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Mechanisms and biological importance of photon-induced bystander responses: do they have an impact on low-dose radiation responses

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Elucidating the biological effect of low linear energy transfer (LET), low-dose and/or low-dose-rate ionizing radiation is essential in ensuring radiation safety. Over the past two decades, non-targeted effects, which are not only a direct consequence of radiation-induced initial lesions produced in cellular DNA but also of intra- and inter-cellular communications involving both targeted and non-targeted cells, have been reported and are currently defining a new paradigm in radiation biology. These effects include radiation-induced adaptive response, low-dose hypersensitivity, genomic instability, and radiation-induced bystander response (RIBR). RIBR is generally defined as a cellular response that is induced in non-irradiated cells that receive bystander signals from directly irradiated cells. RIBR could thus play an important biological role in low-dose irradiation conditions. However, this suggestion was mainly based on findings obtained using high-LET charged-particle radiations. The human population (especially the Japanese, who are exposed to lower doses of radon than the world average) is more frequently exposed to low-LET photons (X-rays or γ-rays) than to high-LET charged-particle radiation on a daily basis. There are currently a growing number of reports describing a distinguishing feature between photon-induced bystander response and high-LET RIBR. In particular, photon-induced bystander response is strongly influenced by irradiation dose, the irradiated region of the targeted cells, and *p53* status. The present review focuses on the photon-induced bystander response, and discusses its impact on the low-dose radiation effect.

Keywords: bystander response; X-rays; low-dose radiation; microbeam; non-targeted effect

INTRODUCTION

X-rays were discovered by Wilhelm Conrad Röntgen in 1895, and he was subsequently awarded the first Nobel Prize in Physics in 1901. Since then, ionizing radiation has been applied in a wide range of fields, including medicine and science. Radiobiology, which is the study of the action of ionizing radiation on living things, began in the early 1900s [1]. Over the past century, accumulating circumstantial evidence has indicated that cellular DNA is the main 'target' of ionizing radiation. Ionizing radiation induces various types of DNA damage. DNA double-strand breaks (DSBs) are a distinctive type of radiation-induced DNA damage, and cells with defective DSB repair genes [2] are hypersensitive to ionizing radiation. Failure in DSB repair results in various detrimental effects such as mutations, transformations, and chromosomal rearrangements. This 'target' paradigm of radiobiology corroborates with the current concept of radiation protection [3]. Additionally, radiation-induced biological effects have been well described by using theoretical models of this paradigm [1, 4, 5].

Over the past two decades, non-targeted effects (or non-DNA-targeted effects), which are not only a direct consequence of radiation-induced initial lesions produced in cellular DNA but also of intra- and inter-cellular communications involving both targeted and non-targeted cells, have been extensively studied and are currently defining a new paradigm in radiobiology. These effects mainly include radiation-induced adaptive

responses, low-dose hypersensitivity, genomic instability, and radiation-induced bystander responses (RIBRs). In addition, gene expression and inverse dose rate effects have also been investigated [6].

A RIBR is generally defined as a cellular response that is induced in a non-irradiated cell that has received bystander signals from directly irradiated cells within an irradiated cell population. However, this definition is highly variable [7]. The RIBR came into the spotlight in the field of radiobiology through the report of Nagasawa and Little [8]. In their study, a significant increase in the frequency of sister chromatid exchanges (SCEs) occurred with doses as low as 0.31 mGy in Chinese hamster ovary (CHO) cells. Although 30% of the cells showed an increased frequency of SCEs at this dose, less than 1% of the nuclei were traversed by α particles. Today, the RIBRs induced by high-linear energy transfer (LET) charged-particle microbeams have been well described in a variety of biological endpoints [9–16]. For example, the mutation frequency in human–hamster hybrid cells after exposure to low doses of α particles, where is an average of less than one particle per cell, was significantly higher than that predicted by linear extrapolation using a high dose, which was due to the bystander response [10]. The yield of damaged bystander cells was independent of the number of charged particles delivered to the targeted cell, when one or four cells in about 5000 cells within the dish were irradiated with 1–15 helium-3 particles (100 keV/μm) [9]. In addition, when 49 cells within a confluent culture of normal human diploid skin fibroblast AG1522 cells were individually hit by one to four particles of ^{40}Ar (~1260 keV/μm) or ^{20}Ne (~380 keV/μm), the production of micronuclei (MN) was 2-fold higher than that in controls, but independent of the number and LET of the particles [17]. On the contrary, 0.036–0.144% of the cells within a confluent culture of normal human skin fibroblast NB1RGB cells were traversed by primary radiation tracks of 5.35-keV X-rays (~6 keV/μm), C-ions (~103 keV/μm), Ne-ions (~380 keV/μm) or Ar-ions (~1260 keV/μm), and MN were induced in a greater fraction of cells than expected based on the fraction of the cells targeted by primary radiation; the resultant effect occurred in a dose-dependent manner [18]. The RIBR has implications in terms of human exposure to very low doses of high-LET charged-particle radiations such as α particles from environmental radon or densely ionizing galactic cosmic rays in space [19–21], although there are some contradictions in dose response.

Evaluation of the biological effects of low-LET, low-dose and/or low-dose-rate ionizing radiation has important implications for radiation protection. The human population, especially the Japanese, who have received lower doses of radon exposures than the world average [22], is more frequently exposed to low-LET photons (X-rays and γ-rays) than to high-LET radiation on daily basis. RIBRs might have important biological consequences under low-dose irradiation conditions where non-hit cells are affected in the irradiated population. However, this suggestion is mainly based on results obtained using high-LET radiation, as previously described. The heterogeneity of the absorbed dose within the irradiated tissues is more relevant for high-LET charged particles than for low-LET photons because the absorbed dose per one hit (one nucleus traversal) for high-LET charged-particle radiations is higher than that for low-LET photons. Current evidence indicates that a feature of bystander response induced by photons differs from that induced by high-LET radiation. Our previous studies using X-ray microbeams demonstrated that X-ray-induced bystander response is largely influenced by the irradiation dose, the irradiated region of the targeted cells, and the *p53* status of the targeted cells and the bystander cells [23–27]. The present review focuses on photon-induced bystander responses and discusses its impact on low-dose radiation response.

DEFINITION OF RADIATION-INDUCED BYSTANDER RESPONSE

The term 'bystander effect' is commonly used in gene therapy; it refers to the killing of several subpopulations of tumor cells by targeting only one 'type' of cells within a heterogeneous cell population [28, 29]. In the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) 2006 Report [29], 'bystander effect' was described using the definition proposed by Djordjevic [30], in which it pertains to the ability of irradiated cells to convey manifestations of damage to neighboring cells that have not been directly irradiated (summarized in [31]). The International Commission on Radiological Protection (ICRP) Publication 99 [20] also described the bystander effect of radiation as the transmission of signals from irradiated to non-irradiated cells in a population, leading to biological changes in the recipient cells. Additionally, 'clastogenic factors' and 'abscopal effect' are considered as *in vivo* bystander response-like phenomena [14, 32]. Briefly, clastogenic factors are released into the blood plasma of irradiated animals and humans and are capable of inducing chromosomal damage in non-irradiated cells. Abscopal effect pertains to the response of a tissue that is physically separate from the region exposed to radiation [29, 31]. Clastogenic factors may be included among the factors that induce an abscopal effect.

The term 'bystander effect' has been broadly interpreted in the past few decades, and it includes the abscopal effect, and occasionally the effect induced by clastogenic factors. RIBR may also be considered as a form of intercellular communication induced by ionizing radiation, regardless of radiation exposure. For example, in an animal study using lead shields to protect a region of the body from direct irradiation, bystander cells or tissues would still receive some scatter dose of radiation. Mancuso *et al.* [33] investigated bystander-related tumor induction in the cerebellum of radiosensitive *Patched-1* (*Ptch1*) heterozygous mice using the shielded

head irradiation method. Induction of medulloblastoma was observed in the brains of mice exposed to shielded irradiation of 3 Gy. In this case, the bystander cells in the brain protected by the lead shield received 0.036 Gy of scattered radiation due to X-ray deflection. Blyth and Sykes [7] presented a comprehensive summary of the definitions of bystander responses and classified radiation-induced bystander or bystander-like signaling responses into three distinct classes that are relevant to human exposure scenarios, i.e., bystander effects, abscopal effects, and cohort effects. Cohort effects are radiation-induced, signal-mediated effects between irradiated cells within an irradiated volume in their working definition, whereas bystander effects and abscopal effects are induced in non-irradiated cells and tissues, respectively.

To determine the relevance of RIBR to the risk of carcinogenesis in conditions of low-dose radiation, the irradiated dose and the location of non-irradiated cells both in and out of the irradiated field should be considered. In this review, RIBR is defined as a cellular response that is induced in a non-irradiated cell that has received bystander signals from directly irradiated cells within an irradiated cell population. The term 'abscopal effect' (or response) is also used to describe an *in vivo* study, regardless of exposure to radiation scatter dose, since most animal studies employ the shielded partial body irradiation method.

DOSE RESPONSE OF PHOTON-INDUCED BYSTANDER/ABSCOPAL RESPONSE

Cellular level study using broadbeams

In vitro bystander responses induced by photons have been primarily studied by using conventional X-ray or γ-ray broadbeams using cell mixing, co-culture, or media transfer methods (reviewed in [14]). The selection of method is based on the difficulty of irradiating specific cells in a cell culture with conventional X-rays or γ-rays, compared with particle radiations that possess a fine track structure and thus provide superior control of directional exposure.

Mothersill, Seymour and co-workers extensively studied the dose response of low-LET photon-induced bystander response using the medium transfer protocol [34–37]. Non-irradiated HPV-transfected human keratinocytes cultured in an irradiated conditioned medium (ICM) derived from ^{60}Co γ-ray-irradiated cells showed a decrease in clonogenic cell survival using a dose range of 0.01–5 Gy, whereas its magnitude was almost unchanged [34]. Additionally, they reported that clonogenic cell death at doses within the range of 0.01–0.5 Gy was caused by the bystander response only. For example, the clonogenic surviving fraction of the cells directly irradiated with 0.01 Gy was almost the same as that of the cells treated with ICM from cells irradiated with 0.01 Gy. Human keratinocyte HaCaT and colon carcinoma SW48 cells cultured in ICM from the ^{60}Co γ-ray-irradiated cells also showed a reduction in cell survival at doses of 0.5–10 Gy and 0.5–5 Gy, respectively [35]. The bystander cell-killing effect was observed in cells cultured in ICM from flasks containing human immortalized keratinocyte HPV-G cells that had been irradiated with 5 mGy–5 Gy [36]. There was also a small but not significant dose–effect relationship using the dose range of 5 mGy–5 Gy. On the other hand, the cell-killing effect was not observed in cells directly irradiated with 5 mGy. Following this report, the bystander cell killing-effect of HPV-G cells cultured in ICM was investigated using low-dose-rate ^{60}Co γ-rays (2.7 and 7.0 mGy/h) [37]. The rate of cell survival significantly decreased at a dose of dose 3 mGy, and reached a plateau at 300 mGy. No significant cell killing due to direct irradiation or as a result of the bystander response was observed when the doses were ≤2 mGy. This result suggests that there is a threshold dose for the RIBR between 2 and 3 mGy.

Jella *et al.* [38] recently reported that HaCaT cells showed a reduction in cell survival and an induction of mitotic cell death after treatment with ICM from cells irradiated with doses of 0.05 and 0.5 Gy. Another group reported the bystander cell-killing effect in the CGL1 human HeLa × skin fibroblast hybrid cell line irradiated at higher doses (1–7 Gy) by using the medium transfer method [39].

Bystander cells may also be capable of enhancing colony-forming efficiency, i.e. the surviving fraction (SF) was >1.0. Shao *et al.* [40] reported that the plating efficiency of non-irradiated bystander human neoplastic salivary duct epithelial HSG cells was enhanced by co-culturing with cells irradiated with 200 kVp X-rays at doses of 1–11 Gy. MN induction in the bystander HSG cells increased when they were co-cultured with donor cells irradiated with X-rays. Baskar *et al.* [41] also showed that the SF of bystander normal human fibroblast GM637H cells was significantly enhanced by treatment with ICM from normal human fibroblast MRC-5 cells irradiated with ^{137}Cs γ-rays at doses of 2.5–20 Gy. SF enhancement might be related to the enhancement of cell growth. Using the co-culture method, Gerashchenko and Howell [42] reported that non-irradiated rat liver epithelial WB-F344 cells that had been co-cultured with cells irradiated with 0.5–20 Gy of ^{137}Cs γ-rays showed enhanced cell growth at doses of >0.5 Gy. Next, cell growth of non-irradiated bystander gap junction intercellular communication (GJIC)-incompetent WB-aB1 cells co-cultured with irradiated cells was compared with that of GJIC-competent bystander WB-G344 cells at doses of 0.5, 1 and 5 Gy [43]. Bystander WB-aB1 cells showed a slightly higher cell growth rate compared with the bystander WB-G344 cells, although the difference was not statistically significant.

The dose response of DSB induction in bystander cells has been extensively investigated. Yang *et al.* [44] showed that the induction of p21^{WAF1} protein and the ratio of cells with γ-H2AX (phosphorylated histone H2AX at Ser139) foci in bystander human diploid skin fibroblast AGO1522 cells co-cultured with X-irradiated cells at doses of 0.1–10 Gy

were ~2-fold higher than that of the control cells. Additionally, the frequency of MN formation in bystander cells also increased at doses of 0.1–10 Gy. Those responses were independent of applied dose range. On the other hand, the bystander cell-killing effect was not detected at 0.1 Gy, whereas this response was detected using doses of 0.5–10 Gy. The reduction in SF was dose-independent at a dose range of 0.5–10 Gy. Sokolov *et al.* [45] also assessed the induction of γ-H2AX foci formation by bystander response in normal human lung fibroblast WI-38 cells using the medium transfer method. Bystander cells with four or more foci per cell were observed after treatment with ICM from cells irradiated with 0.2, 0.6 and 2 Gy of ^{60}Co γ-rays. The duration of media conditioning necessary to elicit the largest bystander response was influenced by the radiation dose, which was 1, 2 and 4 h for 0.2, 0.6 and 2 Gy, respectively. In addition, the ICM from the cells irradiated with 2 Gy was less effective in inducing γ-H2AX foci in bystander cells. Ojima *et al.* [46] reported the dose response of foci formation of phosphorylated ataxia-telangiectasia mutated (ATM) in normal human fibroblast MRC-5 cells directly irradiated with 90 kV X-rays using doses of 1.2–200 mGy. Irradiated cells were fixed 0.05 h after irradiation. The induction of phosphorylated ATM (pATM) foci showed a supra-linear dose–response relationship. The effects induced by doses of 1.2–5 mGy were largely due to RIBR. However, for doses greater than 10 mGy, the effects might be mainly contributed by dose-dependent direct effects and partly by dose-independent bystander response.

To establish the relevance of RIBR to carcinogenesis in the low-dose region, it is important to determine whether the response has been induced in human stem cells. Sokolov and Neumann [47] studied the X-ray-induced bystander response using human bone-marrow mesenchymal stem cells (hMSCs) and embryonic stem cells (hESCs). At doses of 0.2, 2 and 10 Gy, induction of the DNA damage response (which was assessed by the foci formation of 53BP1 or phosphorylated Chk2 (pChk2) and apoptotic cell death) were not significant, either in hMSCs or hESCs treated with ICM. In addition, no robust RIBR was indicated in hMSCs co-cultured with irradiated hMSCs.

The results of these studies are listed in Table 1. The minimal dose to induce a bystander response was 1.2 mGy [46] and 3 mGy [37] at the endpoints of DNA damage induction and clonogenic cell survival, respectively. The relevance of these results to the health effects in the low-dose region are discussed later. One important result is that the human stem cells did not show the RIBR [47]—because the stem cells are considered to be the target cells for carcinogenesis.

Cellular level study using microbeams

Schettino *et al.* [48, 49] investigated the survival response of Chinese hamster V79 cells by irradiating a single cell or all cells within a population using the focused carbon K-shell (C-K) X-ray microbeam (278 eV) at the Gray Cancer Institute (GCI), UK [50]. The relative biological effectiveness (RBE) of ultrasoft C-K X-rays is higher than conventional X-rays and γ-rays. For example, the RBE values for cell inactivation and for induction of DSBs by C-K X-rays are 2.8 ± 0.3 and 2.7 ± 0.3, respectively [51]. In this system, a Fresnel zone plate (FZP) was used to focus the X-ray beam. The beam had a 0.25-μm radius at the sample position [48]. In the dose estimation, a nuclear dose was used, although the energy absorbed volumes were very small. Issues of dose estimation of heterogeneous exposure are extensively described in the International Commission on Radiation Units and Measurements (ICRU) Report 86 [52]. An average quantity such as an absorbed dose cannot adequately describe the different combinations of charged-particle tracks though individual cells or near cells that were not actually hit. Evaluation of microbeam irradiations typically requires information on beam area, position relative to specified targets, fluence, and time between events. When a single cell in about 9×10^3 cells in a dish was targeted, ~10% of the cell population was killed, and the level of SF steeply reached a plateau that was independent of the dose when doses were >0.2 Gy [48] or >0.3 Gy [49] were applied. The reduction of SF showed a dose response <0.2 Gy [48] or <0.3 Gy [49], respectively. Additionally, a significant scatter of the data was observed at doses <0.3 Gy [49]. They suggested that the event that triggers the emission of the bystander signal by the hit cell is an all-or-nothing process.

There are currently two X-ray microbeam facilities in Japan. One is the synchrotron radiation (SR) X-ray microbeam irradiation system developed at the Photon Factory, High Energy Accelerator Research Organization (KEK, Ibaraki) [53–55]. The other is the Microbeam X-ray Cell Irradiation System at the Central Research Institute of Electric Power Industry (CRIEPI, Tokyo) [25, 55]. In the SR X-ray microbeam irradiation system, the SR is reflected at right angles upwards by diffraction through Si(311) to irradiate cells from the bottom of the culture dish. The X-rays have an energy of 5.35 keV. The 1/e attenuation length in water is 288 μm. The range of 5.35-keV photoelectrons is <1 μm. The X-ray microbeam is only formed through a slit, because the SR beam is superior in terms of directivity. Beam size could easily be changed by changing the width of the slit. In the Microbeam X-ray Cell Irradiation System, characteristic Al_K X-rays (1.49 keV) are generated by the focused electron bombardment of an aluminum target and are focused through the FZP. The minimal beam size, which was measured through knife-edge scanning, was 1.8 μm in diameter. The same autostage, cell irradiation dish, and irradiation software were used in both systems.

First, we investigated the dose response of the bystander cell-killing effect using confluent WI-38 cells [23, 25]. The absorbed dose within the microbeam-irradiated region of the cell nucleus was used in these studies, because the irradiated region in the cell nucleus was narrower than the size of cell

Table 1. Dose response of broadbeam photon-induced bystander response *in vitro*

Cells	Method	Endpoint	IR	Investigated dose region (Gy)	Ref
				0.001 0.01 0.1 1 10	
HPV-transfected human keratinocytes	Medium transfer	Clonogenic survival	^{60}Co γ-rays	0.01–5	[34]
HaCaT				0.5–10	[35]
SW48				0.5–5	
HPV-G				0.005–5	[36]
				0.003–5; 0.00004-0.002	[37]
HaCaT	Medium transfer	Clonogenic survival, mitotic cell death	^{60}Co γ-rays	0.05–0.5; 0.005	[38]
CGL1	Medium transfer	Clonogenic survival	X-rays	1–7	[39]
HSG	Co-culture	Clonogenic survival (SF > 1), MN	X-rays	1–11	[40]
Recipient: GM637H Donor: MRC-5	Medium transfer	Clonogenic survival (SF > 1)	^{137}Cs γ-rays	2.5–10; 1	[41]
WB-F344	Co-culture	Cell growth (enhanced)	^{137}Cs γ-rays	1–20	[42, 43]
WB-aB1				0.5–5	[44]
AGO1522	Co-culture	p21^{WAF1} induction, γ-H2AX foci, MN	X-rays	0.1–10	[44]
		Clonogenic survival		0.5–10; 0.1	
WI-38	Medium transfer	γ-H2AX foci	^{60}Co γ-rays	0.2–2	[45]
MRC-5	Direct irradiation	pATM foci	X-rays	0.0012–0.005; 0.2; Direct effect+partly bystander response	[46]
hMSC, hESC	Medium transfer	53BP1/pChk2 foci, apoptosis	X-rays	0.2–10	[47]
hMSC	Co-culture	53BP1/pChk2 foci		0.2–10	

IR = ionizing radiation, solid line = dose range where bystander response was significantly detected, dashed line = dose range where the response was not significantly detected.

nucleus. Bystander cell-killing showed a biphasic relationship with the irradiated dose when the nuclei in five cells in the center of a dish were irradiated. A confluent culture on a microbeam irradiation dish contained about 7×10^5 cells. The reduction in SF reached a plateau when five or more cell nuclei were irradiated with X-ray microbeams. In the study using the 5 μm × 5 μm SR X-ray microbeam [23], the SF significantly decreased at doses of >0.09 Gy and was 0.85 at 1.4 Gy. However, at 1.9 and 4.7 Gy, SF reached a value of ~1.0. At 9.3 Gy, cell survival also decreased. In the study using Al-K X-ray microbeams, a significant bystander cell-killing effect was not detected at doses of 0.12 and 0.23 Gy. SF significantly decreased at doses of ≥0.47 Gy and was 0.88 at 1.2 Gy. However, between doses of 2.3 and 7.0 Gy, the decrease in SF was partially suppressed. At doses of >14 Gy, cell survival levels decreased and reached a plateau. The SF at 21 Gy was 0.88. The X-ray-induced bystander cell-killing effects that reached a plateau or saturation in the higher dose region might have been caused by the induction of complex DNA damage in the targeted cells, as observed by the presence of high-LET-particle radiation. Next, to elucidate the role of *p53* in the soft X-ray-induced bystander cell-killing effect, five cell nuclei of human non-small-cell lung cancer H1299 cells expressing wild-type *p53* (H1299/wt*p53*) or V143A temperature-sensitive mutation in the *p53* gene (H1299/m*p53*-143ts) were irradiated with Al-K X-ray microbeams [26]. The cell-killing effect of H1299/wt*p53* showed a similar dose response to that of WI-38 cells. The SF of H1299/m*p53*-143ts cells also steeply decreased at doses of 0.45 and 1.0 Gy, respectively. However, the decrease in SF remained higher at 5.0 Gy. On the other hand, suppression of the bystander cell-killing effect at 2.0 Gy was also observed in the H1299/m*p53*-143ts cells expressing wild-type *p53* incubated at a permissive temperature of 33°C after irradiation. Thus the suppression of the bystander cell-killing effect at doses of ~2–7 Gy in the cells expressing wild-type *p53* was mainly caused by the activated function of wild-type *p53*.

Maeda *et al.* [24, 27] also reported the dose response of the bystander cell-killing effect of V79 cells using SR X-ray microbeams. In their first study [24], 10 μm × 10 μm beams and 50 μm × 50 μm beams were used to irradiate a cell nucleus and a whole cell, respectively. In these studies, the nuclear-averaged dose was used to compare the effect of nucleus irradiation and whole cell irradiation. In the case of the 10 μm × 10 μm beam, >60% of the cell nucleus was within the irradiated region. In the case of the nucleus-irradiated experiment, when five cell nuclei were irradiated with a dose of ~1 Gy, the SF decreased to 90%, whereas at higher doses, the SF was stable at 96%. On the other hand, in the whole-cell-irradiated case, the SF gradually decreased to 92% and remained stable with higher doses. Next, *hypoxanthine-guanosine phosphoribosyl transferase* (*HPRT*) mutation frequencies in bystander V79 cells were investigated [27]. The mutation frequency in bystander cells significantly decreased, and was lower than the background mutation frequency in the control when five target nuclei were irradiated with a dose of 1 Gy. However, at higher doses, the mutation frequency returned to baseline levels. This biphasic decrease in mutation frequency was similar to that observed in bystander cell death.

Recently, Autsavapromporn *et al.* [18] reported the dose response of MN formation in the normal human skin fibroblast NB1RGB cells using 20 μm × 20 μm of SR X-ray microbeam. When 16 × 16 points within a 15 mm × 15 mm area in the center of dish were irradiated, the frequency of MN increased dose dependently within a dose range of 0.1–1 Gy.

The results of these studies are listed in Table 2. The dose response of the bystander cell-killing effect indicated an exceedingly complicated pattern that depends on the irradiated cell region and cellular *p53* status. Bystander-cell killing of V79 cells showed a biphasic relationship with the irradiated dose when the nuclei in five cells were irradiated with X-ray microbeams as well as WI-38 and H1299/wt*p53* cells, although the V79 cells have mutated *p53* [56, 57]. However, the V79 cells have a residual p53 activity, for example an increase of p53 in the cell nucleus was observed after treatment with both chemical and physical DNA damaging agents [57]. Thus, the cell-killing effect of V79 cells showing a similar dose response to that of wild-type *p53* cells may be caused by the residual *p53* activity, although further experiments are warranted. The minimal dose for the induction of the bystander cell-killing effect was 0.05 Gy [48, 49] when the focused ultrasoft C-K X-ray microbeam was used. On the other hand, the bystander response was not detected in the confluent WI-38 cells irradiated with Al-K X-ray microbeams at doses less than 0.1 Gy. The difference may indicate that the targeted volume within the cell nucleus may affect the bystander effect, as discussed in our previous studies [23, 24].

Animal study

There are fewer reports on the dose response or low dose of bystander/abscopal response induced by low-LET radiation *in vivo* and cultured tissues than reports *in vitro*. Additionally, several studies were performed at doses ≥1 Gy.

In a study of the abscopal effect in a transplanted tumor (which had received a radiation scatter dose), Demaria *et al.* [58] reported a growth delay in the tumor by abscopal effect in BALB/C mice bearing a syngeneic mammary carcinoma, 67NR, in both flanks and treated with the growth factor, Flt-3-L. When only one of two tumors was irradiated with ^{60}Co γ-rays at a single dose of 2 or 6 Gy, the growth of the second non-irradiated tumor was impaired. There was no dose response between 2 and 6 Gy. On the other hand, a dose-dependent abscopal effect was reported in C57BL/6 mice [59]. Lewis lung carcinoma (LLC) was implanted in the midline dorsum of mice. In non-tumor-bearing legs irradiated with five 10-Gy fractions of ^{137}Cs γ-rays, the growth

Table 2. Dose response of X-ray microbeam-induced bystander response *in vitro*

Cells	X-ray microbeam	Endpoint	Dose estimation	Investigated dose region (Gy)	Ref
				0.001 0.01 0.1 1 10	
V79-379A	C-K X-ray	Clonogenic survival	Nuclear dose	0.05–2	[48] [49]
WI-38	SR X-ray 5 × 5 μm	Clonogenic survival	In the irradiated region	0.23–1.6; 9.3; 0.09; 1.9–4.7	[23]
	Al-K X-ray			0.47–1.9; 14-28; 0.12–0.23; 2.3–7.0	[25]
H1299/wt*p53*	Al-K X-ray	Clonogenic survival	In the irradiated region	0.45–1.0; 2.0–5.0	[26]
H1299/mp53-143ts				0.45–5.0	
V79	SR X-ray 10 × 10 μm	Clonogenic survival	Nuclear dose	0.26–1.7; 2.1–3.3; Partly suppressed	[24]
	50 × 50 μm			0.27–3.0; 1.0	
	10 × 10 μm	Clonogenic survival		0.5; 1.5-4.0; Partly suppressed	[27]
		HPRT mutation		1.0; 2.0-4.0	
NB1RGB	SR X-ray 20 × 20 μm	MN	In the irradiated region	0.2-1	[18]

Solid line = dose range where bystander response was significantly detected, dashed line = dose range where the response was not significantly detected or was partly suppressed.

of the LLC tumor was significantly suppressed. The effect decreased when the radiation dose was twelve fractions of 2 Gy each. The dose-dependent abscopal effect was also reported for other endpoints in C57BL/6 mice [60]. When the abdomen of the male mice was irradiated with X-rays, a decrease in the total body bone mineral density (BMD) was observed at doses of 10 and 15 Gy, but not of 5 Gy, 14 days after irradiation. The effect was also observed in mice irradiated at doses of 15 and 20 Gy (7 days after irradiation). The decrease in BMD effect was also observed in the femur and tibia, but not in the lumbar vertebrae, of the mice exposed to a single fraction of 5, 10 and 15 Gy 7 days after irradiation and 15 and 20 Gy 14 days after irradiation.

Mancuso *et al.* recently described dose-dependent abscopal tumorigenesis in *Ptch1*$^{+/-}$ mice [33, 61]. In their previous study [33], induction of medulloblastoma was observed in the brains of the mice exposed to shielded irradiation of 3 Gy. Kaplan–Meier survival analysis of shielded mice showed that the effect at 2 Gy was virtually identical to that in the previous result obtained using 3 Gy [61]. Conversely, no increase in brain tumor mortality was observed in mice irradiated with 1 Gy compared with control mice. Although responses using 2 and 3 Gy resulted in the upregulation of connexin43 (Cx43) in the cerebellum, there was a lack of effect on Cx43 levels after shielded 1 Gy irradiation. On the other hand, dose-dependent induction of apoptotic cell death in the shielded cerebellum was observed at doses of 1–3 Gy.

Several *in vivo* studies clearly indicate that low-dose and low-LET radiation did not induce a bystander response in the mouse spleen [62, 63] and bone marrow [64]. Blyth *et al.* [62] reported that no change in apoptosis or proliferation due to the bystander response was observed in recipient C57BL/6J mice or *pKZ1*$^{+/-}$ transgenic mouse spleen using an adoptive transfer method. In this study, isolated splenic

Table 3. Dose response of photon-induced bystander/abscopal response *in vivo*

Animal	IR	Targeted region	Observed tissues/cells	Endpoint	Investigated dose region (Gy)	Ref
					0.001 0.01 0.1 1 10	
BALB/C	^{60}Co γ-rays	Left flank (67NR tumor)	67NR tumor in right flank	Tumor growth delay	2–6 ◆▶	[58]
C57BL/6	^{137}Cs γ-rays	Right hind leg	LLC tumor in midline dorsum	Tumor growth delay	2 Gy×12–10 Gy×5 ◆▶	[59]
C57BL/6	X-rays	Abdomen	Total body	Loss of BMD	10–20 ◆▶ 5 -->	[60]
			Femur, tibia		5–20 ◆▶	
Ptch1$^{+/-}$ mice	X-rays	Lower body	Cerebellum	Tumor genesis, CX43 expression	2–3 ◆▶ 1 ->	[33, 61]
				Apoptosis	1–3 ◆▶	
C57BL/6J	X-rays	Isolate splenic lymphocytes	Spleen	Proliferation, Apoptosis	0.1–1 <---->	[62]
pKZ transgenic mice	X-rays	Whole body	Spleen	Apoptosis	0.00001–0.001 <>	[63]
C57BL/6	X-rays	Whole body	Bone marrow	p53, p21 induction	0.0017–0.025 <---->	[64]
CBA/Ca					0.0017–0.05 <------>	
C57BL/6, CBA/Ca				Apoptosis (Caspase-3)	0.0017–0.1 <--------->	
				Apoptosis (TUNEL)	0.0017–0.2 <---------->	
CBA/Ca				Cytogenic aberration	0.0017–0.5 <---------->	

IR = ionizing radiation, solid line = dose range where bystander/abscopal response was significantly detected, dashed line = dose range where the response was not significantly detected.

lymphocytes (donor cells) were irradiated with 6-MeV X-rays at doses of 0.1 and 1 Gy. No change in the apoptosis frequency in *pKZ* transgenic mouse spleen was detected at any time after whole-body low-dose X-irradiation (0.01 or 1 mGy), although apoptosis (TUNEL-positive) was induced after irradiation with 1 Gy [63]. Zyuzikov *et al.* [64] reported that there was no bystander response in terms of *p53* and *p21* expression or induction of apoptotic cell death (TUNEL or active-Caspase-3 positive) after *in vivo* X-irradiation of murine bone marrow using a 0–100 mGy range. In addition, the frequency of cytogenetic aberrations did not change 30 days after whole-body irradiation using doses of up to 500 mGy, although bone marrow cells irradiated with a low dose of densely ionizing high-LET α-particles showed a significant expression of chromosomal instability *in vitro* [65] and *in vivo* [66].

The results of these studies are listed in Table 3. No abscopal carcinogenic effects were detected at doses of <1 Gy, even in the radiosensitive *Ptch*$^{+/-}$ mouse. In addition, when the whole body was irradiated with low-dose X-rays of <1 mGy, no bystander response was detected. The importance of the results of these studies in relation to low-dose risk estimates is discussed later.

MECHANISMS OF PHOTON-INDUCED BYSTANDER RESPONSE

The mechanisms of RIBR and its relationship with non-targeted effects have been extensively discussed in several reviews [14–16]. This review will thus mainly focus on the mechanisms of photon-induced bystander response based on our previous studies [23–27]. To elicit a bystander response, at

least two signaling pathways are required: a direct physical connection between cells such as GJIC and the culture medium.

GJIC

Cx43-mediated GJIC is involved in the α-particle-induced bystander signaling in confluent cells [67]. Cx43 expression was observed using mean α-particle doses as low as 1.6 mGy and also in cells exposed to γ-rays at doses of 0.5 and 4 Gy [68]. Autsavapromporn *et al.* [18] reported that the dose-dependent bystander induction of MN by SR X-ray microbeam irradiation was not affected by treatment with an inhibitor of GJIC, whereas heavy-ion-induced bystander MN formation largely depended on the GJIC. In the signaling of abscopal effect *in vivo*, upregulation of Cx43 was also observed in the shielded *Ptch*$^{+/-}$ cerebellum at the doses of >1 Gy [33, 61, 69]. The expression of Cx43 did not increase in the cerebella of mice that were whole-body-exposed to 0.036 Gy compared with that of non-irradiated mice. In our previous studies, in which five cells within a confluent culture of WI-38 cells were irradiated with X-ray microbeams [23, 25], lindane (which is an inhibitor of GJIC) dissolved in a reactive oxygen species scavenger dimethyl sulfoxide (DMSO) partially suppressed bystander cell killing, although the effect was not significantly different from sham-treated [23] or DMSO-treated cells [25]. Sokolov *et al.* [45] reported that γ-H2AX foci formation in bystander cells was completely prevented by lindane at a dose of 0.2 Gy. In their study, WI-38 cells cultured in multi-well slides were irradiated with γ-rays, and non-irradiated WI-38 cells were added to the irradiated cells. Thus, the ratio of irradiated to non-irradiated cells was extremely high. The contribution of GJIC in the bystander signaling may be relevant to the irradiated volume, although there is confusion regarding the dose dependence between *in vitro* and *in vivo* conditions. Abscopal tumorigenesis in the cerebellum of *Ptch1*$^{+/-}$ mice was suppressed when two-thirds of the body was shielded, whereas substantial levels were observed in one-third of body-shielded mice [61].

Nitric oxide (NO) and COX-2

In the case of bystander signaling through the culture medium, possible candidate bystander factor proteins include the redox-modulated tumor necrosis factor-α (TNF-α) or transforming growth factor-β1 (TGF-β1), as reviewed in [16]. Recently, it has been reported that NO is an important factor for photon-induced bystander response. In our previous studies using X-ray microbeams, the bystander cell-killing effect in WI-38 at 0.93 Gy [23] or 0.47 Gy [25] and in H1299/m*p53*-143ts cells at 2 Gy [26] was significantly suppressed in the cells pretreated with aminoguanidine (AG), which inhibits inducible NO synthase (iNOS) and/or carboxy-PTIO (c-PTIO), a scavenger of NO. Additionally, both bystander cell-killing effect and reduction of mutation frequency in bystander cells, which was lower than the baseline levels, were also suppressed in V79 cells pretreated with c-PTIO after 1 Gy of SR X-ray microbeam irradiation [27]. The bystander cell-killing effect in V79 cells was significantly suppressed at the doses of 0.29–1.7 Gy and 0.28–1.7 Gy in the whole-cell-irradiated and the nuclease-irradiated case, respectively [24]. Sokolov *et al.* [45] also reported that γ-H2AX foci formation in bystander WI-38 cells pretreated with AG and c-PTIO was effectively suppressed when the target cells were irradiated with γ-rays at 0.2 Gy and the cell mixing and media transfer methodologies were used. Matsumoto *et al.* [70, 71] observed iNOS accumulation in human glioblastoma T98G cells and A-172/m*p53* cells expressing mutated *p53* irradiated with X-rays at the doses of 3.5 and 2.5 Gy, respectively. The decreased out-of-field cell survival in human prostate cancer DU-145 cells after exposure to intensity-modulated radiation fields at the dose of in-field region 8 Gy was significantly suppressed by pretreatment with AG, despite receiving scatter radiation doses [72]. Further studies showed that iNOS inhibition significantly increased the out-of-field cell survival of AG01522B, MDA-MB-231 (human breast cancer) and DU-154 cells [73] at the dose of in-field region 4 or 8 Gy. NO-induced cellular toxicity is an extremely complex process that involves DNA synthesis inhibition, mitochondrial inactivation, cell membrane lysis, cell cycle arrest, DNA strand break formation, and apoptotic cell death [74]. NO reacts with DNA via multiple pathways and induces DNA damage [75]. Peroxynitrite, $ONOO^-$, oxidizes and nitrifies DNA, which can potentially cause single-strand breaks. Low-level (1 μM) $ONOO^-$ can increase the frequency of γ-H2AX-positive cells [76]. Nitrous anhydride (N_2O_3) could nitrosate amines to form N-nitrosamines, which, after metabolic activation, can alkylate DNA through an indirect mechanism. Nitrosation of primary amines (e.g. in DNA bases) leads to the formation of diazonium ions and subsequent deamination and cross-linking [75]. Additionally, when cells can induce iNOS after irradiation, GJIC may be inhibited by NO (reviewed in [16]). There is growing *in vitro* evidence showing that NO is a chief initiator and/or mediator of photon-induced bystander responses.

The role of COX-2 (also known as prostaglandin endoperoxide synthase-2) in inducing a bystander response was suggested by Matsumoto *et al.* [70] and was indicated using α-particles by Zhou *et al.* [77]. Treatment of bystander cells with a COX-2 inhibitor significantly reduced the α-particle-induced bystander response. Zhou *et al.* [78] described the critical roles of NF-κB/COX-2/prostaglandin E2 (PGE2) and NF-κB/iNOS/NO pathways in generating an α-particle-induced bystander response. Hei *et al.* [15] has provided an excellent review on these signaling pathways. In a previous X-ray-microbeam study [26], H1299/m*p53*-143ts cells that were pretreated with the COX-2 inhibitor and irradiated with 2.0 Gy of X-ray microbeams showed a significant suppression of the bystander cell-killing effect, which suggests that COX-2-mediated signaling plays an essential role in eliciting a photon-induced bystander response.

p53

Several studies have described the relationship between *p53* status and bystander response. However, the proposed role of *p53* in the RIBR differs between reports. In our studies using X-ray microbeams, the bystander cell-killing effect of WI-38 cells and H1299 cells expressing wild-type *p53* showed a parabolic relationship with the irradiated dose of targeted cells; the fraction was steeply reduced and recovered toward to 2.0 Gy [23, 25, 26]. In the co-culture method, H1299/wt*p53* cells irradiated with 2.0 Gy of X-ray microbeams did not induce a bystander cell-killing response in non-irradiated H1299/m*p53*-143ts cells. *p53* can attenuate, or inhibit the induction of iNOS, which catalyzes the conversion reaction of L-arginine into L-citrulline, through the interaction between *p53* and the TATA-binding protein and/or NF-κB, which are essential components for *iNOS* expression (review in [16]). Suppression of the release of bystander signals such as NO mainly depends on the wild-type function of *p53* in X-ray microbeam-irradiated cells.

Matsumoto *et al.* [71] reported that the wild-type *p53* cells cultured in ICM derived from X-irradiated mutated *p53* cells were more radio-resistant than those incubated in fresh medium or in ICM from sham-irradiated mutated *p53* cells. These results indicate that NO, which was generated by radiation-induced iNOS from mutated *p53* cells, can induce radio-resistance in non-irradiated NO recipient wild-type *p53* cells. Additionally, the wild-type *p53* cells treated with a NO-generating agent showed radio-resistance [79]. Mothersill *et al.* [80] tested 13 different cell lines irradiated with 0.5 or 2 Gy of X-rays using medium transfer methods. Cell lines with mutated *p53* demonstrated induced radio-resistance, which was accompanied by low-dose hyper-radiosensitivity, and did not elicit bystander signals that could induce a cell-killing effect. Cell lines with wild-type *p53* did not show increased radio-resistance, but produced bystander signals. Other studies using medium transfer methods in cells irradiated with 0.5 or 5 Gy of γ-rays indicated that the mutated *p53* cell lines elicited bystander signals, although they did not respond to these actual signals [81]. Mothersill *et al.* [82] further reported that both human colon tumor HCT116 cells with wild-type and null *p53* generated bystander signals, although only the wild-type *p53* cells responded to the bystander signals from either cell line. On the other hand, bystander Chang liver cells (wild-type *p53*) showed an increase in MN formation after co-culture with γ-irradiated human hepatoma HepG2 (wild-type *p53*) cells, but not PLC/PRF/5 (mutated *p53*) or Hep3B (*p53* null) cells [83]. Zhang *et al.* [84] reported that in co-culture experiments, cellular *p53* status was independent of the production of bystander mutagenic signals in the cell lines irradiated with 2 Gy of γ-rays and/or the response to these signals, using three human lymphoblastoid cell lines, TK6 (wild-type *p53*), NH32 (*p53* null), and WTK1 (mutated *p53* at codon 237), from the same progenitor.

In the study of abscopal effect *in vivo* [59], LLC (*p53* mutated) was implanted in the midline dorsum of C57BL/6 (wild-type *p53*) or *p53* null mice. When the legs of the *p53* null mice or C57BL/6 mice treated with pifithrin-α (a p53 blocker) were irradiated with five 10-Gy fractions of γ-rays, the growth of the LLC tumor was not suppressed.

The difference in ability to generate and/or respond to bystander signals among mutated *p53* cells might be caused by cell type and/or mutated codons on *p53*. However, in our

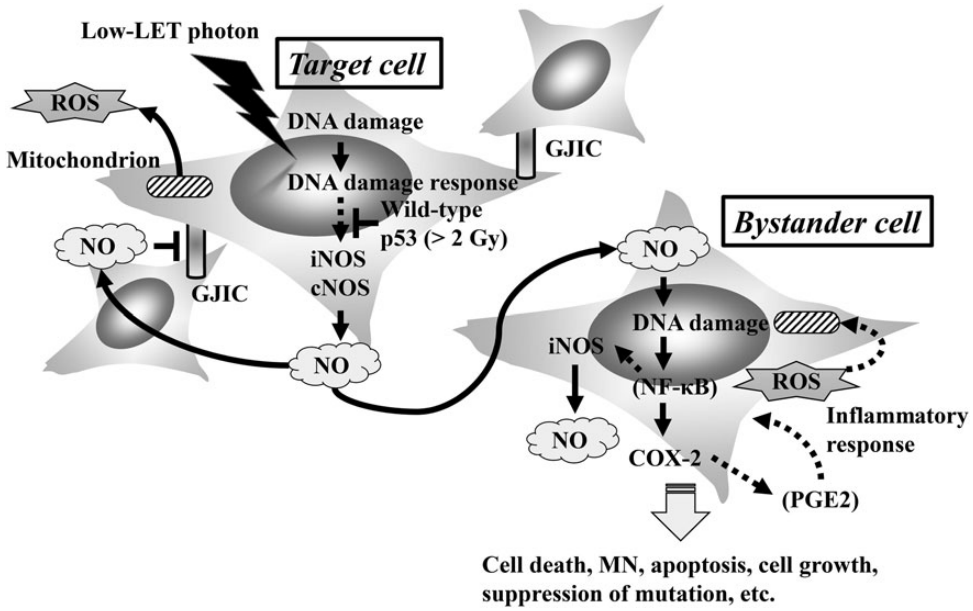

Fig. 1. Schematic drawing of a possible model of photon-induced bystander signaling mainly based on our results obtained using X-ray microbeams [23–27].

microbeam study [26], we clearly showed that the H1299 cells expressing both wild-type and mutated *p53* generate and release bystander signals and can respond to these signals, although signal generation was influenced by the irradiated dose.

Based on the results of our studies, we have generated a model for the photon-induced bystander signaling pathway, as shown in Fig. 1. To resolve current confusion regarding this concept, further experiments confirming the role of *p53* in the photon-induced bystander response *in vitro* and *in vivo* are warranted.

RIBR AND HEALTH EFFECTS OF LOW-DOSE RADIATION

The linear-non-threshold (LNT) model, which is based on the assumption that in the low-dose region, radiation doses greater than zero will increase the risk of excess cancer and/or heritable disease in a simple proportionate manner [85], has been used for the purpose of radiation protection. The ICRP considered that "the LNT model remains a prudent basis for radiological protection at low doses and low dose rates". The LNT model was principally based on data from the Japanese Life Span Study of A-bomb survivors (LSS). The ICRP stated that LSS-based central estimates of radiation cancer risk are reasonably robust and not highly sensitive to choices of risk models [85]. However, risk estimation in epidemiological studies is relatively difficult at doses below a few hundred mSv due to insufficiency of statistical power. Thus, to improve the uncertainty on low-dose risk estimates, it is essential to elucidate the biological mechanisms of radiation action in a low-dose region. RIBR might impart important biological consequences under low-dose irradiation conditions, where non-targeted or non-irradiated cells are affected in the irradiated population. Furthermore, the linear extrapolation of risks from a middle or high-dose region to a low-dose region might underestimate the actual risks of low-dose radiation.

In this review, the dose response of photon-induced bystander/abscopal responses is presented in Tables 1–3. The results are generally confusing; the responses may have been influenced by various factors including cell type, animal strain, endpoints, and method. However, sorting the data related to the radiation-induced carcinogenesis process shows that photon-induced bystander/abscopal responses did not influence the risks of carcinogenesis (Fig. 2). In organisms or tissues exposed to a low dose of radiation, non-irradiated cells begin to appear when irradiation doses are lower than the specific-energy deposition per cell nucleus traversal (hit) $\langle Z_1 \rangle$ [86, 87]. The $\langle Z_1 \rangle$ value of ^{137}Cs γ-rays in HeLa × skin hybrid cells was 0.5–0.6 mGy/Hit, and that of mammography using 30 kVp X-rays was 0.6 mGy/Hit, although the value depends on the size of the cell nucleus [86, 87]. If a bystander or abscopal response was elicited at doses given by a single hit event, radiation-induced cancer risk would indicate a supra-linear relationship with the irradiated dose. However, Kaplan–Meier analysis of survival of brain-shielded radiosensitive *Ptch1*$^{+/-}$ mice showed that the induction of medulloblastoma was significant at doses of 2–3 Gy,

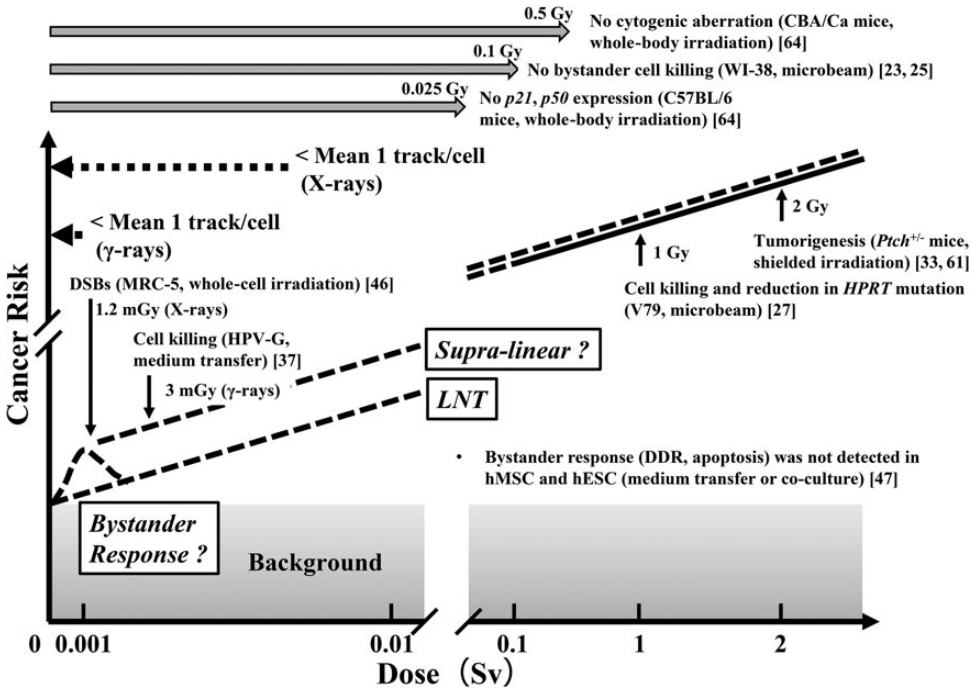

Fig. 2. Schematic representation of the radiation-related cancer risk to very low radiation doses and the photon-induced bystander/abscopal responses, which are partly relevant to the radiation-induced carcinogenesis process.

but not at 1 Gy [33, 61]. Additionally, whole body-irradiated *Ptch1*$^{+/-}$ mice that received a dose of 0.037 Gy did not undergo tumorigenesis [33]. Long-term chromosomal instability was not manifested at doses of ≤500 mGy [64]. X-ray-induced bystander response was not induced in hMSCs and hESCs [47], although the stem cells and/or the progenitor cells are recognized as the targets of radiation carcinogenesis [88]. No bystander cell-killing effect of WI-38 cells was detected at doses of <100 mGy in our previous studies using X-ray microbeams [23, 25], and immortalized human keratinocyte HPV-G cells that received ICM showed a threshold dose of between 2 mGy and 3 mGy of γ-rays [37]. On the other hand, a supra-linear dose response of pATM foci formation in confluent MRC-5 cells was reported at a dose of 1.2 mGy, which is lower than that given by a single track event of X-rays [46]. However, γ-H2AX foci formation in confluent MRC-5 cells showed a linear response relationship to doses ranging from 1.2 mGy to 2 Gy [89]. Persistent pATM and γ-H2AX foci formation at 1.2 mGy was observed in non-dividing confluent cells [89, 90]. The application of 1.2 mGy to induce pATM foci formation as a bystander response did not undergo carcinogenesis based on the observation that the number of cells harboring DNA damages did not increase.

There is a possibility that low-LET and low-dose-rate irradiations also induce a bystander response. The concept of inverse dose-rate effect has been extensively discussed in the literature [91]. However, epidemiological studies on naturally high-background-radiation areas did not show an excessively increased risk for cancer [92, 93]. In our laboratory, Koana *et al*. [94] reported the reduction of spontaneous somatic mutation frequency in *Drosophila* irradiated with 0.2 Gy of X-irradiation at 0.05 Gy/min. In addition, low-dose-rate γ-ray irradiation induced a radioadaptive response mediated by NO [79].

The ICRP 2007 Recommendations [85] indicated that knowledge of the roles of induced genomic instability, bystander cell signaling and adaptive response in the genesis of radiation-induced-health effects is insufficient for radiological protection purposes. In several circumstances, these cellular processes are incorporated into epidemiological risk measurement. The UNSCEAR 2006 Report [29] concluded that the currently available data do not require changes in radiation-risk coefficients for cancer and hereditary effects of radiation in humans. Morgan also stated in his review [95] that non-targeted effects are already encompassed in potential estimates of radiation risk in humans, regardless of the type of individual exposure, namely whole-body or partial-body exposure. The UNSCEAR also described in their white paper that there is little of the coherence required of robust data that can be used confidently for risk assessment [31]. Accumulating knowledge on the photon-induced bystander and abscopal response reviewed in this paper supports their recommendation.

CONCLUSION

Spatio-temporal control of tissue or organ by the cell division, differentiation (especially tissue stem cells), growth, and death are prerequisites for the development and homeostasis of tissues. The generation of photon radiation-induced bystander and abscopal responses do not affect the homeostasis of tissues or the organisms at very low-dose regions, where non-irradiated cells are affected within the irradiated population. On the other hand, in a scenario involving partial tissue exposure to a high dose of photons, a RIBR may occur to sustain the homeostasis of damaged tissue as a whole rather than a set of isolated parts to restore or reset homeostasis [95]. This phenomenon is also known as 'adaptive homeostasis' [95]. The c-Jun N-terminal kinase (JNK)-dependent cell-elimination system is orchestrated by the intercellular communication between normal and aberrant cells and is essential in protecting organisms against deleterious abnormalities such as neoplastic development [96]. 'Cell competition' is one of these processes, and enables the short-range elimination of slowly dividing cells through apoptotic cell death when confronted with a population of faster-growing cells [96, 97]. Thus, cell competition is based on tissue adaptability. In the irradiated population, cell death (including apoptosis) occurs as a bystander response both *in vivo* and *in vitro* (Tables 1–3). In addition, studies on cell competition have identified potential molecular effectors that allow fitness comparisons among cells [97]. Our recent study [27] clearly showed the *HPRT* mutation frequency in bystander cells decreased at a dose at which bystander cell death is induced. NO mediated both a reduction in mutation frequency and cell death. Thus NO might be an effector that activates the elimination of genomically unstable cells as loser cells, sustaining tissue homeostasis against carcinogenesis.

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