Radioprotection 2002 DOI: 10.1051/radiopro:2002007
Vol. 37, n° 3, pages 261 à 282

Invited article

Radiation-induced genomic instability and bystander effects: implications for radiation protection*

J.B. LITTLE[1]

(Manuscript received 15 April 2002, accepted 4 May 2002)

ABSTRACT **Evidence has emerged over the past decade for the existence of two cellular phenomenons which challenge the standard paradigms for the induction of biological effects by ionizing radiation. In both cases, important genetic changes arise in cells that in themselves receive no radiation exposure. In the first, radiation induces a type of transmissible genomic instability in cells that leads to a persistent enhancement in the rate at which genetic alterations including mutations and chromosomal aberrations arise in the descendents of the original irradiated cell after many generations of replication. In the bystander effect, damage signals are transmitted from irradiated to non-irradiated cells in the population, leading to the occurrence of biologic effects in these "bystander" cells. In this review, our current knowledge concerning these two phenomena is described and their potential impact on the estimation of risks of low level radiation exposure discussed.**

RÉSUMÉ **Instabilité génomique et effet « bystander » induit par les rayonnements ionisants : *implications pour la radioprotection.***

Au cours de la dernière décennie est apparue la preuve de l'existence de deux phénomènes cellulaires qui remettent en question les paradigmes classiques concernant l'induction d'effets biologiques par les rayonnements ionisants. Dans les deux cas, d'importantes modifications génétiques surviennent dans des cellules qui n'ont pas été elles-mêmes exposées aux rayonnements ionisants. Dans le premier cas, l'irradiation induit un type d'instabilité génomique transmissible dans les cellules. Elle conduit à une augmentation persistante du taux auquel des altérations génétiques, incluant des mutations et des aberrations chromosomiques, surviennent chez les descendants des cellules irradiées originellement, après plusieurs générations de réplication. Dans l'effet « bystander », des signaux de dommages sont transmis des cellules irradiées aux cellules non-irradiées d'une population cellulaire, conduisant à la survenue d'effets biologiques dans ces cellules. Cette revue décrit *l'état actuel des connaissances pour ces deux types de phénomènes.* Leur impact potentiel sur l'estimation des risques aux faibles doses d'irradiation est discuté.

1. Introduction

It has long been assumed that the important biologic effects of ionizing radiation in mammalian cells are a direct consequence of DNA damage that has not been

* Supported by Research Grant FG02-98Er62685 from the US Department of Energy and Center Grant ES-00002 from the US National Institutes of Health.

[1] Laboratory of Radiobiology, Harvard School of Public Health, Boston MA 02115, USA.

correctly restored by enzymatic repair processes. The DNA molecules are located in the nucleus and carry the genetic information in the cell; mutations are irreversible alterations in the coding sequence of DNA. Genetic changes such as mutations and chromosomal aberrations, which are thought to be early events in the development of cancer, would presumably arise at the site of DNA damage as a consequence of processing during normal DNA replication or enzymatic repair.

Evidence to support this interpretation has been derived from a number of experimental studies carried out over the past several decades. Early experiments with microbeam irradiation utilized a focused beam of high Linear Energy Transfer (LET) particles to irradiate specific cellular substructures (Zirkle and Bloom, 1953). These studies revealed that the nucleus of the cell was the sensitive target for cell killing; cytoplasmic irradiation alone produced little cytotoxic effect. The unique quality of the DNA molecules which carry the genetic information in the cell focused attention on DNA as the important target within the nucleus. This conclusion was strengthened by the discovery of enzymatic DNA repair processes in bacterial cells (Setlow and Carrier, 1964; Hanawalt, 1977) which could greatly modify the cytotoxic effects of radiation. The apparent direct relationship between the modulation of DNA repair capacity and the biological effects of radiation (Hanawalt, 1977) provided a convincing though indirect argument for DNA as the critical target in the cell. Soon afterwards, the existence of such repair processes was demonstrated in mammalian cells, and it has since been shown that a number of repair-deficient mammalian cell lines are highly sensitive to the cytotoxic effects of radiation (Nagasawa and Little, 1983; Abbott *et al.*, 1999; Girard *et al.*, 2000).

Confirmatory evidence for DNA damage as a central factor in the biological effects of radiation was derived from experiments whereby irradiation was localized specifically to DNA. This was accomplished by incubating cells with iodine-125 labeled iododeoxyuridine (^{125}IdUrd) which is incorporated into cellular DNA in place of thymidine. ^{125}I releases a shower of 21 very low energy electrons when it decays to a tellurium atom, which in turn must capture 21 electrons to return to the neutral state. When ^{125}IdUrd is incorporated into cellular DNA, this intense release of energy is confined to a very small region in the DNA molecule within a few base pairs of the site of decay. Such decays were found to be highly mutagenic and cytotoxic (Liber *et al.*, 1983). On the other hand ^{125}I localized within the cytoplasm or associated with the cell membrane had no cytotoxic or mutagenic effects. These studies thus appeared to yield direct evidence for DNA as the critical target for radiation-induced mutagenesis and cell killing. Such findings have led to the development of models for radiation action based on the assumption that dose-dependent effects are related directly to unrepaired or misrepaired DNA damage in irradiated cells. In cell populations

exposed to very low fluences of high LET particles, the dose-dependent risk has been based on the fraction of cells traversed by a track.

Over the past decade, however, data have been emerging which indicate that, when a cell population is exposed to ionizing radiation, biological effects may occur in cells that receive no direct nuclear exposure. By use of a precision microbeam, for example, it has been reported that mutations can arise as a consequence of cytoplasmic irradiation (Wu *et al.*, 1999), though at a significantly lower frequency than is found in cells receiving direct nuclear exposure (Hei *et al.*, 1997). Two phenomenon of considerable recent interest will be described in this review as they may have particular relevance to the assessment of the risk of low-level radiation exposure. They are radiation-induced genomic instability and the bystander effect. In both cases, genetic changes occur in cells that in themselves receive no radiation exposure.

2. Radiation induced genomic instability

The term radiation-induced genomic instability refers to a phenomenon observed in a number of different cellular systems whereby radiation exposure appears to induce a type of instability in individual cells that is transmitted to their progeny, leading to a persistent enhancement in the rate at which genetic changes arise in the descendants of the irradiated cell after many generations of replication. The genetic endpoints studied have included malignant transformation, chromosomal aberrations, specific gene mutations, and cell survival. Typically, this phenomenon has been studied by examining the occurrence of such genetic effects in clonal populations derived from single cells surviving radiation exposure. This phenomenon is illustrated schematically in Figure 1 for the induction of mutations in an irradiated cell population (Little, 2000). Mutations are generally rare events, occurring with frequency around 10^{-5}. By the standard paradigm, a specific mutation would be induced in a small fraction of the irradiated cells (B); this mutation would be transmitted to all of the progeny of that cell. Most cells in the population, however, would not be mutated at that locus (A). The radiation-induced genomic instability phenomenon is shown in C and D; whereas no mutations were induced in the irradiated cells themselves, an increased frequency of mutants arises in the descendants of many of the irradiated cells in the population.

Early evidence for the existence of such a phenomenon was derived from an examination of the kinetics of radiation-induced malignant transformation of cells *in vitro* (Kennedy *et al.*, 1980; Kennedy and Little, 1984). These results suggested that transformed foci did not arise from a single, radiation-damaged cell. Rather, radiation appeared to induce a type of instability in 20–30% of the irradiated cell

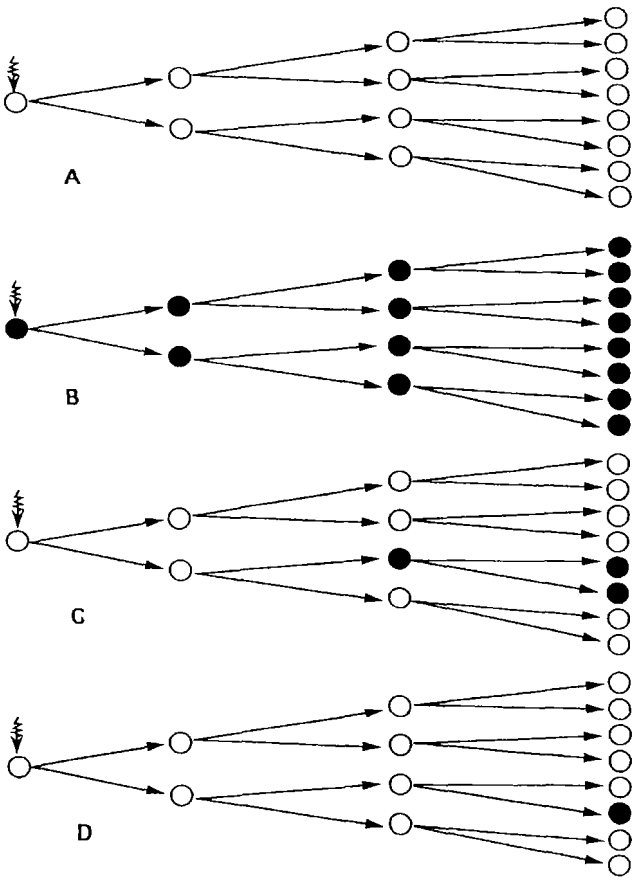

Figure 1 – *Schematic representation of radiation-induced genomic instability. Open circles represent normal wild-type cells, while closed circles represent mutated cells. (B) Example of a cell directly mutated by radiation exposure; the mutation is transmitted to all of its progeny. However, most of the cells in the irradiated population will retain the wild-type phenotype (A). (C and D) Examples of mutations arising as a result of radiation-induced genomic instability. The irradiated cell and its immediate progeny are wild-type, but the frequency with which mutations arise amongst the more distant descendants of the irradiated cell is elevated (Little, 2000).*

Représentation schématique de l'instabilité génomique radio-induite. Les cercles ouverts représentent les cellules normales de type « sauvage », les cercles pleins représentent les cellules mutées. (B) Exemple d'une cellule mutée directement après irradiation ; la mutation est transmise à toute la descendance. Cependant, la plupart des cellules de la population irradiée conservent le phénotype de type « sauvage » (A). (C et D) Exemples de mutation résultant d'une instabilité génomique radio-induite. La cellule irradiée et sa descendance directe sont de type « sauvage », mais la fréquence à laquelle les mutations surviennent chez les descendants de la cellule irradiée est élevée (Little, 2000).

population; this instability enhanced the probability of the occurrence of a second, neoplastic-transforming event. This second event was a rare one, occurring with the frequency of approximately 10^{-6}, and involved the actual transformation of one or more of the progeny of the original irradiated cells after many rounds of cell division. This transforming event occurred with the constant frequency per cell per generation, and had the characteristics of a mutagenic event (Kennedy *et al.*, 1984). Thus, neoplastically transformed foci did not appear to arise from the original irradiated cell but rather from one or more of its progeny. These findings were consistent with the hypothesis that radiation induces genetic instability in cells that enhances the rate at which malignant transformation or other genetic events occur in descendants of irradiated cells after many generations of cell replication.

This hypothesis has subsequently been confirmed in a number of experiment systems for various genetic endpoints (Morgan *et al.*, 1996; Little, 1998; Baverstock, 2000; Romney *et al.*, 2001a). In terms of mutagenesis, approximately 10% of clonal populations derived from single cells surviving radiation exposure showed a significant elevation in the frequency of spontaneously arising mutations as compared with clonal populations derived from non-irradiated cells (Chang and Little, 1992; Little *et al.*, 1997). This increased mutation rate persisted for approximately 30 generations post-irradiation then gradually subsided. Interestingly, the molecular structural spectrum of these late-arising mutants resembles those of spontaneous mutations in that the majority of them are point mutations (Grosovsky *et al.*, 1996; Little *et al.*, 1997), indicating that they arise by a different mechanism from that of direct X-ray-induced mutations which involve primarily deletions. An enhancement of both minisatellite (Li *et al.*, 1992) and microsatellite (Romney *et al.*, 2001b) instability has also been observed in the progeny of irradiated cells selected for mutations at the *thymidine kinase* locus, further evidence that a subpopulation of genetically unstable cells arises in irradiated populations. It is of interest that instability as measured in minisatellite sequences of X-ray-transformed mouse 10T½ cells was markedly enhanced when the cells were grown *in vivo* as compared to prolonged cultivation *in vitro* (Paquette and Little, 1994).

An enhanced frequency of non-clonal chromosomal aberrations was first reported in clonal descendants of mouse hematopoietic stem cells examined 12–14 generations after exposure to alpha radiation (Kadhim *et al.*, 1992). Persistent radiation-induced chromosomal instability has since been demonstrated in a number of other cellular systems (Sabatier *et al.*, 1992; Holmberg *et al.*, 1993; Marder and Morgan, 1993; Kadhim *et al.*, 1995; Little *et al.*, 1997; Ponnaiya *et al.*, 1997). Susceptibility to radiation-induced chromosomal instability differs significantly among cells from different strains of mice (Watson *et al.*, 1996a;

Ponnaiya *et al.,* 1997). It is now clear that genomic instability, both chromosomal and mutational instability, can be induced by high or low LET radiation (Little *et al.,* 1997; Belyakov *et al.,* 1999; Limoli *et al.,* 2000; Evans *et al.,* 2001) and in most cell types. It has been shown recently that long-term instability can be induced by irradiation of cells with single alpha particles from a focused microbeam (Kadhim *et al.,* 2001), supporting earlier observations that the instability phenotype can be activated by low radiation doses, becoming saturated at higher doses (Kadhim *et al.,* 1995; Grosovsky *et al.,* 1996; Little *et al.,* 1997).

Finally, a persistently increased rate of cell death has been shown to occur in cell populations many generations after irradiation (Seymour *et al.,* 1986; Chang and Little, 1992; Belyakov *et al.,* 1999). This phenomenon has been variously referred to as occurring as a result of "lethal mutations" or "delayed reproductive failure", but has been measured as a reduction in the ability of cells to attach and form macroscopic colonies in a classic clonogenic survival assay. In some cellular systems, an increased rate of apoptotic cell death has been shown to accompany this phenomenon (Jamali and Trott, 1996; Limoli *et al.,* 1998; Belyakov *et al.,* 1999). Persistent reproductive failure has been linked to chromosomal instability (Limoli *et al.,* 1998) and malignant transformation (Lewis *et al.,* 2001; Redpath and Gutierrez, 2001), and evidence presented to suggest that DNA is at least one of the critical targets in the initiation of this phenomenon (Limoli *et al.,* 1999). Instability was attenuated by treating the irradiated cells with free radical scavengers or allowing potentially lethal damage to be repaired by confluent holding prior to analyzing the subsequent development of chromosomal instability (Limoli *et al.,* 2001). It has been proposed that oxidative stress perhaps consequent to enhanced, p53-independent apoptosis may contribute to the perpetuation of the instability phenotype in these populations (Limoli *et al.,* 1998; Redpath and Gutierrez, 2001).

Of importance in terms of radioprotection is whether this phenomenon occurs *in vivo* and thus may be related to the induction of cancer. The transmission of chromosomal instability *in vivo* has indeed been demonstrated in several distinct experimental models (Pampfer and Streffer, 1989; Watson *et al.,* 1996b; Ullrich and Davis, 1999), and evidence presented to suggest that instability induced in X-irradiated mouse hematopoietic stem cells may be related to the occurrence of the non-specific genetic damage found in radiation-induced leukemias in these mice (MacDonald *et al.,* 2001).

Another interesting model involves the induction of mouse mammary tumors by radiation. Sensitivity to tumor induction was found to be strain specific and to correlate with the induction of chromosomal instability in mammary epithelial cells irradiated *in vivo* (Ullrich and Davis, 1999). The induction of such instability

was dose dependent. It was subsequently shown that the sensitive, cancer-prone mouse strain (BALB/c) was characterized by reduced expression of the DNA repair enzyme DNA-PKcs, leading to inefficient end rejoining of DNA double strand breaks induced by radiation (Okayasu *et al.,* 2000). This finding is of interest in relation to the recent evidence for the involvement of chromosome telomeres in radiation sensitivity and genomic instability (Bouffler *et al.,* 2001). DNA-PKcs has been shown to play an essential role in telomere function and capping (Gilley *et al.,* 2001; Bailey *et al.,* 2001). A high frequency of telomere fusions occur in DNA-PKcs deficient cells (Gilley *et al.,* 2001); the loss of telomeres has been associated with the development of chromosomal instability in cancer cells (Fouladi *et al.,* 2000). Transmissible instability might thus be a consequence of successive bridge-breakage-fusion cycles resulting from telomere loss.

In sum, it appears well established that ionizing radiation can induce a type of transmissible instability in cells that enhances the probability of the occurrence of multiple genetic effects in the descendants of the surviving cells, sometimes after many generations of replication. Two questions remain to be clarified: what is the event(s) that initiates the process; and how is the signal transmitted over many generations of replication? Current studies are focused on identifying the mechanism for the phenomenon, including the role of oxidative stress, and its importance in terms of the effects of radiation *in vivo.*

3. The bystander effect in irradiated cell populations

The bystander effect of radiation refers to the evidence that damage signals may be transmitted from irradiated to non-irradiated cells in a population, leading to the occurrence of biological effects in cells that receive no radiation exposure. The use of this term has been interpreted broadly, however, as is evidenced by the experimental protocols employed to study such effects *in vitro.* The first protocol employs monolayer cultures of mammalian cells whereby a small fraction of the cells in the population are irradiated, generally by alpha particles, and the biological effect examined in the non-irradiated, neighboring cells. A corollary protocol involves mixing experiments in which irradiated cells are mixed with non-irradiated cells and the biologic effect subsequently measured in the non-irradiated cohort of the population. The second protocol involves the harvesting of conditioned medium from irradiated cultures and incubating this with non-irradiated cells; the bystander cells are thus not in physical proximity to the irradiated cells. Both mixing and medium transfer techniques permit the examination of effects with low LET as well as high LET radiations.

3.1. Monolayer cultures

The experimental model employed in these studies has generally involved the exposure of monolayer cultures of mammalian cells, often confluent or sub-confluent, to very low fluences of alpha particles, fluences whereby only a very small fraction of the nuclei in a cell population will actually be traversed by an alpha particle. This may be accomplished by irradiation from an external source of alpha particles (Metting *et al.,* 1995) or by use of precision microbeam irradiators whereby specific cells can be targeted (Hei *et al.,* 1997; Prise *et al.,* 1998, 2000; Folkard *et al.,* 2001). A grid arrangement has also been employed to protect many cells in a population exposed to relatively high fluences of alpha particles (Lorimore *et al.,* 1998).

The first evidence for this phenomenon was derived from studies of the induction of sister chromatid exchanges (SCE) by very low fluences of alpha particles from an external source (Nagasawa and Little, 1992). It was observed that an enhanced frequency of SCE occurred in 20–40% of the cells exposed to fluences whereby only about 1/1000 to 1/100 cell nuclei were actually traversed by an alpha particle. This finding was later confirmed and evidence presented to suggest that the phenomenon involved secretion of cytokines or other factors by irradiated cells leading to the upregulation of oxidative metabolism in bystander cells (Deshpande *et al.,* 1996; Narayanan *et al.,* 1997, 1999; Lehnert and Goodwin, 1997). It has since been shown that an enhanced frequency of specific gene mutations occurs in bystander cells in populations exposed to very low fluences of alpha particles (Nagasawa and Little, 1999). As a result, the induced mutation frequency per alpha particle track increases at low fluences where bystander as well as directly irradiated cells are at risk for the induction of mutations. This leads to hyperlineality of the dose-response curve in the low dose region (Fig. 3). Studies with microbeam irradiation have provided evidence for an enhanced frequency of micronucleus formation and apoptosis in bystander cells (Prise *et al.,* 1998, 2000; Belyakov *et al.,* 2001), as well as an enhanced frequency of mutations (Zhou *et al.,* 2000, 2001) and malignant transformation (Sawant *et al.,* 2001).

It has also been shown that changes in gene expression occur in bystander cells in monolayer cultures; the expression levels of p53, $p21^{Waf1}$, CDC2, cyclin-B1 and rad51 were significantly modulated in non-irradiated cells in confluent human diploid cell populations exposed to very low fluences of alpha particles (Azzam *et al.,* 1998). These experiments were carried out by western blotting and *in situ* immunofluorescence staining techniques utilizing convocal microscopy. An example of the latter is shown in Figure 2; although only about 1–2% of the cell nuclei was actually traversed by an alpha particle, clusters of cells showed enhanced expression of $p21^{Waf1}$. This phenomenon involved cell-to-cell

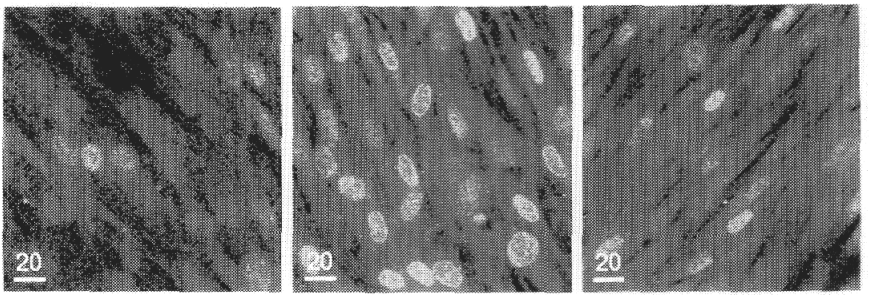

Figure 2 – ***Bystander effect in confluent cultures of normal human diploid fibroblasts, as examined by* in situ *immunofluorescence detection of $p21^{Waf1}$. The panel on the left represents control, non-irradiated cultures, whereas the panel in the center is from a culture irradiated with a 0.3 cGy. Focal areas were observed in which up to 50% of the cells showed enhanced expression of p21, whereas only 1–2% of the nuclei were actually traversed by an α-particle. The panel on the right shows the suppression of the bystander effect by incubation of 0.3 cGy irradiated cultures with Lindane, which inhibits gap junction mediated intercellular communication.***

Effet « bystander » dans des cultures à confluence de fibroblastes humains normaux diploïdes, examinés en immunofluorescence* in situ *pour la détection de la protéine $p21^{Waf1}$. La figure de gauche montre les cultures témoins non irradiées ; la figure du centre montre une culture irradiée à 0,3 cGy. On observe des zones focales dans lesquelles plus de 50 % des cellules sur-expriment p21, alors que seulement 1 à 2 % des noyaux ont été traversés par une particule alpha. La figure de droite montre la suppression de l'effet « bystander » après incubation des cultures de cellules irradiées à 0,3 cGy avec du lindane, qui est un inhibiteur de la communication intercellulaire au niveau des jonctions lâches.

communication *via* gap junctions (Azzam *et al.*, 2001). Examining micronucleus formation, a surrogate measure of DNA damage, provided evidence for DNA damage in bystander cells under these conditions. That the upregulation of the p53 damage response pathway in bystander cells was a consequence of this DNA damage is supported by the observation that p53 was phosphorylated on serine 15 (Azzam *et al.*, 2001).

Interestingly, however, DNA damage in bystander cells appears to differ from that occurring in directly irradiated cells; whereas the mutations induced in directly irradiated cells were primarily partial and total gene deletions, over 90% of those arising in bystander cells were point mutations (Huo *et al.*, 2001). This would be consistent with the evidence that oxidative metabolism is upregulated in bystander cells (Narayanan *et al.*, 1997; Azzam *et al.*, 2002), and has led to the hypothesis that the point mutations are a result of oxidative base damage occurring in bystander cells (Huo *et al.*, 2001). A similar mechanism has been proposed for the observation that localized cytoplasmic exposure from a microbeam irradiator led

to a significant increase in the frequency of point mutations which appeared to involve the generation of reactive oxygen species (Wu *et al.*, 1999).

In earlier studies, it was reported that alpha particle irradiation could induce the intracellular generation of reactive oxygen species (ROS) including the superoxide anion and hydrogen peroxide (Narayanan *et al.*, 1997). This ROS response did not require direct nuclear irradiation, as an ROS response was induced in non-irradiated cells incubated with conditioned medium from alpha irradiated cells. On the other hand, based on the lack of a suppressive effect of DMSO, it has been suggested that reactive oxygen species are not involved in the mutagenic response of bystander cells in monolayer populations following microbeam irradiation (Zhou *et al.*, 2000). In recent experiments (Azzam *et al.*, 2002), the role of oxidative stress has been examined in the modulation of signal transduction and micronucleus formation in bystander cells in confluent monolayer populations of human diploid cells exposed to low fluences of alpha particles. Evidence is presented to support that hypothesis that superoxide and hydrogen peroxide produced by flaving containing oxidase enzymes mediate the activation of several stress inducible signaling pathways as well as micronucleus formation in bystander cells (Azzam *et al.*, 2002). These include the p53 damage response pathway as well as the MAP kinase family of signaling pathways. It has also been reported that nitric oxide may initiate intercellular signal transduction pathways influencing the cellular response to radiation (Matasumoto *et al.*, 2001). Interestingly, this upregulation of oxidative stress in bystander cells is reminiscent of the effect that has been associated with radiation-induced genomic instability (Redpath and Gutierrez, 2001; Limoli *et al.*, 2001), and it has been proposed that the bystander effect may be related to the induction of an inflammatory-type response *in vivo* (Lorimore *et al.*, 2001). The activation of MAP K proteins and their downstream effectors in bystander cells (Azzam *et al.*, 2002) is of particular interest in terms of the recent observation that membrane signaling is involved in the bystander effect in monolayer cultures (Nagasawa *et al.*, 2002).

Bishayee *et al.* (1999) and Howell and Bishayee (2002) developed a three-dimensional tissue culture model to study bystander effects caused by non-uniform distributions of radioactivity. Cells labeled with ^{125}IdUrd were mixed with unlabeled cells and multicellular clusters formed by centrifugation. A decrease in clonogenic survival occurred among the unlabeled cells which, based on inhibitor studies, appeared to depend upon gap junction mediated intercellular communication. Watson *et al.* (2000) transplanted a mixture of irradiated and non-irradiated bone marrow cells in a mouse system that allowed the discrimination between irradiated donor stem cell-derived cells and non-irradiated stem-cell derived cells *in vivo*. They were able to demonstrate chromosomal instability in the progeny of the non-irradiated hematopoietic stem cells, providing a link between

a bystander effect of ionizing radiation and the induction of genomic instability *in vivo*.

These protocols whereby populations of irradiated and non-irradiated cells are mixed together provides some of the characteristic of monolayers in that the bystander and targeted cells are in physical contact. An advantage is that it can be adapted to any type of irradiation, and allows the examination of effects in three-dimensional culture systems as well as *in vivo*.

3.2. Medium transfer experiments

There is a long history of the apparent induction of clastogenic factors by radiation, primarily as measured in the plasma of irradiated individuals. These studies are reviewed in detail by Mothersill and Seymour (2001). These workers have shown more recently that the exposure of cells in culture or explants of tissue to gamma radiation doses as low as 1 cGy can lead to the release of factors into the medium by the irradiated cells; when this conditioned medium is transferred to non-irradiated cells, their cloning efficiency is reduced associated with increased levels of apoptotic cell death. This phenomenon has been associated with early changes in mitochrondrial membrane permeability and the induction of reactive oxygen species (ROS). Lehnert and coworkers (Narayanan *et al.*, 1997; Lehnert and Goodwin, 1997) also showed by medium transfer experiments that extracellular factors including ROS were released by alpha particle irradiated cells, that could lead to the induction of sister chromatid exchanges in non-irradiated cells. Furthermore, they showed that incubation of non-irradiated cells with irradiated culture medium alone led to an enhancement in SCE and ROS in these "bystander cells". On the other hand, other workers have shown this to be a cell mediated response finding no effect of irradiated medium alone (Belyakov *et al.*, 2001; Zhou *et al.*, 2002). Furthermore, Zhou *et al.* (2002) reported that while irradiated cells released cytotoxic factors into the culture medium that killed non-irradiated cells, such factors had little or no effect on mutation induction.

Recently, it has been reported that conditioned medium from alpha particle irradiated cells can stimulate cell proliferation in non-irradiated cells, which was attributed to the promitogenic response to an increase in TGFβ1 acting as a mediator of the increased intracellular ROS observed in bystander cells (Iyer and Lehnert, 2000). Furthermore, an increase in protein levels of AP-endonuclease, a redox and DNA base excision repair protein, were measured in bystander cells but not in directly irradiated cells. This was associated with an increase in cloning efficiency (Iyer and Lehnert, 2002). This finding is of interest as it suggests a possible beneficial bystander effect related to an increase in DNA repair capacity and clonogenic survival, and is thus reminiscent of the earlier finding that

incubation with conditioned medium from plateau-phase cultures facilitated the repair of potentially lethal radiation damage (Little, 1971).

Overall, a clear picture has yet to emerge from the experience with medium transfer experiments. It appears clear that factors are released into the medium by irradiated cells, that can lead to changes in the viability of non-irradiated cells incubated with such conditioned medium. The results from different laboratories, however, are not entirely consistent. Some workers report that incubation with conditional medium harvested from irradiated cultures leads to a reduction in cloning efficiency of the recipient cells (Lyng *et al.,* 2002; Sawant *et al.,* 2002), while others find it is enhanced (Iyer and Lehnert, 2002) or dependent on cell type (Mothersill and Seymour, 1997). The effect of medium irradiation alone is particularly controversial. In terms of genetic effects, one laboratory describes a bystander effect for sister chromatid exchanges in conditioned medium transfer experiments (Lehnert and Goodwin, 1997), whereas another finds little or no evidence for a bystander mutagenic effect under similar conditions (Zhou *et al.,* 2002). The effect appears likely to be mediated by cytokines or reactive oxygen species, but the exact nature of the factor or factors responsible for the biological effects in the non-irradiated, bystander cells remains to be elucidated.

3.3. Conclusions

In sum, these results indicate clearly that damage signals can be transmitted from irradiated to non-irradiated cells. In confluent monolayer cultures, this phenomenon involves gap junction mediated cell to cell communication, and appears to involve both the induction of reactive oxygen species and the activation of extra-nuclear signal transduction pathways. Preliminary evidence suggests a role for membrane signaling. Multiple biological effects may occur in bystander cells including cell killing, the induction of mutations and the modulation of gene expression. Some evidence suggests that regulation of the p53 damage response pathway may be central to this phenomenon. Damage signals may in addition be transmitted through the extracellular medium, also appearing to involve the production of reactive oxygen species. Finally, preliminary studies with a mouse bone marrow stem cell transplant system suggest that the effect may occur *in vivo*.

4. Implications for risk assessment

The relevance of radiation-induced genomic instability to the carcinogenic risk is not yet entirely clear. There is increasing evidence that the development of invasive metastatic cancer involves a series of distinct genetic events some of which can be associated with specific stages in the carcinogenic process (Fearson

and Vogelstein, 1990). A question that arises is how as many as six to eight such genetic events may accumulate in a single cell lineage, given that the frequency of most mutations is about 10^{-5}. Loeb (1991) and others have postulated that early in the process of carcinogenesis a mutation may arise in a gene that is important in maintaining genomic stability, yielding a cell lineage with a mutator phenotype. This phenotype would enhance the frequency with which spontaneous mutations arise in these cells, and thus facilitate the accumulation of the requisite number of genetic events to produce a cancer. Such an example involves hereditary non-polyposis colon cancer which is associated with a germline defect in DNA mismatch repair. While genomic instability is a hallmark of tumor cells, most types of cancer have not been associated with specific DNA repair defects.

The finding that radiation itself may induce an instability phenotype has thus attracted considerable interest. It would suggest that the initial radiation-induced event might be a frequent one involving as many as 10–20% of the population, rather than a rare mutagenic event. This increased level of instability which is transmissible over many generations of cell replication would thus enhanced the rate at which genetic events important to the development of cancer would arise in the cell population. It is not yet clear, however, the extent to which this radiation-induced phenomenon may be of importance in carcinogenesis. The fact that it appears to saturate at fairly low doses (of the order of 10–50 cGy) implies that it could influence the extrapolation to low dose effects. On the other hand, as it may not represent an irreversible carcinogenic event such as mutation, it might be susceptible to modulation by external factors. Clearly, additional research is needed to determine the mechanisms involved in radiation-induced genomic instability, in terms of both the initiating event and how the effect is transmissible for many generations of cell replication, before its implications for the assessment of the carcinogenic risk of irradiation can be clarified.

An important area where this phenomenon could well be of significance involves potential transgenerational effects of irradiation. If exposure to low levels of ionizing radiation induces the instability phenotype in germ cells, it is entirely feasible that this instability could be passed on to the germline of the offspring increasing their susceptibility to cancer or other genetic effects. Indeed, Pils *et al.* (1999) have reported that genomic instability may be passed on to two successive generations of offspring in mice after irradiation of the zygote, and Dubrova and his colleagues (Dubrova *et al.*, 1998; Dubrova and Plumb, 2002) have presented evidence for transmissible germline instability at mouse minisatellite loci. There is also some experimental evidence suggesting the existence of transgenerational effects of radiation in mice, including increased susceptibility to the induction of tumors (Nomura, 1982; Lord *et al.*, 1998; Nomura, 2000), congenital malformations (Lyon and Renshaw, 1988) and other changes (Baulch *et al.*, 2001,

2002). Finally, there is preliminary evidence for the occurrence of enhanced minisatellite instability in the offspring of irradiated human populations (Dubrova *et al.,* 1997). Although the evidence for transgenerational effects of radiation in human populations remains controversial, the radiation induced genomic instability phenomenon would provide a mechanism for such effects.

The bystander effect has clear implications in terms of human exposures to very low fluences of high LET particulate radiation, such as alpha particles from environmental radon or densely-ionizing galactic cosmic rays in space (Brenner and Elliston, 2001). In the case of radon, for example, only a small fraction of a person's bronchial epithelial cells, the presumed target for lung cancer, will be hit each year by an alpha particle arising from residential radon exposure during the person's lifetime. In the past, the genetic or carcinogenic risk has been assumed to be related directly to the number of cell nuclei actually traversed by an alpha particle, thus yielding a linear dose response relationship. The evidence that irradiated cells may transmit damage signals to neighboring non-irradiated cells that result in genetic alterations in these "bystander" cells would invalidate this assumption. Rather, it would suggest that the effect would be greater than predicted for the actual dose received at low particle fluences. This is shown in Figures 3 and 4 for the induction of mutations *in vitro*. When examined at fluences whereby most cells are traversed by one or more alpha particles, the mutagenic effect appears to be a linear function of dose (Fig. 4). When examined at very low particle fluences, however, the dose-response curve is seen to be hyperlinear at low mean doses (Fig. 3), because additional mutations are occurring in bystander cells (Nagasawa and Little, 1999).

Also relevant to the estimation of risks of low level radiation exposure is a phenomenon originally described by Sheldon Wolf and his colleagues called the "adaptive response" (Wolff, 1996). Originally described for the induction of chromosomal aberrations following irradiation in the G_2 phase of the cell cycle (Olivieri *et al.,* 1984), this phenomenon has since been found in a number of experimental systems. In essence, exposure to a very low dose of irradiation (in the range of 1 cGy) renders cells more resistant to a second larger dose of irradiation. A priming dose of 1 cGy, for example, increased the radioresistance of normal human bystander cells (Iyer and Lehnert, 2002). Several recent studies are of interest in relation to this phenomenon. In two quite different experimental systems for the study of malignant transformation *in vitro*, evidence has been presented that the spontaneous transformation frequency is actually reduced by very small doses of radiation (doses as low as 0.1 cGy) (Azzam *et al.,* 1996; Redpath *et al.,* 2001). The frequency of transformation rises rapidly at higher doses. Joiner and his colleagues (Joiner *et al.,* 2001; Mothersill *et al.,* 2002) have examined the cytotoxic effects of radiation at very low doses, and found evidence

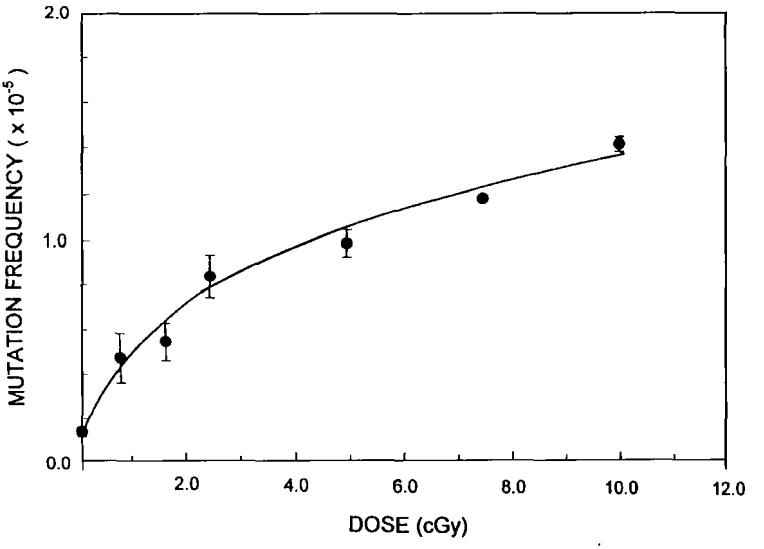

Figure 3 – ***Dose-response relationship for the induction of HPRT mutations in CHO cells by low fluences of alpha particles (Nagasawa and Little, 1999). Over the dose range shown, approximately 3–50% of the cells will be traversed by an alpha particle. In the higher mean dose range (5–10 cGy), the curve becomes a linear function of dose with a slope similar to that in Figure 4. Curvilinear response at lower doses is the result of mutations occurring in non-irradiated bystander cells.***

Relation dose-réponse pour l'induction de mutations au locus HPRT chez des cellules CHO par des fluences faibles de particules alpha (Nagasawa et Little, 1999). Au-delà de la gamme de dose indiquée sur la figure, environ 3 à 50 % des cellules sont traversées par une particule alpha. Dans la gamme de dose moyenne et supérieure, comprise entre 5 et 10 cGy, la courbe devient une fonction linéaire de la dose avec une pente identique à celle de la figure 4. La réponse curvilinéaire aux faibles doses est le résultat des mutations survenant chez les cellules « bystander » non irradiées.

in many cell types for a hypersensitive response to very low doses (<10 cGy), followed by a plateau in sensitivity before killing becomes an exponential function of dose. They have presented preliminary evidence to relate this to DNA repair processes. Finally, evidence for an adaptive response is emerging from studies of several experimental models *in vivo* (Mitchel, personal communication). These include the induction of leukemia and lymphoma in mice (Mitchel *et al.*, 1999) as well as teratogenic effects and the development of heritable germ line mutations. The priming dose in all cases range from 1 to 10 cGy.

When considered as a whole, these emerging results suggest that the risk of low level exposure to ionizing radiation is uncertain, and a simple extrapolation from

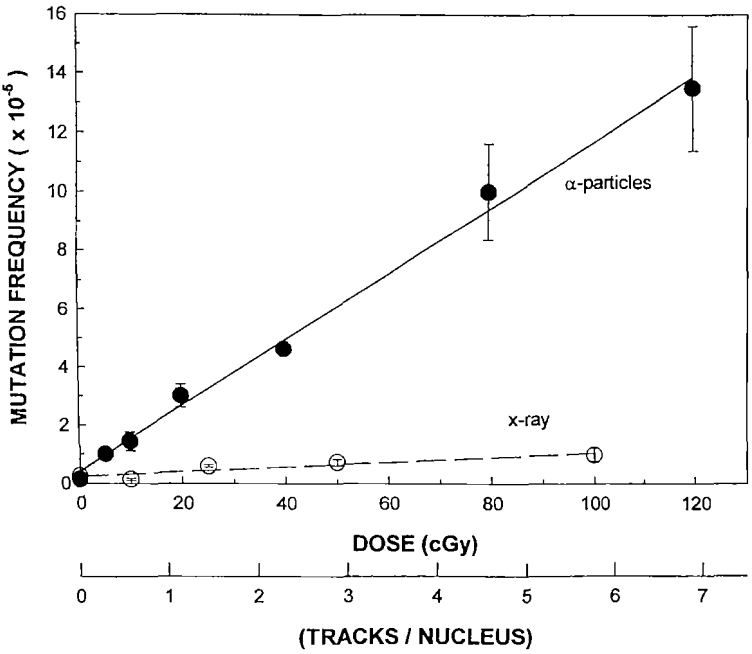

Figure 4 – *Dose-response relationships for the induction of HPRT mutations in CHO cells by alpha particles at mean doses of 5–120 cGy (Nagasawa and Little, 1999). The curve for X-irradiation is shown for comparison.*

Relation dose-réponse pour l'induction de mutations au locus HPRT chez des cellules CHO par des particules alpha à des doses moyennes de 5 à 120 cGy (Nagasawa et Little, 1999). La courbe pour les rayons X est indiquée à titre de comparaison.

high dose effects may not be justified. In some cases, such as the induction of mutations by exposure to very low fluences of high LET particles (Fig. 3), or as reported for the cytotoxic effects of very low doses of X-rays, the effect may be greater than predicted from a linear extrapolation. On the other hand, certain studies of malignant transformation have revealed a reduced effect for very low doses. Evidence suggesting the convergence of these phenomenons is also of interest. Several different studies involving both *in vitro* and *in vivo* assays have shown, that genomic instability may arise in bystander cells, and that the bystander effect may be modulated by the adaptive response.

Overall, these findings imply that the biological effects of radiation in cell populations may not be restricted to the response of individual cells to the DNA damage they receive, but rather that the tissue responds as a whole. A better

understanding of the mechanisms for these phenomenons and how they are interrelated should yield a better understanding of the potential risk to the human population of exposure to low levels of ionizing radiation.

REFERENCES

Abbott D.W., Thompson M.E., Robinson-Benion C., Tomlinson G., Jensen R.A., Holt J.T. (1999) BRCA1 expression restores radiation resistance in BRCA1-defective cancer cells through enhancement of transcription-coupled DNA repair, *J. Biol. Chem.* **274**, 18808-18812.

Azzam E.I., de Toledo S.M., Raaphorst G.P., Mitchel R.E. (1996) Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells, *Radiat. Res.* **146**, 369-373.

Azzam E.I., deToledo S.M., Gooding T., Little J.B. (1998) Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles, *Radiat. Res.* **150**, 497-504.

Azzam E.I., de Toledo S.M., Little J.B. (2001) Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from alpha-particle irradiated to nonirradiated cells, *Proc. Natl. Acad. Sci. USA* **98**, 473-478.

Azzam E.I., de Toledo S.M., Spitz D.R., Little J.B. (2002) Oxidative metabolism modulates signal transduction and micronucleus formation in bystander cells from α-particle-irradiated normal human fibroblast cultures, *Cancer Res.* (in press).

Bailey S.M., Cornforth M.N., Kurimasa A., Chen D.J., Goodwin E.H. (2001) Strand-specific postreplicative processing of mammalian telomeres, *Science* **293**, 2462-2465.

Baulch J.E., Raabe O.G., Wiley L.M. (2001) Heritable effects of paternal irradiation in mice on signaling protein kinase activities in F3 offspring, *Mutagenesis* **16**, 17-23.

Baulch J.E., Raabe O.G., Wiley L.M., Overstreet J.W. (2002) Germline drift in chimeric male mice possessing an F(2) component with a paternal F(0) radiation history, *Mutagenesis* **17**, 9-13.

Baverstock K. (2000) Radiation-induced genomic instability: a paradigm-breaking phenomenon and its relevance to environmentally induced cancer, *Mutat. Res.* **454**, 89-109.

Belyakov O.V., Prise K.M., Trott K.R., Michael B.D. (1999) Delayed lethality, apoptosis and micronucleus formation in human fibroblasts irradiated with X-rays or alpha particles, *Int. J. Radiat. Biol.* **75**, 985-993.

Belyakov O.V., Malcolmson A.M., Folkard M., Prise K.M., Michael B.D. (2001) Direct evidence for a bystander effect of ionizing radiation in primary human fibroblasts, *Br. J. Cancer* **84**, 674-679.

Bishayee A., Rao D.V., Howell R.W. (1999) Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model, *Radiat. Res.* **152**, 88-97.

Bouffler S.D., Blasco M.A., Cox R., Smith P.J. (2001) Telomeric sequences, radiation sensitivity and genomic instability, *Int. J. Radiat. Biol.* **77**, 995-1005.

Brenner D.J., Elliston C.D. (2001) The potential impact of bystander effects on radiation risks in a Mars mission, *Radiat. Res.* **156**, 612-617.

Chang W.P., Little J.B. (1992) Persistently elevated frequency of spontaneous mutations in progeny of CHO clones surviving X-irradiation: association with delayed reproductive death phenotype, *Mutat. Res.* **270**, 191-199.

Deshpande A., Goodwin E.H., Bailey S.M., Marrone B., Lehnert B. (1996) Alpha-particle-induced sister chromatid exchanges in normal human lung fibroblasts: Evidence for an extranuclear target, *Radiat. Res.* **152**, 552-557.

Dubrova Y.E., Plumb M. (2002) Ionising radiation and mutation induction at mouse minisatellite loci. The story of the two generations, *Mutat. Res.* **499**, 143-150.

Dubrova Y.E., Nesterov V.N., Kroucinsky N.G., Ostapenko V.A., Vergnaud G., Giraudeau F., Buard J., Jeffreys A.J. (1997) Further evidence for elevated human minisatellite mutation rate in Belarus eight years after Chernobyl accident, *Mutat. Res.* **381**, 267-278.

Dubrova Y.E., Plumb M., Brown J., Jeffreys A.J. (1998) Radiation-induced germline instability at minisatellite loci, *Int. J. Radiat. Biol.* **74**, 689-696.

Evans H.H., Horng M.F., Ricanati M., Diaz-Insua M., Jordan R., Schwartz J.L. (2001) Diverse delayed effects in human lymphoblastoid cells surviving exposure to high-LET 56Fe particles or low-LET 137Cs gamma radiation, *Radiat. Res.* **156**, 259-271.

Fearson E., Vogelstein B. (1990) A genetic model for colorectal tumorigenesis, *Cell* **61**, 759-767.

Folkard M., Schettino G., Vojnovic B., Gilchrist S., Michette A.G., Pfauntsch S.J., Prise K.M., Michael B.D. (2001) A focused ultrasoft X-ray microbeam for targeting cells individually with submicrometer accuracy, *Radiat. Res.* **156**, 796-804.

Fouladi B., Sabatier L., Miller D., Pottier G., Murnane J.P. (2000) The relationship between spontaneous telomere loss and chromosome instability in a human tumor cell line, *Neoplasia* **2**, 540-554.

Gilley D., Tanaka H., Hande M.P., Kurimasa A., Li G.C., Oshimura M., Chen D.J. (2001) DNA-PKcs is critical for telomere capping, *Proc. Natl. Acad. Sci USA* **98**, 15084-150088.

Girard P.M., Foray N., Stumm M., Waugh A., Riballo E., Maser R.S., Phillips W.P., Petrini J., Arlett C.F., Jeggo P.A. (2000) Radiosensitivity in Nijmegen Breakage Syndrome cells is attributable to a repair defect and not cell cycle checkpoint defects, *Cancer Res.* **60**, 4881-4888.

Grosovsky A.J., Parks K.K., Giver C.R., Nelson S.L. (1996) Clonal analysis of delayed karyotypic abnormalities and gene mutations in radiation-induced genetic instability, *Molec. Cell. Biol.* **16**, 6252-6262.

Hanawalt P.C. (1977) *DNA Repair Processes: An Overview* (W.W. Nichols, D.G. Murphys, Eds.), pp. 1-9. Symposia Specialists, Miami, FL.

Hei T.K., Wu L.-J., Liu S.-X., Vannais D., Waldren C.A., Randers-Pehrson G. (1997) Mutagenic effects of a single and an exact number of α particles in mammalian cells, *Proc. Natl. Acad. Sci. USA* **94**, 3765-3770.

Holmberg K., Fair S., Johansson A., Lambert B. (1993) Clonal chromosome aberrations and genomic instability in X-irradiated human T-lymphocyte cultures, *Mutat. Res.* **286**, 321-330.

Howell R.W., Bishayee A. (2002) Bystander effects caused by nonuniform distributions of DNA-incorporated (125)I, *Micron* **33**, 127-132.

Huo L., Nagasawa H., Little J.B. (2001) *HPRT* mutants induced in bystander cells by very low fluences of alpha particles result primarily from point mutations, *Radiat. Res.* **156**, 521-525.

Iyer R., Lehnert B.E. (2000) Factors underlying the cell growth-related bystander responses to alpha particles, *Cancer Res.* **60**, 1290-1298.

Iyer R., Lehnert B.E. (2002) Alpha-particle-induced increases in the radioresistance of normal human bystander cells, *Radiat. Res.* **157**, 3-7.

Jamali M., Trott K.R. (1996) Persistent increase in the rates of apoptosis and dicentric chromosomes in surviving V79 cells after X-irradiation, *Int. J. Radiat. Biol.* **70**, 705-709.

Joiner M.C., Marples B., Lambin P., Short S.C., Turesson I. (2001) Low-dose hypersensitivity: current status and possible mechanisms, *Int. J. Radiat. Oncol. Biol. Phys.* **49**, 379-389.

Kadhim M.A., MacDonald D.A., Goodhead D.T., Lorimore S.A., Marsden S.J., Wright E.G. (1992) Transmission of chromosomal aberrations and genomic instability after plutonium alpha particle irradiation, *Nature* **355**, 738-740.

Kadhim M.A., Lorimore S.A., Townsend K.M., Goodhead D.T., Buckle V.J., Wright E.G. (1995) Radiation-induced genomic instability: delayed cytogenetic aberrations and apoptosis in primary human bone marrow cells, *Int. J. Radiat. Biol.* **67**, 287-293.

Kadhim M.A., Marsden S.J., Goodhead D.T., Malcolmson A.M., Folkard M., Prise K.M., Michael B.D. (2001) Long-term genomic instability in human lymphocytes induced by single-particle irradiation, *Radiat. Res.* **155**, 122-126.

Kennedy A.R., Little J.B. (1984) Evidence that a second event in X-ray induced oncogenic transformation *in vitro* occurs during cellular proliferation, *Radiat. Res.* **99**, 228-248.

Kennedy A.R., Fox M., Murphy G., Little J.B. (1980) Relationship between X-ray exposure and malignant transformation in C3H 10T1/2 cells, *Proc. Natl. Acad. Sci. USA* **77**, 7262-7266.

Kennedy A.R., Cairns J., Little J.B. (1984) Timing of the steps in transformation of C3H 10T1/2 cells by X-irradiation, *Nature* **307**, 85-86.

Lehnert B., Goodwin E.H. (1997) Extracellular factor(s) following exposure to alpha particles can cause sister chromatid exchanges in normal human cells, *Cancer Res.* **57**, 2164-2171.

Lewis D.A., Mayhugh B.M., Qin Y., Trott K., Mendonca M.S. (2001) Production of delayed death and neoplastic transformation in CGL1 cells by radiation-induced bystander effects, *Radiat. Res.* **156**, 251-258.

Li C.-Y., Yandell D.W., Little J.B. (1992) Evidence for coincident mutations in human lymphoblast clones selected for functional loss of a thymidine kinase gene, *Molec. Carcinogen.* **5**, 270-277.

Liber H.L., LeMotte P.K., Little J.B. (1983) Toxicity and mutagenicity of X-rays and (^{125}I)dUrd or (^{3H})TdR incorporated in the DNA of human lymphoblast cells, *Mutat. Res.* **111**, 387-404.

Limoli C.L., Hartmann A., Shepard L., Yang C.-R., Boothman D.A., Bartholomew J., Morgan W.F. (1998) Apoptosis, reproductive failure and oxidative stress in Chinese hamster ovary cells with compromised genomic integrity, *Cancer Res.* **58**, 3712-3718.

Limoli C.L., Corcoran J.J., Milligan J.R., Ward J.F., Morgan W.F. (1999) Critical target and dose and dose-rate responses for the induction of chromosomal instability by ionizing radiation, *Radiat. Res.* **151**, 677-685.

Limoli C.L., Ponnaiya B., Corcoran J.J., Giedzinski E., Kaplan M.I., Hartmann A., Morgan W.F. (2000) Genomic instability by high and low LET ionizing radiation, *Adv. Space Res.* **25**, 2107-2117.

Limoli C.L., Kaplan M.I., Giedzinski E., Morgan W.F. (2001) Attenuation of radiation-induced genomic instability by free radical scavengers and cellular proliferation, *Free Radic. Biol. Med.* **31**, 10-19.

Little J.B. (1971) Repair of potentially lethal radiation damage in mammalian cells: enhancement by conditioned medium from stationary cultures, *Int. J. Radiat. Biol.* **20**, 87-92.

Little J.B. (1998) Radiation-induced genomic instability, *Int. J. Radiat. Biol.* **6**, 663-671.

Little J.B. (2000) Radiation Carcinogenesis, *Carcinogenesis* **21**, 397-404.

Little J.B., Nagasawa H., Pfenning T., Vetrovs H. (1997) Radiation-induced genomic instability: Delayed mutagenic and cytogenetic effects of X-rays and alpha particles, *Radiat. Res.* **148**, 299-307.

Loeb L.A. (1991) Mutator phenotype may be required for multistage carcinogenesis, *Cancer Res.* **51**, 3075-3079.

Lord B.I., Woolford L.B., Want L., Stones V.A., McDonald D., Lorimore S.A., Papworth D., Wright E.G., Scott D. (1998) Tumour induction by methyl-nitroso-urea following preconceptional paternal contamination with plutonium-239, *Brit. J. Cancer* **78**, 301-311.

Lorimore S., Kadhim M., Pocock D., Stevens D., Papworth D., Goodhead D., Wright E. (1998) Chromosomal instability in the descendants of unirradiated surviving cells after α-particle irradiation, *Proc. Natl. Acad. Sci. USA* **95**, 5730-5733.

Lorimore S.A., Coates P.J., Scobie G.E., Milne G., Wright E.G. (2001) Inflammatory-type responses after exposure to ionizing radiation *in vivo*: a mechanism for radiation-induced bystander effects? *Oncogene* **20**, 7085-7095.

Lyng F.M., Seymour C.B., Mothersill C. (2002) Initiation of apoptosis in cells exposed to medium from the progeny of irradiated cells: A possible mechanism for bystander-induced genomic instability? *Radiat. Res.* **157**, 365-370.

Lyon M.F., Renshaw R. (1988) Induction of congenital malformation in mice by parental irradiation: transmission to later generations, *Mutat. Res.* **198**, 277-283.

MacDonald D., Boulton E., Pocock D., Goodhead D., Kadhim M., Plumb M. (2001) Evidence of genetic instability in 3 Gy X-ray-induced mouse leukaemias and 3 Gy X-irradiation haemopoietic stem cells, *Int. J. Radiat. Biol.* **77**, 1023-1031.

Marder B.A., Morgan W.F. (1993) Delayed chromosomal instability induced by DNA damage, *Mol. Cell Biol.* **13**, 6667-6677.

Matasumoto H., Hayashi S., Hatashita M., Ohnishi K., Shioura H., Ohtsubo T., Kitai R., Ohnishi T., Kano E. (2001) Induction of radioresistance by a nitric oxide-mediated bystander effect, *Radiat. Res.* **155**, 387-396.

Metting N.F., Koehler A.M., Nagasawa H., Nelson J.M., Little J.B. (1995) Design of a benchtop alpha particle irradiator, *Health Phys.* **68**, 710-715.

Mitchel R.E., Jackson J.S., McCann R.A., Boreham D.R. (1999) The adaptive response modifies latency for radiation-induced myeloid leukemia in CBA/H mice, *Radiat. Res.* **152**, 273-279.

Morgan W.F., Day J.P., Kaplan M.I., McGhee E.M., Limoli C.L. (1996) Review: Genomic instability induced by ionizing radiation, *Radiation Res.* **146**, 247-258.

Mothersill C., Seymour C. (1997) Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival or unirradiated cells, *Int. J. Radiat. Biol.* **71**, 421-427.

Mothersill C., Seymour C. (2001) Radiation-induced bystander effects: Past history and future directions, *Radiat. Res.* **155**, 759-767.

Mothersill C., Seymour C.B., Joiner M.C. (2002) Relationship between radiation-induced low-dose hypersensitivity and the bystander effect, *Radiat. Res.* **157**, 526-532.

Nagasawa H., Little J.B. (1983) Comparison of kinetics of X-ray induced cell killing in normal, ataxia telangiectasia and hereditary retinoblastoma fibroblasts, *Mutat. Res.* **109**, 297-308.

Nagasawa H., Little J.B. (1992) Induction of sister chromatid exchanges by extremely low doses of alpha particles, *Cancer Res.* **52**, 6394-6396.

Nagasawa H., Little J.B. (1999) Unexpected sensitivity to the induction of mutations by very low doses of alpha-particle radiation: Evidence for a bystander effect, *Radiat. Res.* **152**, 552-557.

Nagasawa H., Cremesti A., Kolesnick R., Fuks Z., Little J.B. (2002) Involvement of membrane signaling in the bystander effect in irradiated cells, *Cancer Res.* **62**, 2531-2534.

Narayanan P., Goodwin E., Lehnert B. (1997) Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells, *Cancer Res.* **57**, 3963-3971.

Narayanan P.K., LaRue K.E.A., Goodwin E.H., Lehnert B.E. (1999) Alpha particles biological production of Il-8 in human cells, *Radiat. Res.* **152**, 57-63.

Nomura T. (1982) Parental exposure to X-rays and chemicals induces heritable tumours and anomalies in mice, *Nature* **256**, 575-577.

Nomura T. (2000) Transplacental and transgenerational late effects of radiation and chemicals, *Congen. Anomal.* **40**, 554-567.

Okayasu R., Suetomi K., Yu Y., Silver A., Bedford J.S., Cox R., Ullrich R.L. (2000) A deficiency in DNA repair and DNA-PKcs expression in the radiosensitive BALB/c mouse, *Cancer Res.* **60**, 4342-4345.

Olivieri G., Bodycote J., Wolff S. (1984) Adaptive response of human lymphocytes to low concentrations of radioactive thymidine, *Science* **233**, 594-597.

Pampfer S., Streffer C. (1989) Increased chromosome aberration levels in cells from mouse fetuses after zygote X-irradiation, *Int. J. Radiat. Biol.* **55**, 85.

Paquette B., Little J.B. (1994) *In vivo* enhancement of genomic instability in minisatellite sequences of mouse C3H 10T1/2 cells transformed *in vitro* by X-rays, *Cancer Res.* **54**, 3173-3178.

Pils S., Muller W.U., Streffer C. (1999) Lethal and teratogenic effects in two successive generations of the HLG mouse strain after radiation exposure of zygotes – association with genomic instability? *Mutat. Res.* **429**, 85-92.

Ponnaiya B., Cornforth M.N., Ullrich R.L. (1997) Radiation-induced chromosomal instability in BALB/c and C57BL/6 mice: the difference is as clear as black and white, *Radiat. Res.* **147**, 121-125.

Prise K.M., Belyakov O.V., Folkard M., Michael B.D. (1998) Studies of bystander effects in human fibroblasts using a charged particle microbeam, *Int. J. Radiat. Biol.* **74**, 793-798.

Prise K.M., Folkard M., Malcolmson A.M., Pullar C.H., Schettino G., Bowey A.G., Michael B.D. (2000) Single ion actions: the induction of micronuclei in V79 cells exposed to individual protons, *Adv. Space Res.* **25**, 2095-2101.

Redpath J.L., Gutierrez M. (2001) Kinetics of induction of reactive oxygen species during the post-irradiation expression of neoplastic transformation *in vitro*, *Int. J. Radiat. Biol.* **77**, 1081-1085.

Redpath J.L., Liang D., Taylor T.H., Christie C., Elmore E. (2001) The shape of the dose-response curve for radiation-induced neoplastic transformation *in vitro*: evidence for an adaptive response against neoplastic transformation at low doses of low-LET radiation, *Radiat. Res.* **156**, 700-707.

Romney C.A., Paulauskis J.D., Nagasawa H., Little J.B. (2001a) Multiple manifestations of X-ray-induced genomic instability in Chinese hamster ovary (CHO) cells, *Molec. Carcinogen.* **32**, 118-127.

Romney C.A., Paulauskis J.D., Little J.B. (2001b) X-ray induction of microsatellite instability at autosomal loci in human lymphoblastoid WTK1 cells, *Mutat. Res.* **478**, 97-106.

Sabatier L., Dutrillaux B., Martin M.B. (1992) Chromosomal instability, *Nature* **357**, 548.

Sawant S.G., Randers-Pehrson G., Geard C.R., Brenner D.J., Hall E.J. (2001) The bystander effect in radiation oncogenesis: I. Transformation in C3H 10T1/2 cells *in vitro* can be initiated in the unirradiated neighbors of irradiated cells, *Radiat. Res.* **155**, 397-401.

Sawant S.G., Zheng W., Hopkins K.M., Randers-Pehrson G., Lieberman H.B., Hall E.J. (2002) The radiation-induced bystander effect for clonogenic survival, *Radiat. Res.* **157**, 361-364.

Setlow R.B., Carrier W.L. (1964) Disappearance of thymine dimers from DNA: Error-correcting mechanism, *Proc. Natl. Acad. Sci. USA* **51**, 226-231.

Seymour S.B., Mothersill C., Alper T. (1986) High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation, *Int. J. Radiat. Biol.* **50**, 167-179.

Ullrich R.L., Davis C.M. (1999) Radiation-induced cytogenetic instability *in vivo*, *Radiat. Res.* **152**, 170-173.

Watson G.E., Lorimore S.A., Clutton S.M., Kadhim M.A., Wright E.G. (1996a) Genetic factors influencing alpha-particle-induced chromosomal instability, *Int. J. Radiat. Biol.* **69**, 175-182.

Watson G.E., Lorimore S.A., Wright E.G. (1996b) Long-term *in vivo* transmission of alpha-particle-induced chromosomal instability in murine haemopoietic cells, *Int. J. Radiat. Biol.* **69**, 175-182.

Watson G.E., Lorimore S.A., Macdonald D.A., Wright E.G. (2000) Chromosomal instability in unirradiated cells induced *in vivo* by a bystander effect of ionizing radiation, *Cancer Res.* **60**, 5608-5611.

Wolff S. (1996) Aspects of the adaptive response to very low doses of radiation and other agents, *Mutat. Res.* **358**, 135-142.

Wu L.-J., Randers-Pehrson G., Xu A., Waldren C.A., Geard C.R., Yu Z.L., Hei T.K. (1999) Targeted cytoplastic irradiation with alpha particles induces mutations in mammalian cells, *Proc. Natl. Acad. Sci. USA* **96**, 4959.

Zhou H., Randers-Pehrson G., Waldren C.A., Vannais D., Hall E.J., Hei T.K. (2000) Induction of a bystander mutagenic effect of alpha particles in mammalian cells, *Proc. Natl. Acad. Sci. USA* **97**, 2099-2104.

Zhou H., Suzuki M., Randers-Pehrson G., Vannais D., Chen G., Trosko J.E., Waldren C.A., Hei T.K. (2001) Radiation risk to low fluences of alpha particles may be greater than we thought, *Proc. Natl. Acad. Sci. USA* **98**, 14410-14415.

Zhou H., Suzuki M., Geard C.R., Hei T.K. (2002) Effects of irradiated medium with or without cells on bystander cell responses, *Mutat. Res.* **499**, 135-141.

Zirkle R.E., Bloom W. (1953) Irradiation of parts of individual cells, *Science* **117**, 487-496.