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Tracking genomic instability within irradiated and bystander populations

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Abstract

Over the past two decades, our understanding of radiation biology has undergone a fundamental shift in paradigms away from deterministic 'hit–effect' relationships and towards complex ongoing 'cellular responses'. These responses include now familiar, but still poorly understood, phenomena associated with radiation exposure such as genomic instability and bystander effects. Although these responses share some common features (e.g. they occur at high frequency following very low doses, are heterogeneous in their induction and are observed at time points far removed from the initial radiation exposure), the precise relationship between genomic instability and bystander effects remains to be elucidated. This review will provide a synthesis of the known, and proposed, interrelationships among irradiated and bystander cellular responses to radiation. It also discusses our current experimental approach for gaining a clearer understanding of the relationship between damage induction and long-term effects in both irradiated and bystander cells.

Introduction

Since the start of the last century, there have been several challenges made to the belief that the damaging effects of ionising radiation are restricted to those cells and tissues suffering direct nuclear traversal and the associated deposition of energy. These challenges are known as the non-targeted effects of ionising radiation and encompass a range of possibilities. These include the now familiar, but still poorly understood, genomic instability and bystander effects, yet these are precisely the circumstances most relevant to understanding the risk of developing cancer from exposure to radiation.

Genomic instability

Genomic instability is a hallmark of tumorigenic progression, and is observed in the progeny of irradiated and bystander cells as the delayed and stochastic appearance of, for example, de-novo chromosomal aberrations, micronuclei, transformation, gene amplification, gene mutation and reduced plating efficiency (also known as lethal mutations or delayed reproductive cell death) in-vitro and in-vivo (reviewed in Kadhim et al 2004; Morgan 2003a, b; Schwartz 2007). Genomic instability occurs in the progeny of irradiated cells at a frequency that is several orders of magnitude greater than would be expected for mutation in a single gene, implying that a more complex, multigenic phenomenon may underlie the phenotype (Grosovsky et al 1996; Little 1998; Lewis et al 2001; Morgan et al 2002).

Most evidence suggests that the relationship between genomic instability and radiation dose is not strictly linear, but is maximally induced at the lowest doses investigated, including for instance a single alpha particle traversal of a single cell nucleus in a population of cells (Kadhim et al 2001; Moore et al 2005). The expression of genomic instability can depend on several factors, including genotype of the irradiated cell or animal, type of radiation and cell or tissue type (reviewed in *Oncogene*, special issue 22 (45) 2003; *Mutation Research*, special issue (597) 2006; Kadhim et al 2006). A clear mechanistic relationship between these factors and genomic instability has yet to be found; however the introduction of new experimental methods, such as genomics and proteomics, holds promise to advance our understanding of these mechanisms.

Recently, proposals have emerged attempting to connect radiation-induced instability with a specific mechanism. The manifestation of instability end points are ultimately the result of damage to DNA (mutations at target loci, chromosomal rearrangements and alterations, micronuclei, apoptosis, sister chromatid exchange, etc.) and several groups have demonstrated that proteins associated with the repair of DNA may contribute to the instability process, such as DNA-PKcs (Okayasu et al 2000) and p53 (McIlrath et al 2003). Further, certain DNA repair proteins in the non-homologous pathway of double-strand break (DSB) rejoining have been shown to have a second role in the protection of telomeres, the natural ends of chromosomes (Bailey et al 1999, 2004a; Bailey & Goodwin 2004). Cells having mutations in genes coding for these proteins are subject to multiple forms of instability — defective DSB repair, chromosomal end-to-end fusions and joining of unprotected telomeres to radiation-induced DSB ends (Bailey et al 2004b). Indeed, attenuation of normal gene functions, within a selective, but growing, set of critical genes, may contribute to the initiation or perpetuation of instability, as is the case with some heritable genetic disorders such as colorectal cancer, ataxia telangiectasia, Nijmegen breakage syndrome and others, in addition to the controlled experimental data discussed above.

However, several studies suggest that DNA damage itself is not necessarily required to initiate the instability phenotype (Lorimore et al 2003). The evidence comes from several specific characteristics of the instability phenotype in the clonal descendants of single progenitor irradiated and surviving cells. These include the observation that the fraction of surviving clones exhibiting genomic instability vastly exceeds the fraction that would be predicted on the basis of cells traversed by heavy ions and thus suffering DNA damage, as well as the heterogeneous nature of the expression of radiation-induced instability within these clones (Kadhim et al 1992; Holmberg et al 1993, 1995; Grosovsky et al 1996; Morgan 2003a). Additionally, radiation-induced instability is comparably elevated across a wide dose range, including very low doses (Morgan 2003a, b). Recently, we have demonstrated that traversal of a single cell nucleus within a population of normal primary human lymphocytes with a single $^{3}He^{2+}$ ion is sufficient to result in instability in a large fraction of the population (Moore et al, submitted). It is unlikely that the breaks generated by such a single particle traversal directly damaged a DNA repair gene in the irradiated cell, and because the other cells were not irradiated, they were not at risk from direct radiation-induced breaks. Further experimental evidence comes from Morgan's laboratory (Limoli et al 1997), where they investigated the role of DNA strand breakage as the molecular lesion responsible for initiating genomic instability by treatment of cells with five different strand-breaking agents. Their data also confirm that DNA strand breakage per-se does not necessarily lead to chromosomal instability.

It seems probable that epigenetic alterations, such as changes in methylation, acetylation and phosphorylation patterns (El-Osta 2004; Hake et al 2004) or elevated or attenuated oxidative stress (Clutton et al 1996; Lorimore & Wright 2003), which have also been shown to be associated with cancer, may be responsible for the induction of the instability phenotype. Aberrant histone phosphorylation, decondensation and delayed replication timing have also been demonstrated in human tumour cell lines (Smith et al 2001).

One such widely investigated epigenetic mechanism is the hypothesis that oxidative stress is an important early factor in genomic instability (Matsumoto et al 2007). For example, it has been demonstrated that instability may be initiated after high oxidative stress conditions (Limoli et al 2003), that treatment with free radical scavengers can reduce instability in irradiated cells (Limoli et al 2001) and that unstable cells exist in an environment of high oxidative stress (Limoli & Giedzinski 2003). Others have suggested that oxidative stress resulting from inflammatory processes might be a mechanism common to both instability and bystander responses (Lorimore & Wright 2003). Watson et al (1997) reported differential induction of genomic instability by α-particle irradiation in mouse strains that differed in superoxide production, a marker of oxidative stress.

Based on these studies, persistent oxidative stress provides an attractive model by which exposure to irradiation may initiate and perpetuate the instability phenotype. After irradiation and during clonal population outgrowth, low-level oxidative stress initiated by radiation results in new point mutations and DNA strand breaks.

A similar model incorporating a fundamental contribution of ROS to genomic instability and bystander effects has been recently proposed (Morgan 2003c). Contributing to the persistent oxidative stress proposed in this model might be alterations in sub-cellular organelles, such as mitochondria and lysosomes (Lorimore et al 2001). Apparently, processes that affect genomic stability are complex and interlinked, and they cannot be studied in isolation from one another. The recent deluge of descriptions of radiation-like effects in un-irradiated bystander cells provides a good example.

Bystander effects

Bystander effects are defined as radiation-like effects in cells or tissue that have communicated with irradiated cells, but which themselves have not been irradiated. Many innovative systems have been developed to study in-vitro bystander effects, each with distinct differences in the level of communication allowed between irradiated and un-irradiated cells. Grid shielding techniques (Lorimore et al 1998) and microbeam techniques (Ponnaiya et al 2004; Moore et al 2005; Shao et al 2006) allow direct intercellular communication via gap junctions between irradiated and bystander cells before, during and after irradiation. Grid shielding prevents a pre-defined percentage of cells from radiation exposure, while the microbeam uses an automated scanning system to target irradiation to either the nuclei or cytoplasm of any number of individual cells. Co-culture techniques (Geard et al 2002; Zhou et al 2002; Suzuki et al 2004; Hill et al 2005) utilise specialised inserts with porous membranes to create separate irradiated and bystander populations, allowing communication between these populations before, during (not for X-ray) and post-irradiation, only via medium-borne factors.

Medium transfer techniques (Mothersill et al 2001) involve no direct communication between irradiated and bystander cells, only the transfer of medium-borne factors when irradiated medium is removed from irradiated cells and put onto fresh un-irradiated cells.

Furthermore, recent in-vivo studies by Mothersill's group showed that gill tissue from X-ray treated trout and trout exposed to X-ray induced bystander signals in recipient tissues. X-ray exposure increased the expression of the cancer-related protein annexin II. The proteomic changes associated with the bystander effect differed from those associated with direct radiation exposure (Smith et al 2007).

Bystander cells can exhibit a wide range of biological responses, including elevated levels of mutations (Huo et al 2001; Nagasawa et al 2003), chromosomal aberrations (Nagasawa & Little 2002), induction of micronuclei and sister chromatid exchanges, gene amplifications and mutations (Matsumoto et al 2007) and phosphorylation of proteins such as ERK1/2, JNK and p38 (Little et al 2002). Also, similar to genomic instability, many bystander responses appear to saturate at low doses of radiation regardless of LET (Seymour & Mothersill 2000; Little et al 2002; Moore et al 2005). In some cellular systems, the effects are maximally induced by the lowest doses investigated (~10 mGy) (Nagasawa & Little 1992; Schettino et al 2005). Bystander effects have also been reported under conditions where only the cytoplasm is irradiated (Wu et al 1999), and with very low doses of low- (Mothersill & Seymour 2002) or high-LET (Moore et al 2005; Bowler et al 2006) irradiation. At present, there are few data on such effects in whole animals (Watson et al 2000; Xue et al 2002; Lorimore et al 2005); however, it is these studies that will likely have the greatest impact on radiation therapy scheduling and treatment (Mothersill & Seymour 2003, 2006).

Depending on experimental design and cell type investigated, bystander effects may be mediated by either communication via gap junctions (Azzam et al 2001, 2003; Zhou et al 2002) or media-borne factors (Lehnert et al 1997; Mothersill & Seymour 2002; Shanker et al 2006). The nature of the soluble transmitting factor(s) is unknown, but cytokines, including IL-8 (Iyer & Lehnert 2000; Facoetti et al 2006) and transforming growth factor beta (TGF-β) (Portess et al 2007), as well as calcium fluxes (Shao et al 2006) and reactive oxygen species (ROS) (Matsumoto et al 2007), have been suggested as mediators of bystander responses. Moreover, there is also evidence that protein synthesis inhibitors or an inhibitor of inducible nitric oxide synthase (iNOS) (Shao et al 2006, 2008) in irradiated cells can eliminate bystander responses in un-irradiated cells, reflecting possible roles of soluble proteins and nitric oxide (NO). A role for plasma membrane-bound lipid rafts has also been indicated (Nagasawa et al 2002). Furthermore, it is likely that a combination of signalling pathways is involved in the overall bystander responses (Natarajan et al 2007).

Immediate effects in bystander cells and delayed effects in both irradiated and bystander cells share common characteristics: they all appear to saturate at low doses and plateau over an extensive dose range; and ROS have been reported to be involved in all situations (reviewed in Lorimore & Wright 2003). ROS, including pro-inflammatory cytokines, may be important in the initial cellular damage in irradiated (Moore et al 2005) and bystander (Azzam et al 2002; Kashino et al 2004) cells, and may be associated with genomic instability (Lorimore et al 1998; Limoli et al 2003; Moore et al 2005); however, a comprehensive understanding of the relationship between the initial events and the delayed events (genomic instability) in both irradiated and bystander cells has yet to be achieved.

Relationship between early and delayed responses with associated mechanistic considerations in irradiated and bystander cells

Currently there are many bystander studies concentrating on the mechanisms involved in signal production by irradiated cells, and on the manifestation of a bystander effect at early times post-irradiation (Table 1). However, there are fewer studies simultaneously investigating the mechanisms involved in the induction and perpetuation of genomic instability in irradiated and bystander cells, or that track the response several divisions post-irradiation (delayed genomic instability).

The nature of the early events that determine both genomic instability in irradiated cells and in bystander cells is presently not clear. For example, it is not known if the initiating event(s) of genomic instability in irradiated and bystander populations are the same or different. What is clear is that bystander cells receive no direct radiation insult (such as to cause DSBs) and will only be damaged as a result of communication with irradiated cells. We can infer that DSBs must be occurring at delayed times as a result of ongoing cellular processes, similar in both populations, as chromosomal instability is a manifestation of chromosomal breakage and translocations result from misrepair of these breaks (Kadhim et al 2004). Several DNA repair pathways have been identified and many DNA repair genes are known, but which of these are important for processing the lesions that drive genomic instability? The answer to this question relates directly to public health, specifically to the goal of individualising radiation risk assessment. Additionally, the answer will provide mechanistic insights into the molecular processes that generate genomic instability in irradiated and bystander populations.

As described above, both irradiated and bystander cell populations express similar manifestations of chromosomal aberrations, DNA damage, mutations and cell death, the only difference being that bystander cells have not themselves been exposed to any form of radiation. These cells have, though, had 'contact' with irradiated cells. Two main hypotheses exist for how this response occurs. One is that irradiated cells transfer signals to un-irradiated cells via gap-junction communication. Cells would need to be in physical contact, which although highly probable and possible within a population of cells, cannot explain how remote bystander effects have been observed. The other main hypothesis is that irradiated cells actually release soluble signalling factors through the cell membrane, which can diffuse freely to un-irradiated cells.

The potential simultaneous expression of genomic instability and bystander effects means that both phenomena can

Table 1 Brief summary of contributions to the elucidation of genomic instability and bystander effects

Cell type	Radiation type	Experimental design	Manifestation	Initiation/perpetuation?	Outcome	Reference
Reconstructed epidermis and full thickness skin EPI-200, EFT-300	Microbeam	Targeted irradiation	TUNEL apoptosis scoring, MN formation	72 h post-irradiation initiation of damage	Increase in apoptotic and micronucleated cells in un-irradiated (BY) cells up to 1 mm and 0.6 mm, respectively from irradiated tissue	Belyakov et al (2005)
Primary HSF	Microbeam	Targeted irradiation	Surviving fraction	14 days post-irradiation; perpetuation	Non-linear detrimental and adaptive response to irradiation in bystander cells	Frankenberg et al (2006)
Normal HSF AG1522	alpha particle 1 cGy	Medium transfer	γH2AX foci formation, NO measurement	30 minutes post-irradiation; initiation of damage	Excessive DSB positive cells in non-irradiated population Prevented by NO inhibitor	Han et al (2006)
GM10115 hamster-human hybrid cells	X-rays dose 10 Gy	Formation of unstable clone >3 sub-populations post-irradiation	Alkaline comet assay, manual detection	Nature of producing clones means it will be perpetuation of instability	No increase in endogenous DNA damage between chromosomally stable and unstable clones	Morgan et al (2002)
Primary normal human fibroblasts from lung, skin and 180BR	X-rays 0.2 and 2 Gy	—	γH2AX foci formation	Up to 14 days post-irradiation; initiation of damage and perpetuation	Persistence of significant DSBs up to 14 days post-irradiation in some cell types	Rothkamm & Lobrich (2003)
Human glioblastoma	Microbeam	Medium transfer	NO measurement	1 h post-irradiation; initiation	Significant increase in the percentage of cells displaying increased NO production	Shao et al (2003)
Normal HLF WI38	Microbeam and gamma irradiation	Co-culture, cell mixing and medium transfer systems	γH2AX foci	Up to 48 h post-irradiation; initiation	BE but not GI not observed with co-culture at 48 h. As previously, with cell mixing and medium transfer. No linear bystander response	Sokolov et al (2005)
Immortalised M5S mouse cells	X-rays	Direct irradiation	ROS	Up to 14 days post-irradiation; initiation and perpetuation, also delayed response 2 h post-irradiation, initiation of irradiation and bystander effects.	Rapid increased in ROS post-irradiation which decreases at 6 h, baseline level reached within 14 days	Tominaga et al (2004)
Human diploid skin fibroblasts	X-rays doses 0.1–10 Gy	Co-culture system	p21waf1 induction, MN formation, γH2AX foci formation, cell survival, ROS	Dose-dependent increase in the percentage of cells with foci in directly irradiated cells. Fewer cells with foci observed in bystander cells and no dose-dependence but still significantly more than control.		Yang et al (2005)

For each paper, the cell type, radiation quality, experimental design, manifestation of damage, results and whether the group has studied solely the initiation, or also the perpetuation of the phenomena have been identified. BE, bystander effects; GI, genomic instability; HLF, human lung fibroblasts; HSF, human skin fibroblasts; MN, micronuclei.

be studied not only in parallel but using the same end points. Commonly studied endpoints include:

- Firstly, the alkaline comet assay (single-cell gel electrophoresis), a sensitive and widely used technique to quantify many types of DNA damage. This technique responds to single- and double-strand breaks and base damage, and therefore provides a measure of the total extent of damage to single cells as well as the distribution of damaged cells within the population (Collins 2004).
- Secondly, techniques commonly used for specifically detecting DNA DSBs, including γH2AX and 53BP1 (p53 binding protein 1) immunostaining of cell nuclei (Abraham 2002; Stucki & Jackson 2004). These proteins are involved in the initial cellular detection of DNA DSBs, and form repair complexes with other proteins, termed 'foci'. An individual focus is believed to represent the position of a single DSB (Stucki & Jackson 2004).
- Thirdly, cytogenetic analysis of metaphase spreads using Giemsa staining to enable the visualisation of condensed chromosomes and allow quantification of the number and types of chromosomal aberrations due to DNA damage.
- Fourthly, micronuclei (Azzam et al 2002).
- Finally, lethal mutation (Mothersill et al 1998).

Current contributions to mechanistic links

Many groups have studied the induction of genomic instability and bystander effects using one or more of the above biological end points separately or in combination, with the γH2AX and 53BP1 nuclear foci formation being predominantly used for DSB analysis. Table 1 details some of these groups along with their experimental design including their biological system and the type of radiation exposure. However, many studies have been of a single-cell type after exposure to low-LET radiation and no comparisons have been made with high-LET exposures. Other studies have measured and assessed biological responses only immediately post-irradiation using a specific end point. For example, using a co-culture system Yang et al (2005) observed an increase in the formation of γH2AX nuclear foci in human diploid fibroblasts present in bystander cells two hours post-irradiation, as detected by an increase in the percentage of cells with foci at similar levels regardless of dose (0.1–10 Gy), indicating DSBs in the bystander population. In the directly irradiated cells, with increasing dose the percentage of cells with foci increased up to 100% following 5 Gy.

In a study by Sokolov et al (2005) using normal human fibroblasts and three experimental approaches of co-culture, cell mixing and medium transfer, both high- and low-LET exposures were compared. The results of immediate analysis demonstrated similar responses to high- and low-LET irradiation. In both, a bystander response, as visualised by the presence of γH2AX foci, was observed 18 h post-irradiation.

Similarly, a medium transfer study using the same cell type as Sokolov et al (AG1522) supports the finding of a bystander effect a short period of time after irradiation. Han et al (2006) observed an increase in DSB-positive cells as detected by immunoassaying of γH2AX foci. A bystander response was induced utilising the medium transfer experimental method, mediated by factors in the medium that were believed by the group to be nitric oxide. These studies indicate the possibility of a much quicker and more efficient mechanism of eliciting a response with medium transfer than with co-culture of cells, in this particular cell line. This study did not compare differences in radiation quality or responses beyond the immediate time point.

In another study, which used human fibroblasts and X-irradiations (Rothkamm & Lobrich 2003), repair mechanisms were studied by looking at foci formation up to 14 days post-irradiation. Only under the condition where cells were kept at high confluence and exposed to several doses did they still have significant numbers of foci up to 14 days post-irradiation, suggesting the persistence of damage in directly irradiated cells. However, there were no parallel studies for bystander populations.

In summary, all the above examples confirm that to gain a clearer understanding of the relationship between damage induction and long-term effects in both irradiated and bystander cells, experimental research must be designed to study both populations in parallel using the same biological system, approach and end points.

For this we have designed and conducted experiments to allow the study of both genomic instability and bystander effect in parallel (Figure 1) to test the hypothesis that, although directly irradiated cells suffer DNA DSBs, these cells and their descendents do not have to maintain elevated DSB levels to continue to produce a signal communicating genomic instability to bystander cells. In these studies human foetal lung fibroblasts (HF19), in which genomic instability has been previously observed (Kadhim et al 1998), were exposed to either X-rays or α-particles and co-cultured to allow communication between the irradiated and bystander populations by media-borne factors. Cells were then either separated from the two vessels for immediate analysis or were transferred into long-term culture. Several biological end points were used to characterise the initial damage and the perpetuation of a damage response. The end points measured were γH2AX and 53BP1 immunostaining, and single-cell gel electrophoresis (comet) assay. Long-term damage was measured using the same assays in addition to cytogenetics. A summary of currently unpublished results from these ongoing experiments are shown in Figure 2.

Low-LET X-ray

Our data demonstrate that immediately following exposure to low-LET X-ray irradiation, a general damage (i.e. detected by alkaline comet assay) bystander response was observed at all X-ray doses after 1-h co-culture, and persisted up to 18 h with 0.01 Gy, the lowest X-ray dose. Damage was observed as a delayed response (genomic instability) in cells following 1 Gy X-ray in both irradiated and bystander populations, which remained elevated in the bystander population, but not in the irradiated population for the 18-h cultures. De-novo damage, observed as genomic instability in the irradiated population, was observed with 0.1 Gy X-ray in the 18-h cultures.

There were indications of an increased level of DSBs in both X-irradiated and bystander populations only at the low

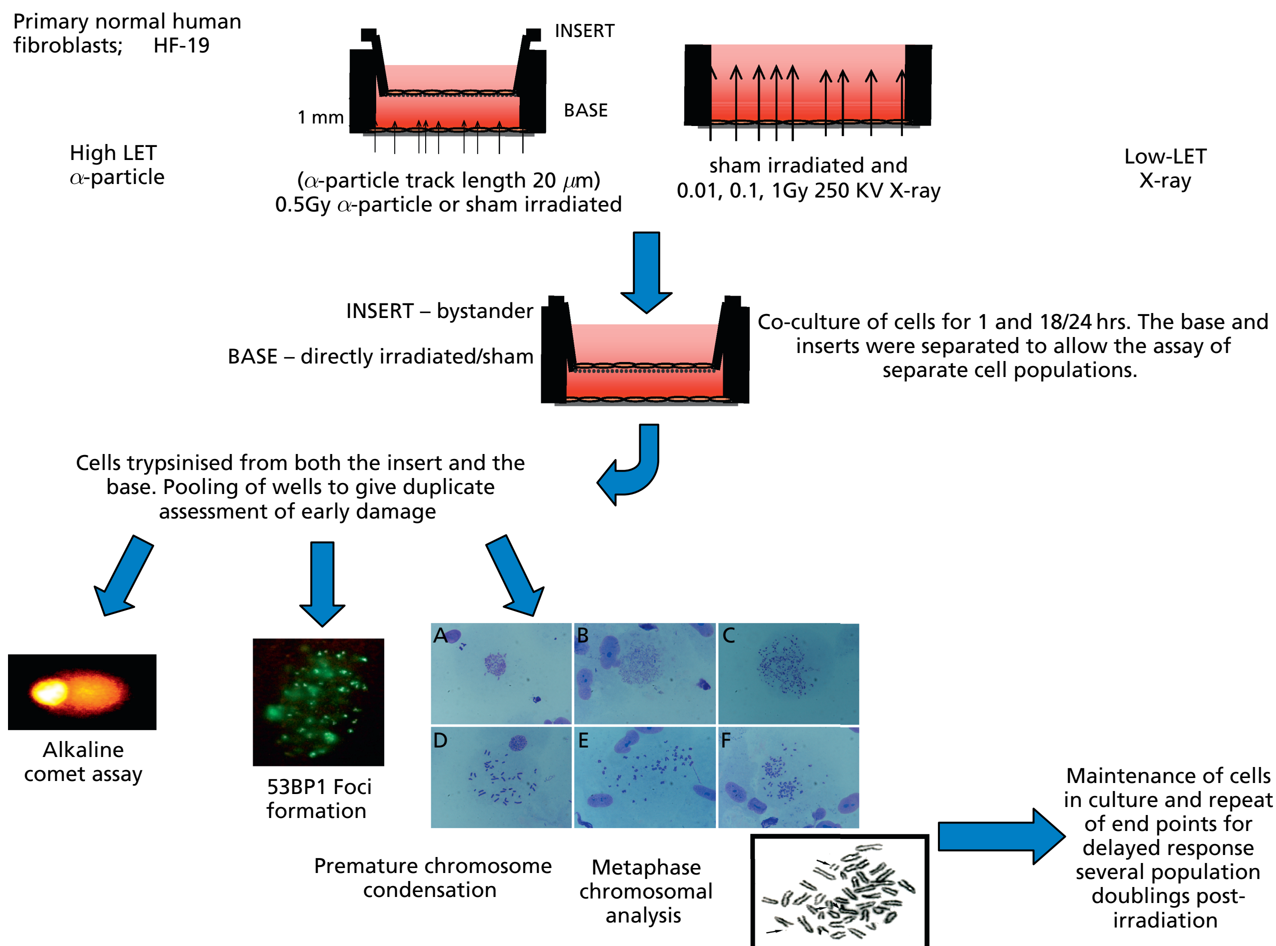


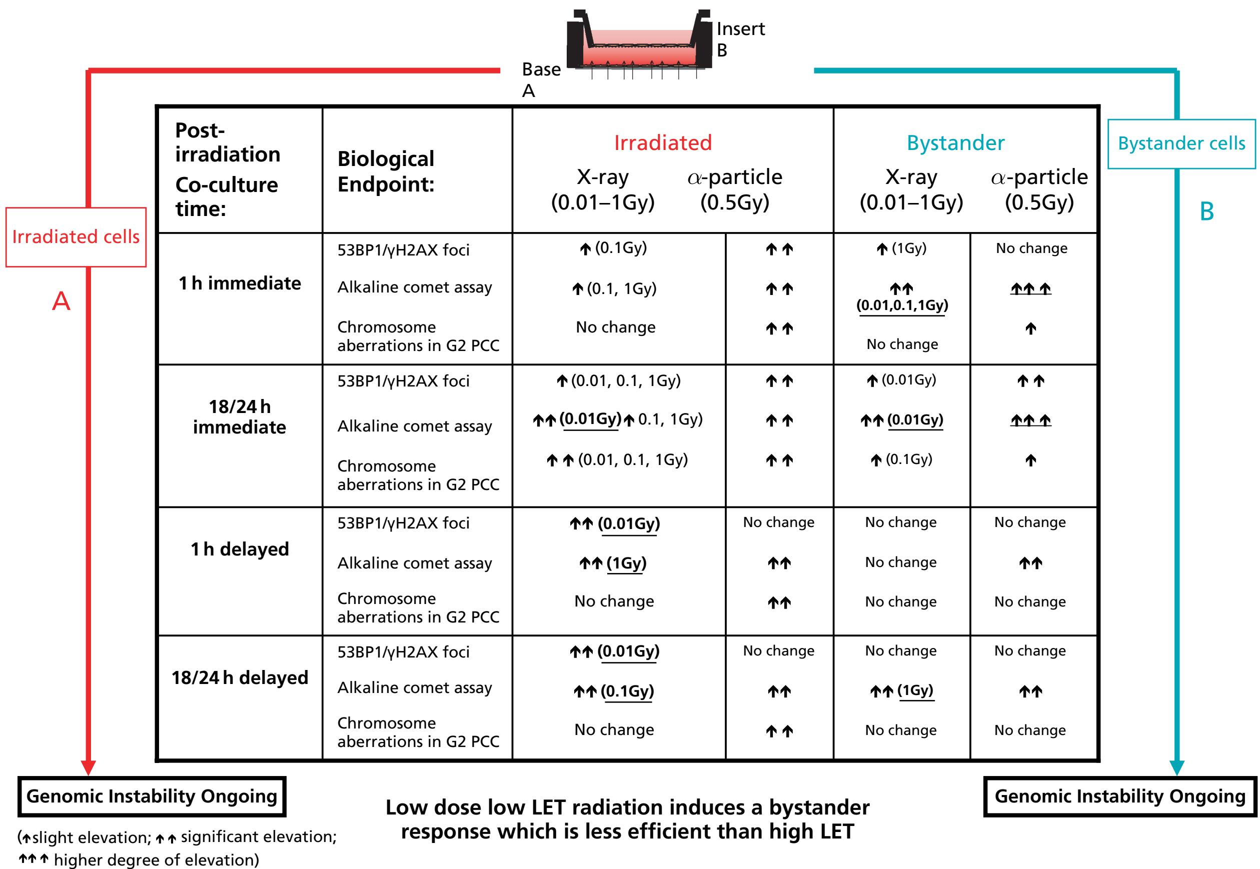
Figure 1 Overview of the experimental design used by our group (Bowler et al 2006). Pre-and during irradiation the experimental design differs between high and low-LET due to the nature of the irradiations and track structure. In co-culture the range of α-particles beyond the base of the irradiation dish is ~ 20 μm and therefore cells cultured in the insert will not be exposed. This is not the case for the X-ray; therefore the dishes were irradiated with X-ray before incubation with inserts. Post-irradiation, both high- and low-LET samples are treated the same, with co-culture communication for 1 and 18/24 h. Following the co-culture period, cells are removed and kept as two separate populations while being assayed for a range of end points; alkaline comet assay (single-cell gel electrophoresis) for the detection of all types of DNA damage including single-strand and double-strand breaks, immunofluorescent staining for 53BP1 foci (an indicator of DNA DSB repair), and chromosomal analysis by PCC (premature chromosome condensation looking at chromosome and chromatid fragments in G2 stage) or metaphase solid staining. In the cases of all end points, a minimum of 100 cells per group were scored. In the case of PCC and 53BP1 foci scoring is carried out manually scored from coded slides. For scoring the alkaline comet assay the Kinetic 5.5 imaging software is used and levels of damage recorded as % tail DNA (Olive & Banath 2006). Cultures are prepared for both populations to be assayed further at delayed times approximately six population divisions post-irradiation and then again at approximately 12 population doublings post-irradiation.

dose of 0.01 Gy immediately post-irradiation. This result suggests that DSBs are not the main lesion for initiating possible genomic instability. At the delayed time point there was no DSB damage in the bystander population, although genomic instability was detected in the irradiated population only with the lowest dose of X-rays. These results demonstrate genomic instability in both irradiated and bystander populations in primary human fibroblasts after low-LET radiation exposure, especially at the lowest dose studied here (0.01 Gy X-ray).

Chromosomal analysis carried out by premature chromosome condensation (PCC) indicates that the main type of lesion that contributes to the chromosomal instability observed in both irradiated and bystander populations is the chromatid break.

High-LET α-particle

Following exposure to 0.5 Gy high-LET α-particles, both irradiated and bystander populations within our co-culture system incurred significant levels of general DNA damage, as detected by the alkaline comet assay. These levels were observed within 1 h and persisted up to 24 h. Damage levels were seen to increase within the bystander population over time, while a decrease, indicating repair, occurred in the irradiated population. No significant increase was observed in DNA DSB levels within the bystander population after 1 h of co-culture, although a clear bystander effect was observed with the alkaline comet assay; however, significant increases in DSBs were observed at later times.



Post-irradiation Co-culture time:	Biological Endpoint:	Irradiated X-ray (0.01–1Gy)	Irradiated α-particle (0.5Gy)	Bystander X-ray (0.01–1Gy)	Bystander α-particle (0.5Gy)
1 h immediate	53BP1/γH2AX foci	↑ (0.1Gy)	↑↑	↑ (1Gy)	No change
	Alkaline comet assay	↑ (0.1, 1Gy)	↑↑	↑↑ (0.01,0.1,1Gy)	↑↑↑
	Chromosome aberrations in G2 PCC	No change	↑↑	No change	↑
18/24 h immediate	53BP1/γH2AX foci	↑ (0.01, 0.1, 1Gy)	↑↑	↑ (0.01Gy)	↑↑
	Alkaline comet assay	↑↑ (0.01Gy) ↑ 0.1, 1Gy)	↑↑	↑↑ (0.01Gy)	↑↑↑
	Chromosome aberrations in G2 PCC	↑↑ (0.01, 0.1, 1Gy)	↑↑	↑ (0.1Gy)	↑
1 h delayed	53BP1/γH2AX foci	↑↑ (0.01Gy)	No change	No change	No change
	Alkaline comet assay	↑↑ (1Gy)	↑↑	No change	↑↑
	Chromosome aberrations in G2 PCC	No change	↑↑	No change	No change
18/24 h delayed	53BP1/γH2AX foci	↑↑ (0.01Gy)	No change	No change	No change
	Alkaline comet assay	↑↑ (0.1Gy)	↑↑	↑↑ (1Gy)	↑↑
	Chromosome aberrations in G2 PCC	No change	↑↑	No change	No change

Figure 2 Some of the results obtained by our group from the HF-19 primary human fibroblast. It allows a comparison between radiation qualities (i.e. both high- and low-LET) of the same three end points (comet assay, foci formation and chromosomal analysis) at both immediate and delayed time points. The wide range of data from these parallel studies enable important links to be made between the initiation and perpetuation of genomic instability and the bystander response across high- and low-LET irradiations.

Genomic instability was observed in the progeny of irradiated and bystander populations up to 28 days post-irradiation as a persistent and significant increase in DNA damage, measured by the alkaline comet assay. These levels of damage were statistically similar in both populations with both showing a significantly increased level in cellular senescence as measured by β-galactosidase levels. There was no significant increase in DNA DSB levels, similarly observed within the low-LET study, supporting the hypothesis that DSBs are not the main lesion contributing to the perpetuation of genomic instability.

Our data demonstrate that following communication post-high or low-LET irradiation, both irradiated and bystander cell populations manifest generic DNA damage as detected by the alkaline comet assay. Damage persisted and was observed at delayed post-irradiation time points. These observations were also supported by chromosomal analysis.

The observation of DSBs (using immunohisochemistry) in the irradiated population, but not in the bystander cells, demonstrated that DNA DSB is not the initial lesion in the bystander response for either radiation type. This is also the case for the progeny of both populations.

Conclusion

Clearly the results of the current studies have provided strong evidence that large fractions of cells surviving exposure to ionising radiation, as well as those cells that are in direct communication with irradiated cells, manifest a number of common features: similar DNA damage levels are observed in multiple end points; both show the absence of a true dose response; and the minimum dose required to induce genomic instability and bystander effects is within the same range. These manifestations were assessed at early times post-irradiation and where communication between irradiated and bystander populations used different biological systems and methods. This makes it very difficult to draw firm conclusions about the specific mechanisms for the induction and perpetuation of genomic instability in either directly irradiated or bystander populations. A clear example can be found in our experiments conducted under conditions designed to elicit responses in both populations at early post-irradiation times and also multiple cell divisions later.

Exposure to radiation in the natural environment is always low-level and apparently unable to initiate low-dose cellular effects, at least not in cells with normal DNA repair capacity. Therefore low-dose cellular effects are not likely to have evolved as a response to radiation. Instead it would seem more probable that radiation triggers one or more stress responses that evolve to combat threats other than radiation. Whether these inappropriately triggered stress responses exasperate or ameliorate the harmful effects of radiation, or perhaps contribute to reported hormetic (beneficial) radiation effects, remains to be elucidated by future investigation.

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