RADIATION AND THE MICROENVIRONMENT – TUMORIGENESIS AND THERAPY

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Abstract | Radiation rapidly and persistently alters the soluble and insoluble components of the tissue microenvironment. This affects the cell phenotype, tissue composition and the physical interactions and signalling between cells. These alterations in the microenvironment can contribute to carcinogenesis and alter the tissue response to anticancer therapy. Examples of these responses and their implications are discussed with a view to therapeutic intervention.

IONIZING RADIATION
Energy from isotopic decay or produced by electromagnetic excitation that is capable of producing ionizations, directly or indirectly, while traversing matter.

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IONIZING RADIATION (IR) is both a carcinogen and a therapeutic agent — low-dose exposure can increase an individual's risk of developing cancer, but when given at high doses it can slow or stop tumour growth (BOX 1). How can IR have such a broad range of effects? Studies into the cellular effects of IR have led scientists to a detailed understanding of the cell cycle, DNA damage, apoptosis and the molecular machines that initiate and execute DNA repair. Cancer radiotherapy relies on two essential components — killing cancer cells while sparing normal tissues. This is achieved in part by taking advantage of the physical attributes of IR that, through sophisticated planning and delivery techniques, make it possible to safely increase the radiation dose to the tumour while limiting the dose to surrounding normal tissues[95]. Further therapeutic benefits can be accrued by understanding and manipulating the biological response of the microenvironment to IR to increase a tumour's sensitivity to radiation or to inhibit deleterious effects, respectively.

Many people assume that one must look no further than IR-induced DNA damage to understand how it functions as a carcinogen and can be used to kill cancer cells. However, IR has many multicellular effects, indicating that additional mechanisms might contribute to the response to and consequences of IR exposure. In an intact organism, all cells are subject to complex regulatory mechanisms that depend on their interactions with the cells and cellular products that comprise their microenvironment. Therefore, the effects of an agent such as IR should not just be considered in terms of isolated cells, but rather that the entire tissue has a role in determining the response of any individual cell to any regulatory or damaging signals.

When cells are exposed to IR, DNA damage induces a stress response through activation or repression of distinct target proteins that primarily function to facilitate DNA repair and prevent the proliferation of damaged cells. Similar to the stress response programme within cells, IR induces multicellular programmes that orchestrate a response to damage at the tissue level[1]. Such programmes are executed by soluble signals such as CYTOKINES, GROWTH FACTORS AND CHEMOKINES, which function on the PARENCHYMA and STROMA to modulate cell behaviours and phenotypes. IR can elicit an 'activated' phenotype in some cells that promotes rapid, persistent stromal remodelling of the EXTRACELLULAR MATRIX (ECM). Remodelling of the ECM occurs through the induction of proteases and growth factors, and the chronic production of REACTIVE OXYGEN SPECIES (ROS). Tissue responses to IR seem to be directed towards limiting damage, inducing repair and restoring tissue homeostasis. However, as with most tissue processes, this response can be disrupted by high doses of radiation, pre-existing conditions such as previous exposure, and the genetic features of the individual (FIG. 1).

In determining how the microenvironment is altered by IR exposure, one must consider how energy interacts with biological matter. The two

Summary

- Exposure to ionizing radiation increases the risk of developing cancer at low doses and is used to control cancer at high doses.
- Ionizing radiation can elicit an 'activated' phenotype in some cells that promotes rapid and persistent remodelling of the extracellular matrix, through the induction of proteases and growth factors, as well as chronic production of reactive oxygen species.
- The rapid and dynamic cell biology that occurs in irradiated tissues indicates the existence of a microenvironment-mediated damage-response programme. Some mechanisms of the ionizing radiation-induced microenvironment include chronic inflammation and persistent production of transforming growth factor-β.
- These cellular and tissue responses to ionizing radiation can have non-targetted effects on non-irradiated cells, such as induction of genomic instability and neoplastic progression.
- The functional consequences of exposing an organism to ionizing radiation are a product of DNA damage, cell loss and altered tissue microenvironments that promote carcinogenesis and might affect responses to anticancer therapies.

primary mechanisms are DIRECT EFFECTS, owing to deposition of energy within a macromolecule, and INDIRECT EFFECTS — the interaction of energy with water to produce ROS. For IRs such as X-RAYS and γ-RAYS, 60% of damage is caused by indirect effects. The probability that IR functions on a sufficient number of similar proteins to elicit a biological response is very small. By contrast, the effects of ROS generation are rapidly amplified through their interactions with lipids, membranes and oxygen. In addition to their self-amplification, ROS are probably crucial mediators of changes in the microenvironment because many proteins have built-in sensors for oxidative stress. So, when an organism is exposed to IR, direct macromolecular damage might be dealt with quickly by tagging proteins for degradation by proteosomes, or by repairing DNA molecules, whereas indirect action through ROS can itself be a signal, can be amplified, and can be persistent.

CYTOKINES, GROWTH FACTORS AND CHEMOKINES
Proteins that convey information between cells, through secretion and interaction with receptors. Signalling by these molecules regulates cell proliferation, differentiation, motility, adhesion and apoptosis.

PARENCHYMA
Organ compartment that performs the function of a tissue — for example, tissue-specific epithelium.

STROMA
Organ compartment serving as the connective tissue framework; includes fibroblasts, immune defense cells and fat cells.

EXTRACELLULAR MATRIX
(ECM). An insoluble protein scaffold on which cells reside. The ECM provides the structure and attachment sites, and signals through cell surface receptors. Epithelial cells, endothelial cells and adipocytes rest on a specialized ECM called the basement membrane. Interstitial ECM is collagen-rich.

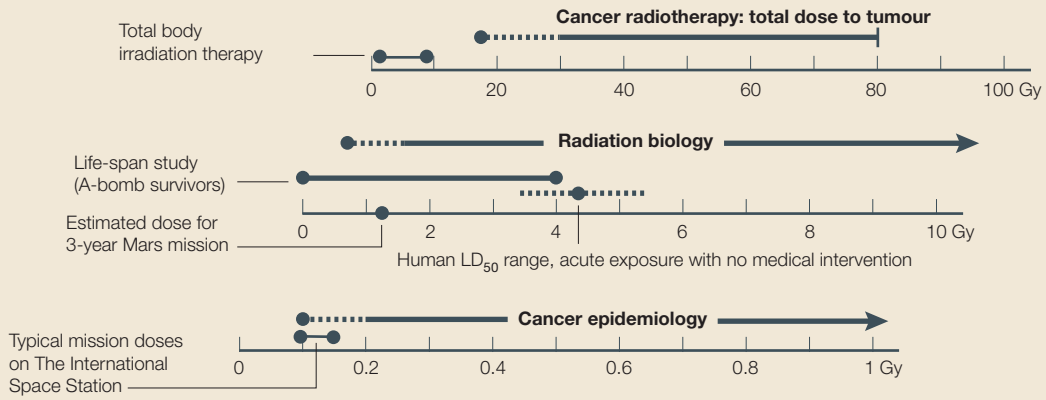
REACTIVE OXYGEN SPECIES
Highly reactive chemical radicals generated as products of oxygen degradation.

Box 1 | Ionizing radiation exposure ranges

Ionizing radiation is used in both diagnostic and therapeutic medical applications, which means that people are exposed over a very large dose range. Ionizing radiation effects are a function of physical attributes of radiation type, dose, and whether the exposure is acute, fractionated or chronic. Biological responses to ionizing radiation exposure also vary depending on age, tissue type, genetic background and physiological status. So, predicting the response to ionizing radiation requires knowledge of both the radiation dosimetry and the biological system.

The diagram shows a comparison of some of the doses of ionizing radiation to which people are exposed, measured in Gray (Gy) — the energy absorbed from 1 joule of energy by 1 kilogram of material. During radiation therapy to treat patients with cancer, tumour tissue can be exposed to over 80 Gy. During irradiation therapy to ablate bone marrow cells, for the treatment of haematogeneous malignancies, patients typically receive 3–10 Gy of total body irradiation. Radiation biology experiments on model organisms and cells typically involve administration of 1–10 Gy, although lower doses are now being studied. The LD_{50} (dose that is lethal to 50% of subjects) for humans is 4.25 Gy, meaning that approximately half of the people who receive an acute whole-body exposure of this amount will die in the absence of medical support.

A lifespan study of atomic bomb survivors who received exposures of up to 4 Gy has been conducted. Epidemiological studies have reported that people who have been exposed to doses greater than 0.5 Gy have a statistically increased risk of developing certain cancers. Because of the high frequency of spontaneous cancers, however, it is difficult to ascertain the possible risk in populations that are exposed to low or chronic radiation. Studies of populations in which the exposure levels are lower — such as for individuals who have undergone medical diagnostic testing, who work in a radiation-related industry or who live in regions of high background radiation — have not demonstrated an increased cancer incidence. A typical International Space Station mission exposes astronauts to only about 0.1 Gy — this is one hundredth of the amount that a patient receives when they undergo total body irradiation to ablate bone marrow during cancer therapy, although the radiation quality in space poses unknown carcinogenic risks. In the figure below, circles embedded in dashed lines indicate the mean and error of a calculated average, a line with two circles at the ends indicates a specific range, a single arrow indicates an open-ended range.



There are many other types of biological processes that elicit similar effects to IR, such as the chronic low-level production of ROS that is generated as a consequence of oxygen metabolism. Both normal homeostatic and stress-activated cellular processes are affected by altered oxidative stress, which influences the regulation of protein kinases and thereby links external stimuli with signal-transduction pathways. Disruption of the balance between pro-oxidants and anti-oxidants can result in a state of oxidative stress that can promote several pathological conditions, including those associated with ageing and cancer. It remains to be determined what levels of acute or chronic irradiation exceed the capacity of a given tissue to maintain homeostasis. Lessons learned from other processes that generate high levels of ROS, such as inflammation and ischaemia/reperfusion, can be useful in identifying potential IR-activated signals.

For the purposes of brevity, we will highlight transforming growth factor-β (TGFβ; BOX 2) as one mediator of the IR response that is found in the microenvironment. TGFβ is involved in, and is an important mediator of, the microenvironment's response to IR. This has revealed new facets of TGFβ biology and is an example of how extracellular signalling orchestrates multicellular responses to damage that occurs at the molecular level.

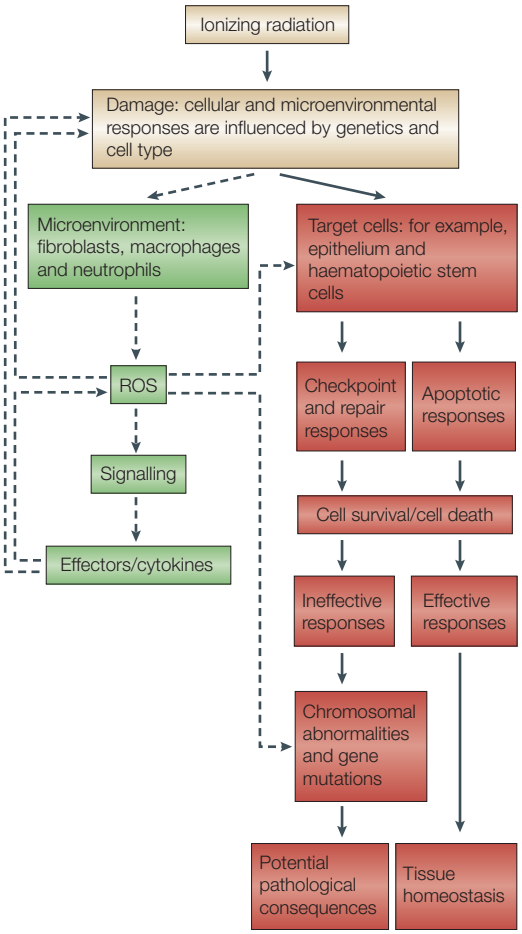


Figure 1 | **Ionizing radiation, the microenvironment and cellular responses.** Ionizing radiation damages both the tissue microenvironment (green boxes) and tumour/target cells (red boxes) — each of these components has a different effect on tissue homeostasis. In target cells, such as epithelial and haematopoietic stem cells, ionizing radiation activates cell-cycle checkpoints and apoptotic programmes. When these processes are ineffective and target cells survive, they can propagate chromosome abnormalities and mutations that lead to tumorigenesis. Concomitantly, cells in the microenvironment, such as fibroblasts and immune cells, respond to ionizing radiation by altering their production of soluble growth factors, cytokines, reactive oxygen species (ROS) and extracellular matrix proteins. These signals normally have effects on damaged target cells to limit neoplastic potential. However, alterations in these activities can promote tumour formation through the pathways that are indicated by dashed lines. For example, in response to ionizing radiation, fibroblasts and macrophages can permanently arrest in an activated state that continuously generates growth factors and ROS — these can affect the function of not only normal epithelial and haematopoietic cells, but also factiliate tumorigenesis by cells that carry genetic alterations. These signals from the microenvironment can persist for long periods (that is, weeks to months), contributing additional damage or perturbations that promote malignant phenotypes.

The response of the microenvironment

The behaviour of individual cells is dictated by their interactions with each other, such that tissue function is the result of a coordinated multicellular behaviour. A multicellular unit of function, such as the epithelium of a particular tissue, integrates diverse signals through the microenvironment. This microenvironment-mediated control is particularly important for stem cells, where combination and interactions with stromal elements provide the STEM-CELL NICHE[2,3]. In the steady state, locally acting signals that originate from the microenvironment maintain most stem cells in proliferative quiescence. For the purposes of this discussion and its focus on cancer, we will consider the radiation responses of the microenvironment separately from the responses in 'target cells' (that is, potentially tumorigenic parenchymal cells). Most human cancers arise from epithelial or haematopoietic cells, so 'non-target' stromal cells include cell types such as all variants of fibroblasts, endothelial cells, adipocytes, pericytes, myofibroblasts, cells of the immune system and inflammatory cells. The microenvironment of a given target cell is defined as the range of such cells and their products — the ECM and soluble signals (including growth factors, cytokines and chemokines) — that can function on the target cell. Cells from the microenvironment can regulate the parenchyma by interacting directly (cell–cell contact) with target cells and/or by secreting regulatory molecules that stimulate or inhibit target-cell proliferation and differentiation.

Many cytokines have been shown to be induced by IR. The most significant among them are epidermal growth factor (reviewed in REF. 4), pro-inflammatory cytokines (reviewed in REF. 5) and fibroblast growth factor (reviewed in REF. 6). Furthermore, activation of TGFβ is an early and persistent event in tissues that have been exposed to both high and low doses

DIRECT EFFECTS
Interaction of energy with matter, resulting in ionization.

INDIRECT EFFECTS
Interaction of energy with water, resulting in production of reactive oxygen species.

X-RAYS
Sparsely ionizing radiation that has similar effects to γ-rays, and is used in radiotherapy. X-rays result from electron energy-transitions with the atom or through the deceleration of high kinetic-energy electrons.

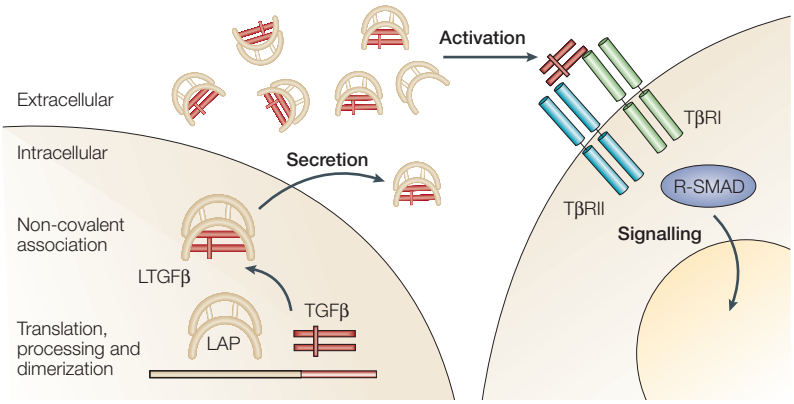
γ-RAYS
Sparsely ionizing radiation that has similar effects to X-rays, and is used in radiotherapy. γ-Radiation results from excited and unstable nuclei of radioactive materials.

STEM-CELL NICHE
The restricted, specialized microenvironment that mediates stem-cell expansion and differentiation.

Box 2 | **Biology of transforming growth factor-β**

Transforming growth factor-β1 (TGFβ1) is a multifunctional mediator of both homeostasis and injury responses. TGFβ regulates the fate and function of multiple cell-types by controlling both proliferation and apoptosis. It also regulates the character of the microenvironment through the control of extracellular matrix deposition and composition[89]. All cells express receptors for TGFβ (TβRI and TβRII in the diagram). The biological activity of TGFβ1 is constrained by its secretion as a latent complex (LTGFβ), which consists of TGFβ non-covalently associated with its processed amino-terminal pro-segment, the latency-associated peptide (LAP). Once LTGFβ is secreted by cells, TGFβ must be released from LAP before it can bind to its cell-surface receptors. Interaction of TGFβ with its receptors activates signal transduction pathways, mediated by R-SMAD, which initiate a tissue-wide response to damage in several physiological processes. TGFβ activation often amplifies the events that are associated with the release of TGFβ from LAP, thereby perpetuating its bioactivity.

The LTGFβ complex is very stable. In solution, the protein requires a pH of below 3 or above 11, or high heat, to release TGFβ (activation)[90]. Physiological activation mechanisms include protease cleavage and integrin-mediated disruption of the latent complex. Ionizing radiation also induces the release and activation of TGFβ in both tissues and cells. Studies into this mechanism showed that oxidation of recombinant LTGFβ by reactive oxygen species in a cell-free system causes the release and activation of TGFβ1 (REF. 12). In comparison to other activation mechanisms, this redox activation is unique in that it occurs independently of any other protein or cell requirement. More importantly, with regard to the understanding of TGFβ biology, this mechanism endows TGFβ with the ability to function, respectively, as a sensor and signal of oxidative stress. The wide distribution of LTGFβ1 would allow oxidative stress to elicit tissue-wide TGFβ activation, which would orchestrate cellular responses to damage (FIG. 1) as well as systemically recruit additional cells that mediate tissue repair.



of IR[7–9]. TGFβ1 is secreted in a latent complex[10] that is widely distributed throughout the microenvironment. The ECM therefore serves as a reservoir for this cytokine[11]. A protein redox switch activates latent TGFβ1, allowing it to function as an extracellular sensor of oxidative stress[12] (BOX 2). In addition to its role in homeostatic growth control, TGFβ has a more complex role in regulating tissue responses to damage, the failure of which could contribute to the development of cancer. The most intriguing of the recent mouse models of TGFβ function is a fibroblast-specific conditional knockout of TGFβ signalling created by Moses and colleagues. Mice with a fibroblast-specific knockout of the TGFβ type II receptor rapidly develop epithelial tumours[13]. The development of intraepithelial neoplasia in prostate tissues and invasive squamous-cell carcinoma of the forestomach were accompanied by an increased abundance of stromal cells. The authors suggest that this is partly owing to the dysregulation of hepatocyte growth factor production, but it is clear that TGFβ signalling functions both directly and indirectly to suppress tumorigenesis. Furthermore, studies using *Tgfβ1*-knockout mice have shown that IR-induced epithelial apoptosis and phosphorylation of p53 are severely compromised[14]. This indicates that radiation-induced extracellular signalling has a direct and crucial impact on the cellular response to DNA damage.

Studies of irradiated tissues have shown that the stem-cell compartment is most sensitive to damage, as shown by the selective apoptosis of these cells in response to IR (reviewed in REF. 15). It has been postulated that this mechanism might serve to eliminate potentially neoplastic cells from the organism. However, in some tissues there is a regenerative recruitment of progenitor cells to re-establish the stem-cell compartment. How does the irradiated microenvironment contribute to the regulation of stem cells? In the intestine, TGFβ concentrations have been observed to increase in the proliferative zone where stem cells reside[16], and this cytokine has been postulated to regulate the exit of cells in the intestinal crypt from the cell cycle and their subsequent differentiation[17]. Furthermore, treatment of the intestine with TGFβ3 reduces loss in the sensitive stem-cell compartment following irradiation[18]. These data indicate that one function of radiation-induced TGFβ is to protect the stem-cell compartment.

In the haematopoietic system, the orderly production of blood cells is regulated by feedback mechanisms that involve complex interactions between the stem cells and progenitor cells, and the products of their microenvironment. Following exposure to moderate doses of IR, locally acting signals stimulate stem-cell proliferation, resulting in re-population of the depleted haematopoietic system. Following this, inhibitory signals restore proliferative quiescence[19]. IR-induced granulocyte-colony-stimulating factor (G-CSF) and granulocyte–macrophage-colony-stimulating factor (GM-CSF) elicit recruitment of haematopoietic progenitor cells from the peripheral blood[20]. Consistent with the requirement for a context-specific microenvironment, a combination of anti-apoptotic cytokines provides radioprotection when given shortly after lethal irradiation[21]. Indeed, the concept that, rather than being an outcome of DNA damage, bone marrow failure following lethal IR doses might be the consequence of an IR-induced microenvironment or, alternatively, the failure of a normal microenvironment, is supported by the rescue of lethally irradiated mice by soluble factors or non-irradiated stromal cells[22].

IR-induced inflammation

There are many examples in which inflammation, through production of ROS and/or reactive nitrogen species (RNS) by tissue macrophages or neutrophils,

results in collateral damage in parenchymal cells to promote tumorigenesis[23]. Recent studies have shown that IR induces the accumulation of macrophages that have the phenotype of activated phagocytes, accompanied by MARGINATION and the infiltration of tissues by neutrophils[24,25]. These are classical signs of an inflammatory response.

The ability of IR to induce inflammation might contribute to its carcinogenic activity. Consistent with this, acute myeloid leukaemia is reproducibly induced by irradiation of mice but not when germ-free mice are irradiated under sterile conditions. Transferring the mice to conventional housing restores the inducibility of the leukaemia[27]. In another study, the induction of inflammation did not affect the incidence of myeloid leukaemia in non-irradiated mice, but significantly increased the incidence of leukaemia in irradiated mice[26]. These studies clearly implicate inflammation as a microenvironment-based component of IR-mediated leukaemogenesis.

Inflammation-associated increases in ROS production can increase the rate of DNA mutation while simultaneously altering the secretion of cytokines by macrophages — this can compromise normal immuno–haematopoietic regulatory circuits. There is direct evidence that such changes occur and persist, as the Japanese survivors of the atomic bomb still experience sub-clinical levels of inflammation 50 years after exposure to IR[28,29]. Taken together, the experimental and clinical findings of a pro-inflammatory response to IR provide a plausible mechanistic framework for understanding the observations of persisting CLASTOGENIC factors that are characteristic of ongoing oxidative processes in the peripheral blood after various exposures to IR[30].

IR-induced genomic instability is defined as non-clonal DNA damage that arises or increases several cell-generations after exposure to IR. It is characterized by a number of delayed adverse responses, including chromosomal abnormalities, gene mutations and cell death. Wright and colleagues showed that chromosomal instability occurs in the clonal descendants of haematopoietic stem cells after irradiating mouse[31] or human[32] bone marrow with α-PARTICLES. Because cells that are irradiated by α-particles are defined by a Poisson distribution of individual particle traversals, there is an inevitable proportion of non-irradiated cells in the surviving population. The calculated expected proportions of irradiated and non-irradiated cells indicate that the number of clonogenic cells that transmit chromosomal instability is greater than the number that are expected to survive being hit by the particles. This observation raised the possibility that IR-induced genomic instability *in vivo* might not only result from direct DNA damage by IR, but also that the ongoing production of damaged cells might result from the production of ROS/RNS or pro-inflammatory cytokines by macrophages and/or other cells within the tissue[25,33]. This was tested in cultured cells by interposing a grid between cells and the α-particle source, shielding some cells from irradiation. A comparison of cells cultured together, with or without the grid, revealed similar levels of genomic instability — α-particle-induced chromosomal instability occurred in both the progeny of irradiated and the shielded, non-irradiated stem cells. These studies showed, unexpectedly, that instability can arise from interactions between irradiated and non-irradiated cells[34].

Macrophage activity is controlled by specific signals that stimulate their development into discrete phenotypes, differing in terms of receptor expression, effector function and cytokine production. The T-helper-1-type, interferon-γ-dependent classical activation of macrophages is a well-recognized feature of cellular immunity, and these 'M1 macrophages' secrete various cytokines, such as interleukin 1(IL-1), IL-6 and tumour-necrosis factor-α (TNF-α), as well as ROS and RNS[35]. However, macrophages can also be activated by the T-helper-2-type cytokines IL-4 and IL-13. This produces a distinctive macrophage phenotype that is associated with tissue repair, TISSUE REMODELLING and immunoregulation — these macrophages are classified as 'alternatively activated' or 'M2 macrophages'[35–38]. However, macrophages show considerable heterogeneity in their phenotypes and effector functions, and the M1 and M2 phenotypes reflect the extremes of a continuum of activation states[39]. Interestingly, the genotype dependency of the expression of delayed cytogenetic damage post-irradiation[33] correlates with M1-type and M2-type responses of genotype-dependent macrophage phenotypes in irradiated tissues[25,40]. These findings offer new insights into the signalling processes underlying the genotype-dependent expression of IR-induced chromosomal instability and its potential contribution to genotype-dependent IR-induced malignancy.

There are also significant differences in the expression of IR-induced chromosomal instability when *in vitro* or *in vivo* sources of the same types of haematopoietic cells are compared[41]. The irradiation of cells *in vivo* results in fewer cells with chromosomal defects, and far fewer chromosome abnormalities per cell, compared with *in vitro*-derived irradiated cells. One possible explanation for the difference might be the presence of *in vivo* tissue-defense mechanisms that recognize and remove aberrant cells. Apoptosis is generally regarded as a non-inflammatory process[42,43] and so would reduce the number of damaged cells *in vivo*. However, in some circumstances, such as the acute injury response that occurs following IR, the resulting enzymatic activity associated with phagocytosis of damaged cells can increase the release of inflammatory cytokines as well as DNA-damaging free radicals[44–46]. Wright and colleagues have postulated that the delayed appearance of genomically unstable haematopoietic cells in irradiated mice is consistent with a long-lived tissue reaction to injury by irradiation that is characteristic of an inflammatory response acting in a 'bystander' fashion[25].

Radiation-induced carcinogenesis

Various studies have shown that IR leads to a rapid, global and persistent activation of the microenvironment. So, it has been postulated that the microenvironmental changes that are induced by IR could

MARGINATION
Blood vessels outlined with cuffs of neutrophils.

CLASTOGENIC
Biological factors that increase markers of DNA damage such as mutation frequency, chromosome aberrations or sister chromatid exchange.

α-PARTICLES
Short range, high linear-energy transfer radiation from isotopic decay that gives rise to clusters of ionized molecules.

TISSUE REMODELLING
Activation process of extracellular matrix (ECM) degradation and production that affects the turnover and composition of ECM proteins. This process accompanies wound healing, inflammation and large scale apoptotic events, such as mammary gland involution. It can be induced by ionizing radiation, either subclinically or at a pathological level, preceding fibrosis.

promote the progression of pre-existing initiated cells to malignancy. Therefore, the ability of IR to alter the stroma would be considered a third class of carcinogenic action that is distinct from mutation or mitotic stimuli[47]. This hypothesis of IR action is supported by the emerging concept that cells other than epithelial cells, and mechanisms other than genetic alteration, influence the process of carcinogenesis.

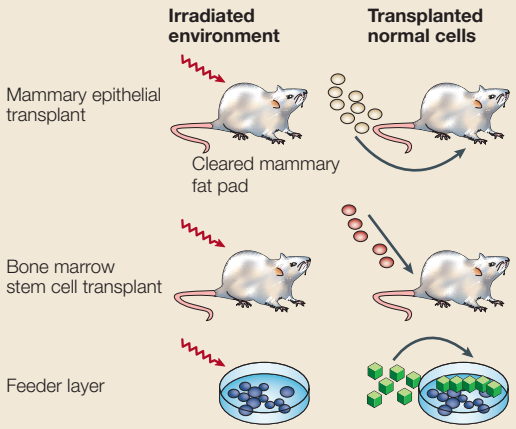
The effect of an irradiated microenvironment on tumour progression has been tested by creating radiation chimeric models or tissues (BOX 3). To test whether the IR-induced microenvironment alters breast cancer progession, M.H.B.H. took advantage of the postnatal development of the mouse mammary gland. Non-irradiated mammary epithelial cells that harbour a mutation in both alleles of the *Trp53* gene are often weakly tumorigenic if implanted subcutaneously in nude mice or in 3-week-old mammary fat pads[48]. The frequency of tumour formation increased fourfold when mutant-p53 mammary epithelial cells were transplanted to fat pads of hosts that had been irradiated. Furthermore, the irradiated stroma increased the ability of these cells to establish tumours even as long as two weeks after exposure to IR. Furthermore, these tumours were biologically distinct as they were considerably larger than the few that arose in non-irradiated hosts. As hemi-body irradiation resulted in tumorigenesis by the implanted p53-mutant cells only on the irradiated side, this effect seems to be primarily due to an altered stromal microenvironment. Similarly, an immortal myogenic cell line forms tumours far more rapidly in irradiated than in non-irradiated host muscle[49]. Although the effect of pre-irradiation on tumour formation was persistent and dose-dependent, the cells were still capable of forming large amounts of muscle when re-implanted into a non-irradiated muscle. Yuan and colleagues used three-dimensional co-culture to model the interactions of irradiated fibroblasts with mammary epithelial cells[94,95]. The authors conclude that chronic IR exposure induces a senescence-like phenotype that significantly perturbs mammary ductal morphogenesis. Upregulation of multiple secreted matrix metalloproteinases by irradiated fibroblasts causes epithelial cells to grow into enlarged cystic structures, which develop further and become disorganized cell masses on inactivation of cellular death pathways. Furthermore, breast cancer cells grown in association with irradiated fibroblasts show increased malignant behaviour and growth.

When considering stromal influences on tumorigenesis, another relevant observation is the uncommon, but well validated, development of leukaemia in donor ALLOGENEIC bone marrow cells following transplantation into patients with leukaemia or aplastic anaemia. This indicates that the transplant recipient's bone marrow stroma either elicits a malignant phenotype or induces transformation[50]. A role for factors produced by the bone marrow stromal microenvironment in haemato-poietic malignancy is supported by experimental studies in which irradiated stromal cells have been shown to aid the survival of irradiated stem cells and contribute to the selection and proliferation of a malignant clone[51,52] (BOX 3). The frequency of transformation of non-irradiated growth

ALLOGENEIC
When donor and recipient tissues share the same major histocompatibilty complex antigens.

Box 3 | **Radiation chimeric models**

To examine how radiation-induced signalling from the microenvironment affects putative target cells, several *in vivo* and cell culture 'radiation chimaera' models have been studied. These allow investigation of how the crosstalk between irradiated and non-irradiated tissue compartments affect function. The female mammary gland is unique among glands in that the epithelium develops postnatally from a rudiment that can be removed from the inguinal glands at approximately 3 weeks of age. Surgical removal of the parenchyma results in a gland-free mammary fat pad, referred to as a 'cleared fat pad', which is suitable for receiving donor tissue at the time of clearing or later[91]. Transplantation of normal mammary epithelial cells or tissue fragments produces ductal outgrowths that fill the fat pad and are nearly indistinguishable from an intact gland. In addition, mouse mammary epithelial cell lines, like the COMMA-D cell line, retain the ability to proliferate and undergo ductal morphogenesis *in vivo*[92]. Mice with cleared mammary glands can therefore be irradiated and then receive normal COMMA-D cells, to study the effects of the irradiated stroma on normal cell function (see figure)[48]. Most functional tests of bone marrow stem cells have used ionizing radiation to ablate the autologous bone marrow before transplantation of donor bone marrow or putative stem cells. These models can also be used to study the effects of the irradiated bone marrow microenvironment on haematopoietic stem cell function and leukaemogenesis. Long-term *in vitro* culture of bone marrow cells requires the presence of bone marrow stromal cells — these are frequently irradiated to mimic autologous bone marrow transplantation[93]. Studies in this cell system have shown that irradiation prevents stem cell replication but stimulates the production of growth factors and cytokines that can promote differentiation or proliferation.



factor-dependent stem cells is significantly increased by co-culture with irradiated bone marrow stromal cell lines[53] or by transplantation into irradiated SYNGENEIC mice[54,55]. These effects seem to be because of the activation of signalling pathways that alter cell adhesion and increase the production of cytokines and growth factors such as TGFβ and/or nitric oxide by the irradiated stroma, resulting in the production of high levels of ROS by the co-cultured haematopoietic cells[56]. TGFβ has also been shown to actively promote metastasis in carcinoma models[57]. Increased TGFβ production can elicit altered stromal phenotypes that promote neoplasia. TGFβ-induced conversion of fibroblasts to myofibroblasts can break down tissue barriers to malignant invasion[58,59]. Radiation-induced myofibroblast differentiation[60], which is TGFβ-dependent, could function in a similar manner. So, TGFβ activation by IR might promote, rather than limit, pre-malignant tumour growth in some contexts.

Tissue microenvironments that foster neoplastic behaviour are also observed following exposure to other agents, as a result of physiological processes, or by transgenic manipulation. Different carcinogens vary by the extent to which the stroma mediates their carcinogenic and mutagenic potential. Zarbl and colleagues have shown that mammary tumours that arise in rats after *N*-nitroso-*N*-methylurea treatment facilitate tumorigenesis in cells with pre-existing *Hras* mutations, rather than through direct induction of new mutations[61]. Recent studies have shown that *N*-nitroso-*N*-methylurea-treated rat mammary stroma actively supports the malignant progression of non-exposed epithelial cells[62]. By contrast, another carcinogen, 7,12-dimethylbenzanthracene, does not promote tumour progression when only the stroma is exposed[63].

Whether these differences are a function of the model, the species or the carcinogen remains to be determined. Experimental models have also demonstrated that carcinogenesis is increased by wounding[64], overexpression of platelet-derived growth factor[65], and misregulation of proteases such as stromelysin[66] or matrix metalloproteinase 2 (REF. 67). Such events are non-mutagenic but seem to promote neoplasia by releasing the suppression of malignant target cells. Understanding how the perturbation of stromal–epithelial interactions by IR and other carcinogens contributes to malignancy should provide new strategies for decreasing the risks of accidental, occupational and therapeutic exposure.

Radiation therapy

The mechanisms that control the therapeutic efficacy of IR have classically focused on the ability of IR to kill cancer cells while sparing normal tissues. However, there are several lines of clinical evidence that support the role of tissue remodelling and multi-cellular responses to IR as a significant mechanism of clinical effect.

For breast cancer, IR therapy is used in conjunction with surgery to dramatically reduce the risk for local recurrence within the primary site. After radiotherapy, the risk for true recurrent tumours — that is, those that are derived from the primary tumour — is initially the greatest within the first few years after treatment. However, there is a persistent risk for true local recurrence of 1–2% per year even years later. So, when true local recurrence occurs, it indicates that the original cancer cells have remained in a quiescent state within the irradiated tissue. The mechanism by which this occurs is not well understood. Analysis of breast tissue specimens from patients who have undergone radiation therapy indicates that these mechanisms are not limited to an acute time frame or restricted to cancer cells[68–70]. Significant and persistent remodelling of ECM composition occurs after IR exposure. Animal studies demonstrate rapid and chronic loss of hyaluronic acid[71], induction of collagen remodelling[72], as well as persistent changes in collagen production by irradiated cells[73]. Epithelial abnormalities, as well as atypical stromal fibroblasts and vascular changes in irradiated breast, indicate that the response to IR involves all tissue compartments. Furthermore, residual cancer cells are often observed in irradiated tissues[74]. So, why do these not form tumours? It seems that the therapeutic effects of IR result not only from the susceptibility of cancer cells themselves to this treatment, but also from the its formation of a stroma that is non-permissible for tumour re-growth[75].

Radiation therapy induces global changes in tissues that may prevent seeding or growth of tumours. One study showed that women with breast cancer who had not yet experienced metastatic disease and who received bone radiation to mid-thoracic vertebrae subsequently developed fewer vertebral lesions compared with women who did not receive radiation[76]. Studies in patients with prostate cancer also demonstrated dose-dependent IR-induced changes in bone that decreased the risk of metastasis[77].

The microvasculature is essential for tumour growth and has been widely discussed as a crucial target in controlling tumorigenesis. Recent studies by Fuks and colleagues indicate that the endothelial response to IR determines its effects in both normal tissues and tumours[78,79]. The loss of endothelial cells following high-dose irradiation in an experimental mouse model was required for normal-tissue toxicity in the gut as well as for tumour control, and could be modulated by basic fibroblast growth factor production. The role of the endothelial response in IR-mediated tissue toxicity is not well understood, but might be a target for manipulation to improve the response of normal tissue to radiation.

The effects of IR on the normal tissue microenvironment limit the radiation dose that can be applied to tumours. Several experimental models, however, support the concept that IR-mediated toxicity might be decreased by limiting TGFβ signalling. Studies in rodents have shown that inhibition of TGFβ signalling can limit the amount of IR-induced damage that occurs in normal tissues[80–82]. Limiting the predominantly stromal production of TGFβ during radiotherapy seems to block the deleterious cytokine cascades that stimulate inflammation and stromal remodelling[72,83].

SYNGENEIC
Genetically identical — for example, fully inbred mouse strains.

Future directions

In cancer, as in life, understanding the context is the key to understanding the consequences. An active role for the microenvironment during carcinogenesis has recently gained prominence, both because it reveals new aspects of tissue biology and because it represents a novel target for prevention and therapy. It seems that the rapid and dynamic cellular effects that occur in irradiated tissues are the result of a microenvironment-mediated damage-response programme[84,85]. This programme coordinates individual cell responses through extracellular signalling. In normal tissues, extracellular signals that are induced by IR have the potential to modify the risk of carcinogenesis by eliminating damaged cells and suppressing neoplastic behaviour. However, in some animal models that are exposed to significant doses of radiation there is evidence that genomic instability and carcinogenesis might be augmented when the cellular response to IR disrupts or persistently alters communication between cells or among different cell-types (reviewed in REFS 1,86–88).

The challenge for the future is to understand not only how the tumour co-opts normal cells to support growth, but how tissues contend with damage that might induce or expand neoplastic potential. The contribution of microenvironment signalling to radiation effects at high (that is, therapeutic) doses could function, in part, by modifying the local tissue to impede tumour recurrence or metastasis. At low (that is, environmental, diagnostic and occupational) doses or under chronic exposure conditions, the microenvironment might provide a potential offset of direct DNA damage by IR. Placing radiation damage at the cellular level into the context of a dynamic multicellular system will provide a better basis for predicting radiation health effects in humans.

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Competing interests statement
The authors declare no competing financial interests.

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