Adaptive and Bystander Effects Induced in Human and Rodent Cell Populations Exposed to Low Dose/Fluence Ionizing Radiation

Edouard I. Azzam, Sonia M. de Toledo and Perumal Venkatachalam

Department of Radiology, UMDNJ - New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103
Correspondence to azzamei@umdnj.edu

Abstract – *In an effort to understand low-dose effects and their potential impact on risk from ionizing radiation, we have investigated the modulation of gene expression and induction of DNA damage in human and rodent cells exposed to low doses of γ-rays or very low fluences of α-particles. Cells pre-exposed to a low γ-ray dose were protected from the DNA damaging and killing effects induced by a subsequent acute challenge exposure to γ-rays. Furthermore, a low dose chronic exposure to γ-rays decreased the frequency of micronucleus formation and neoplastic transformation to a level below the spontaneous rate in human and rodent cells respectively. In contrast, in cell cultures exposed to fluences of α-particles by which a small fraction of the nuclei were traversed by a particle track, stressful effects were transmitted from irradiated to adjoining non-irradiated bystander cells. The mechanisms underlying these effects and their relative contribution to the overall risk to ionizing radiation will be discussed.*

I. INTRODUCTION

Living organisms are continually exposed to low-level ionizing radiation (IR) from natural sources with radon gas being the prime source. Due to its alpha-particle emitting decay products, radon gas has been considered to be the single largest naturally occurring environmental hazard[1]. In addition, the human population is likely to be subjected to man-made sources of radiation from nuclear weapons development, electrical energy generation by nuclear power reactors and the clean-up of sites associated with such activities. In addition, with radiology's explosive growth in the past decade, an increasing number of individuals are exposed to radiation for diagnostic procedures including computed tomography (CT). Based on the current increase in CT examinations in the past decade, by the year 2010, one in every four individuals residing in the USA will have a CT scan annually, with the possibility of several repeats in the patient's lifetime[2, 3]. As a result, there is a particular public and scientific interest in characterizing the biological effects of IR in the low dose/fluence range at which these latter activities are likely to occur. Specifically, focus is on characterizing the molecular and biochemical mechanisms underlying such effects.

Currently, for the purposes of radiation protection, the deleterious effects of radiation are assumed to have no dose threshold and to show a linear dose response, with low dose-rate exposures resulting in reduced effects by about a factor of two. The effects of sequential doses are assumed to be additive[4]. One consequence of a linear, no threshold hypothesis is the assumption that exposure to any dose of radiation, however small, can potentially result in detrimental health effects. However, increasing experimental evidence in human and other mammalian cells shows that cellular exposure to doses as low as 0.01 Gy from low linear energy transfer (LET) radiation induces a protective mechanism that reduces the amount of chromosomal damage caused by a subsequent exposure[5]. Importantly, exposure to doses below 10 cGy was shown, in some instances, to reduce the level of chromosomal damage due to endogenous oxidative processes[6]. This phenomenon, termed adaptive response, has been shown to be dependent upon the dose rate, expression time, culture conditions, cell and tissue type, stage of the cell cycle, and the endpoint measured[7]. The observations of adaptive responses in mammalian cells mirror the evidence for the existence of radiation-inducible DNA repair systems in prokaryotes and lower eukaryotes[8] and hence support the concept that its existence is evolutionarily conserved.

In contrast to radiation-induced adaptive responses, several bystander effect studies, mainly in cells exposed to high LET radiations such as α-particles, have shown that biological stress responses, including genetic effects, can occur in cells that received no radiation exposure; such effects presumably occur as a result of signals transmitted from irradiated cells[9]. Widespread experimental evidence now indicates that IR traversal through the nucleus of a cell is not a prerequisite to produce genetic damage or a biological response. Bystander cells in a population that are in the vicinity of directly targeted cells or recipient of growth medium from irradiated cell cultures have been shown to respond to the

radiation exposure[10]. Significant levels of genetic changes and lethality have been observed in bystander cells of varying genetic background, lineage and organ origin when such cells were in the neighborhood of cells targeted by α-particles.

While evidence for both adaptive and bystander effects has been well established, a clear understanding of the basic biochemical and molecular processes by which they occur is only beginning to emerge. In the present paper, we will describe aspects of our research focusing on characterizing the adaptive and bystander responses and elucidating the molecular mechanisms underlying these effects. The experimental model for these experiments is confluent monolayer cultures of normal human or mouse cells exposed to low dose/low dose rate γ-rays or very low fluences of α-particles from a conventional broad-beam irradiator.

II. ADAPTIVE RESPONSES TO γ-RAY EXPOSURES

In early studies, we have tested the influence of low γ-ray doses delivered at low dose rate on expression of the adaptive response in quiescent normal human diploid skin fibroblasts (AG1522 cells). The endpoint of micronucleus (MN) formation was used as a measure of radiation-induced chromosomal breaks in cytochalasin-treated cells (this treatment enables the cells to undergo nuclear division while inhibiting cytoplasmic division, hence ensuring that micronuclei are scored in cells that have undergone one nuclear division only). The data in Figure 1 indicate that the level of MN formation, in cells exposed to a 0.5 Gy at low dose rate (0.002 Gy/min) prior to being exposed to an acute 4 Gy challenge dose, is significantly lower than in cells exposed to the challenge dose only. These results indicate that adapted cells are better protected against DNA damage that leads to chromosomal breaks and MN formation. When a 5h incubation period at 37°C separated the priming and challenge doses, hence allowing more time for expression of the adaptive process(es), even less MN formation occurred following the test dose.

In more recent experiments, we have used human fibroblasts grown in a 3-dimensional architecture that mimic cell growth *in vivo* and measured chromosomal damage and changes in the expression of stress related proteins following exposure to a single small dose (10 cGy) of γ-rays delivered at variable dose rates. Compared to sham-manipulated controls, the data in Figure 2 indicate a significant increase in MN in cells exposed to 10 cGy delivered acutely. When the dose was protracted over 24h, the residual level of MN was significantly reduced. Importantly, when the dose was delivered over 48h, the level of MN formation in the exposed cells was reduced to a level below the spontaneous rate. This pattern of MN formation correlated with the pattern of changes in the phosphorylation of serine15 in the p53 protein. The p53 protein is activated and stabilized in response to a wide range of cellular stresses. Its activation is associated with phosphorylation of its serine15 residue. Similar to the micronucleus formation data, a significant increase in serine15 phosphorylation was observed in cells exposed to an acute dose of 10 cGy. When the dose was protracted over 48 h, the level of serine15 phosphorylation was lower than detected in sham-manipulated control cells (data not shown).

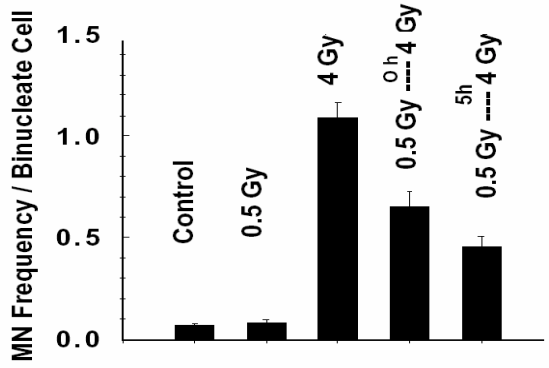

Figure 1. Frequency of micronucleus formation in confluent, density inhibited AG1522 normal human diploid fibroblasts exposed to γ-rays (0.5 Gy at 0.002 Gy/min and or 4 Gy at 1.8 Gy/min).

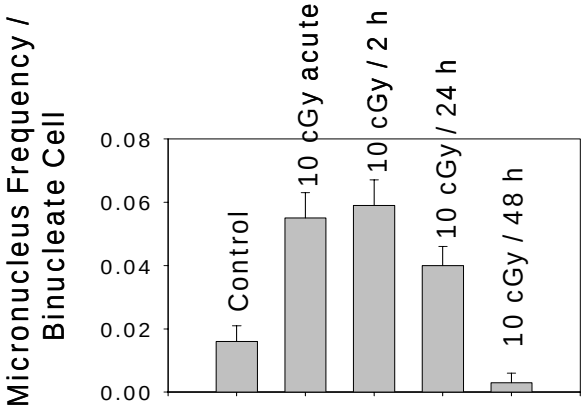

Figure 2. Frequency of micronucleus formation in AG1522 normal human diploid fibroblasts exposed to 10 cGy from γ-rays at various dose rates.

While the above results (Figures 1 & 2) clearly support the expression of radio-protective mechanisms that result in reduced residual DNA damage in human cells exposed to low dose/low dose rate γ-radiation, it is of great interest to characterize the effect on the carcinogenic risk in cells exposed under similar protocols.

A model system suitable for the study of the effects on carcinogenesis of a radiation-induced adaptive response is the C3H 10T½ mouse embryo fibroblast "transformation assay". In this assay, non-transformed cells in tissue culture can be transformed into demonstrably malignant cells by exposure to IR. Using this system, the data in Table 1, indicate that when C3H 10T½ cells are challenged by a large acute γ-ray dose of 4 Gy, the transformation frequency was increased about 10-fold over the spontaneous frequency detected in control cells. However, when the challenged cells were pre-exposed, 3.5 h earlier, to a 0.1 Gy low dose rate (0.002 Gy/min) priming dose, risk was not increased as predicted by the linear no threshold (LNT) hypothesis; it was actually decreased by 2- to 3-fold[11]. These results (Table 1) mirror the data described in Figure 1 using human cells and support the induction of radioprotective mechanisms against radiation damage in mouse embryo fibroblasts. The decrease in the transformation frequency was associated with a decrease in MN formation (Table 1), presumably reflecting error-free repair of chromosomal damage. These results are inconsistent with the assumptions used in radiation protection, specifically that the cumulative cancer risk from two sequential exposures can never be less than one alone. They indicate that cells can adapt when exposed to low chronic doses and such adapted cells are both better able to correctly repair lesions resulting from a subsequent exposure and thus less likely to be neoplastically transformed from that second exposure.

Treatment	Transformation frequency x 10^{-3} per viable cell (±SD)	Percentage of binucleated cells with micronuclei (±SD)
Control	0.4 (0.4)	11.5 (0.75)
4 Gy	4.1 (0.5)	85.3 (2.30)
0.1 Gy	1.6 (0.7)	16.2 (0.73)
0.1 to 4 Gy	2.2 (0.6)	81.5 (1.99)

Table 1. Pre-exposure to a chronic adapting dose (0.1 Gy) reduces micronucleus formation and neoplastic transformation in C3H 10T½ mouse embryo fibroblasts challenged by an acute dose of 4 Gy from γ-rays.
(→ indicates incubation at 37°C for 3.5 h).

In subsequent experiments, we have examined the consequences for risk resulting from the small dose exposure alone[6]. In these experiments, cells were exposed to small doses (from 0.001 to 0.1 Gy) of γ-rays delivered chronically (0.002 Gy/min), and rather than assayed immediately after the irradiation for the endpoint as was done for the experiments described in Figures 1 & 2 and Table 1, the cells were incubated for 24 h after the exposure following which they were assayed for neoplastic transformation. Contrary to the predictions of the LNT hypothesis that any dose, no matter how small, increases the cancer risk, the data in Table 2 indicate that exposure to chronic doses in a range from 0.1 to 10 cGy reduces the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T½ cells. The level of significance of these results was maintained when the data for the various radiation exposures were pooled and compared to control. In contrast, when cells were assayed immediately following the low dose chronic exposures (1-10 cGy), the neoplastic frequencies were not significantly different from the control spontaneous frequency (data not shown) suggesting that time is needed for expression of radioprotective mechanisms (e.g. inducible DNA repair, induction of cell death).

A dose of 0.1 cGy is approximately equivalent to the annual non-radon dose received from background radiation (but delivered more quickly in the experiments described in Table 2). Such dose is also in the range of a typical occupational exposure and represents, on average, about one track per cell that is hit[12], the lowest possible dose a cell can receive. Hence, the data in Table 2 imply that any single track through any one of these cells, whether from background radiation or other exposure, reduces the risk of spontaneous neoplastic transformation in that cell. These results, in rodent cells, therefore show that a single low dose, in the background or occupational dose range, can in some circumstances induce processes, which reduce, rather than increase, the risk of neoplastic transformation. Since human cancer risks from exposure to high doses of IR have been well established, these results suggest that exposure of mammalian cells to low doses could induce molecular signaling processes that are different from those induced by high doses.

Treatment	Number of transformed foci/number of assay flasks	ρ
Control	46 / 85	–
0.1 cGy + 24 h holding	5 / 27	2.4×10^{-2}
1 cGy + 24 h holding	5 / 42	7.8×10^{-4}
10 cGy + 24 h holding	6 / 41	2.4×10^{-3}
Summed data: 0.1+1+10 cGy with 24 h holding	16 / 110	1.9×10^{-5}

Table 2. The effect of low chronic (0.002 Gy/min) doses on spontaneous transformation frequency.

III. THE ALPHA-PARTICLE INDUCED BYSTANDER EFFECT

The radiation-induced bystander effect has been broadly defined as referring to the occurrence of biological effects in unirradiated cells as a result of exposure of other cells to IR[10, 13]. A bystander effect induced in cell cultures exposed to α-particles was initially described by Nagasawa and Little[14]. An enhanced frequency of sister chromatid exchanges in 20-40% of Chinese hamster ovary cells was observed in cultures exposed to fluences by which only 0.1-1% of the cells' nuclei were actually traversed by a particle track. These results indicated that the target for genetic damage by α-particles is much larger than the nucleus or in fact than the cell itself. This was subsequently confirmed by others for the same endpoint in human fibroblasts[15]. Since, it has been shown that an enhanced frequency of specific gene mutations can also occur in bystander cells present in cultures exposed to very low fluences of α-particles[16, 17]. Also, an enhanced frequency of micronucleus formation and apoptosis in bystander cells was observed[18, 19], and *in vitro* neoplastic transformation experiments have shown that bystander cells neighboring irradiated cells are also at risk[20]. The latter studies thus suggest that, under some conditions, mutations and chromosomal aberrations induced in bystander cells may lead to tumourigenesis.

Using gene expression as an endpoint, it was also shown that stress effects are transmittable from irradiated to non-irradiated cells. It was found, by flow cytometry, that p53 levels were induced by α-particle irradiation in a greater fraction of cells than were hit by a particle track[21]. We have further developed these studies and examined up-regulation of stress sensitive proteins in a variety of human and rodent cell types using *in situ* immunodetection techniques[22]. The representative data in Figure 3 describe the expression of the stress sensitive protein $p21^{Waf1}$ in control and irradiated normal human fibroblasts. The $p21^{Waf1}$ protein is a p53 downstream effector that regulates the cellular growth cycle; its expression is increased in cells that undergo DNA damage. Confluent density-inhibited cultures were exposed to a mean dose of 0.3 cGy from α-particles in the presence or absence of the gap-junction-inhibitor lindane. The latter chemical disrupts intercellular communication that occurs through gap-junction channels that allow the exchange of small molecules among contiguous cells. Based on microdosimetric calculations, at a mean dose of 0.3 cGy, about 2% of the cells in the exposed culture would be expected to be traversed through the nucleus by an α-particle. The data in Figure 3 indicate that a significantly greater fraction than 2% of the cells up-regulate $p21^{Waf1}$. Interestingly, up-regulation of $p21^{Waf1}$ occurred in aggregates of neighboring cells, supporting the view that damage signals were communicated from irradiated to bystander cells. This view was supported when the cultures were exposed to 0.3 cGy in the presence of lindane. The *in situ* immunofluorescence data in Figure 3 show clearly that treatment of the exposed cultures with lindane resulted in inhibition of the aggregate pattern of $p21^{Waf1}$ induction (Figure 3, right panel) that typically occurs in control irradiated cultures (Figure 3, mid panel). In irradiated cultures treated with lindane, $p21^{Waf1}$ was induced primarily in single cells. These data thus implicate gap-junction intercellular communication in the bystander $p21^{Waf1}$ response observed after exposure to fluences where a very small fraction of cell nuclei in the exposed culture is traversed by an α-particle.

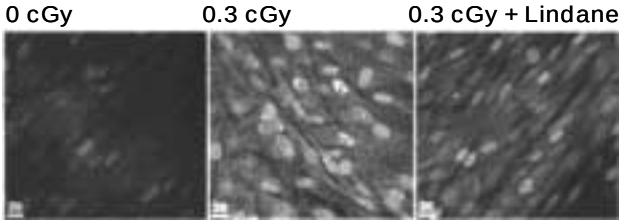

Figure 3. *In situ* immunofluorescence detection of $p21^{Waf1}$ expression in non-irradiated, lindane-treated (40 μM), and irradiated AG1522 cultures exposed to 0.3 cGy α-particles in the presence or absence of lindane.

To investigate whether the bystander induction of the stress-inducible $p21^{Waf1}$ protein (Figure 3) is associated with higher levels of DNA damage than expected after cellular exposure to low fluences of α-particles, we measured the frequency of micronucleus formation in confluent cultures of AG1522 fibroblasts held in confluence for 3 h after the exposure. Compared to control, non-exposed cells, the data in Figure 4 indicate a 3-fold increase in the induction of micronuclei after exposure to mean doses in the range of 1-3 cGy, and only a 4-fold increase after exposure to 10 cGy. At a mean dose of 10 cGy, 10-fold more cells in the population experience a nuclear traversal by an α-particle than by 1 cGy. Therefore, the magnitude of the response at low fluences suggests that non-traversed bystander cells were also subject to DNA damage. To investigate the involvement of gap-junction intercellular communication in the response, lindane was added to the cultures 2 h prior to exposure and remained for 3h thereafter. A highly significant reduction in the frequency of micronucleus formation was observed in cultures exposed to 1 or 2 cGy. At 10 cGy lindane did not reduce the frequency of micronucleus formation in confluent cultures exposed to this same mean dose (Figure 4). These data thus suggest that DNA damage may be the signal for the bystander induction of $p21^{Waf1}$ in low fluence exposed confluent cell cultures. However, both effects may also be independent consequences of signals communicated from irradiated to bystander cells.

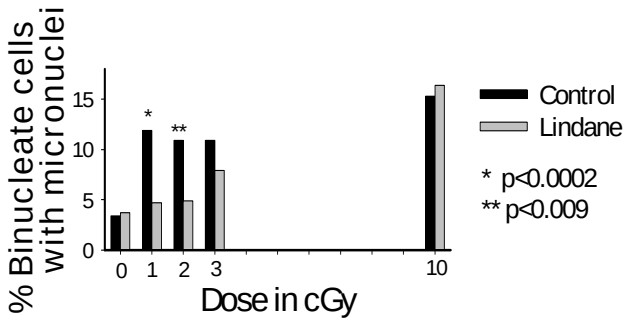

Figure 4. Micronucleus formation in α-particle exposed confluent, density-inhibited AG1522 normal human fibroblast cultures. The cultures were irradiated in the presence or absence of the intercellular communication inhibitor lindane. *P* values were determined by the chi-square test.

The induction of micronuclei and the up-regulation of the stress sensitive $p21^{Waf1}$ protein in bystander cells neighboring α-particle irradiated cells is in contrast to the above observations with low dose/low dose-rate γ-irradiated cells (Figures 1 & 2 and Tables 1 & 2) whereby a γ-ray dose as little as 0.1 cGy has been reported to induce a protective mechanism against endogenous damage or a subsequent challenge radiation exposure. As discussed above, micronuclei arise predominantly from un-rejoined DNA double–strand breaks[23] which have been strongly implicated in the process of cancer development in humans[24]. If DNA damage were to occur in bystander cells *in vivo*, and these cells survive such damage, these observations in α-particle exposed cultures would significantly impact on the assessment of cancer risk due to low fluence exposures. However, in contrast to these data, cell growth and protective bystander effects were also reported[25, 26]. Furthermore, cells recipient of conditioned medium from irradiated cell cultures became resistant to the lethal effects of a subsequent challenge dose of radiation[27, 28]. In addition, analyses have shown that lung cancer rates in U.S. Counties, with or without correction for smoking, decrease with increasing radon exposure, in sharp contrast to the increase predicted by the linear no-threshold theory[29].

IV. CONCLUSION

Some of the mechanisms (e.g. gap-junction intercellular communication, oxidative metabolism) that underlie the bystander effect have been also implicated in the adaptive response to IR and in some cases the same endpoint (e.g. cell death) has been used to examine expression of either phenomenon. However, classical adaptive response protocols involving low LET radiation are clearly distinct from those of bystander studies conducted mainly with high LET radiation. In the adaptive response, cells are pre-exposed to a small dose of low LET radiation prior to a challenge dose of the same type of radiation. In contrast, cells traversed by an α-particle receive a substantial dose (10-70 cGy) and undergo a complex type of DNA damage. While similar mediators may modulate the endpoint (e.g. viability) in both phenomena, the occurrence of opposite effects such as of pro-survival rather than cytotoxic effect may reflect changes in concentration of the inducing factor(s). For example, reactive oxygen species have been shown to be a double-edged sword capable of inducing both proliferative or cell death effects depending on their concentration. However, the bystander effect and adaptive response could also be mediated by distinct mechanisms/mediating factors; induction of an adaptive response to low LET IR protected against bystander damage induced by α-particles[30]. While, DNA damage was shown to be unequivocally induced in bystander cells, the adaptive response implicates the involvement of DNA repair and up-regulation of antioxidation resulting in reduced residual DNA damage.

Human epidemiology alone has been unable to resolve the issue of whether there are low dose thresholds or whether there is an increased risk at very low doses. Such inability does not mean that these effects do not occur *in vivo*. *In vitro* cell models provide a unique opportunity to control confounding factors and address in controlled studies the relevance to risk of chronic low dose low LET irradiation, α-particle irradiation and variability among populations.

ACKNOWLEDGEMENT

The authors are very grateful to Dr. R.E.J. Mitchel and Dr. John B. Little for their mentorship; much of the adaptive and bystander response experiments described in this manuscript were generated with their guidance. We also thank Dr. Prasad V.S.V. Neti for various discussions. Research Grants FG02-02ER63447 from the U.S. Department of Energy, 1RO1-CA92262-01A1 from the National Institutes of Health and 02-1081-CCR-S2 from the New Jersey Commission on Cancer Research supported this investigation.

REFERENCES

1. BEIR-VI Health Effects of Exposure to Radon (BEIR VI). Washington, D.C.: National Academy Press, 1998.
2. BRENNER, D. J., ELLISTON, C. D., HALL, E. J., AND BERDON, W. E. Estimates of the Cancer Risks from Pediatric CT Radiation Are not Merely Theoretical: Comment on "Point/Counterpoint: In X-Ray Computed Tomography, Technique Factors Should Be Selected Appropriate To Patient Size. Against the Proposition". *Med Phys*, **28**, 2387 (2001).

3. BAKER, S. R. Musings at the Beginning of the Hyper-CT Era. *Abdominal Imaging*, **28**, 110 (2003).
4. 'UNSCEAR' (United Nations Scientific Committee on the Effects of Atomic Radiation) Report to the General Assembly, United Nations. New York, 1988.
5. WOLFF, S. Failla Memorial Lecture. Is Radiation All Bad? The Search for Adaptation. *Radiat Res*, **131**, 117, (1992).
6. AZZAM, E. I., DE TOLEDO, S. M., RAAPHORST, G. P., AND MITCHEL, R. E. 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, (1996).
7. SHADLEY, J. D. Chromosomal Adaptive Response in Human Lymphocytes. *Radiat Res*, **138**, S9, (1994).
8. SAMSON, L. AND CAIRNS, J. A New Pathway For DNA Repair in Escherichia Coli. *Nature*, **267**, 281, (1977).
9. AZZAM, E. I., DE TOLEDO, S. M., AND LITTLE, J. B. Oxidative Metabolism, Gap Junctions and the Ionizing Radiation-Induced Bystander Effect. *Oncogene*, 22, 7050, (2003).
10. LITTLE, J. B., AZZAM, E. I., DE TOLEDO, S. M., AND NAGASAWA, H. Bystander Effects: Intercellular Transmission of Radiation Damage Signals. *Radiat Prot Dosimetry*, **99**,159, 2002.
11. AZZAM, E. I., RAAPHORST, G. P., AND MITCHEL, R. E. Radiation-Induced Adaptive Response For Protection Against Micronucleus Formation And Neoplastic Transformation In C3H 10T1/2 Mouse Embryo Cells. *Radiat Res*, **138**, S28, (1994).
12. BOND, V. P. AND FEINENDEGEN, L. E. What Is a 'Low Dose' of Radiation? *Int. J. Radiat. Biol.*, **53**, 1, (1988).
13. MOTHERSILL, C. AND SEYMOUR, C. Radiation-Induced Bystander Effects: Past History and Future Directions. *Radiat Res*, **155**, 759, (2001).
14. NAGASAWA, H. AND LITTLE, J. B. Induction of Sister Chromatid Exchanges by Extremely Low Doses of Alpha-Particles. *Cancer Res*., **52**, 6394, (1992).
15. Deshpande, A., Goodwin, E. H., Bailey, S. M., Marrone, B. L., and Lehnert, B. E. Alpha-particle-induced sister chromatid exchange in normal human lung fibroblasts - Evidence for an extranuclear target. Radiat. Res., *145:* 260-267, 1996.
16. NAGASAWA, H. AND LITTLE, J. B. Unexpected Sensitivity to the Induction of Mutations by Very Low Doses of Alpha-Particle Irradiation: Evidence for a Bystander Effect. *Radiat. Res*., **152**, 552, (1999).
17. ZHOU, H. N., RANDERS-PEHRSON, G., AND HEI, T. K. Studies of Bystander Mutagenic Response Using a Charged Particle Microbeam. *Radiation Research*, **153**,234, (2000).
18. PRISE, K. M., BELYAKOV, O. V., FOLKARD, M., AND MICHEAL, B. D. Studies on Bystander Effects in Human Fibroblasts Using a Charged Particle Microbeam. *Int. J. Radiat. Biol*., **74**, 793, (1998).
19. AZZAM, E. I., DE TOLEDO, S. M., SPITZ, D. R., AND LITTLE, J. B. Oxidative Metabolism Modulates Signal Transduction and Micronucleus Formation in Bystander Cells from Alpha-Particle-Irradiated Normal Human Fibroblast Cultures. *Cancer Res,* **62**, 5436, (2002).
20. SAWANT, S. G., RANDERS-PEHRSON, G., GEARD, C. R., BRENNER, D. J., AND HALL, E. J. 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, (2001).
21. HICKMAN, A. W., JARAMILLO, R. J., LECHNER, J. F., AND JOHNSON, N. F. Alpha-Particle-Induced P53 Protein Expression in a Rat Lung Epithelial Cell Strain. *Cancer Res*., **54**, 5797, (1994).
22. AZZAM, E. I., DE TOLEDO, S. M., AND LITTLE, J. B. Direct Evidence for the Participation of Gap-Junction Mediated Intercellular Communication in the Transmission of Damage Signals from Alpha-Particle Irradiated to Non-Irradiated Cells. *Proc. Natl. Acad. Sci. USA*, **98**, 473, (2001).
23. Fimognari, C., Sauer-Nehls, S., Braselmann, H., and Nusse, M. Analysis of radiation-induced micronuclei by FISH using a combination of painting and centromeric DNA probes. Mutagenesis, *12:* 91-95, 1997.
24. KARRAN, P. DNA Double Strand Break Repair In Mammalian Cells. *Curr Opin Genet Dev*, **10**, 144, (2000).
25. IYER, R. AND LEHNERT, B. E. Factors Underlying the Cell Growth-Related Bystander Responses to Alpha Particles. *Cancer Res*., **60**, 1290, (2000).
26. GERASHCHENKO, B. I. AND HOWELL, R. W. Flow Cytometry as a Strategy to Study Radiation-Induced Bystander Effects in Co-Culture Systems. *Cytometry*, **54A***:* 1, (2003).
27. IYER, R. AND LEHNERT, B. E. Alpha-Particle-Induced Increases in the Radioresistance

of Normal Human Bystander Cells. *Radiat Res*, **157**, 3, (2002).

28. MATSUMOTO, H., HAYASHI, S., HATASHITA, M., OHNISHI, K., SHIOURA, H., OHTSUBO, T., KITAI, R., OHNISHI, T., AND KANO, E. Induction of Radioresistance by a Nitric Oxide-Mediated Bystander Effect. *Radiat Res*, **155**, 387, (2001).
29. COHEN, B. L. Problems in the Radon vs Lung Cancer Test of the Linear No-Threshold Theory and a Procedure for Resolving Them. *Health Phys, **72***, 623, (1997).
30. SAWANT, S. G., RANDERS-PEHRSON, G., METTING, N. F., AND HALL, E. J. Adaptive Response and the Bystander Effect Induced by Radiation In C3H 10T(1/2) Cells In Culture. *Radiat Res*, **156**, 177, (2001).