,** 555–564.
Emerit I. (1994). *Free Radic. Biol. Med.,* **16,** 99–109.
Emerit I, Khan SH and Esterbauer H. (1991). *Free Radic. Biol. Med.,* **10,** 371–377.
Emerit I, Levy A, Cernjavski L, Arutyunyan R, Oganesyan N, Pogosian A, Mejlumian H, Sarkisian T, Gulkandanian M, Quastel M, Goldsmith J and Riklis E. (1994). *J. Cancer Res. Clin. Oncol.,* **120,** 558–561.
Emerit I, Levy A, Pagano G, Pinto L, Calzone R and Zatterale A. (1995). *Human Genet.,* **96,** 14–20.
Emerit I, Oganesian N, Arutyunian R, Pogossian A, Sarkisian T, Cernjavski L, Levy A and Feingold J. (1997). *Mutat. Res.,* **377,** 239–246.
Fei P, Bernhard EJ and El-Deiry WS. (2002). *Cancer Res.,* **62,** 7316–7327.
Futaki M and Liu JM. (2001). *Trends Mol. Med.,* **7,** 560–565.
Giles KM, Hart SP, Haslett C, Rossi AG and Dransfield I. (2000). *Br. J. Haematol.,* **109,** 1–12.
Goh K and Sumner H. (1968). *Radiat. Res.,* **35,** 171–181.
Gorgojo L and Little JB. (1989). *Int. J. Radiat. Biol.,* **55,** 619–630.
Gorospe M, Cirielli C, Wang X, Seth P, Capogrossi MC and Holbrook NJ. (1997). *Oncogene,* **14,** 929–935.
Gregory CD. (2000). *Curr. Opin. Immunol.,* **12,** 27–34.
Grosovsky AJ, Parks KK, Giver CR and Nelson SL. (1996). *Mol. Cell Biol.,* **16,** 6252–6262.
Hall EJ. (2001). *Phys. Med.,* **17,** 21–25.
Harper K, Lorimore SA and Wright EG. (1997). *Exp. Hematol.,* **25,** 263–269.
Hayashi T, Kusunoki Y, Hakoda M, Morishita Y, Kubo Y, Maki M, Kasagi F, Kodama K, Macphee DG and Kyoizumi S. (2003). *Int. J. Radiat. Biol.,* **79,** 129–136.
Hibner U and Coutinho A. (1994). *Cell Death Differ.,* **1,** 33–37.
Hickman AW, Jaramillo RJ, Lechner JF and Johnson NF. (1994). *Cancer Res.,* **54,** 5797–5800.
Hollowell JG and Littlefield LG. (1967). *J. SC Med. Assoc.,* **63,** 437–442.
Holmberg K, Meijer AE, Auer G and Lambert BO. (1995). *Int. J. Radiat. Biol.,* **68,** 245–255.
Holmberg K, Meijer AE, Harms-Ringdahl M and Lambert B. (1998). *Int. J. Radiat. Biol.,* **73,** 21–34.
Ishikawa Y, Wada I and Fukumoto M. (2001). *J. Environ. Pathol. Toxicol. Oncol.,* **20,** 311–315.
Iwamoto KS, Fujii S, Kurata A, Suzuki M, Hayashi T, Ohtsuki Y, Okada Y, Narita M, Takahashi M, Hosobe S, Doishita K, Manabe T, Hata S, Murakami I, Itoyama S, Akatsuka S, Ohara N, Iwasaki K, Akabane H, Fujihara M, Seyama T and Mori T. (1999). *Carcinogenesis,* **20,** 1283–1291.
Iyer R and Lehnert BE. (2000). *Cancer Res.,* **60,** 1290–1298.
Kadhim MA. (2002). Bystander mediated genomic instability of individual radiation track. *Abstracts of the Forty-ninth Annual Meeting of the Radiation Research Society*: Oak Brook, IL, USA, P.84.

Kadhim MA, Lorimore SA, Hepburn MD, Goodhead DT, Buckle VJ and Wright EG. (1994). *Lancet,* **344,** 987–988.
Kadhim MA, Macdonald DA, Goodhead DT, Lorimore SA, Marsden SJ and Wright EG. (1992). *Nature,* **355,** 738–740.
Kadhim MA, Marsden SJ, Goodhead DT, Malcolmson AM, Folkard M, Prise KM and Michael BD. (2001). *Radiat. Res.,* **155,** 122–126.
Knudsen LE, Loft SH and Autrup H. (2001). *Mutat. Res.,* **482,** 83–88.
Lefevre SH, Vogt N, Dutrillaux AM, Chauveinc L, Stoppa-Lyonnet D, Doz F, Desjardins L, Dutrillaux B, Chevillard S and Malfoy B. (2001). *Oncogene,* **20,** 8092–8099.
Lehnert BE and Goodwin EH. (1997a). *Cancer Res.,* **57,** 2164–2171.
Lehnert BE and Goodwin EH. (1997b). *Environon. Health Perspect.,* **105** (Suppl. 5)**,** 1095–1101.
Li CY, Yandell DW and Little JB. (1992). *Mol. Carcinogen.,* **5,** 270–277.
Li CY, Yandell DW and Little JB. (1994). *Mol. Cell Biol.,* **14,** 4373–4379.
Limoli CL, Day JP, Ward JF and Morgan WF. (1998a). *Photochem. Photobiol.,* **67,** 233–238.
Limoli CL, Hartmann A, Shephard L, Yang CR, Boothman DA, Bartholomew J and Morgan WF. (1998b). *Cancer Res.,* **58,** 3712–3718.
Limoli CL, Kaplan MI, Giedzinski E and Morgan WF. (2001). *Free Radic. Biol. Med.,* **31,** 10–19.
Limoli CL, Kaplan MI, Phillips JW, Adair GM and Morgan WF. (1997). *Cancer Res.,* **57,** 4048–4056.
Little JB. (1998). *Int. J. Radiat. Biol.,* **74,** 663–671.
Little JB. (2000). *Carcinogenesis,* **21,** 397–404.
Little JB, Gorgojo L and Vetrovs H. (1990). *Int. Radiat. Oncol. Biol. Phys.,* **19,** 1425–1429.
Little JB, Nagasawa H, Pfenning T and Vetrovs H. (1997). *Radiat. Res.,* **148,** 299–307.
Littlefield LG, Hollowell Jr JG and Pool Jr WH. (1969). *Radiology,* **93,** 879–886.
Lloyd DC and Moquet JE. (1985). *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.,* **47,** 433–444.
Lorimore SA, Coates PJ, Scobie GE, Milne G and Wright EG. (2001). *Oncogene,* **20,** 7085–7095.
Lorimore SA, Kadhim MA, Pocock DA, Papworth D, Stevens DL, Goodhead DT and Wright EG. (1998). *Proc. Natl. Acad. Sci. USA,* **95,** 5730–5733.
Lorimore SA and Wright EG. (2003). *Int. J. Radiat. Biol.,* **79,** 15–25.
Lyng FM, Seymour CB and Mothersill C. (2000). *Br. J. Cancer,* **83,** 1223–1230.
Lyng FM, Seymour CB and Mothersill C. (2002). *Radiat. Res.,* **157,** 365–370.
Maeda H and Akaike T. (1998). *Biochemistry (Moscow),* **63,** 854–865.
Marder BA and Morgan WF. (1993). *Mol. Cell Biol.,* **13,** 6667–6677.
Meyn MS. (1997). *Curr. Top Microbiol. Immunol.,* **221,** 71–148.
Mohrenweiser HW and Jones IM. (2000). *Radiat. Res.,* **154,** 722–723, discussion 723–724.
Morgan WF, Hartmann A, Limoli CL, Nagar S and Ponnaiya B. (2002). *Mutat. Res.,* **504,** 91–100.
Mothersill C, Crean M, Lyons M, McSweeney J, Mooney R, O'Reilly J and Seymour CB. (1998). *Int. J. Radiat. Biol.,* **74,** 673–680.
Mothersill C, Kadhim MA, O'Reilly S, Papworth D, Marsden SJ, Seymour CB and Wright EG. (2000a). *Int. J. Radiat. Biol.,* **76,** 799–806.
Mothersill C, Rea D, Wright EG, Lorimore SA, Murphy D, Seymour CB and O'Malley K. (2001). *Carcinogenesis,* **22,** 1465–1471.
Mothersill C and Seymour C. (1997). *Int. J. Radiat. Biol.,* **71,** 421–427.
Mothersill C and Seymour CB. (1998). *Radiat. Res.,* **149,** 256–262.
Mothersill C and Seymour C. (2000). *Radiat. Biol. Radioecol.,* **40,** 615–620.
Mothersill C, Stamato TD, Perez ML, Cummins R, Mooney R and Seymour CB. (2000b). *Br. J. Cancer,* **82,** 1740–1746.
Mothersill CE, O'Malley KJ, Murphy DM, Seymour CB, Lorimore SA and Wright EG. (1999). *Carcinogenesis,* **20,** 2273–2278.
Nagar S, Smith LE and Morgan WF. (2003). *Cancer Res.,* **63,** 324–328.
Nagasawa H and Little JB. (1992). *Cancer Res.,* **52,** 6394–6396.
Nagasawa H and Little JB. (1999). *Radiat. Res.,* **152,** 552–557.
Narayanan PK, Goodwin EH and Lehnert BE. (1997). *Cancer Res.,* **57,** 3963–3971.
Nathan C and Shiloh MU. (2000). *Proc. Natl. Acad. Sci. USA,* **97,** 8841–8848.
Nebert DW. (2000). *Clin. Chem. Lab. Med.,* **38,** 857–861.
Neriishi K, Nakashima E and Delongchamp RR. (2001). *Int. J. Radiat. Biol.,* **77,** 475–482.
Pampfer S and Streffer C. (1989). *Int. J. Radiat. Biol.,* **55,** 85–92.
Pant GS and Kamada N. (1977). *Hiroshima J. Med. Sci.,* **26,** 149–154.
Parchment RE and Natarajan K. (1992). *Cytotechnology,* **10,** 93–124.
Parsons WB, Watkins CH, Pease GL and Childs DS. (1954). *Cancer Genet. Cytogenet.,* **7,** 179–189.
Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF and Ponder BA. (2002). *Nat. Genet.,* **31,** 33–36.
Phillipson RP, Tobi SE, Morris JA and McMillan TJ. (2002). *Free Radic. Biol. Med.,* **32,** 474–480.
Ponnaiya B, Cornforth MN and Ullrich RL. (1997). *Radiat. Res.,* **147,** 121–125.
Prise KM, Belyakov OV, Folkard M and Michael BD. (1998). *Int. J. Radiat. Biol.,* **74,** 793–798.
Puck T and Marcus P. (1956). *J. Exp. Med.,* **103,** 653–666.
Rich T, Allen RL and Wyllie AH. (2000). *Nature,* **407,** 777–783.
Rosin MP, Anwar WA and Ward AJ. (1994). *Cancer Res.,* **54,** 1929s–1933s.
Scott D. (1969). *Cell Tissue Kinet.,* **2,** 295–305.
Seymour C and Mothersill C. (1992). *Mutat. Res.,* **267,** 19–30.
Seymour CB and Mothersill C. (2000). *Radiat. Res.,* **153,** 508–511.
Seymour CB, Mothersill C and Alper T. (1986). *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.,* **50,** 167–179.
Shacter E, Beecham EJ, Covey JM, Kohn KW and Potter M. (1988). *Carcinogenesis,* **9,** 2297–2304.
Shiloh Y. (2001). *Curr. Opin. Genet. Dev.,* **11,** 71–77.
Simons JW. (1995). *Crit. Rev. Rev. Oncogenesis,* **6,** 261–273.
Tappel AL. (1973). *Fed. Proc.,* **32,** 1870–1874.
Thompson LH and Suit HD. (1969). *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.,* **15,** 347–362.
Trott KR and Hug O. (1970). *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.,* **17,** 483–486.
Uchimura E, Watanabe N, Niwa O, Muto M and Kobayashi Y. (2000). *J. Leukocyte Biol.,* **67,** 780–784.

Ullrich RL and Ponnaiya B. (1998). *Int. J. Radiat. Biol.,* **74,** 747–754.
Wallace M, Coates PJ, Wright EG and Ball KL. (2001). *Oncogene,* **20,** 3597–3608.
Wang S, Guo M, Ouyang H, Li X, Cordon-Cardo C, Kurimasa A, Chen DJ, Fuks Z, Ling CC and Li GC. (2000). *Proc. Natl. Acad. Sci. USA,* **97,** 1584–1588.
Watson GE, Lorimore SA, Clutton SM, Kadhim MA and Wright EG. (1997). *Int. J. Radiat. Biol.,* **71,** 497–503.
Watson GE, Lorimore SA, Macdonald DA and Wright EG. (2000). *Cancer Res.,* **60,** 5608–5611.
Watson GE, Lorimore SA and Wright EG. (1996). *Int. J. Radiat. Biol.,* **69,** 175–182.
Watson GE, Pocock DA, Papworth D, Lorimore SA and Wright EG. (2001). *Int. J. Radiat. Biol.,* **77,** 409–417.
Weitberg AB, Weitzman SA, Destrempes M, Latt SA and Stossel TP. (1983). *N. Engl. J. Med.,* **308,** 26–30.
Weitzman SA and Gordon LI. (1990). *Blood,* **76,** 655–663.
Weitzman SA and Stossel TP. (1981). *Science,* **212,** 546–547.
Wright EG. (1999). *J. Pathol.,* **187,** 19–27.
Wright EG. (2002). *Radiation and Homeostasis: International Congress Series*. Vol. 1236. Sugahara T, Nikaido O and Niwa O (eds). Elsevier: Amsterdam, pp. 271–281.
Wu LJ, Randers-Pehrson G, Xu A, Waldren CA, Geard CR, Yu Z and Hei TK. (1999). *Proc. Natl. Acad. Sci. USA,* **96,** 4959–4964.
Yu J, Zhang L, Hwang PM, Rago C, Kinzler KW and Vogelstein B. (1999). *Proc. Natl. Acad. Sci. USA,* **96,** 14517–14522.
Zhou BB and Elledge SJ. (2000). *Nature,* **408,** 433–439.
Zhou H, Randers-Pehrson G, Waldren CA, Vannais D, Hall EJ and Hei TK. (2000). *Proc. Natl. Acad. Sci. USA,* **97,** 2099–2104.
Zhou H, Suzuki M, Randers-Pehrson G, Vannais D, Chen G, Trosko JE, Waldren CA and Hei TK. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 14410–14415.