Chapter 35

# Genomic Instability and the Spectrum of Response to Low Radiation Doses

C. Mothersill, C. Seymour
*McMaster University, Hamilton, ON, Canada*

## Chapter Outline



## 1. INTRODUCTION TO LOW RADIATION–DOSE EFFECTS

### 1.1 Background to the Controversy

Few scientific fields are as divisive as the area of low radiation–dose effects. In 2005, two groups even analyzed the same data sets and came to opposite conclusions [1,2]. Accusations of "cherry-picking" to support a viewpoint abound, and sometimes it would appear that belief systems take the place of science. The purpose of this chapter is to question why this should be so? Why have we no definite answers about radiation health risks after low-dose/dose-rate exposure? Why are beliefs so entrenched? Most importantly, how can we remove the rhetoric from both sides and replace it with rational argument and scientific fact?

### 1.2 Epidemiology Is a Blunt Tool

Key to understanding the issue is to understand how we estimate risk at present and what might be wrong with this system. The perception that the system itself is wrong has led to much of the polarization, with some groups arguing that the risks are grossly overestimated and others arguing they are underestimated [3,4,5]. The first problem is that all our risk estimates use the epidemiological data from Hiroshima/Nagasaki cohorts as the "gold standard" and use cancer as the end point. The relationship is usually considered to "fit" the linear nonthreshold (LNT) relationship between dose, which is estimated and weighted using factors to correct for relative biological effectiveness (RBE) and dose rate, and effect (cancer) [6]. The A-bomb cohorts however do not give much information below 100 mGy and the exposure was acute. The dose rate and RBE correction factors are themselves highly controversial having been estimated using inbred mice or cultured cells [7,8]

**Genome Stability.** http://dx.doi.org/10.1016/B978-0-12-803309-8.00035-5

which may not represent human response or the induction of effects in genetically diverse populations in the environment [9]. There is also a huge disagreement about the doses actually received by the Japanese victims, the contribution of neutrons, the impact of genetics, war and malnutrition, and so on [10–13]. There is also a controversy about the reliability of the underlying radiobiological studies which purport to give the mechanistic support to LNT—for example, the work of Calabrese [4] suggests that there was a very big political component to enshrining and defending LNT as the way dose and effect are related. The usual representation of this relationship is shown in Fig. 35.1 where the low-dose region is depicted as having multiple possible dose–response relationships. The purpose of this chapter is not to try to resolve this debate, but rather to suggest a totally new way of looking at low-dose risk, which focuses on response rather than dose. Central to this concept is the acceptance of the paradigm shift which has occurred in low-dose radiobiology since 1990 [14,15]. While this shift is recognized in radiobiology, it has yet to be accepted as having any relevance to radiation protection [16,17].

## 1.3 Targeted and Nontargeted Effects

The key new research which is driving the paradigm shift is the recognition that in addition to targeted (direct) effects of radiation, there is a totally different set of mechanisms operating in cells which are not the result of direct damage resulting from ionizing energy deposition in DNA in targeted cells, but rather occur as a result of signaling or other mechanisms operating at the system level [18]. These so-called *nontargeted* effects (NTEs) occur in cells, organs, or organisms which have not received a direct deposition of ionizing radiation energy (see Fig. 35.2). NTEs have been extensively reviewed by these authors and many others [14,15]. They can broadly be divided into genomic instability (GI) and bystander effects with subcategories of adaptive/hormetic-type responses, generally seen as beneficial, and stress responses leading to damaging effects such as increases in mutation, related to reactive oxygen species (ROS) generally seen as adverse [18]. Fig. 35.3 represents a possible way to visualize these multiple effects.

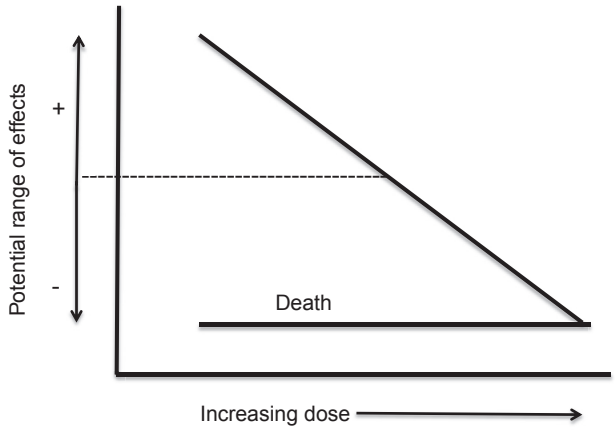


**FIGURE 35.1 The concept of a wide spectrum of possible outcomes in the low-dose region.** These may be positive or negative. The possibilities decrease as the dose increases until death is the only possible outcome.

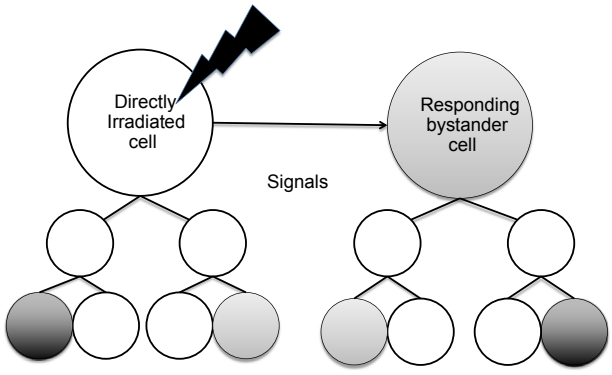


**FIGURE 35.2 Nontargeted and nonclonal effects transmitted horizontally (bystander effects) or vertically (genomic instability effects in progeny).** Light gray depicts adaptive responses and dark gray depicts stress responses.

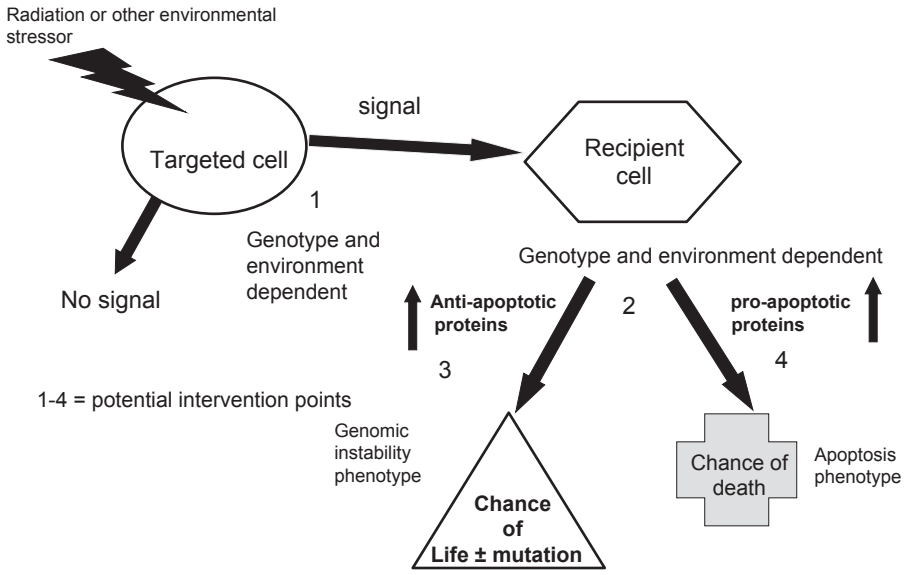


**FIGURE 35.3 Possible outcomes in the low-dose region, where nontargeted effects may or may not occur.** Numbers 1–4 indicate points where it might be possible to modulate the outcome.

## 1.4 Genomic Instability

GI is a concept that describes delayed genetic alterations observed in the progeny of the exposed cells many generations after the initial radiation insult [15,19,20]. The importance of the discovery of GI lies in the recognition that cell populations surviving radiation exposure and their progeny which show no evidence of mutations or altered fitness will not necessarily behave normally. Up to the 1980s, the central dogma in radiobiology was that all the damage was put into the cell by the ionizing radiation. If the cell was able to repair the damage and undergo successful mitosis—at least five times—then it (and its progeny) carried no residual damage and the population was as fit as if never irradiated [21]. The key paradigm changing finding which came from several independent studies was that the progeny of irradiated cells which have survived according to the earlier criteria show evidence of de novo, nonclonal effects, meaning that the damage was not induced by the initial energy deposition from radiation.

One of the first publications suggesting that the progeny of apparently recovered irradiated cells might also be at risk was published by Seymour et al. [19]. The team observed lethal mutations in the distant progeny of cells exposed to photons from a Cobalt-60 source. They suggested that the appearance of lethal mutations might require many successful generations to be expressed. Those investigations were reinforced by reports from Streffer and colleagues, who showed chromosomal aberrations at the second mitosis after X-irradiating a two-cell mouse embryo [22]. The Streffer group subsequently showed that 29% of the aberrations were carried from the first to the second mitosis [23]. Similar data to those presented by the Seymour group and the Streffer group were published by Mendonca and colleagues [24] where delayed cell death started after 10 successful divisions; and by Kadhim and colleagues [20], who compared the effect of X-rays against alpha particles. These studies were performed in bone marrow stem cells—which meant that clonal lineages could be followed—and revealed that alpha particles were more effective at inducing nonclonal aberrations than X-rays. Later studies revealed persistent levels of ROS in cells showing GI, suggesting that oxidative stress plays a role in perpetuating the insult [25,26].

Although GI occurs as a consequence of direct radiation exposure, it occurs in distant progeny and represents what is now referred to as the vertical transmission of radiation effects. There are several reports indicating it might also involve bystander effects (described later) because the damage in the progeny is nonclonal and therefore cannot be attributed to DNA mutations only [20,27,28]. This damage can also be induced using bystander protocols such as medium transfer, meaning no direct energy deposition is needed to trigger GI [29,30].

## 1.5 Bystander Effects

Another broad category of NTEs is bystander effects [31,32]. They can occur in vivo and in vitro, and refer to the horizontal transmission of radiation damage—as opposed to the vertical transmission seen in GI experiments. Bystander effects refer to effects seen in cells, tissues, or organisms which receive some type of a signal from an irradiated cell, tissue, or organism. Various protocols, possible mechanisms, and models have been extensively reviewed and readers are referred to [15,18,33,34] for both the history and mechanisms under consideration. The fundamental point of interest in this chapter is

that these effects occur in the absence of a dose of radiation to the cell, tissue, or organism being examined and dominate the dose–response in the low-dose region [35,36]. What does this mean for radiation protection? How can we estimate low-dose radiation risk if unirradiated organisms merely living in a proximity to irradiated organisms [37,38,39] display the same type of effect as the directly irradiated organisms? In particular, if bystander mechanisms underlie low-dose effects and can trigger GI, does this lend support to the contention that we are underestimating low-dose risk? This position has been put forward by several authors (reviewed in [40]). However, others have found evidence for adaptive and hormetic responses [41,42,43], suggesting bystander effects could be a form of a homeostatic mechanism. Table 35.1 lists effects documented immediately after direct irradiation, in distant progeny (GI) and in bystander populations.

## 1.6 Adaptive/Hormetic Effects

As suggested before and in Table 35.1, the literature on NTEs documents what might be classed as desirable effects as well as adverse effects. GI is generally regarded as an undesirable effect because it involves genetic alterations [44,45]. Bystander effects were initially observed as an increase in genetic damage [46] or a decrease in cell clonogenicity [47,48]; however, several reports indicate that bystander responses may not always be harmful. Early reports of protective bystander effects in vitro include the work by Azzam and colleagues [49,50] where they showed that bystander signals induced the reduction of neoplastic transformations. There is also evidence of bystander effects inducing adaptive responses which are discussed by some authors [51]. These include the protection afforded by exposure to irradiated medium against radiation-induced cell death, GI, and micronucleus formation. Other studies looking at the bystander proteome indicated that bystander signals may confer beneficial effects by upregulating protective proteins in both rainbow trout and medaka fish exposed to bystander signals [38,52]. In mammals, similar protective effects were seen in the unirradiated left brain hemisphere of healthy Wistar rats [53]. Many authors have suggested that low doses of radiation are actually protective [49,50] and bystander mechanisms are suggested as underlying factors in radiation hormesis [54,55]. It is important at this point to distinguish between adaptive responses, hormesis, and adaptation at the individual and population levels. Adaptive responses generally refer to individuals and the responses mounted by their body systems in response to encountering a new and hazardous threat. Adaptive responses can include behavioral or physiological changes which make the individual better able to survive a future or ongoing encounter with the hazard [56]. Hormesis, on the other hand, is already there as part of the dose response [57,58]. The hormetic dose range is the range where the substance or physical agent is *not* a hazard but is present at optimal levels which if exceeded can become hazardous [59]. Examples are trace metals, which are essential for health but toxic in high amounts. Adaptation is used more as a term to describe the evolution of populations, which come to live with a hazard due to the natural selection of those genotypes or phenotypes that are most able to cope with

**TABLE 35.1 List of End points Which Have Been Shown to Change in Directly Irradiated Cells, in Bystander Cells, and in Distant Progeny**

| Endpoint Changes | Directly Irradiated | Bystander | Distant Progeny |
|---|---|---|---|
| Reproductive death | X | X | X |
| Chromosomal aberration | X | X | X |
| Mutation | X | X | X |
| Mini/microsatellite | X | | X |
| Micronucleus frequency | X | X | X |
| Gene expression | X | X | X |
| Protein expression | X | X | |
| Apoptosis | X | X | X |
| Transformation | X | X | X |
| Mitochondrial function | X | X | X |
| Calcium | X | X | |
| ROS | X | X | X |

the altered environment. Using the example of radiation exposure, low doses may be within a natural hormesis zone and therefore be beneficial, or they may be in the adaptive zone and lead to mechanisms being induced which protect against subsequent exposures. They may also result in adaptations at the population level when radiation exposure is chronic [60,61]. Of course, a confounding factor leading to much confusion is that the dose range for different zones and transition points may be different for different species and different individuals of the same species. A key issue here is that human radiation protection seeks to protect every individual from any adverse consequence and currently does not recognize the hormesis zone, while environmental radiation protection seeks to protect populations and ecosystem structure [62] and does acknowledge at least that there are thresholds for harm if not actually beneficial doses. This leads to widely different concepts and perceptions of risk and of strategies for protection.

### 1.7 Generic Stress Responses

GI is often regarded as resulting from a "generic stress response" [63,64]. However, what exactly this means is not very clear. Stress is defined by Selye [65] as "the nonspecific response of the body to any demand for change." Stress is considered by Selye and many others to be necessary to trigger appropriate responses to the stressor. Calabrese et al. [66] sought to clarify the generic stress response by pointing out that there are many terms across many disciplines for what is a common occurrence in biology, that is, a small dose of a stressor can induce an adaptive response to a large dose of the same or in some cases a different stressor. The chapter went further to show that opposite effects can occur after low-dose exposures compared to high-dose exposures leading to "U"- or "J"-shaped nonlinear dose–response relationships. In radiobiology, we recognize "oxidative stress" as a generic stress resulting from excess ROS and leading to DNA, mitochondrial, and cell membrane damage [67,68]. Oxidative stress is often cited as a mechanism for deleterious low-dose effects [69]. However, others argue that the amount of ROS generated by low doses is so small in relation to that generated by oxidative metabolism, that it could not account for low-dose damage and that anyway it all gets repaired [70]. What is not considered here is again the concept of individual variation in the ability to tolerate and repair oxidative stress–induced DNA or membrane damage. Also not considered is the energy cost of repair and the dependence of repair on nutritional factors, time, age, and metabolic rate [71,72]. Other forms of stress that are thought to be associated with radiation include immune system stress and the mounting of an inflammatory response [73,74]. Both are thought to result from GI-induced changes in the bone marrow stem cells [75]. While direct doses needed to generate bone marrow stem cell damage are relatively high, bystander effects can occur at very low doses of the order of few milligray and appear to be either fully expressed or not expressed at all in the area affected by the signal, saturating at extremely low doses to the signal-generating cells [36,76,77]. This means that in theory at least, a very low dose of radiation could turn on a bystander effect in the tissue, which in certain phenotypes could lead to immune compromise and inflammatory responses. Such a mechanism has been proposed to explain the ill health seen in atomic test veterans, people who suffer from CFIDS (chronic fatigue and immune deficiency syndrome), people exposed to depleted uranium, and victims of radiation accidents, where the calculated doses are considered much too low to account for the observed level and variety of illnesses [78]. In the environment, similar mechanisms might also help to explain the reported high level of mutations in butterflies and birds from Chernobyl and Fukushima [79,80], where again, doses and dose rates are considered much too low to cause biological effects [81,82], and radiation as a cause of these phenomena is vehemently denied.

## 2. CONCEPT OF UNCERTAINTY

This chapter does not seek to suggest that low doses are universally "bad" or universally "good." Rather, we seek to suggest that both response extremes and everything in between *can* occur. What actually happens will depend on the context at the individual and population levels. Indeed, it is possible that an adverse outcome studied at one organization level (eg, cell death) could be beneficial if considered at a higher level of organization. This type of thinking, while obvious and accepted generally in scientific theory, does not help to resolve the debate about risk and leads to people on both sides citing papers which present sound science as "evidence" in support of their belief. Our conclusion after many years of study is that everything is true and nothing is true. In other words, the only thing to do is to accept uncertainty after low-dose exposure and move on to see where this acceptance will take us in trying to address the issues surrounding low-dose exposures to ionizing radiation. Fig. 35.4 is an attempt to convey this idea.

### 2.1 Spectrum of Effects

The key point is to recognize that there is a spectrum of low-dose effects and that we currently do not know the drivers that determine which ultimate outcome prevails. This is not the same as saying that the probability of random damage exists

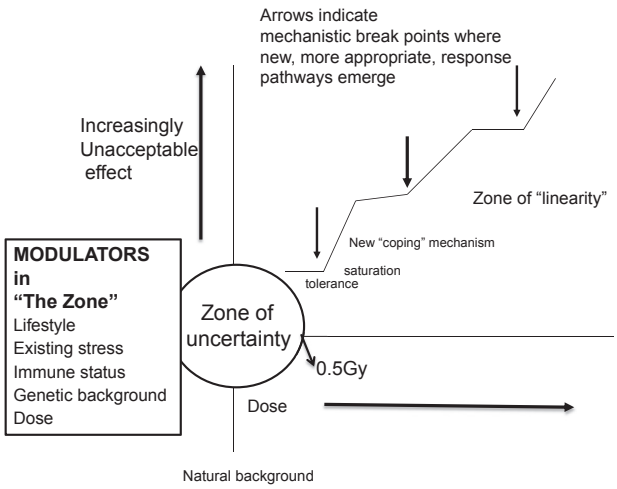


**FIGURE 35.4 The concept of an unpredictable "zone" in the dose–effect relationship where factors in addition to dose determine the response.** With an increasing dose, the unpredictable zone gives way to a series of emergent responses which optimize the outcome.

because that type of statistical analysis is concerned with estimating the chance of a mutation leading to cancer occurring and becoming fixed in DNA [83]—that is, it is a target theory-driven and dose-driven hypothesis. It is, however, what we use in radiation protection [84]. The suggestion that we are presenting is rather that a spectrum of effects ranging from truly beneficial to truly harmful can occur and could occur in the same biological system depending on factors including dose selection that impact the outcome.

## 2.2 Spectrum of Responses

Similarly, a spectrum of responses to the outcome(s) also can occur. These may enhance or reduce the impact, whether positive or negative, of that outcome. Examples could include a nutritional status or smoking that could impact energy delivery for repair or could compromise checkpoint proteins such as p53 [85]. Early research into factors associated with the generation of GI did identify several scenarios which favored the turning on of the phenotype in the progeny of irradiated cells. These included background genetics, time post exposure, the presence of other stressors, the availability of glucose or lactate, the point in the cell cycle of initial irradiation to progenitor cells, and the number of progenitor cells that were irradiated [86,87]. However, there were also sudden failures of hitherto reliable protocols for measuring GI that were never resolved. There was also a considerable interlaboratory variation where a protocol could not be transferred or replicated in another laboratory even by the same individual. With hindsight, these difficulties, many of which were never recorded and remain anecdotal, strongly suggest that there was an underlying randomness in the system or that we did not appreciate all the factors which determined which response prevailed. Either way, it suggests that in addition to a spectrum of effects of radiation exposure, there is also a spectrum of responses. Responses to bystander signals are also determined by genetic, epigenetic, environmental, and unknown factors [88], making it very complicated and beyond the ability of most modeling approaches to resolve [89]. In the paragraphs that follow, some of the literature which has been produced in support of what are considered to be key factors for predicting likelihood of beneficial or adverse outcomes following low-dose exposures is reviewed.

## 2.3 Individual Variation

The individual variation in radiosensitivity has been recognized for many years in radiotherapy and many susceptibility genes mainly associated with faulty DNA repair are known. In low-dose radiobiology, susceptibility genes are associated with extreme reactions to UV or ionizing radiation, these again mostly involve DNA repair. In the field of NTE, genetics is also known to play a role with susceptible and resistant strains of mice, cell lines, and human explants, all documented [90,91,92]. System-level variability is harder to study but bacterial populations have been shown to demonstrate cross-resistance to multiple stressors including heat and radiation [93]. Species-sensitivity distributions (SSDs) discussed later are also an ecological approach being used to determine action levels for the protection of ecosystems. However, just because the individual variation and underlying genetics have been identified as important, they do not address the problem of the *extent* to which genetics contributes to the outcome, or whether it is a determining factor always, sometimes, or ever.

## 2.4 The Role of Genetic Background

Proponents of the "old radiobiology" which holds that energy deposited in DNA causes strand breaks and represents the key way radiation causes damage obviously extend this theory to then suggest that damage to critical targets in DNA underlies the harm caused by ionizing radiation, and that individuals with compromised genetics are therefore most at risk because they cannot repair or detect or otherwise deal with the damage [21]. There is no doubt that at high doses of radiation, this rationale is sound and well proven but after low-dose exposure, it is likely to be far less important due to the myriad other mechanisms such as homeostasis, hormesis, adaptive mechanisms, and system-level responses. While there is probably a spectrum of gene strengths or gene dosages [94,95], it is more likely that protein-level changes induced by the radiation stress predominate meaning that enzyme kinetics, energy budgets, cofactors, and the presence or absence of activators and inhibitors are more likely to determine the outcome [96,97].

## 2.5 The Role of Other Stressors

One of the key issues of concern in radiation protection, particularly of non-human biota, is the problem of multiple stressors [98]. Currently, radiation is regulated as a stand-alone agent, but it is well recognized that in reality, humans and nonhumans are exposed to many stressors such as heavy metals PCBs, heat, and drought. It is one thing to realize this is an issue but quite another one to find a way of regulating in a multiple stressor environment. When DNA was the key target and double-strand breaks—the key damaging lesion, it made sense to regulate radiation separately, but now that NTEs are recognized as key low-dose effects, we have to understand how GI and bystander effects might be modulated by the presence of other stressors. Given the complexity of the issue and the few studies which address it [99,100], it is likely that a move to response- rather than dose-driven protection strategies is necessary to move forward on this issue.

## 2.6 The Role of Lifestyle Factors

A major gap in our knowledge concerning NTEs is the lack of information about these effects in humans. In vivo studies so far are limited to a few mouse strains, fish, and tadpoles [38,39,101]. There is research using human cell lines [102], explants [103], and bone marrow cultures [104], and some work have been done on patients with metal implants [105]. However, none of these studies were conducted with a view to looking at lifestyle factors. The explant studies of normal human bladder done by this group [106] did suggest that smoking could impact bystander effects (reducing the strength of the signal), but the numbers were very small. It appears logical though that factors leading to an increased cancer risk such as smoking might act to increase radiation-induced GI, and this is an area needing investigation. Epidemiological-type studies recording lifestyle information but monitoring GI or bystander effects as well as cancer incidence are needed.

## 2.7 Species-Sensitivity Distribution

This was previously referred to in the context of individual variation in radiation response. SSD is a method being developed to try to get a picture of ecosystem sensitivity to radiation. According to the US Environmental Protection Authority, "Species-sensitivity distributions (SSDs) are cumulative probability distributions of toxicity values for multiple species." For the environmental risk assessment, the chemical concentration that may be used as a hazard level can be extrapolated from SSD using a specified percentile of the distribution (http://www.epa.gov). Basically for radiation studies, SSD is calculated using the data in the literature contained in the FREDERICA database concerning radiation dose response for non-human species and builds a graph of the species present in a habitat and plots their radiosensitivity using $EDR_{10}$ (effective dose rate causing effects in 10% of the population) or $HDR_5$ (hazardous dose rate affecting 5% of species at the 10% level) [107]. Most effects in radioecology concern mortality or reproductive endpoints of the effects but there is no reason that this approach could not be used to "rank" human cells/cell lines, tissues, or individuals using NTE end points. A key benefit is that this approach is effect rather than dose driven, and thus could pull out system-level effects.

# 3. SEARCH FOR DETERMINATORS

Much of the preceding discussion depends on being able to measure NTE reliably in a wide variety of systems at multiple levels of organization. In the next part of this chapter, the broad categories of approaches to this are discussed. Fig. 35.5 summarizes key things we know about GI and bystander effect mechanisms. From these data, attempts have been made to develop reliable determinants of response, both in the directly hit cell/tissue/organism, its progeny, and in bystanders. There are several types of determinants which is now considered.

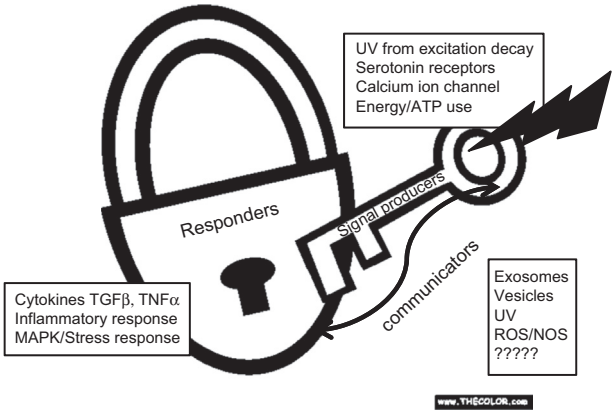


**FIGURE 35.5** **Key factors involved in the mechanisms of the bystander effect grouped into signal production in the directly irradiated cell; communication of information between the targeted cell and bystanders; response transduction in bystander cells.**

## 3.1 Bioindicators

Bioindicator is a term taken from environmental toxicology and is defined as "an organism or biological response that reveals the presence of the pollutants by the occurrence of typical symptoms or measurable responses. These organisms (or communities of organisms) deliver information on alterations in the environment or the quantity of environmental pollutants by changing in one of the following ways: physiologically, chemically or behaviourally" [108].

A very simple bioindicator in radiobiology could be reproductive death measured using clonogenic assays [109] which can be applied to assess the level of GI in progeny or the strength of bystander signals. Population-level bioindicators could include ion fluxes through membrane channels, the integrity of gap junctions in cell membranes, or the coordinated behavior and function of mitochondria in cell populations, all of which have been documented in the literature.

## 3.2 Biomarkers

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."

This means that the biomarker is a surrogate or marker for dose or exposure. In radiobiology, γH2AX is often referred to as a surrogate for radiation dose because it reflects the level of DNA breaks [110]. Dicentric chromosomes are also used as surrogates for dose but do not report reliably below 2 Gy [111]. Thus, biomarkers are more removed from the actual effect on a system, especially if it is complex and not linearly related to dose. This is the situation with NTE.

## 3.3 Biosensors

A Biosensor is defined as an analytical device, used for the detection of an analyte, that combines a biological component with a physicochemical detector. Generally, it is used to describe assays such as ELISA which use enzymes to cause reactions that make it easy to measure color change. However, the reporter assay developed to detect the strength of bystander signal could qualify as a biosensor assay [90] as could the use of fin clips, blood cells, or samples of embryos or sperm that can be incubated to allow the release of bystander signals into medium which is then assayed to determine signal strength. When used with populations of cells, sperm, or embryos, the biosensor approach can provide information about population-level responses to an insult, and thus is a useful response-driven approach for the use in nonlinear dose–response situations such as the detection of GI induction or bystander effects [112].

## 3.4 Signals

The ideal way to measure bystander NTE would be to know what the signal is that goes from an exposed cell or organism to another nonirradiated cell or organism. However, despite almost 20 years of looking, the actual signal or signals remain elusive. Fig. 35.5 referred to earlier shows what we know about the nature of the signal, and clearly, exosomes (possibly containing miRNA), UV or visible light, calcium, serotonin, TGFβ, p53, ROS, and NOS are all reported to be involved, but what transmits the information is unclear, and while events involved in signal production are well understood, and the

response in the recipient cell is also well documented, we know little about the processes of signal transmission. It is also controversial whether there are multiple signals. The works carried out in 2007, 2012, and 2015 from our laboratory suggest that contrary to a popular belief, the signal(s) may be physical rather than chemical [113,114,115]. This work suggests that UVA is emitted by irradiated cells and that if the signal is not emitted, the bystander effect does not occur. The UVA signal is emitted from all cell types, but not all cells respond to the UVA signal. Other evidences supporting physical emissions from cells include the work by Papineni and colleagues who measure bioluminescence coming from irradiated cells [116]. However, the evidence supporting a role for exosomes or larger vesicles is equally compelling [117,118,119]. This leads us to believe that multiple mechanisms may exist.

### 3.5 System-Level Responses

In ecology, it has been known for many years that ecosystems work as a result of complex interactions between elements of the system. Surprisingly, in radiobiology, while this concept is well known to physiologists, radiation action was thought to involve stand-alone actions on individual cells with no communication [21]. Independent survival was a concept enshrined in target theory and its main tool of analysis—the clonogenic survival curve [21]. This all changed in the mid-1990s with several demonstrations of interdependent death and survival of irradiated cells [30]. Of course, as with most "discoveries," there was a body of research that was ignored or forgotten because it did not fit. Chief among this was the work using spheroids where cooperative repair could be demonstrated [120]. Mole [121] also suggested using modeling approaches that the proximity of at least two cells was necessary for carcinogenesis to occur. This ran contrary to the conventional wisdom that cancer originated in a single damaged cell—the clonal origin of cancer theory [122]. The application of system biology tools in radiobiology started with the realization that the microenvironment was important and that bystander effects existed [123]. Signaling is now recognized as a major factor determining the coordinated response of system elements, especially after low doses. This hierarchical theory holds that system-level signaling optimizes the system-level response to a challenge affecting lower levels of organization.

### 3.6 Emergent Effects

Emergence is defined as a process whereby larger entities, patterns, and regularities arise through interactions among smaller or simpler entities that themselves do not exhibit such properties. Emergence is central in theories of integrative levels and of complex systems. In system radiobiology, it refers to responses to radiation in tissues, organs, individuals, and populations that are not predictable from the behavior of individual irradiated cells [124]. Integrating complexity theory into radiobiology and radiation protection is one of the most exciting challenges in the field. Since the discovery of GI and bystander effects, it has become apparent that not only do cells not act alone but that outcomes considered at the cellular level such as death of the cell may lead to radically different consequences at higher levels of organization and if time is factored in to the experiments. New mechanisms and new responses may emerge which are not measurable at the level of the individual cell. In radioecology, the recognition of this phenomenon of emergence has led to a search for so-called "system-level biomarkers" to try to quantify impacts in complex systems. Perhaps, the act of mounting a bystander response could be considered such a biomarker?

## 4. CONCLUSIONS

The aim of this chapter is to consider critically what impact NTEs have on our understanding of radiation risk and what might be a way forward to reconcile the fiercely opposing views about the benefits and hazards associated with low-dose exposure. We suggest that we need to accept that a spectrum of effects occurs after low-dose exposure which cannot be predicted in relation to dose. We suggest that a response-driven approach should be considered and a search for a reliable system and individual-level determinants of response is necessary.

## GLOSSARY

**Adaptive response** A less-damaging effect of a large dose of radiation if a small dose is administered some hours before.

**Bystander effect** The occurrence of radiation-type effects or responses in cells, tissues, organs, or organisms which were not irradiated but received signals from irradiated entities.

**Hormesis** A phenomenon where low doses of physical and chemical agents which are toxic at higher doses can be protective or "good for you."

**Nontargeted effects** Effects occurring in the absence of direct energy deposition in DNA.

## LIST OF ACRONYMS AND ABBREVIATIONS

**DNA** Deoxyribonucleic acid
**EDR** Effective dose rate
**ELISA** Enzyme-linked immunosorbent assay
**GI** Genomic instability
**γH2AX** H2A histone family, member X serine phosphorylated
**HDR** Hazardous dose rate
**LNT** Linear nonthreshold
**miRNA** Micro-ribonucleic acid
**NTE** Nontargeted effects
**PCB** Polychlorinated biphenyl
**RBE** Relative biological effectiveness
**ROS** Reactive oxygen species
**SSD** Species-sensitivity distribution
**TGFβ** Transforming growth factor beta
**UV** Ultraviolet light

## ACKNOWLEDGMENTS

We thank the Canadian National Science and Engineering Research Council and the Canada Research Chairs Program for financial support. We are especially grateful to Alan Cocchetto of the National CFIDS Foundation Inc. for financial support and inspiration.

## REFERENCES

[1] National Research Council. Committee to assess health risks from exposure to low levels of ionizing radiation: BEIR VII- phase 2. Washington (DC): National Academies Press; 2005.
[2] Tubiana M. Dose–effect relationship and estimation of the carcinogenic effects of low doses of ionizing radiation: the joint report of the Académie des Sciences (Paris) and of the Académie Nationale de Médecine. Int J Radiat Oncol 2005;63(2):317–9.
[3] Averbeck D. Does scientific evidence support a change from the LNT model for low-dose radiation risk extrapolation? Health Phys 2009;97(5):493–504.
[4] Calabrese EJ. On the origins of the linear no-threshold (LNT) dogma by means of untruths, artful dodges and blind faith. Environ Res 2015;142:432–42.
[5] Castillo H, Schoderbek D, Dulal S, Escobar G, Wood J, Nelson R, et al. Stress induction in the bacteria *Shewanella oneidensis* and *Deinococcus radiodurans* in response to below-background ionizing radiation. Int J Radiat Biol 2015;15:1–33.
[6] Preston RJ, Boice Jr JD, Brill AB, Chakraborty R, Conolly R, Hoffman FO, et al. Uncertainties in estimating health risks associated with exposure to ionising radiation. J Radiol Prot 2013;33(3):573–88.
[7] Task Group on Radiation Quality Effects in Radiological Protection, Committee 1 on Radiation Effects, International Commission on Radiological Protection. Relative biological effectiveness (RBE), quality factor (Q) and radiation weighting factor (w(R)). A report of the International Commission on Radiological Protection. Ann ICRP 2003;33(4):1–117.
[8] Wrixon AD. New ICRP recommendations. J Radiol Prot 2008;28(2):161–8.
[9] Brechignac F. Protection of the environment: how to position radioprotection in an ecological risk assessment perspective. Sci Total Environ 2003;307(1–3):35–54.
[10] Fujikawa K, Hasegawa Y, Matsuzawa S, Fukunaga A, Itoh T, Kondo S. Dose and dose-rate effects of X rays and fission neutrons on lymphocyte apoptosis in p53(+/+) and p53(−/−) mice. J Radiat Res 2000;41(2):113–27.
[11] Dobson RL, Kwan TC. The tritium RBE at low-level exposure – variation with dose, dose rate, and exposure duration. Curr Top Radiat Res Q 1978;12(1–4):44–62.
[12] Stewart AM, Kneale GW. A-bomb survivors as a source of cancer risk estimates: confirmation of suspected bias. In: Baverstock KF, Stather JW, editors. Low-dose radiation: biological bases of risk assessment. London, New York: Taylor and Francis; 1989. p. 354–63.
[13] Kerr GD. Workshop report on atomic bomb dosimetry – residual radiation exposure: recent research and suggestions for future studies. Health Phys 2013;105(2):140–9.
[14] Brooks AL. Thirty-sixth Lauriston S. Taylor Lecture on radiation protection and measurements—from the field to the laboratory and back. Health Phys 2013;105(5):407–21.
[15] Mothersill C, Seymour C. Changing paradigms in radiobiology. Mutat Res 2012;750(2):85–95.
[16] Morgan WF, Bair WJ. Issues in low dose radiation biology: the controversy continues. A perspective. Radiat Res 2013;179(5):501–10.
[17] National Academies Press. Health risks from exposure to low levels of ionizing radiation: BEIR VII phase 2. (USA): National Academies Press; 2006.
[18] Tang FR, Loke WK. Molecular mechanisms of low dose ionizing radiation-induced hormesis, adaptive responses, radioresistance, bystander effects, and genomic instability. Int J Radiat Biol 2015;91(1):13–21.

[19] Seymour C, Mothersill C, Alper T. High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation. Int J Radiat Biol 1986;50(1):167–79.
[20] Kadhim MA, MacDonald DA, Goodhead DT, Lorimore SA, Marsden SJ, Wright EG. Transmission of chromosomal instability after plutonium α-particle irradiation. Nature 1992;355(6362):738–40.
[21] Elkind MM, Whitmore GF. The radiobiology of cultured mammalian cells. New York: Gordon and Breach; 1967.
[22] Pampfer S, Streffer C. Increased chromosome aberration levels in cells from mouse fetuses after zygote X-irradiation. Int J Radiat Biol 1989;55(1):85–92.
[23] Weissenborn U, Streffer C. Analysis of structural and numerical chromosomal aberrations at the first and second mitosis after X irradiation of two-cell mouse embryos. Radiat Res 1989;117(2):214–20.
[24] Mendonca MS, Kurohara W, Antoniono R, Redpath JL. Plating efficiency as a function of time postirradiation: evidence for the delayed expression of lethal mutations. Radiat Res 1989;119(2):387–93.
[25] Clutton S, Townsend K, Walker C, Ansell J, Wright E. Radiation-induced genomic instability and persisting oxidative stress in primary bone marrow cultures. Carcinogenesis 1996;17(8):1633–9.
[26] Limoli C. Attenuation of radiation-induced genomic instability by free radical scavengers and cellular proliferation. Free Radic Biol Med 2001;31(1):10–9.
[27] Seymour C, Mothersill C. All colonies of CHO-K1 cells surviving γ-irradiation contain non-viable cells. Mutat Res 1992;267(1):19–30.
[28] Chang WP, Little JB. Delayed reproductive death as a dominant phenotype in cell clones surviving X-irradiation. Carcinogenesis 1992;13(6):923–8.
[29] Seymour CB, Mothersill C. Delayed expression of lethal mutations and genomic instability in the progeny of human epithelial cells that survived in a bystander-killing environment. Radiat Oncol Investig 1997;5(3):106–10.
[30] Mothersill C, Seymour C. Survival of human epithelial cells irradiated with cobalt 60 as microcolonies or single cells. Int J Radiat Biol 1997;72(5):597–606.
[31] Nagasawa H, Little JB. Induction of sister chromatid exchanges by extremely low doses of alpha-particles. Cancer Res 1992;52(22):6394–6.
[32] Mothersill C, Seymour CB. Medium from irradiated human epithelial cells but not human fibroblasts reduces the clonogenic survival of unirradiated cells. Int J Radiat Biol 1997;71(4):421–7.
[33] Mothersill C, Seymour C. Radiation-induced bystander effects: past history and future directions. Radiat Res 2001;155(6):759–67.
[34] Hei TK, Zhou H, Ivanov VN, Hong M, Lieberman HB, Brenner DJ, et al. Mechanism of radiation-induced bystander effects: a unifying model. J Pharm Pharmacol 2008;60(8):943–50.
[35] Seymour CB, Mothersill C. Relative contribution of bystander and targeted cell killing to the low-dose region of the radiation dose – response curve. Radiat Res 2000;153(5):508–11.
[36] Schettino G, Folkard M, Michael BD, Prise KM. Low-dose binary behavior of bystander cell killing after microbeam irradiation of a single cell with focused C(K) X rays. Radiat Res 2005;163(3):332–6.
[37] Surinov BP, Isaeva VG, Dukhova NN. Postirradiation volatile secretions of mice: syngeneic and allogeneic immune and behavioral effects. Bull Exp Biol Med 2004;138(4):384–6.
[38] Mothersill C, Bucking C, Smith RW, Agnihotri N, O'Neill A, Kilemade M, et al. Communication of radiation-induced stress or bystander signals between fish in vivo. Environ Sci Technol 2006;40(21):6859–64.
[39] Audette-Stuart M, Yankovich T. Bystander effects in bullfrog tadpoles. Radioprotection 2011;46(6):S497.
[40] Morgan WF. Non-targeted and delayed effects of exposure to ionizing radiation: I. Radiation-induced genomic instability and bystander effects in vitro. Radiat Res 2012;178(2):AV223–236.
[41] Smith RW, Mothersill C, Hinton T, Seymour CB. Exposure to low level chronic radiation leads to adaptation to a subsequent acute X-ray dose and communication of modified acute X-ray induced bystander signals in medaka (Japanese rice fish, *Oryzias latipes*). Int J Radiat Biol 2011;87(10):1011–22.
[42] Azzam EI, Little JB. The radiation-induced bystander effect: evidence and significance. Hum Exp Toxicol 2004;23(2):61–5.
[43] Calabrese EJ. Hormesis: improving predictions in the low-dose zone. EXS 2012;101:551–64.
[44] Wright EG. Manifestations and mechanisms of non-targeted effects of ionizing radiation. Mutat Res 2010;687(1–2):28–33.
[45] Kaup S, Grandjean V, Mukherjee R, Kapoor A, Keyes E, Seymour CB, et al. Radiation-induced genomic instability is associated with DNA methylation changes in cultured human keratinocytes. Mutat Res 2006;597(1–2):87–97.
[46] McAllister KA, Lorimore SA, Wright EG, Coates PJ. In vivo interactions between ionizing radiation, inflammation and chemical carcinogens identified by increased DNA damage responses. Radiat Res 2012;177(5):584–93.
[47] Mothersill C, Seymour C. Radiation-induced bystander effects: are they good, bad or both? Med Confl Surviv 2005;21(2):101–10.
[48] Sawant SG, Randers-Pehrson G, Geard CR, Brenner DJ, Hall EJ. The bystander effect in radiation oncogenesis: I. Transformation in C3H 10T1/2 cells in vitro can be initiated in the unirradiated neighbors of irradiated cells. Radiat Res 2001;155(3):397–401.
[49] Azzam EI, Toledo SMD, Raaphorst GP, Mitchel REJ. Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells. Radiat Res 1996;146(4):369–73.
[50] Ko M, Lao X-Y, Kapadia R, Elmore E, Redpath J. Neoplastic transformation in vitro by low doses of ionizing radiation: role of adaptive response and bystander effects. Mutat Res 2006;597(1–2):11–7.
[51] Matsumoto H, Takahashi A, Ohnishi T. Radiation-induced adaptive responses and bystander effects. Biol Sci Space 2004;18(4):247–54.
[52] Smith RW, Wang J, Mothersill CE, Hinton TG, Aizawa K, Seymour CB. Proteomic changes in the gills of wild-type and transgenic radiosensitive medaka following exposure to direct irradiation and to X-ray induced bystander signals. Biochim Biophys Acta 2011;1814(2):290–8.

[53] Smith RW, Wang J, Schültke E, Seymour CB, Bräuer-Krisch E, Laissue JA, et al. Proteomic changes in the rat brain induced by homogenous irradiation and by the bystander effect resulting from high energy synchrotron X-ray microbeams. Int J Radiat Biol 2013;89(2):118–27.
[54] Choi VWY, Cheung ALY, Cheng SH, Yu KN. Hormetic effect induced by alpha-particle-induced stress communicated in vivo between zebrafish embryos. Environ Sci Technol 2012;46(21):11678–83.
[55] Mosse IB. Genetic effects of ionizing radiation – some questions with no answers. J Environ Radioact 2012;112:70–5.
[56] Hamada N, Maeda M, Otsuka K, Tomita M. Signaling pathways underpinning the manifestations of ionizing radiation-induced bystander effects. Curr Mol Pharmacol 2011;4(2):79–95.
[57] Kudryasheva N, Rozhko T. Effect of low-dose ionizing radiation on luminous marine bacteria: radiation hormesis and toxicity. J Environ Radioact 2015;142:68–77.
[58] Szumiel I. Radiation hormesis: autophagy and other cellular mechanisms. Int J Radiat Biol 2012;88(9):619–28.
[59] Hoffmann G, Stempsey W. The hormesis concept and risk assessment: are there unique ethical and policy considerations? Hum Exp Toxicol 2008;27(8):613–20.
[60] Marquis O, Miaud C, Ficetola GF, Bocher A, Mouchet F, Guittonneau S, et al. Variation in genotoxic stress tolerance among frog populations exposed to UV and pollutant gradients. Aquat Toxicol 2009;95(2):152–61.
[61] Geras'kin S, Evseeva T, Oudalova A. Effects of long-term chronic exposure to radionuclides in plant populations. J Environ Radioact 2013;121:22–32.
[62] Bradshaw C, Kapustka L, Barnthouse L, Brown J, Ciffroy P, Forbes V, et al. Using an ecosystem approach to complement protection schemes based on organism-level endpoints. J Environ Radioact 2014;136:98–104.
[63] Burkhalter MD, Rudolph KL, Sperka T. Genome instability of ageing stem cells—induction and defence mechanisms. Ageing Res Rev 2015;23:29–36.
[64] Ermolaeva MA, Schumacher B. Systemic DNA damage responses: organismal adaptations to genome instability. Trends Genet 2014;30(3):95–102.
[65] Selye H. The stress of life. New York: McGraw-Hill; 1956.
[66] Calabrese EJ, Bachmann K, Bailer J, Bolger M, Borak J, Cai L, et al. Biological stress response terminology: integrating the concepts of adaptive response and preconditioning stress within a hormetic dose-response framework. Toxicol Appl Pharmacol 2007;222(1):122–8.
[67] Nikitaki Z, Hellweg CE, Georgakilas AG, Ravanat J-L. Stress-induced DNA damage biomarkers: applications and limitations. Front Chem 2015;3:35.
[68] Indo HP, Yen H-C, Nakanishi I, Matsumoto K, Tamura M, Nagano Y, et al. A mitochondrial superoxide theory for oxidative stress diseases and aging. J Clin Biochem Nutr 2015;56(1):1–7.
[69] Caputo F, Vegliante R, Ghibelli L. Redox modulation of the DNA damage response. Biochem Pharmacol 2012;84(10):1292–306.
[70] Ulsh BA. Checking the foundation: recent radiobiology and the linear no-threshold theory. Health Phys 2010;99(6):747–58.
[71] Kojima S. Involvement of ATP in radiation-induced bystander effect as a signaling molecule. Yakugaku Zasshi 2014;134(6):743–9.
[72] Oblong JE. The evolving role of the NAD+/nicotinamide metabolome in skin homeostasis, cellular bioenergetics, and aging. DNA Repair (Amst) 2014;23:59–63.
[73] Schaue D, Micewicz ED, Ratikan JA, Xie MW, Cheng G, McBride WH. Radiation and inflammation. Semin Radiat Oncol 2015;25(1):4–10.
[74] Rastogi S, Boylan M, Wright EG, Coates PJ. Interactions of apoptotic cells with macrophages in radiation-induced bystander signaling. Radiat Res 2013;179(2):135–45.
[75] Lorimore SA, Coates PJ, Scobie GE, Milne G, Wright EG. Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects? Oncogene 2001;20(48):7085–95.
[76] Liu Z, Mothersill CE, McNeill FE, Lyng FM, Byun SH, Seymour CB, et al. A dose threshold for a medium transfer bystander effect for a human skin cell line. Radiat Res July 2006;166(1 Pt. 1):19–23.
[77] Rothkamm K, Löbrich M. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. Proc Natl Acad Sci USA 2003;100(9):5057–62.
[78] Wahab M, Nickless E, Najar-M'kacher R, Parmentier C, Podd J, Rowland R. Elevated chromosome translocation frequencies in New Zealand nuclear test veterans. Cytogenet Genome Res 2008;121(2):79–87.
[79] Hiyama A, Nohara C, Kinjo S, Taira W, Gima S, Tanahara A, et al. The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly. Sci Rep 2012;2:570–80.
[80] Mousseau TA, Moller AP. Genetic and ecological studies of animals in Chernobyl and Fukushima. J Hered 2014;105(5):704–9.
[81] Featherstone S. The swallows of Fukushima. Sci Am 2015;312(2):74–81.
[82] Garnier-Laplace J, Geras'kin S, Della-Vedova C, Beaugelin-Seiller K, Hinton T, Real A, et al. Are radiosensitivity data derived from natural field conditions consistent with data from controlled exposures? A case study of Chernobyl wildlife chronically exposed to low dose rates. J Environ Radioact 2013;121:12–21.
[83] Brenner DJ, Doll R, Goodhead DT, Hall EJ, Land CE, Little JB, et al. Cancer risks attributable to low doses of ionizing radiation: assessing what we really know. Proc Natl Acad Sci USA 2003;100(24):13761–6.
[84] Breckow J. Linear-no-threshold is a radiation-protection standard rather than a mechanistic effect model. Radiat Environ Biophys 2006;44(4):257–60.
[85] Furukawa K, Preston DL, Lönn S, Funamoto S, Yonehara S, Matsuo T, et al. Radiation and smoking effects on lung cancer incidence among atomic bomb survivors. Radiat Res 2010;174(1):72–82.
[86] Morgan WF. Is there a common mechanism underlying genomic instability, bystander effects and other nontargeted effects of exposure to ionizing radiation? Oncogene 2003;22(45):7094–9.

[87] Mothersill C, Seymour C. Mechanisms and implications of genomic instability and other delayed effects of ionizing radiation exposure. Mutagenesis 1998;13(5):421–6.
[88] Tawn EJ, Whitehouse CA, Winther JF, Curwen GB, Rees GS, Stovall M, et al. Chromosome analysis in childhood cancer survivors and their offspring—no evidence for radiotherapy-induced persistent genomic instability. Mutat Res 2005;583(2):198–206.
[89] Ballarini F, Alloni D, Facoetti A, Mairani A, Nano R, Ottolenghi A. Modelling radiation-induced bystander effect and cellular communication. Radiat Prot Dosimetry 2006;122(1–4):244–51.
[90] Mothersill C, Rea D, Wright EG, Lorimore SA, Murphy D, Seymour CB, et al. Individual variation in the production of a 'bystander signal' following irradiation of primary cultures of normal human urothelium. Carcinogenesis 2001;22(9):1465–71.
[91] Kamiya K, Ozasa K, Akiba S, Niwa O, Kodama K, Takamura N, et al. Long-term effects of radiation exposure on health. Lancet 2015;386(9992):469–78.
[92] Ponnaiya B, Cornforth MN, Ullrich RL. Radiation-induced chromosomal instability in BALB/c and C57BL/6 mice: the difference is as clear as black and white. Radiat Res 1997;147(2):121–5.
[93] Cox MM, Battista JR. *Deinococcus radiodurans*—the consummate survivor. Nat Rev Microbiol 2005;3(11):882–92.
[94] Li G, Ho VC. p53-dependent DNA repair and apoptosis respond differently to high- and low-dose ultraviolet radiation. Br J Dermatol 1998;139(1):3–10.
[95] Hall EJ, Giaccia AJ. Radiobiology for the radiologist. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
[96] Lee K-F, Weng JT-Y, Hsu PW-C, Chi Y-H, Chen C-K, Liu IY, et al. Gene expression profiling of biological pathway alterations by radiation exposure. Biomed Res Int 2014;40:83–7.
[97] Choudhury A, Cuddihy A, Bristow R. Radiation and new molecular agents part I: targeting ATM-ATR checkpoints, DNA repair, and the proteasome. Semin Radiat Oncol 2006;16(1):51–8.
[98] Salbu B. Multiple stressors—a challenge for the future. J Environ Monit 2005;7(6):539.
[99] Mothersill C, Smith RW, Heier LS, Teien H-C, Land OC, Seymour CB, et al. Radiation-induced bystander effects in the Atlantic salmon (salmo salar L.) following mixed exposure to copper and aluminum combined with low-dose gamma radiation. Radiat Environ Biophys 2014;53(1):103–14.
[100] Korkaric M, Behra R, Fischer BB, Junghans M, Eggen RI. Multiple stressor effects in *Chlamydomonas reinhardtii* – toward understanding mechanisms of interaction between effects of ultraviolet radiation and chemical pollutants. Aquat Toxicol 2015;162:18–28.
[101] Morgan WF. Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. Radiat Res 2003;159(5):581–96.
[102] Ryan LA, Smith RW, Seymour CB, Mothersill CE. Dilution of irradiated cell conditioned medium and the bystander effect. Radiat Res 2008;169(2):188–96.
[103] Pinho C, Timotin E, Wong R, Sur RK, Hayward JE, Farrell TJ, et al. Assessing patient characteristics and radiation-induced non-targeted effects in vivo for high dose-rate (HDR) brachytherapy. Int J Radiat Biol 2015;1:1–41.
[104] Kadhim M, Lorimore S, Townsend K, Goodhead D, Buckle V, Wright E. Radiation-induced genomic instability: delayed cytogenetic aberrations and apoptosis in primary human bone marrow cells. Int J Radiat Biol 1995;67(3):287–93.
[105] Coen N, Kadhim MA, Wright EG, Case CP, Mothersill CE. Particulate debris from a titanium metal prosthesis induces genomic instability in primary human fibroblast cells. Br J Cancer 2003;88(4):548–52.
[106] Mothersill C. p53 protein expression and increased SSCP mobility shifts in the p53 gene in normal urothelium cultured from smokers. Carcinogenesis 1997;18(6):1241–5.
[107] Garnier-Laplace J, Alonzo F, Adam-Guillermin C. Establishing relationships between environmental exposures to radionuclides and the consequences for wildlife: inferences and weight of evidence. Ann ICRP 2015;44(1 Suppl.):295–303.
[108] McCarty LS, Power M, Munkittrick KR. Bioindicators versus biomarkers in ecological risk assessment. Hum Ecol Risk Assess 2002;8(1):159–64.
[109] Puck TT. Action of x-rays on mammalian cells. J Exp Med 1956;103(5):653–66.
[110] Vandevoorde C, Franck C, Bacher K, Breysem L, Smet MH, Ernst C, et al. γ-H2AX foci as in vivo effect biomarker in children emphasize the importance to minimize x-ray doses in paediatric CT imaging. Eur Radiol 2014;25(3):800–11.
[111] Romm H, Wilkins RC, Coleman CN, Lillis-Hearne PK, Pellmar TC, Livingston GK, et al. Biological dosimetry by the triage dicentric chromosome assay: potential implications for treatment of acute radiation syndrome in radiological mass casualties. Radiat Res 2011;175(3):397–404.
[112] Kong EY, Choi VWY, Cheng SH, Yu KN. Some properties of the signals involved in unirradiated zebrafish embryos rescuing α-particle irradiated zebrafish embryos. Int J Radiat Biol 2014;90(12):1133–42.
[113] Le M, McNeill FE, Seymour C, Rainbow AJ, Mothersill CE. An observed effect of ultraviolet radiation emitted from beta-irradiated HaCaT cells upon non-beta-irradiated bystander cells. Radiat Res 2015;183(3):279–90.
[114] Mothersill C, Smith RW, Fazzari J, McNeill F, Prestwich W, Seymour CB. Evidence for a physical component to the radiation-induced bystander effect? Int J Radiat Biol 2012;88(8):583–91.
[115] Mothersill C, Moran G, McNeill F, Gow MD, Denbeigh J, Prestwich W, et al. A role for bioelectric effects in the induction of bystander signals by ionizing radiation? Dose-Response 2007;5(3):214–29.
[116] Papineni RV. X-ray irradiation induced bio luminescence: ex vivo and endoscopic imaging of radiobioluminescence. Cancer Res 2014;74(19):4921.
[117] Jella KK, Rani S, O'driscoll L, McClean B, Byrne HJ, Lyng FM. Exosomes are involved in mediating radiation induced bystander signaling in human keratinocyte cells. Radiat Res 2014;181(2):138–45.
[118] Al-Mayah AHJ, Irons SL, Pink RC, Carter DRF, Kadhim MA. Possible role of exosomes containing RNA in mediating nontargeted effect of ionizing radiation. Radiat Res 2012;177(5):539–45.

[119] Albanese J, Dainiak N. Modulation of intercellular communication mediated at the cell surface and on extracellular, plasma membrane–derived vesicles by ionizing radiation. Exp Hematol 2003;31(6):455–64.
[120] Sutherland RM, Durand RE. Radiation response of multicell spheroids–an in vitro tumour model. Curr Top Radiat Res Q 1976;11(1):87–139.
[121] Mole RH. Radiation-induced acute myeloid leukaemia: an unusually valuable experimental model for testing basic assumptions about the process of carcinogenesis. In: Iversen OH, editor. Theories of carcinogenesis. Washington (DC): Hemisphere Publishing Co.; 1988. p. 133–41.
[122] Fialkow PJ. Clonal origin of human tumors. Biochim Biophys Acta 1976;458(3):283–321.
[123] Lorimore SA, Wright EG. Radiation-induced genomic instability and bystander effects: related inflammatory-type responses to radiation-induced stress and injury? A review. Int J Radiat Biol 2003;79(1):15–25.
[124] Baverstock K. Radiation-induced genomic instability: a paradigm-breaking phenomenon and its relevance to environmentally induced cancer. Mutat Res 2000;454(1):89–109.