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Commentary on radiation-induced bystander effects

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The paradigm of genetic alterations being restricted to direct DNA damage after exposure to ionizing radiation has been challenged by observations in which effects of ionizing radiation arise in cells that in themselves receive no radiation exposure. These effects are demonstrated in cells that are the descendants of irradiated cells (radiation-induced genomic instability) or in cells that are in contact with irradiated cells or receive certain signals from irradiated cells (radiation-induced bystander effects). Bystander signals may be transmitted either by direct intercellular communication through gap junctions, or by diffusible factors, such as cytokines released from irradiated cells. In both phenomena, the untargeted effects of ionizing radiation appear to be associated with free radical-mediated processes. There is evidence that radiation-induced genomic instability may be a consequence of, and in some cell systems may also produce, bystander interactions involving intercellular signalling, production of cytokines and free radical generation. These processes are also features of inflammatory responses that are known to have the potential for both bystander-mediated and persisting damage as well as for conferring a predisposition to malignancy. Thus, radiation-induced genomic instability and untargeted bystander effects may reflect interrelated aspects of inflammatory type responses to radiation-induced stress and injury and contribute to the variety of the pathological consequences of radiation exposures. *Human & Experimental Toxicology* (2004) **23,** *91–94*

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The paradigm of genetic alterations being restricted to direct DNA damage after exposure to ionizing radiation has been challenged by observations in which nonirradiated cells exhibit responses typically associated with direct radiation exposure as a consequence of contact with irradiated cells or after receiving certain signals from irradiated cells. The reported responses, mainly but not exclusively for fibroblasts, include increases or decreases in damage-inducible and stress-related proteins, increases or decreases in reactive oxygen species, cell death or cell proliferation, induction of mutations and chromosome aberrations and chromosomal instability.

These so-called bystander effects may reflect at least two separate mechanisms for the signal transfer. One mechanism, reported in studies of densely ionizing high LET radiation, depends on gap junction intercellular communication stimulating a damage signalling pathway mediated by the tumour suppressor gene product p53 and its downstream target CDKN1A/p21, a protein involved in cell cycle checkpoint function.[1,2] Other studies of both high LET and sparsely ionizing low LET irradiation implicate a second mechanism in which irradiated cells secrete cytokines, such as TGF-beta or IL-8, or other factors that act to increase intracellular levels of reactive oxygen species in unirradiated cells.[3–8] Cytogenetic damage mediated by both mechanisms does not demonstrate a linear relationship to dose but is maximally induced by the lowest doses investigated ($\sim$1 cGy). Potentially related to the mechanisms mediating damage and not requiring gap junctional communication is the finding that medium in which certain cells have been irradiated contains an activity, probably a protein, that produces cytotoxic effects in nonirradiated cells.[7–13]

Although most reported effects are damage responses, alpha-irradiated normal human lung fibroblasts produce a promitogenic bystander signal (attributed to the cytokine TGF-beta1), which is associated with increased intracellular reactive oxygen species in unirradiated cells but decreased cellular levels of p53 and CDKN1A/p21[6] and after both alpha- and gamma-irradiation a radioadaptive bystander activity is present in the supernatant medium.[14,15]

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Experimental evidence for bystander interactions *in vivo* is provided by a study in which mixtures of irradiated and nonirradiated haemopoietic cells were transplanted using a sex mismatch congenic transplantation protocol, such that cytogenetic scoring could distinguish not only host-derived cells from donor-derived cells but also cells derived from the irradiated or nonirradiated donor stem cells.[16] Using this system in which relatively few stem cells were transplanted, chromosome aberrations were documented in the descendants of nonirradiated stem cells. Evidence that these effects are not restricted to experimental models is provided by a recent report of a 35-year old man accidentally exposed to acute high-dose total body neutron radiation who received a stem cell transplant from his HLA-identical sister. In monitoring this patient, chromosomal instability in donor female cells was demonstrated consistent with a bystander effect of the neutron exposure.[17]

Prior to the recent studies of bystander effects, there are numerous reports that a transferable clastogenic factor capable of causing chromosome breaks in unirradiated lymphocytes was present in plasma after radiotherapy but with considerable interindividual variation in both production and response. Clastogenic factors in plasma have also been obtained from atomic bomb survivors, Chernobyl liquidators and from patients with a variety of chromosome instability syndromes and inflammatory disorders (reviewed in references 18–20). These clastogenic factors are produced via superoxide and also induce the production of superoxide and this may be the explanation of their persistence over many years. Their clastogenic activity may be related to the formation of lipid peroxidation products,[21] inosine nucleotides[22] and cytotoxic cytokines.[23] Potentially related to these clastogenic factors, there is a body of radiotherapy data concerning so-called abscopal effects of radiation, where responses are noted in unrelated organs or tissues that are not irradiated and more specific effects where radiation pneumonitis may develop in a contralateral nonirradiated lung. These responses indicate the potential for unexpected effects at the edges of and beyond conventional radiation fields.

A second untargeted effect of irradiation that challenges conventional models is the high frequency of chromosomal abnormalities, gene mutations and in some cases reproductive cell death occurring in unirradiated cells that are the descendants of cells irradiated many generations previously. There is accumulating evidence that these manifestations of radiation-induced genomic instability may be a consequence of, and in some cell systems may also produce, bystander interactions involving intercellular signalling, and the production of cytokines and free radicals.[20,24–29] From a mechanistic point of view, these are also features of inflammatory responses and such responses may be protective or damaging depending on context, but do have the potential for both persisting and bystander-mediated damage. The well-documented increases in malignancy in the A-bomb survivors have recently been supplemented by reports of increases in circulatory, digestive and respiratory system diseases[30] and cardiovascular disease.[31] Given that inflammatory responses may confer a predisposition to malignancy and be risk factors for the development of many clinical conditions, including atherosclerosis, the demonstration of significant increases in inflammatory activity that are still demonstrable in the blood of the A-bomb survivors[32,33] lends support to the conclusion that radiation injury may predispose to a wider range of health consequences than was previously thought. If indirectly affected cells can contribute to the adverse effects of irradiation at low doses, this has an important implication not only for mechanistic studies but also for risk assessment. If responses to nontargeted effects increase the probability of a cell surviving with genomic damage this may increase risk at low doses. However, a cell death response would deviate from a linear-no-threshold model in a protective direction. The potential consequences of untargeted effects appear to represent a balance between the production of toxic factors and the response to such factors. Both signal production and signal response may be significantly influenced by genetic and cell/tissue type-specific factors and until the underlying mechanisms are better understood it is difficult to see how general principles can be extracted to comment on risk.

Recently, it has been reported that changes in the sequence of unstable tandem-repeat sequences (minisatellites) can be seen in the offspring of male mice exposed to radiation, and that these changes occur at a frequency far greater than can be accounted for by conventional mutation rates or the number of radiation damage sites in the DNA.[34–36] As the effects also include elevated mutation frequencies in the unirradiated female allele in the offspring,[37,38] it has to be concluded that a mechanism exists in male germ cells of mice that can extend the consequences of radiation damage to DNA sequences that have not been damaged directly and must be considered as evidence for a genomic instability induced by radiation. Analysis of germline mutation rate at human minisatellites among children born in areas of the

Mogilev district of Belarus heavily polluted after the Chernobyl accident provides evidence that the effect may be relevant to human exposures.[39] The consequences of such a process are far from clear as minisatellites are not part of coding sequences. However, there is evidence that they may affect the expression of adjacent structural genes.[40–46] In one study of the offspring of irradiated male mice there was evidence of a perturbed haemopoietic system, an increase in chromosomal aberrations and enhanced vulnerability to secondary exposure to a chemical leukaemogen.[47] These results demonstrated an interaction between germline-mediated radiation effects and somatic cell chemical exposure that could involve bystander interactions.

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