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Acknowledgements

The authors would like to acknowledge the participants of the National Cancer Institute Diagnostic Criteria for Hereditary non-Polyposis Colorectal Cancer and Microsatellite Instability meeting, which led to the development and publication (reference 6) of the revised Bethesda guidelines.

Competing interests statement

The authors declare that they have no competing financial interests.

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OPINION

Radiation-induced bystander effects — implications for cancer

Carmel Mothersill and Colin B. Seymour

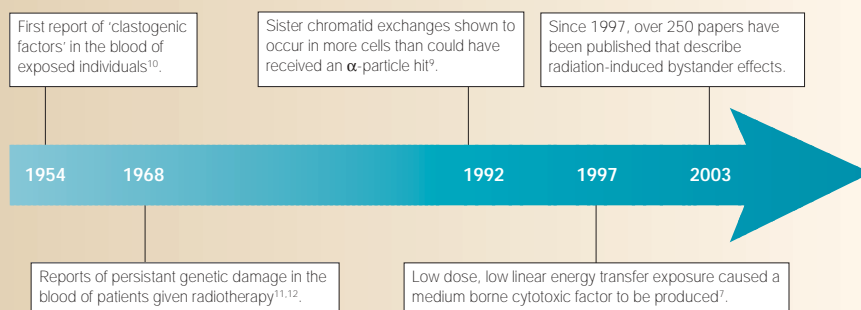
The term radiation-induced bystander effects describes a situation where cells that have not been directly exposed to ionizing radiation behave as though they have been exposed: they die or they show chromosomal instability and other abnormalities. The bystander cells might be immediately adjacent or might be some distance away from the exposed cell. Although the nature of the communication system that is involved in producing these responses is not yet known, there is strong evidence for a chemical signalling process that transmits information from the irradiated cell to neighbouring cells. The bystander effect has several important implications for radiation protection, radiotherapy and diagnostic radiology.

Radiation-induced bystander effects (RIBE) have been described in the literature as far back as 1954, when it was reported by Parsons that factors that cause damage to chromosomal structures —

clastogenic factors — could be detected in the blood of irradiated patients¹⁰ (see TIMELINE). RIBE are effects that occur in cells that have not themselves been irradiated, but that have received a signal from an irradiated cell. As a result of this signal, the unirradiated cell might behave as though it had been irradiated — by dying or showing signs of genetic instability (BOX 1).

The growth in research investigating these effects has been exponential since the late 1990s. Advances in the related field of radiation-induced genomic instability (which can be defined as high levels of non-clonal mutations in the progeny of apparently healthy cells that have survived radiation exposure) and the recent data concerning dose thresholds for DNA repair have led to a paradigm shift from DNA-damage/mutation-centred radiobiology and radiation carcinogenesis to a recognition that the target for radiation damage is not only DNA. At low doses, which are of key concern for carcinogenesis, the nature

Timeline | Key events in the study of radiation-induced bystander effects



This Timeline shows how radiation-induced bystander effects were documented in the literature as early as 1954, but were not integrated into mainstream radiobiological studies until over 40 years later.

might need to be re-structured, both conceptually and in practice, with far more emphasis on individual susceptibility and environmental or lifestyle factors.

In the therapeutic arena, the biological effects of the radiation beam will cover a wider area than the physical beam, and the concept of a biological penumbra might need to be considered (FIG. 2). New multi-field radiotherapy treatments such as intensity-modulated radiotherapy (IMRT) might also need to be reviewed in the light of bystander effects. These treatments increase the number of radiation fields, so decreasing the size of the dose to some normal tissues, but having the effect of increasing the volume of tissue that is exposed to low levels of radiation. As a result of RIBE, the effect of this increase in irradiated-tissue volume cannot be predicted by the current LNT hypothesis, which only relates dose to effect and not irradiated volume to effect. The bystander effect might also have implications for diagnostic radiology, as it occurs at doses that might be exceeded during complex investigations.

There might also be implications for fractionated-radiotherapy schedules, as the direct (irradiated cells) and indirect (bystander cells) effects should be independent. It should be possible to develop different types of drugs, novel radiation sensitizers and protective agents to modify the two types of response, as direct and indirect effects of radiation are mechanistically different and therefore provide new intervention pathways. In each fraction of radiotherapy given there will be a combination of direct and bystander effects, each controlled by different mechanisms. It is important to work out the relative contribution of each mechanism to overall outcome and to develop drugs that are targeted specifically at each mechanism.

of the response to radiation, which is determined by a combination of genetic and epigenetic factors, is now considered by many radiobiologists to be as important as dose. At low doses (doses below 0.5 Gy), actual dose seems to be irrelevant. This is important in radiation-protection terms, as it is at odds with the traditionally accepted linear relationship between cause and effect.

Radiation protection is a scientific field that seeks to predict biological effects of low doses of radiation by extrapolating from known epidemiological data sets that mainly relate to high-dose effects. The main source of information is from data that have been collected about the Japanese atomic bomb survivors. Standards and guidelines regarding acceptable doses to the general public and to radiation workers are developed and reviewed by assuming a linear no-threshold (LNT) hypothesis, which relates dose to biological effect. The LNT hypothesis states that the dose–effect relationship is linear even at very low doses, meaning that, in theory, the lowest dose imaginable has a finite probability, however small, of causing a biological effect. No threshold below which radiation has no effect is assumed in this hypothesis. All types of radiation are regulated in this way and certain weighting factors (radiation-quality factors) are used to account for the differences in the damaging properties of the different radiation types. All radiation exposures to individuals are limited using the guidelines, except medical exposures (diagnostic and therapeutic), where the benefit to the individual is assumed to outweigh the potential risk. The effect of a radiation dose no longer depends on the amount of energy deposition, but, instead, on the cellular response to that energy deposition; that is, how much and what kind of signal is generated and, in turn, how the bystander cells

respond to this signal. In practice, therefore, bystander effects challenge some precepts of the LNT hypotheses.

Limiting radiation-induced cancer is a key aim of radiation-protection guidelines. Consideration of RIBE in this context is important, because their existence means that there is no direct correlation between the number of cells that are exposed to radiation and the number of cells that are at risk of showing effects such as mutation, chromosomal damage or apoptosis. Instead, any ultimate effects depend on complex, and potentially modifiable, interactions between the irradiated cell and the bystander cells (FIG. 1). There is no longer any single cell that is at risk from radiation damage; instead, the risk is spread among bystander cells. So, the consequence of the bystander effect is that the target model of radiation damage — which assumes that there is a specific target with which radiation interacts — is not tenable at low doses. Therefore, a simple dose–effect relationship cannot be sustained. One implication is that radiation protection

Box 1 | The bystander effect — an analogy

Imagine a lecturer in an old-fashioned tiered lecture theatre, full of attentive students. The lecturer turns the lights off, and throws oranges into the audience. When the lecturer puts the lights back on, she notices that some of the audience members are bruised. The lecturer makes the assumption of cause and effect — that the bruised audience were hit by the fruit. She works out a whole theory of risk estimates, based on the probability of being an audience member hit by an orange. At some stage a student attempts to replicate the experiments, but leaves the lights on. She notices that some of the audience members hit by the oranges are completely undamaged, but that when they are hit by the orange they throw their arms out wide, injuring their neighbours. These injured neighbours, in turn, might (or might not) strike out at one or both of their neighbours, introducing a response that is not directly proportional to the amount of fruit thrown. The student establishes that the bruised audience members are bystanders that have not been hit by an orange, and so the risk is no longer directly proportional to the number of oranges thrown. This is analogous to the radiation story, where at doses of radiation below 0.5 Gy most radiation effects are expressed in bystander cells. At higher radiation doses there is a complex mix of both direct and indirect effects.

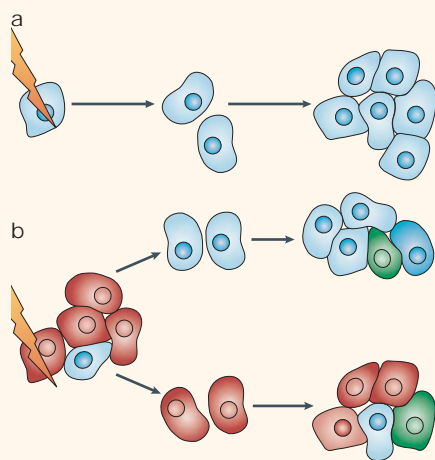


Figure 1 | Two views of how radiation effects are perpetuated. a | In the traditional clonal view, all the damage is introduced into the cell by the actual traversal of the nucleus by a track of ionizing radiation. If the track causes a mutation, this is passed to all progeny. **b** | In the new hypothesis, many different effects such as chromosome damage and mutations are induced in cells that are not hit directly by the radiation beam, and these effects are often not apparent until several generations after the exposure.

In the 1990s, key papers were published showing that cell cultures that were exposed to very low doses of α particles — large, heavily charged particles that can cause ionization — produced more damage than was attributable to the dose itself^{1,2}, and that filtered medium harvested from radiation-treated cells could cause cell death in cultures that had not been exposed to radiation³. It is now known that, in addition to cell death, carcinogenesis and cell mutation can be caused in these unexposed cell populations^{4,5,6}. Bystander cells seem to be the population at risk of delayed effects, such as genomic instability, which are associated with many cancers^{7,8,9}. Organs or individuals who are exposed to low-dose radiation, either environmentally or from scattered radiation from a therapeutic or diagnostic beam, or cells on the edge of a radiotherapy radiation field, are at the highest relative risk of bystander effects. This is because the percentage of cell death that is attributable to the direct effect of radiation increases as the dose increases, and the relative importance of the bystander effect, which saturates at a low dose, decreases. Serum that is harvested from patients with cancer who have been treated with radiotherapy also causes cell death and clastogenic effects in unexposed cells in culture^{10,11,12}. The molecules that are involved in these processes are unknown, but we have shown that exposure to

bystander factors causes high, transient increases in intracellular calcium (FIG. 3) and long-term, irreversible increases in reactive oxygen species (ROS), which are maintained in progeny^{13,14}. The bystander effect cannot be attributed to direct radiation damage to DNA, so must, in part at least, have an epigenetic component; the role of epigenetic effects in cancer is coming under increasing scrutiny.

Cell-signalling mechanisms might also be involved in RIBE, and during the transduction process, transmission of small proteins or peptides between exposed and non-exposed cells seems to occur. As the factor(s) that are responsible for the bystander effect are unknown, it is difficult to differentiate between bystander-signal production and bystander-signal response. As noted above with the calcium pulse and the ROS, it is puzzling that some effects are permanent whereas others are not. Further details of the background literature in the field can be found in recently published specialist reviews^{15,16}.

Given the intense interest in low-dose radiation exposure as a cause of cancer, and its widespread use as a diagnostic and therapeutic tool for treatment of the same disease, this article aims to explore the subject of RIBE and to consider the challenge of relating radiation dose and effect to the traditional DNA-damage/mutation-centred concept of radiation-induced carcinogenesis.

Measurement of RIBE

As the signal(s) are as yet unknown, the measurement of effect has to be indirect; that is, response has to be measured in adjacent cells or following exposure of unirradiated cells to medium from irradiated cells. Typically, RIBE are studied *in vitro* using cell cultures in one of three ways. One approach is to use a particle accelerator that is focused using microscope optics to deliver high-energy particles to a specific cell and then measure the effects in other cells in the culture. Using this approach, bystander effects have been demonstrated both in isolated and communicating cells and even in three-dimensional tissue fragments^{4,5,17}. Another method is to harvest culture medium from irradiated cells and controls and measure the effects of medium-borne signals on unexposed recipient cells^{3,18,19}. The third approach is based on probability and involves exposing cultures to very low doses of α particles, such that every cell could not receive an α particle 'hit'^{1,2}.

In vivo experimental approaches include total-body irradiation of animals followed by examination of tissues after death or of

cultured-tissue pieces *ex vivo*²⁰; observation of chromosome damage in re-populating lymphocytes following bone-marrow ablation and transplantation with opposite-sex bone marrow²¹; study of serum from patients who have had radiotherapy; and recording of out-of-field (abscopal) effects in radiotherapy.

Response can be measured as death of cells, early apoptotic cascade events, mutation, transformation to a malignant phenotype or genomic instability. There are anecdotal reports of proliferation responses but the vast bulk of the literature deals with death or mutation/chromosomal aberration responses.

Mechanisms of RIBE

The basic features of RIBE and their possible consequences are shown in FIGS 1,2. Delineating the mechanisms of RIBE and associated genomic instability is proving to be a challenge for radiobiologists. These mechanisms are highly relevant to other scientists, as they represent a conceptual shift from DNA-damage-centred radiation-induced carcinogenesis and therapy to a much more complex, integrated and iterative mechanism of carcinogenesis, where response, rather than dose, is the main determinant of outcome^{22,23,24}. It might be helpful to adopt a 'holistic' approach to the understanding of the process, at least until mechanisms and controls become clearer. For example there are many inducers of RIBE, which have many possible consequences in different cell systems. The precise consequence of a specific trigger might depend on various factors.

The mechanisms by which individual cells respond to the radiation-induced signal(s) are conventional; we understand much of the mechanistic basis of apoptosis, genomic instability and chromosomal changes. Calcium release and downstream apoptotic events can be measured^{13,14,27}. Altered levels of proteins that are associated with the above effects and with a generalized stress response have also been detected^{14,25,27}. Repair processes can modulate any or all of these responses^{3,9,25,26}. Large RIBE are seen in DNA-repair-deficient cell lines, leading to the suggestion that p53/ATM (ataxia telangiectasia mutated) pathways of cellular damage control are involved in expression of at least some bystander effects^{25,26}.

Medium transfer between responding and non-responding cell lines has clearly shown that signal production by an irradiated cell and response to that signal by a recipient cell can be distinguished as separate processes²⁰. Both

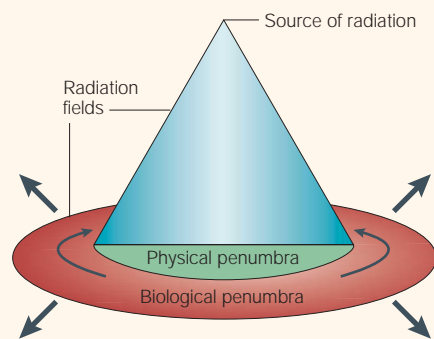


Figure 2 | Biological effects of a radiation beam. The biological effects of radiation spread beyond the field that gets directly hit by ionizing radiation tracks — known as the physical penumbra. The limits of spread — known as the biological penumbra — are unknown and might be systemic.

processes seem to be p53-independent, although there is evidence that the response in fibroblast cells might require a functional p53 pathway. Azzam *et al.*²⁷ have linked the NAD(P)H oxidase/nuclear factor of κ B (NF- κ B) pathway to the bystander effect in human lung fibroblasts. They investigated the role of oxidative metabolism in the upregulation/activation of stress-inducible signalling pathways as well as induction of micronucleus formation in bystander cells. Enzyme-activity assays indicated that exogenous superoxide dismutase became significantly associated with the cells. ROS that were apparently derived from a flavin-containing oxidase enzyme (presumably an NAD(P)H oxidase) seemed to be key contributors to the bystander-induced upregulation of p53 and WAF1 (also known as p21) as well as micronucleus formation, as evidenced by the inhibition of these effects with diphenyliodonium. Rapid activation of NF- κ B, and of several other stress-inducible signalling pathways, also occurred in bystander cells from cultures of human cells that were exposed to low fluences of α -particles.

This group clearly showed involvement of the p53 damage-response pathway in cells that were exposed to very low fluences of α particles (1cGy), whereas we have used human-papillomavirus-transfected keratinocytes, which are TP53-null, to show a pronounced bystander effect³. On the other hand, SW48 cells, which, although derived from a colon tumour, express wild-type TP53, have a very strong RIBE signal, whereas PC3 (prostate cancer) cells, which express a TP53 mutant, have a weak or absent signal. It might be that the presence of wild-type TP53 facilitates the transduction of the signal, but that in its absence, alternative p53-independent pathways transduce the signal.

The signal and the initial response. Despite many years of intense research, we still do not know what the signal is or even whether it is simply a chemical or a more complex entity/pathway. Candidate bystander factors are many and varied, but none fit all the facts. Some, such as long-lived radicals, are still too short lived; some are too large in molecular size (current estimates put the factor at less than 1000 daltons (C.B.S. and C.M., unpublished observations)); and inhibitors of other candidates do not inhibit the bystander effect. This lack of understanding of the nature of the signal precludes many approaches to understanding how it is produced by radiation.

There is now some evidence to indicate that the tissue or cell community orchestrates the response to the signal. All cells that are exposed to the signal do not undergo the same final response, but they do all seem to produce an initial response of calcium release into the interior of the cell¹⁴ (FIG. 3) and, once activated, the cells can produce signals that affect other cell populations, causing further signal production in these cells¹⁸. Strong bystander effects can be produced in epithelial cell systems and in bone-marrow cultures. Both these cell types communicate more than fibroblasts to coordinate cell function in the body. It is tempting to speculate that the production of bystander signals might be a very primitive form of a cellular-coordination response similar to that seen in colonial invertebrates and sessile plant species, which often secrete toxic or growth-controlling substances in spatially defined regions in relation to environmental threats from other encroaching colonies of the same or different species^{28,29,30}. Although reductionist approaches will, in time, sort out the 'how' of RIBE, it is likely that to understand the 'why', we will need to think and experiment inductively.

In some ways, bystander effects are very similar to cytokine-mediated effects and might involve, but do not always require, gap-junction-mediated transfer of factors from cell to cell. Signal production seems to be genetically controlled, at least in part. Certain mouse and human genotypes produce a signal that causes calcium release, apoptosis and other forms of reproductive death in reporter cells, whereas others do not produce this signal^{20,21}. *In vitro* and *ex vivo*, signal production is detected using a known responding cell line, because the actual nature of the signal is unknown and cannot, therefore, be assayed for directly. So, signal production can only be discussed

in terms of the response it produces in the cell line. Whether the same signal can produce different responses in different recipient genotypes or whether different signals, which can induce different pathways, are produced by different genotypes, remain intriguing and as yet unanswered questions.

Other unanswered questions relate to whether non-responding cells are non-responders because they do not get a signal or because they can not or do not respond to it. Some *in vitro* work indicates that there are no clear-cut answers. Clearly, if a cell lacks an apoptotic mechanism of any kind, or if that mechanism is suppressed, that cell will not respond to an external signal to undergo apoptosis. However, thresholds might also exist that define types or levels of response. A recent paper by Rothkamm and Lobrich³¹, which shows that there are thresholds of radiation dose below which DNA repair is not activated in response to radiation exposure, might be a relevant

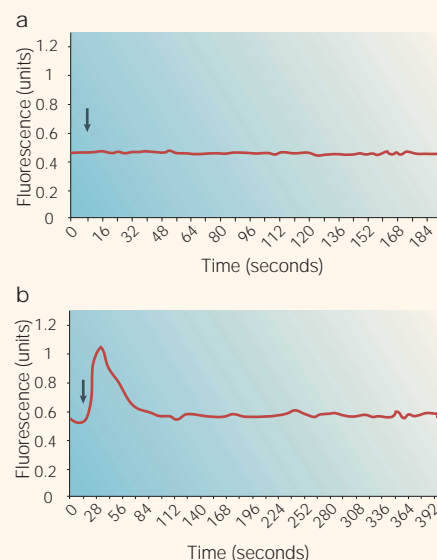


Figure 3 | Exposure of unirradiated cells to culture medium harvested from irradiated cells causes release of calcium.

a | Unirradiated human keratinocytes were exposed to medium that was harvested from keratinocytes that had not been irradiated — this is the control. No changes in intracellular calcium levels were seen; the confidence intervals (not shown) were ± 0.06 fluorescent units.

b | Unirradiated human keratinocytes were exposed to medium that was harvested from keratinocytes that had been irradiated with 0.5 Gy ionizing radiation. A rapid flux of calcium was seen within about 30 seconds of exposure. The calcium release lasted about 2 minutes; the confidence intervals (not shown) were ± 0.2 fluorescent units. The culture medium contains a factor or factors that causes this calcium pulse. Figure adapted from Ref. 39.

example. This is an interesting paper, but it is not clear whether the authors were examining direct or indirect effects. Recent experiments in our laboratory that used DNA-repair-deficient cell lines have led us to conclude that different repair pathways are involved in the modification of direct and bystander effects. Studies of adaptive responses, where a very small radiation dose can make cells more resistant to subsequent exposure to a high dose³², also need to be carefully examined for what insights they might give. Fractionating any dose of radiation into two or more doses generally causes less damage than the same dose given all at one time. However, two doses of a bystander factor that was contained in medium harvested from irradiated cells showed no such damage sparing³³.

The fundamental implication of the existence of RIBE is that they provide a new way of considering the life or death decisions that are made by individual cells in the context of survival and function of the organ as a whole and, ultimately, the organism. An understanding of mechanisms that are involved in this control of cancer at the organ level requires not only an understanding of the factors that determine life or death of the individual cell, but also of how life or death decisions at this level are communicated and impact on functional integrity at a higher level of organization.

Dose–effect relationship. Another characteristic feature of RIBE is that there is no apparent increase in effect with increasing radiation dose. In toxicology, saturable mechanisms such as this are well known, but radiation biology, and particularly radiation protection, is built on the concept of linearity. So, there is an assumption that the number of DNA lesions increases in direct proportion to the amount of ionizing energy that is deposited in the cell³⁴. This allows the extrapolation from high to low doses to be used to project risk. Non-linear responses due to repair processes are accepted, but the idea of a process that significantly affects cell death and survival but that only impacts the low-dose region of the survival curve and seems to be mainly responsible for effects in this low-dose region challenges the rationale behind radiation protection at the mechanistic level.

RIBE and inflammatory responses. Although many characteristics of RIBE are reminiscent of immune or inflammatory responses, and cytokines or cytokine-like processes are candidate bystander factors, it is important to distinguish between bystander effects

and the well-documented inflammatory responses that are seen systemically after high-dose radiotherapy. RIBE are induced by radiation doses that are below levels necessary to induce inflammation of the kind associated with tissue injury. Bystander effects are unique and important processes that do not seem to involve any of the known cytokines. In some ways, they can be thought of as analogous to a primitive immunological response, but it is important to emphasize that they remain a unique and much more generalized process. Inflammation *in vivo* is the appropriate response of specialized blood and endothelial cells to contain actual damage and mount an appropriate response. The bystander effect, however, amplifies levels of damage, which should not pose a problem for the tissue at all and spreads the effect, rather than controlling it. A key point here is whether RIBE are part of a controlled response to a damage signal or whether they are uncontrolled and insidious low-dose effects that cause widespread long-term harm. It is very important to determine whether the field is of any overall relevance to assessment of cancer risk in human populations. It is clearly a demonstrable effect of low-dose exposure, but whether it is part of a natural homeostatic mechanism or not is unknown at present.

RIBE and genomic instability. The suspected mechanistic association between RIBE and the induction and perpetuation of genomic instability is the reason that cancer researchers are getting interested in this field. In the late 1990s, it was recognized that experiments that were designed to look at RIBE also produced high levels of genomic instability. Direct evidence that cells that were affected by bystander signals were genetically unstable came from two early sources. We showed that medium from irradiated cells could cause delayed death/lethal mutations in recipient cells⁷. Lorimore *et al.*⁸ showed that chromosomal instability could be induced in cells that were shielded from lethal doses of α irradiation. The instability, therefore, had to come not from direct α -particle-induced DNA damage, but from signals from the irradiated cells. It is now suspected that bystander signals actually drive the whole process of radiation-induced genomic instability, making it an epigenetically driven phenomenon. The actual mechanism of induction and perpetuation of non-clonal genomic instability remains unknown. There is good evidence that progeny of irradiated cells maintain high levels of ROS for many cell

generations^{14,36,37}, but how this is maintained and why it is not selected out during expansion of cell populations is unknown. Even clonal expansions retain the characteristic features of the post-irradiation colony (C.B.S. and C.M., unpublished observations). This perpetuation without any sign of clonal selection can be neatly explained if all or even some of the post-irradiation population and progeny are secreting bystander signals continuously, as seems to be the case^{14,38}. Bearing in mind that these cells do not actually have radiation-induced DNA lesions, this would ensure that all cells that respond to the signal behave in a coordinated manner and this would make selection irrelevant, or at least not related in any simple way, to ‘fitness’. The signals could induce ROS in recipient cells, leading to further activation of bystander-type effects. This would have the effect of increasing the frequency of oxidative damage in DNA, which is already known to be mutagenic.

As an epigenetic perpetuator of genomic instability, RIBE will facilitate the generation of mutator phenotypes and specific mutations in the population, which could be carcinogenic. These could lead to a high chance of secondary or downstream effects such as gene amplification and microsatellite/minisatellite instabilities, which are clearly clonal and DNA-damage based.

Implications and future directions
The consideration of RIBE in risk assessment is controversial, as it requires modification of the conceptual basis for the existing methods that are used to determine radiation risk. It is no longer possible to define low-dose risk in terms of DNA damage causing a potentially carcinogenic mutation in a cell. This means that the current method of extrapolating risk from well-characterized high-dose data — such as the cohort of atomic-bomb survivors — to low-dose situations, although operationally useful, might not be conceptually sound. Which way the risk curve will go (towards higher or lower risk) at low doses is not known.

RIBE, although induced by very low doses of radiation, must itself be controlled. Risk of cancer induction by radiation does not seem to be significantly greater after low-dose exposure, although it would be hard to detect because cancer has a multifactorial aetiology. The implication of data that indicate genetic background as a principal factor in determining response is that radiation risk might now need to be analysed in terms of genetic susceptibility, and not absorbed dose. Making this change

would not only facilitate the integration of environmental protection with radiation protection, but would also open up new strategies for protection of the public and the environment from the totality of cancer-causing agents, rather than from individual agents that are each controlled separately.

For cancer therapy, the consideration of bystander effects could perhaps allow an alternative view to the hypothesis that radiation destroys cancers by introducing lethal DNA damage into clonogenic stem cells, and might lend support to nascent ideas that are based more on radiation-induced changes in the population dynamics of both tumour and normal cells. Also, these concepts, especially in radiotherapy, allow a framework for consideration of more complex biological mechanisms (for example, signal-transduction pathways, cell-membrane-associated radiation effects and intercellular communication).

There is much debate about the importance of the RIBE in radiation-induced normal-tissue damage and tumour response, and this comes from clear-cut clinical observations of infield dose-effect relationships in almost all patients (tumours and normal tissues). Although there are only occasional cases of radiation damage outside the field, there is a growing awareness among cancer physicists of vague radiotherapy-associated malaise such as fatigue, which might or might not be associated with systemic effects of radiation. The lack of marked 'out-of-field' effects does indicate a 'reset' mechanism or controlling process that can limit bystander effects at the systemic level.

The future priorities in the field are to identify the bystander factor, assuming it is a factor and not a process! Such identification would have great impact in the field of radiation protection; for example, protective inhibitors or activators of the factor in different genotypes could be developed. Adjuvant drugs for the treatment of out-of-field or systemic effects of radiotherapy could be developed. The priorities for scientists studying cancer risk from radiation are to develop new risk models that incorporate bystander effects and to show bystander effects *in vivo*, so that the implications for humans who are exposed to low doses can be assessed. Given the known genetic susceptibility of certain genotypes, it will be important to identify the genes that are involved in producing and controlling bystander effects. Other key research priorities are to determine whether the same factor is produced after γ - and α -radiation exposures and whether gap junctions are necessary for communicating bystander signals.

It is very important to determine how much of the mechanism(s) of RIBE are dictated by genetic versus epigenetic factors. For the regulators of radiation protection, it is crucial to establish whether RIBE are of any relevance at all to the assessment of cancer risk in human populations.

Nothing is known about whether bystander effects are specific to radiation or whether chemicals might also produce bystander effects. Technical problems make these experiments difficult to carry out, given the systemic distribution of most toxic chemicals in the body. With regard to radiotherapy and diagnostic radiology, it is important to assess the relative contribution of bystander and direct radiation effects using different therapy schedules and different radiation types. Most research work in the field has been done with high-energy protons, α particles and cobalt 60 γ rays, but radiotherapy and diagnostic radiology use different energies of X-rays. Finally, bystander effects occur in all the species we have looked at, from prawns to people. Worms, sponges and slime moulds communicate and coordinate multicellular activities using secreted chemicals¹⁵. This could indicate that what we now call 'the bystander effect' or something similar occurred very early in evolutionary terms and that it has a purpose that crosses species boundaries. It will be very exciting to find out if bystander signals work in other ways to promote advantageous or adverse outcomes at the tissue level and to determine whether they are indeed random killing effects or part of a complex and ancient control mechanism that facilitates adaptation to new environmental challenges.

Carmel Mothersill and Colin B. Seymour are in the Department of Medical Physics and Applied Radiation Science Unit at McMaster University, Nuclear Research Building, 1280 Main Street West, Hamilton, Ontario L8S4K1, Canada.

**Correspondence to C.M.
e-mail: mothers@mcmaster.ca**

doi:10.1038/nrc1277

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Acknowledgements

The authors acknowledge support from the following sources in Ireland and Europe: the Science Foundation Ireland; Saint Luke's Institute of Cancer Research; the Cancer Research Advancement Board; and the European Union Radiation Protection Programme.

Competing interests statement

The authors declare that they have no competing financial interests.

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