The Radiation Response in Cells Not Directly Traversed by High Charge and High Energy Particles: The Bystander Effect of Space Radiation

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ABSTRACT

Developing a thorough understanding of the biological processes mediating the effects of low doses/low fluences of space radiation is relevant towards reducing the uncertainty in estimating the risk of their health hazards. During deep space travel, astronauts are exposed to a spectrum of energetic particles, among which are low fluences of high atomic number (Z) and high energy (E) (HZE) particles. These particles are densely ionizing and upon interaction with cells, cause severe oxidative injury and result in biological responses that are far less understood than those observed following irradiation with sparsely ionizing X and γ rays. When cultured cell populations are exposed to low doses from HZE particles, a significant fraction of the cells are not traversed by a primary radiation track or its secondaries, yet oxidative stress is observed in nearby cells that were not irradiated. Furthermore, the stressful effects in these cells have been shown to persist in their progeny. The occurrence of such effects expands the target at risk and amplifies the biological effects resulting from direct irradiation. If these effects also to occur in the tissues of astronauts, then the elevated oxidative stress in progeny cells could disrupt physiological processes and may result in detrimental effects during space missions and long after mission completion. Oxidative stress promotes genomic instability and is intimately linked with increased risk of age-related diseases. While this paper summarizes a recent review of bystander effects induced by HZE particles, it stresses the need for studies of the cross-talks between biological responses to HZE particles and other types of space radiation within intact tissues, in order to better understand the response of the entire biological system.

INTRODUCTION

During deep space exploration, many factors, including gravitational changes and exposure to ionizing radiation, induce cellular oxidative stress in astronauts. The space radiation environment is dynamic and primarily consists of protons, helium ions and high charge and high energy (HZE) particles, making it unique from the background radiation that humans encounter on earth. Although HZE ions constitute only a small component of galactic cosmic rays, their high biological effectiveness makes them a significant contributor to the effective dose received during missions in space. As HZE nuclei are highly charged, they are densely ionizing and therefore possess strong oxidizing power (*1*). Upon impact with biological material, they cause clustered oxidative damage in DNA and other molecules (*2*). For these reasons, the National Aeronautics and Space Administration (NASA) is greatly concerned about long-term health risks to astronauts (*3*), particularly during long-duration missions when exposure to ionizing radiation would easily exceed the applicable guidelines (*4, 5*). The oxidative damage of nucleic acids, proteins, and lipids is directly linked to aging, cardiovascular disease, neurodegenerative disorders, and cancer among other pathologies (*6, 7*). In particular, recent work in cell culture models have shown that oxidative stress is not restricted to cells traversed by ionizing radiation but is propagated to nearby non-traversed cells (i.e. bystander cells). Therefore, understanding the various steps involved in HZE particle-induced cellular responses that lead to short- and long-term oxidative stress is important for evaluating the risk of health hazards during prolonged space travel and/or after return to earth. Elements of these steps have been described in a recent review on oxidative injury by targeted and non-targeted exposures to HZE ions (*8*).

Non-targeted effects: A new paradigm

It has long been considered that the important biological effects of ionizing radiation in a cell population are a direct consequence of DNA damage occurring in the directly irradiated cells, commonly referred to as the "targeted" cells: unrepaired or misrepaired DNA damage in these cells is responsible for the genetic effects of radiation. Presumably, no effect would be expected in cells in the population that receive no direct

radiation exposure, with such cells being referred to as "non-targeted" or bystander cells. However, during the past 25 years, a large body of experimental evidence has challenged the concept that radiation traversal through the nucleus of a cell is the only prerequisite for the production of genetic damage or an important biological response. In a landmark study in 1992, Nagasawa and Little showed that when less than 1% of nuclei in a monolayer cell culture are traversed by a densely ionizing α particle track, more than 30% of the cells experience an increased frequency of sister-chromatid exchanges (*9*). Such findings were unexpected and led to a paradigm shift in understanding radiation effects (*10*). Since then, numerous cell culture experiments (almost exclusively monoculture) have shown that cells in the vicinity of directly irradiated cells may respond to the radiation exposure through redox-modulated intercellular communication pathways that propagate the oxidative stress initially originated in the irradiated cells (*11-14*). Together, these studies denote that cell populations exposed to ionizing radiation respond as an integrated unit rather than separate individual cells that have been irradiated. Upregulation of stress-responsive genes and proteins, genetic and epigenetic changes, induction of cell cycle checkpoints and cell killing arise in both irradiated and neighboring bystander cells, and the effects occur in various cell types of human and rodent origin at different stages of growth (reviewed in (*15-17*)). In addition to direct and indirect modes of intercellular communication, oxidative metabolism and DNA repair processes also mediate these effects (*18, 19*); however, the exact molecular steps involved have not been defined (*20*). Remarkably, perturbations in oxidative metabolism (*e.g.*, disruption of the balance between oxidant production and antioxidant defense) occur not only in irradiated and bystander cells but also in their progeny (Figure 1). Clearly, the induction of oxidative injury in progeny of irradiated (*21-23*) and bystander cells (*24, 25*) has important relevance to risk of health hazards, including neurodegeneration, cardiovascular diseases and cancer (*26*).

Figure 1

Bystander cells

Direct (e.g. gap-junctions) and/or indirect (e.g. diffusible factors) mediate the propagation of harmful or protective molecules between targeted and non-targeted cells

Progeny

death

survival

↑ Oxidative stress:
- Activation of oxidases/nitric oxide synthases leading to protein oxidation/lipid peroxidation
- Mitochondrial dysfunction leading to perturbations in oxidative metabolism
- Genetic/epigenetic changes leading to chromosomal rearrangements, enhanced mutation rate, etc.
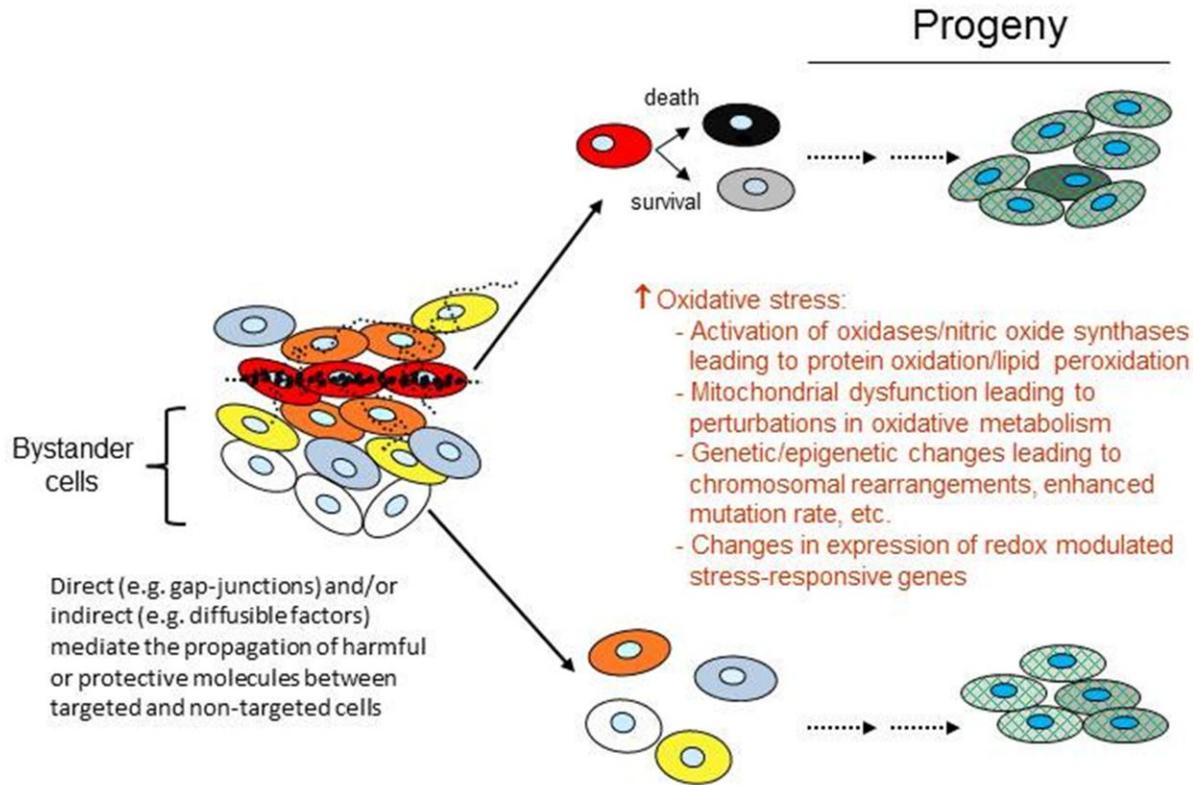- Changes in expression of redox modulated stress-responsive genes

Figure 1. HZE-particle irradiation induces targeted and non-targeted effects. Intercellular communications via direct or indirect modes lead to propagation of stress-inducing molecules from cells traversed by an HZE particle (cells in red) or the resulting secondary radiations such as delta electrons (cells in orange or yellow) causing induction of oxidative stress in bystander cells (blue). The progeny of cells traversed by radiation and the progeny of bystander cells may also experience oxidative stress. Cells in white are non-affected cells.

Although classic *in vitro* radiation-induced bystander effects have been extensively investigated over the past 20 years, such interactions between irradiated and non-irradiated cells had been suggested from observations made decades earlier. Numerous experiments had shown that blood plasma from individuals undergoing radiotherapy or from individuals who were accidentally irradiated had a clastogenic effect on normal non-exposed cells (*27, 28*). Strikingly, levels of the inflammatory markers C-reactive protein (CRP), interleukin-6 (IL-6), and sialic acid were found to be increased in survivors of the A-bomb long after the event (*29, 30*). Moreover, increasing evidence indicates that inflammatory cells in circulating blood of patients that received partial body irradiation may also induce DNA damage at sites that are distant from the irradiated target (*31*), thus contributing to 'out-of-field' or abscopal effects (*32-35*). Hence, the effects of localized energy deposition events in a cell cannot be assessed independently of neighboring cells. In the context of exposure to low mean absorbed doses of radiation, non-targeted stressful effects are likely to develop in cells exposed to densely rather than sparsely ionizing radiations (*24*). For sparsely ionizing radiation (*e.g.* γ rays delivered at low dose rate) protective (*36-38*) rather than harmful (*39*) responses may be induced.

Experimental strategies

Various *in vivo* and *in vitro* experimental strategies are being adopted to investigate non-targeted effects of low doses/low fluences of ionizing radiations, including HZE particles found in space. They involve partial body irradiation of rodents, monolayer cell cultures exposed to electromagnetic radiations or very low fluences of energetic ions, and co-cultures of irradiated cells with non-irradiated cells (Figure 2).

Figure 2

a Direct & indirect modes of communication

b IR Medium Transfer — Indirect communication
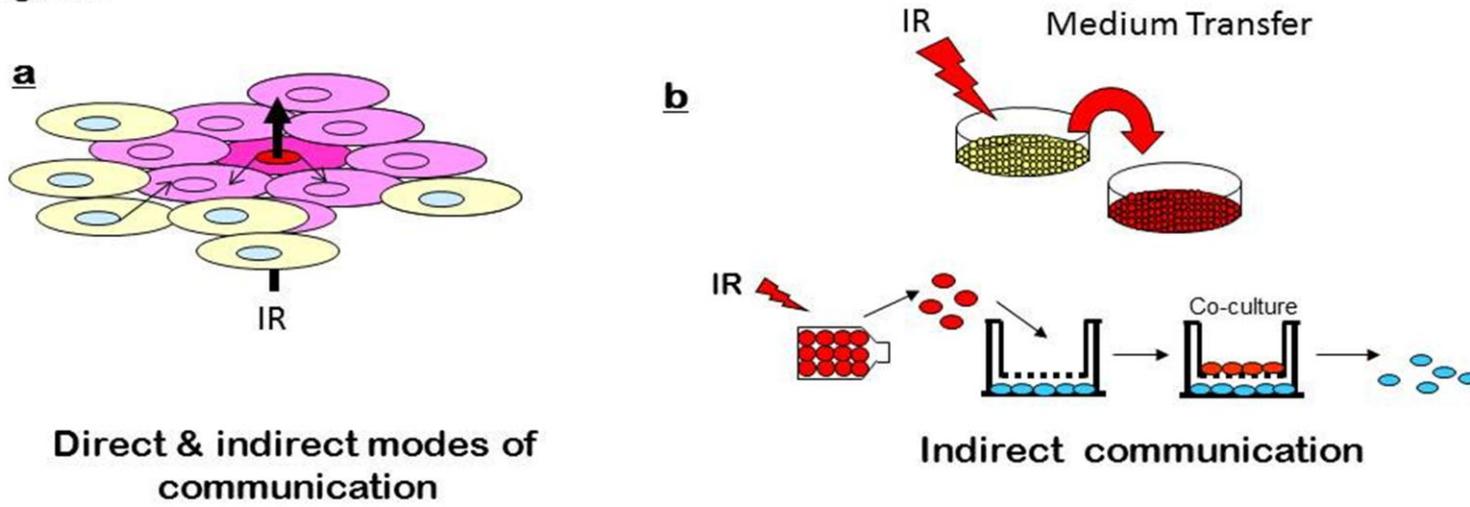
c Abscopal Effect HZE

Figure 2. Experimental approaches used in studies of non-targeted effects of ionizing radiation. (a) *In vitro* studies where monolayer cell cultures are exposed to low fluences of particulate ionizing radiations (IR) or to particulate or electromagnetic radiations from a microbeam; direct and indirect modes of communication may mediate non-targeted effects in this approach; (b) *in vitro* studies where diffusible factors mediate non-targeted effects; (c) *in vivo* approach where immune/inflammatory responses mediate non-targeted (abscopal) effects. Note: The physical and biological parameters that characterize 'non-targeted effects' are likely to depend on a number of factors, such as dose, dose rate, radiation quality, and cell phenotype.
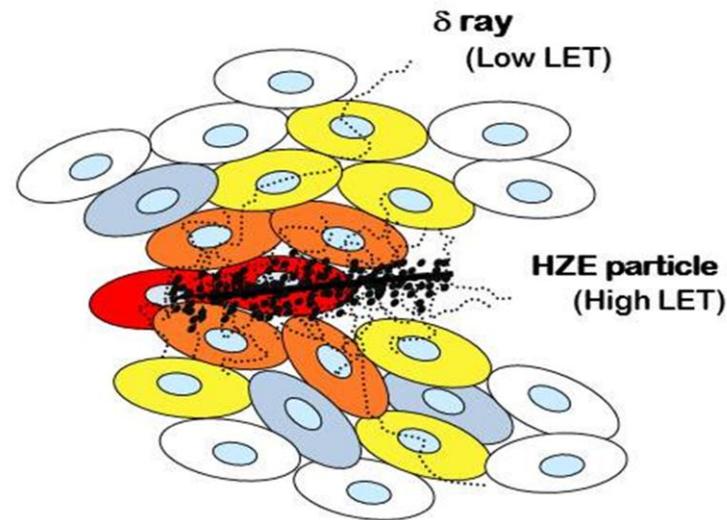
Figure 3

Figure 3. The track structure and apparent bystander effects. Delta rays resulting from the interaction of primary HZE particles with the target materials travel a significant distance and may deposit a biologically significant dose in cells in the vicinity.

When examining non-targeted effects induced by HZE ions, for the *in vivo* strategy and in monolayer cell culture studies, the effects of secondary radiation have to be considered when interpreting non-targeted oxidative effects. Cells presumed to be bystander might be hit by secondary electrons generated as a result of the interaction of the primary HZE particles with biological matter (Figure 3). Whereas the scheme in Figure 3 is representative of δ electrons, other secondary radiations including heavy fragments, α-particles, protons, photons and neutrons may be generated; however, the absorbed doses due to these particles is very small compared to that of the δ electrons (the readers may wish to refer to expert reviews on this topic). Monte-Carlo simulations using programs such as FLUKA and MCNPX multi-particle transport codes are greatly enhancing our understanding of the contribution of secondary radiations in HZE particle-induced bystander effects (*40, 41*). These studies allow estimation of the fraction of cells that are not traversed by fragmentation products and also the fraction of cells that are traversed by these products together with the doses received. Importantly, they permit quantification of the radial distribution of the secondary particles, as was recently shown (*41*).

Compared to studies with irradiated monolayer cell cultures, the use of co-culture strategy eliminates complications in interpreting bystander responses attributed to secondary radiations. In the co-culture strategy, secondary radiations due to fragmentation of the primary HZE particle are absent as the co-cultured cells originate from separate growth vessels (*42, 43*). Further, this strategy readily permits investigation of phenotypic and genotypic traits (e.g. altered oxidative metabolism and genetic susceptibility to radiation-induced damage) of cells donating or receiving the bystander signal. On the other hand, effects of rapidly propagating factors from irradiated cells may be missed because adequate time is required after irradiation to establish the co-culture. Importantly, such experiments should be stringently controlled for variations in oxygen tension, pH, temperature and dilution effects during handling of cells, as these parameters affect cellular physiology, the propagated molecules and the modulated signaling pathways.

Space radiation-induced non-targeted effects

Studies of bystander effects in cell cultures exposed to low fluences of HZE particles are only emerging, and conflicting data have been reported. In early experiments with a microbeam, stressful effects were shown to be propagated from HZE particle-irradiated cells to contiguous bystander cells (*44*). In addition, when HZE particle-irradiated cells were co-cultured with bystander cells in a manner in which they shared only the growth medium, stressful responses were also induced in the bystander cells and were similar in nature to those generated in the targeted cells (*45-48*). With relevance to cancer risk, oxidative stress and DNA damage persisted in distant progeny of bystander cells that had been in contiguous co-culture with HZE particle-irradiated cells (*24, 25*). However, transferring the growth medium from irradiated cultures to recipient bystander cells (*49, 50*), or the targeting of a small number of cells in a population with HZE particles delivered by a microbeam (*51*), did not induce bystander effects measured by a variety of endpoints in several cell types. Numerous factors may underlie the absence of observable effects in these cases, including timing of endpoint measurement, dilution of the inducing factor and the metabolic state/redox environment of the recipient cells.

A clear demonstration of HZE particle-induced bystander effects was recently generated by Gonon et. al. (*41*). In a study involving normal human cells maintained in culture, the authors investigated the upregulation of stress markers in confluent cells exposed to 1000 MeV/u iron ions (linear energy transfer (LET) ~151 keV/µm) or 600 MeV/u silicon ions (LET ~50 keV/µm) at mean absorbed doses as low as 0.2 cGy, wherein on a statistical basis,1-3% of the cells were traversed through the nucleus by a primary particle. Adopting *in situ* studies, the authors have observed significantly greater increases in 53BP1 foci formation, a marker of DNA damage, than expected from the number of primary particle traversals. Their observations highlighted the importance of radiation quality in the induced effect. By simulations with the FLUKA multi-particle transport code, they showed that fragmentation products, other than electrons, in cell cultures exposed to HZE particles comprise <1 % of the absorbed dose. Further, the radial spread of dose due to secondary heavy ion fragments is confined to approximately 10-20 µm. Hence, they concluded that the latter are unlikely to significantly contribute to stressful effects in cells not targeted by primary HZE particles.

Interestingly, a dose response model that takes into account bystander effects provided an improved fit, compared to the targeted effect model in analyses of Harderian gland tumors induced in mice exposed to heavy ions (*52*). These data probably represent the most comprehensive study for tumor induction by heavy ions. In other studies, Weil *et al.* (*53*) have suggested circulating inflammatory cytokines and non-irradiated parenchymal cell responses to oxidative stress as contributing factors to the unexpected high relative biological effectiveness of energetic iron ions in inducing hepatocellular carcinoma.

Mechanisms of radiation-induced bystander effects

Investigations of the mechanisms mediating the radiation-induced bystander effects have mainly focused on the role of transmissible factor(s) generated by irradiated cells, direct intercellular communication via gap-junctions, oxidative metabolism and DNA repair. In the first instance, it has been suggested that secreted transforming growth factor beta 1 (TGF-β1) (*54, 55*), Il-8 (*56*) or prostaglandins (*57*) in the medium of α-particle irradiated cell cultures may have a role in mediating bystander responses. Network analysis of gene expression data has identified a number of genes coding for potential extracellular signaling molecules that were up-regulated in both irradiated and bystander fibroblasts. These included genes previously implicated in bystander response, such as IL8, IL1A and IL1B, as well as new candidates, such as IL6, IL33, LIF (leukemia inhibitory factor gene) and FGF2 (fibroblast growth factor 2) (*58*).

Evidence for the involvement of gap junction intercellular communication (GJIC) in propagation of bystander effects has been derived from studies with high and low LET radiations (*44, 59-66*). Gap-junctions were shown to mediate the propagation of stressful effects not only between targeted and non-targeted cells, but also among the targeted cells (*67*). The intercellular channels that comprise gap-junctions are formed by *connexin* proteins (*68*). Manipulation (↓ ↑) of connexin expression/gap-junction gating by chemical agents, forced connexin expression by transfection, and connexin gene knockout studies provide substantial evidence for the participation of gap-junctions in radiation-induced bystander effects (*12*).

An indication that reactive oxygen species (ROS) are involved in the induction of bystander effects was suggested when the induction of sister chromatid exchanges (SCEs) in bystander cells was inhibited by the antioxidant superoxide dismutase (SOD), a superoxide radical scavenger (*54*). Subsequent studies using more direct approaches have shown that low doses of α-particles initiate the intracellular production of ROS (superoxide anions and hydrogen peroxide) in human cells through involvement of the plasma membrane bound NADPH-oxidase (*69*). These studies suggested that the ROS response did not require direct nuclear or even cellular hits by α-particles (*69*). Oxidative metabolism has also been implicated in toxic bystander effects observed in media transfer experiments involving γ-radiation (*11, 70, 71*). Treatment of the irradiated cultures with the antioxidants L-lactate and L-deprenyl (*11, 70, 71*) or with drugs that inhibit collapse of mitochondrial membrane potential prevented the cytotoxic effects from irradiated cell conditioned medium (*71*). Further, critical molecules that participate in inflammatory responses/redox-modulated events, such as cyclooxygensae-2 (COX-2), were shown to participate in bystander effects (*57*).

The involvement of DNA repair in bystander effects was implied by studies with Chinese hamster ovary cells bearing an xrs-5 (X-ray sensitive-5) mutation, which reduces the ability to repair DNA double strand breaks. The latter cells were sensitized relative to wild type cells to the formation of chromosome aberrations caused by bystander effects induced by a low fluence of α-particles (*72*). Further, Rad9, which is involved in DNA repair and cell cycle checkpoints, was shown to participate in the bystander response to radiation exposure. Mouse embryonic stem (ES) cells null for the RAD9 gene demonstrated enhanced bystander micronuclei formation and apoptosis relative to wild-type *Rad9* controls (*73*).

Conclusions

In vitro and *in vivo* observations have provided strong evidence indicating that molecular events leading to various biological effects, including genetic damage, can be transmitted from irradiated to non-irradiated cells. Preliminary evidence points to a crucial role of plasma membrane originating effects where gap-junctions and critical

enzymes such as COX-2 and numerous kinases are located. Further, the expression of connexin proteins has been reported to be modulated by the cellular redox environment and by redox-sensitive soluble factors released by cells exposed to ionizing radiation (e.g. tumor necrosis factor alpha(TNF-α), Il-1β) (*74, 75*). However, it is also possible that specific bystander effects are regulated by some mechanism(s) and not by others; this may depend on cell type, cellular growth state, type of radiation and the biological endpoint being measured. It is attractive to speculate that extensive cross-talks between the various mediating mechanisms will occur.

The lack of clear knowledge about non-targeted responses has been singled out by the U. S. National Academies (*3*) as one of the important factors limiting the prediction of radiation health risks associated with space exploration. This is because during deep space travel, only a small fraction of cells in the human body experiences extremely dense ionizations created along the tracks of the traversing particles in a given day (*76*). Moreover, the radiation traversals that create these densely ionizing tracks are likely to be separated in tissue location and time (*39*).

Due to our limited knowledge of biological changes in cells traversed by primary HZE particles and in those in the vicinity, scientific councils (*77, 78*) have postponed defining the exposure limits for a Mars mission until more data on the effects of exposure to heavy ions are obtained. Further studies of non-targeted effects involving variations in dose rate and exposure to mixed beams of low and high linear energy transfer (LET) radiations (e.g., low fluence protons and HZE particles) are essential towards setting these limits. However, such investigations are currently limited. The availability of ground-based facilities such as the NASA Space Radiation Laboratory (NSRL) at the Brookhaven Laboratory capable of generating broad beams of HZE particles and protons will greatly support these efforts. The cross-talk between signaling events induced by the dense and sparse ionizations are likely to have significant effects on the biological response. In cell populations, and potentially tissue systems, exposed to low fluences of HZE ions, protective mechanisms (*e.g.*, enhanced DNA repair, increased antioxidant potential) induced by low LET secondary radiations may mitigate stressful effects propagated from adjacent cells traversed by primary particles (*79*). Conversely, the non-irradiated cells in the exposed population may transmit rescuing signals to the irradiated

cells (*80*). In this context, experiments evaluating various biological endpoints following exposure of cell cultures to a specific dose of HZE particles delivered either acutely or at very low dose rate would be highly informative of the cross talk that is occurring. This is critical as astronauts are usually exposed to heterogeneous ionizing radiation fields. A prior exposure to a low dose of low-LET radiation may induce protective processes, thereby attenuating the damaging effects of a subsequent exposure to high-LET ionizing radiation and ensuing harmful bystander effects, at least in the short-term. Additional studies addressing this aspect as was done in tissue cultures experiments involving space radiation (*43*) or alpha particles such as those emitted by radon and its progeny (*81, 82*) would enhance our understanding of the biological effects of the mixed fields of radiation that astronauts are likely to encounter.

In summary, bystander effect studies have led to a paradigm shift in our understanding of classic target theory. They are enhancing our general understanding of intercellular communication under stress conditions. The outcome may contribute to both radiation protection and radiotherapy.

References

1. Tobias CA. Failla Memorial lecture. The future of heavy-ion science in biology and medicine. Radiat Res 1985; 103:1-33.
2. Hada M, Georgakilas AG. Formation of clustered DNA damage after high-LET irradiation: a review. J Radiat Res (Tokyo) 2008; 49:203-10.
3. Managing Space Radiation Risk in the New Era of Space Exploration. Washington, D.C.: The National Academies Press; 2008.
4. Schimmerling W, Wilson JW, Cucinotta F, Kim MH. Evaluation of risk from space radiation with high-energy heavy ion beams. Phys Med 1998; 14 Suppl 1:29-38.
5. Cucinotta FA. Once we know all the radiobiology we need to know, how can we use it to predict space radiation risks and achieve fame and fortune? Phys Med 2001; 17 Suppl 1:5-12.
6. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408:239-47.
7. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol 1956; 11:298-300.
8. Li M, Gonon G, Buonanno M, Autsavapromporn N, De Toledo SM, Pain D, et al. Health Risks of Space Exploration: Targeted and Non-targeted Oxidative Injury by High Charge and High Energy Particles. Antioxidants and Redox Signaling 2013; in press.
9. Nagasawa H, Little JB. Induction of sister chromatid exchanges by extremely low doses of α-particles. Cancer Res 1992; 52:6394-6.
10. Little JB. Lauriston S. Taylor lecture: nontargeted effects of radiation: implications for low-dose exposures. Health Phys 2006; 91:416-26.
11. Mothersill C, Stamato TD, Perez ML, Cummins R, Mooney R, Seymour C. Involvement of energy metabolism in the production of 'bystander effects' by radiation. Brit J Cancer 2000; 82:1740-6.
12. Azzam EI, de Toledo SM, Little JB. Oxidative metabolism, gap junctions and the ionizing radiation-induced bystander effect. Oncogene 2003; 22:7050-7.
13. Hei TK, Zhou H, Ivanov VN, Hong M, Lieberman HB, Brenner DJ, et al. Mechanism of radiation-induced bystander effects: a unifying model. The Journal of pharmacy and pharmacology 2008; 60:943-50.
14. Prise KM, O'Sullivan JM. Radiation-induced bystander signalling in cancer therapy. Nat Rev Cancer 2009; 9:351-60.
15. Morgan WF. Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects *in vivo*, clastogenic factors and transgenerational effects. Radiat Res 2003; 159:581-96.
16. Hamada N, Maeda M, Otsuka K, Tomita M. Signaling pathways underpinning the manifestations of ionizing radiation-induced bystander effects. Current molecular pharmacology 2011; 4:79-95.

17. Blyth BJ, Sykes PJ. Radiation-induced bystander effects: what are they, and how relevant are they to human radiation exposures? Radiat Res 2011; 176:139-57.

18. Azzam EI, de Toledo SM, Spitz DR, Little JB. Oxidative metabolism modulates signal transduction and micronucleus formation in bystander cells from alpha-particle-irradiated normal human fibroblast cultures. Cancer Res 2002; 62:5436-42.

19. Nagasawa H, Wilson PF, Chen DJ, Thompson LH, Bedford JS, Little JB. Low doses of alpha particles do not induce sister chromatid exchanges in bystander Chinese hamster cells defective in homologous recombination. DNA Repair (Amst) 2008; 7:515-22.

20. Ghandhi SA, Sinha A, Markatou M, Amundson SA. Time-series clustering of gene expression in irradiated and bystander fibroblasts: an application of FBPA clustering. BMC Genomics 2011; 12:2.

21. Chang WP, Little JB. Delayed reproductive death in X-irradiated Chinese hamster ovary cells. Int J Radiat Biol 1991; 60:483-96.

22. Kadhim MA, Macdonald DA, Goodhead DT, Lorimore SA, Marsden SJ, Wright EG. Transmission of chromosomal instability after plutonium α-particle irradiation [see comments]. Nature 1992; 355:738-40.

23. Mothersill C, Moriarty MJ, Seymour CB. Radiobiologic response of CHO-KI cells treated with vitamin A. Acta Radiologica Oncology 1986; 25:275-80.

24. Buonanno M, De Toledo SM, Pain D, Azzam EI. Long-term consequences of radiation-induced bystander effects depend on radiation quality and dose and correlate with oxidative stress. Radiat Res 2011; 175:405-15.

25. Ponnaiya B, Suzuki M, Tsuruoka C, Uchihori Y, Wei Y, Hei TK. Detection of chromosomal instability in bystander cells after Si490-ion irradiation. Radiat Res 2011; 176:280-90.

26. Morgan WF, Day JP, Kaplan MI, McGhee EM, Limoli CL. Genomic instability induced by ionizing radiation. Radiat Res 1996; 146:247-58.

27. Hollowell JG, Jr., Littlefield LG. Chromosome damage induced by plasma of x-rayed patients: an indirect effect of x-ray. Proc Soc Exp Biol Med 1968; 129:240-4.

28. Goh K, Sumner H. Breaks in normal human chromosomes: are they induced by a transferable substance in the plasma of persons exposed to total-body irradiation? Radiat Res 1968; 35:171-81.

29. Hayashi T, Kusunoki Y, Hakoda M, Morishita Y, Kubo Y, Maki M, et al. Radiation dose-dependent increases in inflammatory response markers in A-bomb survivors. Int J Radiat Biol 2003; 79:129-36.

30. Neriishi K, Nakashima E, Delongchamp RR. Persistent subclinical inflammation among A-bomb survivors. Int J Radiat Biol 2001; 77:475-82.

31. Martin OA, Redon CE, Nakamura AJ, Dickey JS, Georgakilas AG, Bonner WM. Systemic DNA damage related to cancer. Cancer Res 2011; 71:3437-41.

32. Jain MR, Li M, Chen W, Liu T, De Toledo SM, Pandey BN, et al. In vivo space radiation-induced non-targeted responses: late effects on molecular signaling in mitochondria. Current molecular pharmacology 2011; 4:106-14.

33.   Mole RH. Whole body irradiation; radiobiology or medicine? Br J Radiol 1953; 26:234-41.

34.   Ohba K, Omagari K, Nakamura T, Ikuno N, Saeki S, Matsuo I, et al. Abscopal regression of hepatocellular carcinoma after radiotherapy for bone metastasis. Gut 1998; 43:575-7.

35.   Tamminga J, Kovalchuk O. Role of DNA damage and epigenetic DNA methylation changes in radiation-induced genomic instability and bystander effects in germline in vivo. Current molecular pharmacology 2011; 4:115-25.

36.   Azzam EI, de Toledo SM, Raaphorst GP, Mitchel RE. Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells. Radiat Res 1996; 146:369-73.

37.   Olivieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 1984; 223:594-7.

38.   Redpath JL, Antoniono RJ. Induction of an adaptive response against spontaneous neoplastic transformation in vitro by low-dose gamma radiation. Radiat Res 1998; 149:517-20.

39.   Held KD. Effects of low fluences of radiations found in space on cellular systems. Int J Radiat Biol 2009; 85:379-90.

40.   Lebel EA, Rusek A, Sivertz MB, Yip K, Thompson KH, Tafrov ST. Analyses of the secondary particle radiation and the DNA damage it causes to human keratinocytes. J Radiat Res 2011; 52:685-93.

41.   Gonon G, Groetz. JE, detoledo SM, Howell RW, Fromm M, Azzam EI. Non-Targeted Stressful Effects in Normal Human Fibroblast Cultures Exposed to Low Fluences of High Charge, High Energy (HZE) Particles: Kinetics of Biologic Responses and Significance of Secondary Radiations. Radiat Res 2013; 179:000-.

42.   Buonanno M, de Toledo SM, Azzam EI. Increased frequency of spontaneous neoplastic transformation in progeny of bystander cells from cultures exposed to densely-ionizing radiation. PloS one 2011; 6: art. no. e21540.

43.   Yang H, Magpayo N, Held KD. Targeted and non-targeted effects from combinations of low doses of energetic protons and iron ions in human fibroblasts. Int J Radiat Biol 2011; 87:311-9.

44.   Shao C, Furusawa Y, Kobayashi Y, Funayama T, Wada S. Bystander effect induced by counted high-LET particles in confluent human fibroblasts: a mechanistic study. FASEB J 2003; 17:1422-7.

45.   Fournier C, Becker D, Winter M, Barberet P, Heiss M, Fischer B, et al. Cell cycle-related bystander responses are not increased with LET after heavy-ion irradiation. Radiat Res 2007; 167:194-206.

46.   Yang H, Anzenberg V, Held KD. The time dependence of bystander responses induced by iron-ion radiation in normal human skin fibroblasts. Radiat Res 2007; 168:292-8.

47.   Yang H, Anzenberg V, Held KD. Effects of heavy ions and energetic protons on normal human fibroblasts. Radiatsionnaia biologiia, radioecologiia / Rossiiskaia akademiia nauk 2007; 47:302-6.

48.   Wakatsuki M, Magpayo N, Kawamura H, Held KD. Differential Bystander Signaling Between Radioresistant Chondrosarcoma Cells and Fibroblasts After X-Ray, Proton, Iron Ion and Carbon Ion Exposures. Int J Radiat Oncol Biol Phys 2012.

49.   Groesser T, Cooper B, Rydberg B. Lack of bystander effects from high-LET radiation for early cytogenetic end points. Radiat Res 2008; 170:794-802.

50.   Sowa MB, Goetz W, Baulch JE, Pyles DN, Dziegielewski J, Yovino S, et al. Lack of evidence for low-LET radiation induced bystander response in normal human fibroblasts and colon carcinoma cells. Int J Radiat Biol 2010; 86:102-13.

51.   Fournier C, Barberet P, Pouthier T, Ritter S, Fischer B, Voss KO, et al. No evidence for DNA and early cytogenetic damage in bystander cells after heavy-ion microirradiation at two facilities. Radiat Res 2009; 171:530-40.

52.   Cucinotta FA, Chappell LJ. Non-targeted effects and the dose response for heavy ion tumor induction. Mutat Res 2010; 687:49-53.

53.   Weil MM, Bedford JS, Bielefeldt-Ohmann H, Ray FA, Genik PC, Ehrhart EJ, et al. Incidence of acute myeloid leukemia and hepatocellular carcinoma in mice irradiated with 1 GeV/nucleon (56)Fe ions. Radiat Res 2009; 172:213-9.

54.   Narayanan PK, Goodwin EH, Lehnert BE. Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells. Cancer Res 1997; 57:3963-71.

55.   Shao C, Folkard M, Prise KM. Role of TGF-beta1 and nitric oxide in the bystander response of irradiated glioma cells. Oncogene 2008; 27:434-40.

56.   Narayanan PK, LaRue KEA, Goodwin EH, Lehnert BE. Alpha particles induce the production of interleukin-8 by human cells. Radiat Res 1999; 152:57-63.

57.   Zhou H, Ivanov VN, Gillespie J, Geard CR, Amundson SA, Brenner DJ, et al. Mechanism of radiation-induced bystander effect: role of the cyclooxygenase-2 signaling pathway. Proc Natl Acad Sci U S A 2005; 102:14641-6.

58.   Ivanov VN, Zhou H, Ghandhi SA, Karasic TB, Yaghoubian B, Amundson SA, et al. Radiation-induced bystander signaling pathways in human fibroblasts: a role for interleukin-33 in the signal transmission. Cell Signal 2010; 22:1076-87.

59.   Azzam EI, de Toledo SM, Gooding T, Little JB. Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluences of alpha particles. Radiat Res 1998; 150:497-504.

60.   Zhou HN, Suzuki M, Randers-Pehrson R, Chen G, Trosko J, Vannais D, et al. Radiation risk at low doses may be greater than we thought. Proc Natl Acad Sci USA 2001; 98:14410-5.

61.   Azzam EI, de Toledo SM, Little JB. 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 2001; 98:473-8.

62. Bishayee A, Rao DV, Howell RW. Evidence for pronounced bystander effects caused by nonuniform distributions of radioactivity using a novel three-dimensional tissue culture model. Radiat Res 1999; 152:88-97.

63. Harada K, Nonaka T, Hamada N, Sakurai H, Hasegawa M, Funayama T, et al. Heavy-ion-induced bystander killing of human lung cancer cells: role of gap junctional intercellular communication. Cancer Sci 2009; 100:684-8.

64. Mancuso M, Pasquali E, Leonardi S, Tanori M, Rebessi S, Di Majo V, et al. Oncogenic bystander radiation effects in Patched heterozygous mouse cerebellum. Proc Natl Acad Sci U S A 2008; 105:12445-50.

65. Suzuki M, Tsuruoka C. Heavy charged particles produce a bystander effect via cell-cell junctions. Biol Sci Space 2004; 18:241-6.

66. Vance MM, Wiley LM. Gap junction intercellular communication mediates the competitive cell proliferation disadvantage of irradiated mouse preimplantation embryos in aggregation chimeras. Radiat Res 1999; 152:544-51.

67. Autsavapromporn N, de Toledo SM, Buonanno M, Jay-Gerin JP, Harris AL, Azzam EI. Intercellular communication amplifies stressful effects in high-charge, high-energy (HZE) particle-irradiated human cells. J Radiat Res (Tokyo) 2011; 52:408-14.

68. Harris AL. Emerging issues of connexin channels: biophysics fills the gap. Q Rev Biophys 2001; 34:325-472.

69. Lehnert BE, Goodwin EH. Extracellular factor(s) following exposure to alpha particles can cause sister chromatid exchanges in normal human cells. Cancer Res 1997; 57:2164-71.

70. Lyng FM, Seymour CB, Mothersill C. Production of a signal by irradiated cells which leads to a response in unirradiated cells characteristic of initiation of apoptosis. Br J Cancer 2000; 83:1223-30.

71. Lyng FM, Seymour CB, Mothersill C. Oxidative stress in cells exposed to low levels of ionizing radiation. Biochem Soc Trans 2001; 29:350-3.

72. Nagasawa H, Little JB. Bystander effect for chromosomal aberrations induced in wild-type and repair deficient CHO cells by low fluences of alpha particles. Mutat Res 2002; 508:121-9.

73. Zhu A, Zhou H, Leloup C, Marino SA, Geard CR, Hei TK, et al. Differential impact of mouse Rad9 deletion on ionizing radiation-induced bystander effects. Radiat Res 2005; 164:655-61.

74. Weill D, Gay F, Tovey MG, Chouaib S. Induction of tumor necrosis factor alpha expression in human T lymphocytes following ionizing gamma irradiation. J Interferon Cytokine Res 1996; 16:395-402.

75. Hosoi Y, Miyachi H, Matsumoto Y, Enomoto A, Nakagawa K, Suzuki N, et al. Induction of interleukin-1beta and interleukin-6 mRNA by low doses of ionizing radiation in macrophages. Int J Cancer 2001; 96:270-6.

76. Cucinotta F, Nikjoo H, Goodhead DT. The effect of delta rays on the number of particle traversals per cell in laboratory and space exposures. Radiat Res 1998; 150:115-19.

77. Guidance on radiation received in space activities. Report No. 98. Bethesda: National Council on Radiation Protection and Measurements; 1994.

78.   A Strategy for Research in Space Biology and Medicine in the New Century. Washington, D.C.; 1998.

79.   Elmore E, Lao XY, Kapadia R, Redpath JL. Threshold-type dose response for induction of neoplastic transformation by 1 GeV/nucleon iron ions. Radiat Res 2009; 171:764-70.

80.   Chen S, Zhao Y, Han W, Chiu SK, Zhu L, Wu L, et al. Rescue effects in radiobiology: unirradiated bystander cells assist irradiated cells through intercellular signal feedback. Mutat Res 2011; 706:59-64.

81.   Sawant SG, Randers-Pehrson G, Metting NF, Hall EJ. Adaptive response and the bystander effect induced by radiation in C3H 10T(1/2) cells in culture. Radiat Res 2001; 156:177-80.

82.   Zhou H, Randers-Pehrson G, Geard CR, Brenner DJ, Hall EJ, Hei TK. Interaction between radiation-induced adaptive response and bystander mutagenesis in mammalian cells. Radiat Res 2003; 160:512-6.