### Abstract

The mechanisms underlying the unequivocal association between ionizing radiation and the development of leukemia remain unknown. Recent studies present a rather complex picture where, in addition to damage induced directly in target stem cells, a variety of genotype-dependent cellular interactions may also make important contributions to determining overall outcome. The new findings indicate that, contrary to conventional radiobiological models, all adverse effects need not necessarily be induced at the time of irradiation and that indirect effects mediated through the microenvironment may promote pre-existing potentially leukemic cells as a consequence of genotype-dependent, inflammatory-type responses to radiation-induced tissue injury. Genetic factors also significantly influence the number of irradiated cells that survive with potential to express radiation-induced damage and the genetic background producing the more effective apoptotic response would more effectively eliminate unstable and potentially malignant cells.

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Early publications indicating a link between medical radiation exposures and leukemia were those reporting excess leukemia in patients treated with radiotherapy for benign diseases such as ankylosing spondylitis\(^1,2\) and metropathia haemorrhagica\(^3,4\) and more recently the development of secondary leukemia has become a complication of cancer therapy. Studies of patients who have received radiotherapy in the absence of chemotherapy indicate that acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) are the most common disorders with less acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) and no evidence for an increased incidence of chronic lymphocytic leukemia (CLL).\(^5,6\)

The first systematic experimental studies of therapeutically-relevant doses were undertaken in the 1950s when lifetime studies of large numbers of irradiated mice were conducted.\(^7,8\) The most comprehensive information is available for murine lymphomas as these are induced by radiation in most mouse strains and commonly result in the spread of malignant cells into the blood. Acute myeloid leukemias are induced only in certain strains of mice and although deletions involving one homologue of chromosome 2 are characteristic of the disease neither the induction of a chromosome 2 aberration nor the presence of a chromosome 2 aberrant clone specifically predict disease development in the AML model.\(^9\) A number of studies point to chromosomal instability contributing to disease development and the question arises as to how such instability arises in irradiated mice that are genetically susceptible to radiation-induced AML.\(^10\)

**DNA as a target for radiation-induced lesions**

Chromosome aberrations and gene mutations induced by ionizing radiation are conventionally attributed to the DNA being irreversibly changed immediately after exposure, either during the processing and enzymatic repair of the damage or during DNA replication. Consequently, the progeny of a single irradiated cell would be expected to show any transmissible radiation-induced genetic change in all cells i.e. the effect would be clonal. This can be readily observed in experimental studies and it has been widely accepted that lethal or mutational changes take place at the time of radiation exposure. As malignant transformation is generally regarded as being
initiated by a gene mutation or a chromosomal aberration, the initiating lesion for malignant transformation has been similarly attributed to DNA damage in a directly irradiated target cell.

Recently, the view that radiation-induced deposition of energy in the nucleus of an irradiated cell leads to all the adverse consequences of radiation exposure has been challenged by observations in which effects of ionizing radiation are demonstrated in cells that are not themselves irradiated but are the descendants of irradiated cells (radiation-induced genomic instability) or in cells that have communicated with irradiated cells (radiation-induced bystander effects). Radiation-induced genomic instability is characterized by the appearance of a number of delayed non-clonal effects in the clonal progeny of irradiated cells, including chromosomal aberrations, gene mutations and cell death and similar effects have been associated with radiation-induced bystander effects.11,12

Radiation-induced genomic instability

It is difficult to explain the phenomenon of radiation-induced genomic instability by invoking a mutator phenotype arising from mutation in a genome maintenance gene as the frequency of induction is orders of magnitude greater than that of radiation-induced mutations.15-15 Although the results of many studies suggest that radiation-induced genomic instability is not a direct consequence of a radiation-induced mutation in a particular gene, the expression of the instability phenotype is strongly influenced by genetic factors. Studies of various laboratory mouse strains demonstrate that CBA strains may be regarded as susceptible and the C57BL/6 strain as relatively resistant to the induction of chromosomal instability in the bone marrow as well as being susceptible (CBA) and resistant (C57BL/6) to radiation-induced AML.16 The induction of chromosomal instability in some, but not all, samples of irradiated human bone marrow suggests that this might be attributed to inter-individual differences in genetic predisposition.14

The basis of this genotype-dependent expression of delayed chromosomal aberrations seems to lie in the observation that in populations expressing the chromosomal instability phenotype, cytogenetic aberrations and cell death were inversely related, i.e. the more resistant C57BL/6 genotype (with respect to chromosomal instability) exhibited a greater proportion of apoptotic cells. This observation is consistent with the genotype-dependent differences in responses to direct irradiation where, for the same radiation dose, C57BL/6 bone marrow has a greater frequency of apoptotic cells and subsequent reduction in tissue cellularity than CBA/Ca bone marrow.17,18

Thus, genetic factors significantly influence the number of irradiated cells that survive with potential to express radiation-induced damage and the genetic background that produces the more effective apoptotic response would more effectively eliminate unstable and potentially malignant cells. Interestingly, chromosomal instability with the same genotype-dependency is demonstrable after exposure to the benzene metabolite hydroquinone.19 In addition to ionizing radiation, benzene exposure is associated with acute myeloid leukaemia (AML) and exposure of CBA strains of mice to either radiation or benzene leads to the development of AML.20 These results are consistent with the proposal that genotype-dependent chromosomal instability induced by either agent may contribute to AML development by increasing the number of genetic lesions in haemopoietic cells.

Cellular interactions in radiation-induced genomic instability

A number of studies have pointed to an association between non-targeted radiation effects and free radical-mediated processes and certain bystander effects have been attributed to the secretion of cytokines or other factors that induce an elevation in intracellular levels of reactive oxygen species in unirradiated cells. Although, one cannot exclude some transmissible unknown memory of irradiation in particular cell systems, there are reasons for attributing many expressions of delayed effects in haemopoietic cells to mechanisms involving inter-cellular signalling.

An early indication that the chromosomal instability phenotype in vitro may involve cellular interactions came from the observation that more clonogenic cells than were actually irradiated by a Poisson distribution of alpha-particles generated colonies expressing chromosomal instability.15 This discrepancy, indicating that cells exhibiting instability in vitro could be derived from non-irradiated stem cells, was subsequently confirmed by direct experiment.21 By using a congenic CBA/Ca bone marrow transplantation protocol in which mixtures of cytogenetically distinguishable irradiated and non-irradiated bone marrow were transplanted into opposite sex recipients, chromosome aberrations were
demonstrated in descendants of both irradiated and non-irradiated stem cells in vivo for many months post-transplantation.\textsuperscript{22,23} These studies also revealed that there was less cell production than expected from the irradiated stem cells. The deficit of cells derived from irradiated stem at all times after transplantation can be explained by the phenomenon of delayed reproductive death, a well-documented manifestation of the radiation-induced genomic instability phenotype.\textsuperscript{24} Thus, this study demonstrated the in vivo persistence of the chromosomal instability phenotype in the descendants of the transplanted irradiated hematopoietic stem cells and additionally a delayed bystander-induced instability in the progeny of the non-irradiated stem cells. In a clinical context, chromosomal instability in opposite sex donor cells has been reported in a study of a radiation accident victim treated by allogeneic transplantation.\textsuperscript{25}

A potential mechanism underlying these experimental and clinical observations is suggested by macrophages exhibiting characteristics in common with activated inflammatory macrophages after whole body irradiation.\textsuperscript{26} Inflammation is essentially a protective process that has evolved to deliver leukocytes and plasma proteins to sites of injury but, if not resolved in a timely fashion, has the ongoing potential for activated macrophages to induce damage in neighbouring cells. However, macrophages are remarkable for the diverse activities in which they engage and their various activities are controlled by specific signals that stimulate their development into discrete phenotypes differing in terms of receptor expression, effector function and cytokine secretion.\textsuperscript{27} M1 functional subsets display pro-inflammatoryatory activities and M2 functional subsets exhibit repair and remodelling patterns of function, although a strict M1/M2 distinction is an over-simplification\textsuperscript{27} and in response to changes in their tissue environment, macrophages can change the pattern of functions that they express.\textsuperscript{28} Preliminary studies comparing macrophages obtained from the mouse strains that exhibit high (CBA/H) or low (C57BL/6) susceptibility to radiation-induced bone marrow chromosomal instability and AML suggest an association with M1-like (pro-inflammatory) and M2-like (repair/remodelling) states, respectively.

In considering in vivo responses to radiation exposure, a case can be made for the tissue microenvironment contributing genotype-dependent secondary cell damage as a consequence of an ongoing inflammatory-type response secondary to radiation-induced injury. This type of mechanism may also underlie the reports of transferable clastogenic factor capable of causing chromosome breaks in unirradiated lymphocytes being present in the blood of irradiated animals or radiotherapy patients, atomic bomb survivors and Chernobyl liquidators but with considerable inter-individual variation in both production and response; similar findings have also been obtained in studies of patients with a variety of chromosome instability syndromes and inflammatory disorders.\textsuperscript{29} Taken together, a number of studies indicate that the radiation-induced genomic instability phenotype in vivo may reflect the responses to ongoing production of damaging signals in the microenvironment rather than the presence of intrinsically unstable cells.

Interactions between the microenvironment and stem cells have been implicated in the progression of normal to leukaemic haemopoiesis from both clinical and experimental observations. These various findings may reflect changes in stromal cell regulation that alter the overall growth and phenotypic characteristics of stem cells\textsuperscript{30,31} and oxidative stress-related genotoxicity in stem cells due to exposure to nitric oxide released by the host stromal cells.\textsuperscript{32} There is also experimental evidence that inflammatory responses can contribute to the development radiation-induced leukaemias in RFM and C3H mice.\textsuperscript{33,34}

Clearly, microenvironmentally-mediated, inflammatory-like processes as a consequence of radiation exposure introduce yet more complexity when considering mechanisms underlying radiation leukaemogenesis and it should also be noted that the well-documented increases in malignancy in the Japanese A-bomb survivors have recently been supplemented by reports of increases in cardiovascular, gastrointestinal and respiratory system diseases\textsuperscript{35,36} associated with persisting inflammation.\textsuperscript{37,38} Many studies have now identified radiation-induced changes in the tissue microenvironment that affect cell phenotype, tissue composition and the physical interactions and signalling between cells. All these various alterations can contribute to malignancy and alter the tissue response to anticancer therapy.\textsuperscript{39}

Radiation exposure and the promotion of initiated target cells

An association between inflammation and leukaemia may be particularly relevant to the aetiology of childhood ALL, where an abnormal response to common infections is accepted by many as playing a decisive role in disease devel-
A number of investigations are consistent with the view that the leukaemia is fetal in origin with a variable and often protracted latency and the need for further postnatal events to produce clinical disease. This is endorsed by the finding that leukaemic fusion genes are present in normal newborn infants at a rate that exceeds the cumulative risk of leukaemia by two orders of magnitude. Thus, the question arises as to how this biological and epidemiological evidence relates to standard radiation risk estimates of approximately one third of childhood/young adult leukaemia cases being linked to natural background ionizing radiation. The explicit assumption in conventional radiobiological models is that the radiation produces an initiating lesion at the time of exposure. However, because radiation damage is random it would not be expected to produce such specific translocations in the relevant target cells of such large numbers of individuals. Thus, for most exposures radiation is likely to be promoting pre-existing initiated cells. A similar argument has recently been advanced to suggest that virtually all of the characteristics of the excess absolute risk for ALL and probably CML and young-at-exposure cases of acute myelogenous leukaemia in the Japanese A-bomb survivor cohort can be explained by a small fraction of predisposed people who carry clonally expanded preleukemic cells and that those who did not have substantial numbers of such cells had a low risk of developing leukaemia. Overall, a number of observations can be used to argue that radiation implicated as causal may, in fact, be promoting the acquisition of secondary genetic changes in pre-existing initiated cells rather than inducing specific initiating lesions. Non-targeted effects and delayed tissue responses are candidates for the underlying mechanism(s).

References