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Untargeted effects of ionizing radiation: Implications for radiation pathology

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Abstract

The dogma that genetic alterations are restricted to directly irradiated cells has been challenged by observations in which effects of ionizing radiation, characteristically associated with the consequences of energy deposition in the cell nucleus, arise in non-irradiated cells. These, so called, untargeted effects are demonstrated in cells that have received damaging signals produced by irradiated cells (radiation-induced bystander effects) or that are the descendants of irradiated cells (radiation-induced genomic instability). Radiation-induced genomic instability is characterized by a number of delayed adverse responses including chromosomal abnormalities, gene mutations and cell death. Similar effects, as well as responses that may be regarded as protective, have been attributed to bystander mechanisms. Whilst the majority of studies to date have used in vitro systems, some adverse non-targeted effects have been demonstrated in vivo. However, at least for haemopoietic tissues, radiation-induced genomic instability in vivo may not necessarily be a reflection of genomically unstable cells. Rather the damage may reflect responses to ongoing production of damaging signals; i.e. bystander responses, but not in the sense used to describe the rapidly induced effects resulting from direct interaction of irradiated and non-irradiated cells. The findings are consistent with a delayed and long-lived tissue reaction to radiation injury characteristic of an inflammatory response with the potential for persisting bystander-mediated damage. An important implication of the findings is that contrary to conventional radiobiological dogma and interpretation of epidemiologically-based risk estimates, ionizing radiation may contribute to malignancy and particularly childhood leukaemia by promoting initiated cells rather than being the initiating agent. Untargeted mechanisms may also contribute to other pathological consequences.

Keywords: Radiation; Genetic instability; Bystander effects; Clastogenic factors; Inflammation; Cell signalling

1. Introduction

Chromosome aberrations, gene mutations and cell death 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 most of the 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

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damage in a directly irradiated cell that leads to the emergence of a pre-neoplastic clone of "initiated" cells that, with time, accumulate further mutations to produce a fully malignant clone.

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 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 delayed chromosomal aberrations and gene mutations, reduced plating efficiency and delayed cell death. Radiation-induced bystander effects are generally demonstrated very rapidly after irradiation but are characterized by appearing in non-irradiated cells that are in close proximity to irradiated cells or have received damaging signals from more distant irradiated cells. Reported bystander effects include increases or decreases in damage-inducible and stress-related proteins, increases or decreases in reactive oxygen species, cell death or cell proliferation, cell differentiation, radioadaptation, induction of mutations and chromosome aberrations and chromosomal instability. These phenomena have recently been extensively reviewed [1–9] and only a brief overview is provided here.

2. Radiation-induced delayed genomic instability

An early indication for delayed radiation responses was the finding of loss of reproductive potential and cell death among the clonal progeny of irradiated cells [10–13]. More recently, it was demonstrated that the loss of reproductive potential persists for many generations and possibly indefinitely in established cell lines [14–20]. The terms lethal mutations and delayed reproductive death are used interchangeably for this delayed death phenotype and the phenomenon is now generally regarded as a manifestation of radiation-induced genomic instability.

An early report of chromosomal instability as a delayed effect of radiation was a study of skin fibroblast cultures obtained from fetuses derived from X-irradiated zygotes [21] but general attention was drawn to the phenomenon by investigations designed to study the effects of α-particle irradiation on haemopoietic stem cells [22]. In these investigations a clonogenic cell culture assay was used to obtain clonal cell populations derived from mouse bone marrow stem cells that had been irradiated with approximately one alpha particle traversal per cell. Cytogenetic analyses revealed that, as expected, clonal aberrations were rare ($\leq$1%) but, unexpectedly, up to half the colonies contained cells with karyotypic abnormalities with up to one in five metaphases exhibiting single or multiple non-identical aberrations; i.e. the aberrations were non-clonal; a high frequency of chromatid-type aberrations was consistent with an ongoing generation of cytogenetic aberrations during colony development. Similar results were obtained using human haemopoietic stem cells [23]. Subsequently, radiation-induced chromosomal instability was reported for cultures of human skin fibroblasts that had been exposed to heavy ions [24] and clonal cultures of X-irradiated primary human lymphocytes [25]. A feature of the lymphocyte studies was that sub-clones in the clonal cultures were found to have heterogeneous late appearing aberrations and some of these sub-clones underwent sequential changes to progressively more aberrant karyotypes with growth in culture [25–27]. An inducible chromosomal instability phenotype was also demonstrated in a hamster-human hybrid cell line several generations after expanding single-cell colonies that had survived X-irradiation and in these cells the types of chromosomal rearrangements observed suggested that chromosome fusion, followed by bridge breakage and re-fusion was contributing to the observed instability [28].

There are now many reports of radiation-induced chromosomal instability in a variety of established cell lines and some primary cells in culture and the topic has been the subject of several recent reviews [1–7]. In some situations, the same cell types have been irradiated with different types of radiation and differences in the types of cytogenetic abnormalities have been recorded [24,29,30]. A common feature of the various studies seems to be lack of evidence for a conventional dose-response relationship as instability is induced at the lowest doses investigated (including a single alpha particle traversal) with no increased expression after higher doses. There is also a general trend for densely-ionizing radiation to be a more effective inducer than sparsely-ionizing radiation. The radiation-induced chromosomal instability phenotype is not universally expressed in mammalian cells [31–35] and expression depends strongly on genotype, at least for some primary cells [36,37]. Significant inter-individual variation in the expression of instability in vivo has also been noted even in those inbred strains of mice that may express high levels of instability [38,39]. It should be noted that if unstable chromosome aberrations commonly resulted in

cell death as demonstrated by some studies [28,40–42] then lack of significant chromosomal instability would not necessarily exclude the existence of a state of instability if cell death and gene mutations have not been studied.

Another issue relevant to in vivo expression of instability is that there are likely to be important differences between the rather artificial conditions of cells in culture and cells in their normal tissue environment. Comparing the same types of haemopoietic cells obtained from in vitro or in vivo sources there are very significant differences in the expression of radiation-induced chromosomal instability; the in vivo data revealing far fewer cells demonstrating abnormalities and much less damage per cell than in vitro data [39]. One possible explanation for such a large difference might be the cellular defence mechanisms in vivo that have evolved to recognize and remove aberrant cells. Whatever the precise explanation for this difference and for the various reported differences in the expression of chromosomal instability and other endpoints of genomic instability that may be attributed to cell type or genotype or other, as yet poorly understood, factors, it is clear that there is a need for some caution in drawing generalized conclusions from limited data in individual studies.

Using established cell lines, persisting high frequencies of late-arising mutations in the hypoxanthine-guanine-phosphoribosyl-transferase (*hprt*) locus have been detected in a relatively large fraction (approximately 10%) of the cell population with no evidence of a conventional dose-response relationship [43–45]. Radiation-induced delayed mutations are not restricted to studies of established cell lines as mutations in *p53* and *hprt* genes have been demonstrated in, respectively, primary murine mammary epithelium [46] and haemopoietic cells [47]. The mutation spectrum of *hpr*t mutations arising as a consequence of induced instability is more like that of spontaneously arising mutations than of conventional radiation-induced mutations [45] and in an extensive clonal analysis of delayed *hprt* mutations it was found that approximately 25% of the clones showed evidence of chromosomal instability. Furthermore, in these clones a four to eight-fold increase in thymidine kinase (*tk*)mutations was also demonstrated suggesting that a persistent elevation in genome-wide mutation frequency can be associated with chromosomal instability [48]. Consistent with a globally operating mechanism, clones with delayed *tk* mutations also exhibit increased frequencies of mutations in other regions of the genome [49–51]. It is not known whether delayed gene mutations, chromosome aberrations and death are manifestations of a single common underlying mechanism although it is a tempting speculation.

3. Radiation-induced bystander effects

Over the last 10 years many bystander responses have been reported. They include damage-inducible stress responses [52–55], sister chromatid exchanges [56–58], micronucleus formation [59,60], apoptosis [59], gene mutation [61–63], chromosomal instability [64] and transformation of rodent cells in vitro [65,66]. Whilst many of the studies have concentrated on genome damage endpoints, there have also been reports of other effects being induced in bystander cells including increased cell proliferation [67,68] and release of growth inhibitory factors [69]. A protective adaptive response has also been reported, where bystander cells that are subsequently irradiated are more radioresistant than cells not exposed to bystander signals [70,71]. Bystander induction of terminal differentiation with loss of proliferative potential [72] may also be regarded as a protective response. Thus, it seems that there are both damaging and protective cell signals that are encompassed within the general field of bystander effects and that the potential consequences of these effects reflect a balance between the type of bystander signals produced and the responses of cell populations to such signals, both of which may be significantly influenced by cell type and genotype [9].

The first cytogenetic effect attributed to a bystander mechanism was of sister chromatid exchanges in cultures of Chinese hamster ovary (CHO) cells [57] and normal human lung fibroblasts [58] when very few cells were actually traversed by an alpha particle. An enhanced frequency of *hprt* mutations was also demonstrated in bystander CHO cells [61]. Mutations induced in directly irradiated cells are largely partial and total gene deletions, those in bystander cells are, like spontaneous mutations and the delayed mutations characteristic of radiation-induced genomic instability [45], primarily point or small scale mutations [73].

Although bystander effects are generally regarded as rapidly induced responses there are reports of bystander-mediated transformation in cell culture models. One study used medium transfer [66], the other, microbeam irradiation [65]. In the medium transfer experiment, exposing unirradiated human CGL1 cells to medium from cells irradiated with 5 or 7 Gy resulted in a nearly four-fold increase in the frequency from 6.3×10^{-6} in unirradiated controls to 2.3×10^{-5}. In the microbeam experiment, when 10% of the cells on a dish were exposed to alpha particles the resulting frequency of induced transformation was at least that observed when

every cell on the dish was exposed to the same number of alpha particles.

The various studies of bystander effects indicate that, like genomic instability, densely-ionizing radiation tends to be a more effective inducer than sparsely-ionizing radiation and the effects saturate at low doses. Thus, non-targeted effects have the potential to introduce discontinuities into dose-response relationships at low doses. Using the data for bystander-mediated transformation in rodent cells [65], attempts have been made to produce quantitative models relevant to low dose risk. One that assumes the oncogenic bystander response is a binary "all or nothing" phenomenon in a small sensitive subpopulation of cells (and that cells from this sensitive subpopulation are also very sensitive to direct hits from alpha particles generally resulting in a directly hit sensitive cell being inactivated) suggests that, at least for alpha particle-induced oncogenic transformation, bystander effects are important only at doses below about 0.2 Gy. At still lower doses, bystander effects may dominate the overall response, possibly leading to an underestimation of low-dose risks extrapolated from intermediate doses, where direct effects dominate [74]. However calculations of the ratio of the lung cancer risk among persons exposed to residential doses of radon daughters to that among underground miners exposed to higher doses suggest that the oncogenic bystander effect cannot be making a significant contribution to radon-induced lung carcinogenesis in humans [75]. At the present time, because the data are fragmentary and may be inconsistent it is difficult to see how general principles can be extracted to comment on risk.

4. Mechanisms underlying untargeted radiation effects

It is difficult to explain the phenomenon of radiation-induced genomic instability by invoking a mutator phenotype arising from mutation in genome maintenance genes. Typically in a population of mammalian cells approximately one in 10^6 will carry a mutation in any given gene and this increases approximately 10-fold after exposure to 1 Gy X-rays such that 0.001% of surviving clonogenic cells will transmit any particular mutation [76,77]. The situation with the induced instability phenotype is that approximately 10% of surviving cells produce clones that exhibit delayed hypoxanthine phosphoribosyl-transferase (*hprt)* mutations [45,47,78] and a similar or much greater proportion (in some cases up to ∼50%) produce clones exhibiting chromosomal instability [22,23,28]. Although, one cannot exclude some persisting unknown 'memory' of irradiation in particular systems, there are reasons for attributing many expressions of delayed abnormalities to mechanisms involving cell:cell interactions, similar to the intercellular signalling in radiation-induced bystander effects. A number of studies have pointed to an association between induced chromosomal instability and free radical-mediated processes [40,79,80]. These various associations point to increases in damaging signals of the type that produces spontaneous damage or decreased ability to deal with such damage and would be consistent with a bystander-type mechanism. The first clue for this type of mechanism came from the observation that chromosomal instability in vitro was expressed in the progeny of more clonogenic haemopoietic stem cells than were actually traversed by an alpha particle [22]. This discrepancy, indicating that cells exhibiting instability could be derived from non-irradiated stem cells, was subsequently confirmed by direct experiment using the presence or absence of a shielding grid [64].

In experiments designed to model the mixture of irradiated and non-irradiated cells in the in vitro studies of α-irradiated haemopoietic stem cells, a congenic bone marrow transplantation protocol was designed in which mixtures of neutron-irradiated and non-irradiated bone marrow, distinguishable by a cytogenetic marker, were transplanted into opposite sex recipients. Donor origin of cells could be confirmed by the sex difference and descendants of irradiated or non-irradiated stem cells by the cytogenetic marker. Using this three-way marker system, chromosome instability was demonstrated in descendants of both irradiated and non-irradiated stem cells [38]. As cells derived from irradiated or non-irradiated donor stem cells could be unequivocally identified, it is clear that chromosomal instability in descendants of non-irradiated stem cells many months after transplantation must be attributed to an indirect cell interaction mechanism.

In vitro evidence consistent with cell interactions producing chromosomal instability is provided by investigations of delayed effects in the Chinese hamster–human hybrid GM10115 cell line [28,40]. In these cells a high level of recombination characterizes the chromosomal instability and using the comet assay to assess DNA strand breaks, no significant difference between clones derived from non-irradiated cells and radiation-induced chromosomally unstable clones was found. The proposed explanation was that radiation induces conditions and/or factors that stimulate the production of reactive oxygen species producing a pro-oxidant environment that cycles over multiple generations, promoting chromosomal recombination and other phenotypes associated with genomic instability [81]. Subsequently, a

"death-inducing effect" of exposing GM10115 cells to medium in which unstable GM10115 cells had been cultured was implicated in the delayed death associated with the chromosomally unstable clones [82].

There are two general classes of experiment in which bystander responses have been demonstrated. One has exploited the ability of bystander signals to be transferred from irradiated cells to unirradiated cells by medium transfer, the other has investigated response of cells to low fluences of alpha particles where the majority of cells have not been irradiated. These two approaches also seem, broadly, to reflect two types of mechanism; the former, dependent on the release of diffusible signalling molecules [68,83], the latter on gap junction intercellular communication [53,54,67]. In both mechanisms, oxidative metabolism and stress-inducible proteins have been implicated in the signalling process [55,56,84] and it seems probable that differences between these two mechanisms may be related not only to cell type but also to cell density and other cell context aspects of the in vitro systems used in the various studies. Overall, there are many similarities between the proposed mechanisms underlying bystander effects and induced instability and, at least in the haemopoietic system, bystander effects can be both a cause and a consequence of induced instability [4]. Increasingly, the two manifestations of untargeted radiation effects are being considered as linked processes. Thus, in addition to targeted effects of damage induced directly in cells by irradiation, a variety of untargeted effects may also make important short-term and long-term contributions to determining overall outcome after radiation exposures (Fig. 1).

Consideration of the design and the results of the congenic transplantation experiment outlined above, led to the conclusion that mononuclear phagocytic cells with the characteristics of phagocytes found in inflammatory conditions were the likely source of bystander signals [38]. Subsequent studies have provided evidence for non-targeted in vivo mechanisms that result in such activated macrophages [85]. In haemopoietic tissue 24 h or more after irradiation there are macrophages that are activated by ultrastructural criteria, exhibit increased levels of nitric oxide synthase, increased capacity to produce superoxide and increased lysosomal enzyme

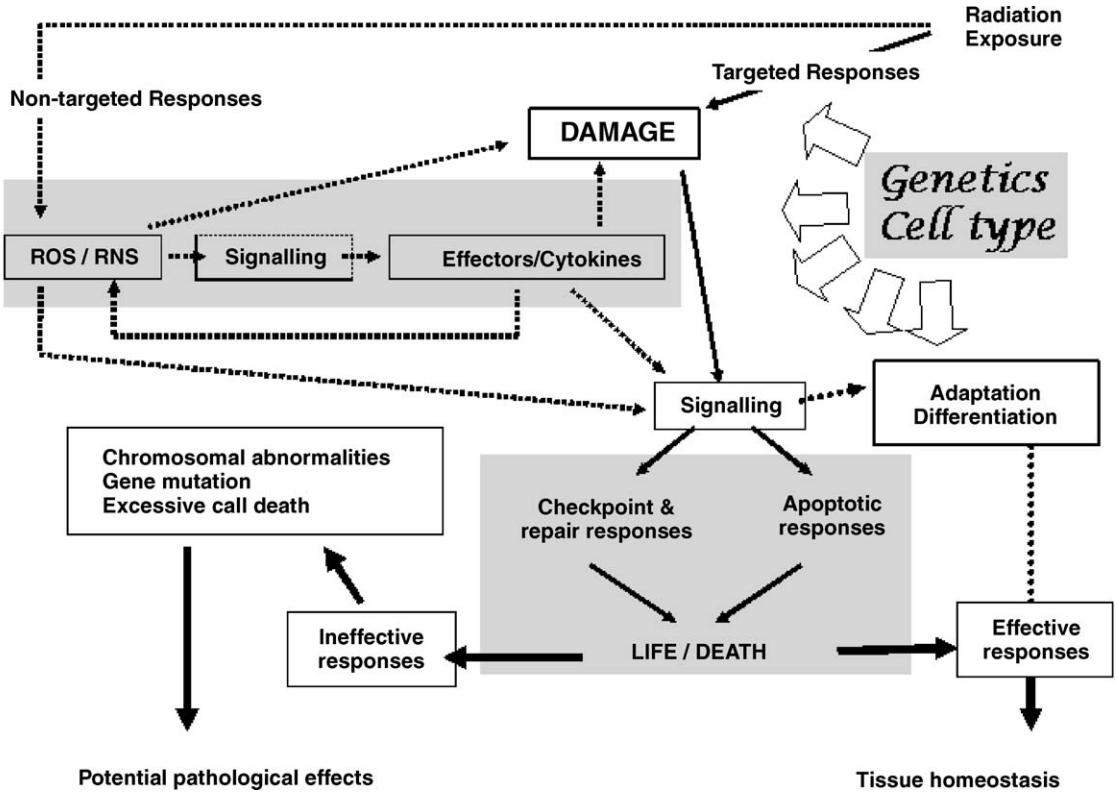

Fig. 1. A schematic representation of how targeted and untargeted effects result in damage that induces checkpoint and repair (with the possibility of misrepair) and apoptotic responses and how untargeted effects have the potential for persisting damage due to free radicals stimulating cytokine production that, in turn, generate more free radicals. Poorly understood processes that include cell type-specific and genetic factors govern the decision-making processes that, in a context dependent manner, may result in an overall damaging or protective response.

activity that correlated directly with phagocytosis of apoptotic cells. A number of features of this study indicated that the macrophage activation is not a direct effect of radiation but rather a delayed response correlating with the recognition and engulfment of apoptotic cells. That macrophage activation was associated with the phagocytic clearance of radiation-induced apoptotic cells was confirmed using p53−/− mice that lack p53-dependent radiation-induced apoptosis. In addition to the macrophage activation, neutrophil margination in blood vessels and splenic infiltration was observed These are classic features of inflammation that are also reported to occur in the thymus post-irradiation [86].

Macrophage activation and neutrophil migration are essentially protective processes that have evolved to deliver leukocytes and plasma proteins to sites of injury but, if not terminated in a timely fashion, have the potential for bystander-mediated and persisting damage. Thus, a case can be made for the tissue microenvironment contributing secondary cell damage as a consequence of an ongoing inflammatory-type response secondary to radiation-induced injury. This type of mechanism may also underly the earlier reports of clastogenic factors in the plasmas of irradiated animals or radiotherapy patients [87]. Clastogenic factors are produced via superoxide and also induce the production of superoxide [88] and have also been obtained from atomic bomb survivors and Chernobyl liquidators [89–91] and from patients with a variety of chromosome instability syndromes and inflammatory disorders [88,92]. Clearly inflammatory processes as a consequence of radiation exposure introduces yet more complexity to considering delayed and indirect effects of radiation with implications for radiation pathology; in particular the mechanisms of radiation carcinogenesis and leukaemogenesis.

5. Inflammation as a contributory factor to malignancy and other diseases

Increasingly, the conventional view that cancers arise as a consequence of DNA mutations in a single target cell is acknowledged as too simplistic due to the growing awareness of the significant role of the tissue microenvironment in the developmental regulation of normal and neoplastic cells. Cell growth, differentiation and death are directed in large part by extracellular signaling arising from multiple interactions of cells with other cells and with the extracellular matrix; these interactions are in turn modulated by complex regulatory mechanisms including, for example, the actions of cytokines and growth factors. This microenvironmental control is particularly important for stem cells where combination with stromal elements provide the “stem cell niche” [93–99]. It is well established that the microenvironment may significantly influence the growth of pre-neoplastic cells and either aid or inhibit tumour development [100–104]. Potentially damaging signals generated in the microenvironment are also implicated as an early and possibly initiating factor in malignant transformation.

It is now well established that both normal homeostatic and stress-activated cellular processes are affected by altered redox potential acting via the regulation of protein kinases and thereby linking external stimuli with signal transduction pathways [105–109]. A disruption of the balance between prooxidants and antioxidants can result in a state of oxidative stress able to promote several pathological conditions, including those associated with ageing and cancer [110–116]. The best understood examples of tumours arising as a consequence of damaging signals generated in the microenvironment are those that arise in a setting of chronic inflammation where the production of reactive oxygen species and/or reactive nitrogen species by tissue macrophages or neutrophils results in collateral damage in adjacent cells [111,117–119]. As well as direct mutation, reactive oxygen species generation can also lead to changes in DNA methylation, a common occurrence in many cancers that leads to epigenetic alterations in gene expression [120].

In addition to the specific examples of tumours associated with inflammation, there is a substantial body of evidence for altered stromal function associated with inflammatory processes contributing in the early stages of malignancy. A particularly relevant example is radiation leukaemogenesis where, in mouse models, acute myeloid leukaemia is reproducibly induced by irradiation but not when the mice are re-derived and housed under sterile conditions. Transferring the mice to conventional housing restores the leukaemia inducibility [121]. In an unrelated study of a mouse model of radiation-induced leukaemia, the induction of inflammation did not affect the incidence of myeloid leukemia in unirradiated mice but significantly increased the incidence of leukaemia in irradiated mice [122]. These studies clearly implicate inflammation as microenvironmental component of radiation leukaemogenesis. A role for microenvironmental factors is also supported by studies in which bone marrow stromal cells have been shown to aid the survival of irradiated stem cells and contribute to the selection and proliferation of a malignant clone [123,124]. This is particularly well demonstrated by the frequency of transformation of unirradiated growth factor-dependent cells being significantly increased by co-culture with irradiated bone marrow stromal cell lines [125–127] or by transplantation into irradiated syngeneic

mice [128,129]. These effects appear to be due to activation of signalling pathways responsible for changes in adhesion and growth factor production [130] and for the release of cytokines, such as TGF-beta [123] and/or nitric oxide [131] by the irradiated stroma resulting in the co-cultured haemopoietic cells expressing high levels of reactive oxygen species [123]. Relevant to consideration of stromal influences is the uncommon occurrence of leukaemia in donor cells following allogeneic marrow transplantation for leukaemia or aplastic anaemia in irradiated recipients [132–136] and many human and animal studies of leukaemia and myelodysplasia have shown functional abnormalities in stromal cells [123,130,137–141].

Our recent studies of the CBA model of radiation leukaemogenesis have provided a link between stromal changes, inflammatory-type processes and stromal-mediated DNA damage [85]. An important finding of this study was that macrophage activation was related to the phagocytic clearance of the wave of apoptosis in haemopoietic tissues a few hours after irradiation. The in vivo finding that engulfment of apoptotic cells produces such inflammatory-type responses is unexpected, since removal of apoptotic cells is generally considered to be a silent process and many in vitro studies have suggested it to be actively anti-inflammatory [142–144]. However, a variety of studies now indicate that apoptotic cell removal can indeed produce inflammatory-type processes and altered release of regulatory cytokines as well as DNA-damaging free radicals [144]. In addition, the phagocytic uptake of apoptotic cells can result in further apoptosis by soluble signals that induce Fas-mediated and also Fas-independent apoptosis of bystander cells [145]. These data suggest the existence of complex regulatory feedback loops associated with phagocytic clearance (Fig. 2).

Additional mechanisms underlying the association between inflammatory processes and tumour initiation and/or progression are being rapidly identified. For instance, production of CSF-1, the macrophage regulatory cytokine, by tumour cells is required for recruitment of macrophages into the tumour and promotes progression and metastasis [146]. Expression of MMP-9 by infiltrating stromal cells potentiates tumour formation in an HPV16 model of squamous carcinoma [147], regulates vasculature in neuroblastoma by recruiting pericytes [148] and induces angiogenesis in pancreatic islet cell tumours [149]. Most recently, the NF-κB transcription factor has been shown to be one of the key signalling pathways that link inflammation and tumourigenesis [150,151]. In addition, links have been established between inflammatory processes and modulation of p53 functions. For example, the pro-inflammatory cytokine, macrophage migration inhibitory factor (MIF) inhibits p53 function [152,153] whereas interferon-α and -β induce p53 gene transcription, increase p53 protein levels and augment p53 responses to stress [154]. Clearly, inhibition or activation of p53 response by the microenvironment will be an important determinant of outcome to cellular stress and injury (Fig. 2).

6. Radiation and childhood leukaemia

The rapidly accumulating evidence linking inflammation to the promotion of malignancy may be particularly relevant to childhood leukaemia. The cumulative risk of any child developing leukaemia before the age of 15 years is around one in 2000 [155]. More than 200 genes have been identified that are involved in chromosomal translocations in childhood leukaemia but many are rare and certain genes predominate. These include MLL in infant acute lymphoblastic leukaemia and TEL

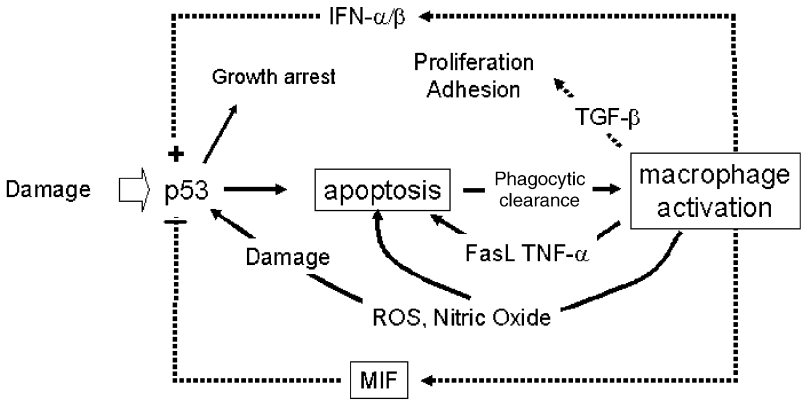

Fig. 2. A schematic representation of the types of feedback loops associated with apoptotic cell removal that produce both inhibition or activation of p53 response and inflammatory processes leading to altered release of regulatory cytokines and DNA-damaging free radicals.

and AML1 in childhood acute lymphoblastic leukaemia [155]. Analysis of pairs of identical twins with concordant leukaemias shows that leukaemic cells from both twins share the same breakpoints in the TEL and AML1 genes or in the MLL gene. For the same breakpoints to be present in the same genes the generation of the fusion gene must have occurred once, in a single stem cell, in one twin in utero and its descendants must spread to the other twin via the shared placenta. Further evidence that childhood leukaemia can originate before birth comes from PCR analysis of neonatal blood spots (Guthrie cards) where primers for specific fusion genes, designed for each patient, can detect as few as 1–20 cells carrying such translocations. The presence of the same fusion gene sequence in a neonatal blood spot as is in the patient's leukaemic cells at diagnosis provides unequivocal evidence that leukaemia has been initiated prenatally, probably by formation of the fusion gene itself [156–158]. The conclusions from these studies are that leukaemia is fetal in origin in all cases of infant leukaemia (with fusions of the MLL gene), in the majority of most cases of the common form of childhood acute lymphoblastic leukaemia (with TEL-AML1), and in about half of cases of childhood acute myeloblastic leukaemia (TEL-AML1 or AML-ETO). The studies of neonatal blood, identical twins, and normal newborn cord bloods clearly reveal a frequent prenatal origin and an early or initiating role for specific chromosome translocations. Further, they provide evidence for a variable and often protracted latency and the need for further postnatal exposures and/or genetic 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. [155,159].

Approximately one third of childhood leukaemia cases have been linked to natural background ionizing radiation [160] with the explicit assumption that the radiation produces the initiating lesion at the time of exposure. However, because radiation damage is random it would not be expected to produce such specific translocations in such large numbers of individuals. In addition, there is recent evidence suggesting that most human fetal lymphoid precursor cells are insensitive to registering damage expressed as chromosome aberrations [161] and earlier reports that murine B lymphocyte precursors are extremely sensitive to the lethal effects of ionzing radiation [162,163]. Taken together, these observations can be used to argue that environmental agents implicated as "causal" may, in fact, be promoting the acquisition of secondary genetic changes rather than inducing specific initiating lesions (Fig. 3). Untargeted effects provide a different biological paradigm for radiation effects that is compatible with such a promotional model. Specifically, a pathophysiologically relevant mechanism is suggested by observations of inflammatory responses as a delayed and long-lived effect of tissue response to radiation injury. Inflammation is known to have the potential for both bystander-mediated and persisting damage, as well as for conferring a predisposition to malignancy.

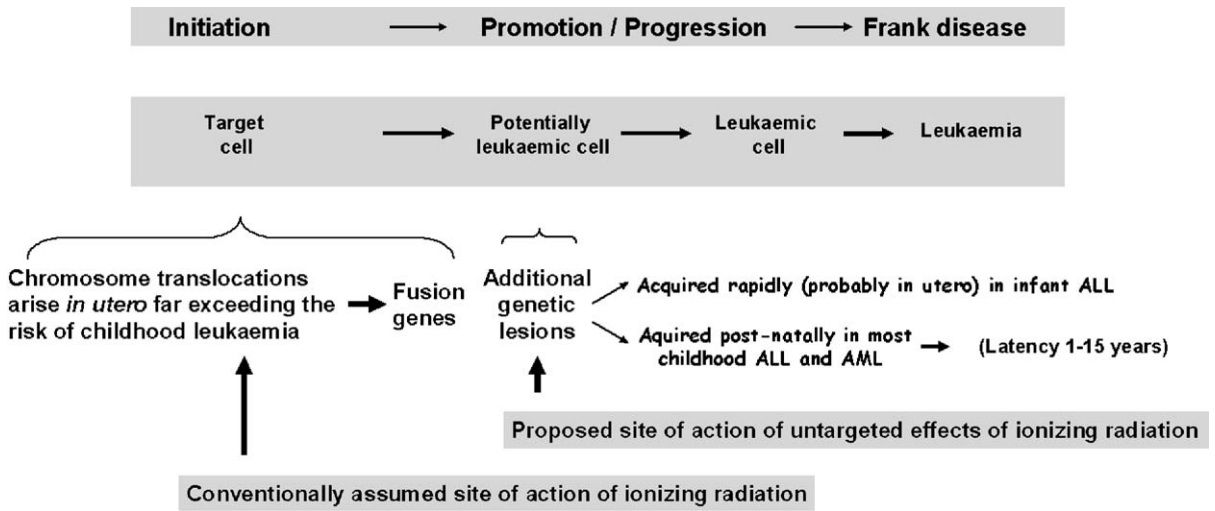

Fig. 3. A number of studies reveal a frequent prenatal origin and an early or initiating role for specific chromosome translocations in childhood leukaemia. Further, the incidence of the resultant leukaemic fusion genes exceeds the cumulative risk of leukaemia by two orders of magnitude. These findings can be used to argue that environmental agents implicated as "causal" may, in fact, be promoting the acquisition of secondary genetic changes rather than inducing specific initiating lesions. Thus, contrary to general assumptions, if ionizing radiation has a role in childhood leukaemia, then in most cases it may be in promotion rather than initiation.

Speculatively, it may provide a unifying concept for the contributions to disease pathogenesis of a diverse range of environmental agents implicated in childhood leukaemia.

The well-documented increases in leukaemia and other cancers in the Japanese A-bomb survivors have recently been supplemented by reports of increases in cardiovascular, digestive and respiratory system diseases [164,165]. Given that inflammation contributes to ageing and a variety of pathological conditions [110,115] and is a significant risk factor for atherosclerosis [166,167], the inflammatory activity that is currently demonstrable in the blood of the A-bomb survivors [168,169] lends support to the proposal that untargeted consequences of radiation injury that have these persisting inflammatory characteristics may predispose to a wide range of health consequences.

7. Conclusions

The major adverse consequences of radiation exposures are attributed to DNA damage in irradiated cells that have not been correctly restored by metabolic repair processes. However, the dogma that genetic alterations are restricted to directly irradiated cells has been challenged by observations in which effects of ionizing radiation, characteristically associated with the consequences of energy deposition in the cell nucleus, arise in non-irradiated cells. These, so called, untargeted effects are demonstrated in cells that are the descendants of irradiated cells (radiation-induced genomic instability) or in cells that have communicated with irradiated cells (radiation-induced bystander effects). The phenotypic expression of untargeted effects reflects a balance between the type of bystander signals produced and the responses of cell populations to such signals, both of which may be significantly influenced by cell type and genotype.

Whilst the majority of studies to date have used in vitro systems, some adverse non-targeted effects have been demonstrated in vivo. However, in haemopoietic tissues such effects can be attributed to the microenvironment contributing secondary damage as a consequence of inflammatory-type processes that arise as a consequence of the tissue response to radiation injury. These indirect responses have significant implications for extrapolating in vitro findings to the in vivo situation and for mechanistic studies of radiation-associated pathologies including leukaemia. At least for haemopoietic tissues, it is likely that, in vivo, radiation-induced genomic instability may not necessarily be a reflection of genomically unstable cells but rather the responses of cells to ongoing production of damaging signals that in turn are not simply radiation-induced bystander signals but mediated by unresolved inflammation.

On the basis primarily of in vitro findings, it has been widely discussed that untargeted effects of radiation pose major paradigm-breaking challenges to current views of the mechanisms of radiation-induced DNA damage and malignant transformation [170]. However, on the basis of in vivo findings, it is possible that delayed and indirect effects of radiation may reflect another aspect of the relationship between inflammation and malignancy with the significant implication that ionizing radiation may have an important role as a promotional factor in the development of some malignancies. In addition, the inflammatory nature of untargeted mechanisms may contribute to a range of other pathological outcomes.

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