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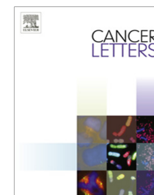

Mini-review

Radiation-induced bystander effect: Early process and rapid assessment

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ABSTRACT

Radiation-induced bystander effect (RIBE) is a biological process that has received attention over the past two decades. RIBE refers to a plethora of biological effects in non-irradiated cells, including induction of genetic damages, gene expression, cell transformation, proliferation and cell death, which are initiated by receiving bystander signals released from irradiated cells. RIBE brings potential hazards to normal tissues in radiotherapy, and imparts a higher risk from low-dose radiation than we previously thought. Detection with proteins related to DNA damage and repair, cell cycle control, proliferation, etc. have enabled rapid assessment of RIBE in a number of research systems such as cultured cells, three-dimensional tissue models and animal models. Accumulated experimental data have suggested that RIBE may be initiated rapidly within a time frame as short as several minutes after radiation. These have led to the requirement of techniques capable of rapidly assessing RIBE itself as well as assessing the early processes involved.

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1. Introduction

Since the discovery of X-ray by Röntgen in 1895, ionizing radiation has been used in both diagnostic and therapeutic medical applications although its biological effects have not been fully understood. Extensive researches have been carried out on victims of atomic bomb explosions, occupational and accidental radiation exposure in the past century and have aroused the general awareness among general citizens on the potential benefits and risks of ionizing radiation [1]. Despite the vast benefits derived from various medical applications, radiation can be harmful and is well established as a carcinogen to living organisms [2].

Previous works in the past three decades have established that the main biological "target" of radiation was the cell nuclei, while DNA damages, or more precisely the subsequent genetic changes due to mis-repaired or un-repaired DNA damages, were deemed the most important biological effect. Presumably, no effects would occur in cells not traversed by radiation. This dogma has been widely adopted by the radiation protection agencies. However, this dogma has been challenged by scientific findings since 1990s, exemplified by the occurrence of genetic changes in non-irradiated cells in a partially irradiated cell population [3–7]. Such a non-targeted phenomenon, called radiation-induced bystander effect (RIBE), implies radiation risks to cells or tissues which have not been irradiated, e.g., when the body is only partially exposed to irradiation [8,9]. Subsequent investigations on RIBE employed various research strategies, including partial irradiation of cell populations, tissues or animal models with conventional broad-field or microbeam irradiation, medium transfer, co-culturing with irradiated cells in separate inserts, mixed co-culturing with irradiated cells, etc. [10–12]. As time goes by, RIBE has been reported to induce various biological effects including sister chromatid exchange (SCE), micronuclei (MN), DNA double strand breaks (DSBs), gene locus mutation, neoplastic, even tumor formation, etc. [11–13].

Studies on mechanisms underlying RIBE have identified possible signaling pathways. For example, irradiated cells would release RIBE signal(s) which "attacked" the neighboring or even distant cells either through cellular gap-junction intercellular communication (GJIC) or through diffusion in the medium [9,11,13]. Oxidative stress plays a very important role in the generation, release and propagation of these signal(s) [9,11,13]. The involved signaling pathways which might mediate RIBE transduction were reported in subsequent studies. The mitogen-activated protein kinases (MAPK) signal pathway, nuclear factor kappa B/prostaglandin-endoperoxide synthase (cyclooxygenase) 2 (NF-κB/COX-2) pathway, nitric oxide (NO) related signal pathway, inflammation-related signal pathways, etc., were found to be an integral part of RIBE transduction [9,12–14]. The nature of extracellular RIBE signaling molecule(s) has been explored in the past years. Accumulated evidences indicated that the molecules(s) being released by irradiated cells and acting as possible extracellular RIBE signals include NO, transforming growth factor beta 1 (TGF-β1), tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), IL-8, etc. [9,12].

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The ionizing radiations to which common people are exposed come from sources in our natural environment such as radon progeny, and from medical activities and some unpredicted accidents. The radiation dose involved in these conditions is mostly very low [15]. Even in the recent Fukushima-Daiichi nuclear power plant disaster (which occurred on 11 March, 2011) which was rated 7 on the International Nuclear Events Scale (INES) scale, medical checks harvested on 28 August, 2011 of 3514 workers who had worked at the plant since 11 March, 2011 showed that only 124 of them had received radiation doses above 100 mSv [16]. When compared to the direct effects of irradiation, RIBE is very weak in the medium to high dose range [1,17]. However, in the low-dose range, RIBE could not be ignored [10,15,17,18]. Recently, Yang et al. confirmed that RIBE dominated the response in the cell population irradiated with high-energy protons and iron ions at low doses (5 cGy) [19]. Therefore, when considering the low-dose radiation exposures relevant to public health, assessment of health risk caused by both the direct effect and RIBE would be indispensable, e.g., for relevant risk assessment or for planning the necessary medical treatments.

The presence of RIBE increased the health risk of low dose radiation exposure since more cells in addition to the directly irradiated cells were indirectly damaged by the radiation. During radiotherapy, RIBE would increase the possibility of genetic changes and even tumor induction in tissues beyond the "targeted" tumor. In the investigation of RIBE, some rapid assessment or testing methods have been established and these methods have helped radiobiologists understand the occurrence of RIBE within a short time frame (such as 1–2 h) post irradiation and to further explore the possible mechanisms of RIBE. These rapid assessment or testing methods will also help the health risk assessment after some unplanned exposure such as nuclear accidents or assault of "dirty bombs".

2. Rapid assessment of RIBE

RIBE had been assessed with SCE testing [3], MN testing [5], cell death or clonal survival assay, gene locus mutation [7,20–22], etc. For these biological endpoints, relative long time periods would be required, e.g., for the formation of chromosome aberration, cell apoptosis, cell clones and mutations. At least 1–2 days or even more than 1–2 weeks would be needed with these methods. Besides, extra biological factors might be involved in the relative long process of RIBE measurement, and these extra factors might make it more difficult to identify the mechanisms of RIBE. Fortunately, continued research studies on RIBE had established methods with assessment time frame of several hours. Through these techniques, radiobiologists had been able to detect the promptly recruited DSB damage sensors or repair factors, specific genes expression, cell cycle relevant proteins, or transcription factors in the bystander cells to assess their risk. Significantly, some research groups had been able to observe fast occurrence of RIBE in even less than 5 min after irradiation [23–25]. Apparently, knowing more details of the early process of RIBE will be very helpful in better understanding how the RIBE signals are generated and released, and how they modulate the bystander cells.

2.1. Phosphorylation of H2AX

DSB is the most important lesion among different DNA damages [26,27]. The research presented by Little's group showed higher yields of chromosome aberration (MN and SCE) [28,29] and mutation [30] in bystander cells deficient in DSB repair, when compared to those in wild-type cells and cells deficient in base excision repair. These results also insinuated that DSBs were induced in bystander cells as a result of RIBE.

Phosphorylation of the H2AX protein, one member of the histone families, on serine 139 (γ-H2AX) has been known as one of the early responses of cells to DSBs induced by various stimulating factors [27,31,32]. Sedelnikova et al. proved that each discrete γ-H2AX focus contained a single DNA DSB, and suggested that the assessment by counting the number of γ-H2AX foci in the cell nuclei was the most sensitive method to evaluate the DSBs in cells [33]. In 2005 three groups independently reported their researches about assessing RIBE with γ-H2AX immunofluorescence [34–36]. Using a co-cultured system with multi-well inserts, Yang et al. detected significant γ-H2AX formation in bystander human fibroblasts cultured in the inserts after X-ray irradiation [34]. In a full confluent human skin fibroblast population, Hu et al. detected more γ-H2AX positive cells than the number of cells hit by low-dose α particles, which was attributed to the existence of RIBE [36]. Sokolov et al. presented more definite proofs of RIBE-induced DSBs in cultured human fibroblasts with γ-H2AX immunofluorescence and the co-localization of γ-H2AX foci and other DSB-related factors, such as phosphorylated ataxia-telangiectasia mutated kinase (ATM), 53BP1 and components of the MRN complex after irradiation with prescribed numbers of α particles from a microbeam facility [35].

In the following years, more research groups used this technique to assess RIBE in their research models [23,37–41]. Yang et al. observed a two- to threefold increase in the number of γ-H2AX foci in bystander cells sharing the medium with cells, which had been irradiated with iron ions in a co-cultured system with multi-well inserts, as early as 1 h after irradiation and lasted at least 24 h [42]. The temporal change of γ-H2AX foci formation in the bystander cells, which constituted half of the fully confluent cell population and which were shielded from irradiation, showed distinct increase in the number of foci over the control as early as 10 min post irradiation and reached the maximum at 30 min [24,25]. In an artificial human tissue model, Sedelnikova et al. detected 4-6-fold increase in the number of γ-H2AX positive cells in the bystander cell population, as far as 2.5 mm away from the plane of irradiation precisely performed with a microbeam facility, and as early as 0.5 h post irradiation [43]. In *in vivo* animal models, RIBE-induced γ-H2AX formation and Rad 51 expression were detected in the lead-shielded bystander skin [44] or cerebellum [45] of mice at about 6 h after X-ray exposure. On one hand, the γ-H2AX immunofluorescence technique allowed rapid detection of RIBE-induced genetic damages. On the other hand, the correspondence between the numbers of γ-H2AX foci and DSB enabled detailed assessment on the extent of damages from RIBE.

2.2. 53BP1

The tumor suppressor p53-binding protein 1, known as 53BP1, is a protein that in humans encoded by the TP53BP1 gene, and 53BP1 is required for p53 accumulation and cell cycle checkpoint in response to DSB repair [46]. Sokolov et al. remarked that 53BP1 foci could be induced in bystander human skin fibroblasts, and these foci were co-localized with γ-H2AX foci in bystander cells, which were co-cultured with cells irradiated by α particles from a microbeam facility or which received the conditioned medium harvested from a γ-ray irradiated cell population [35]. Tartier et al. used 53BP1 foci to assess RIBE induced by precise irradiation on cell nucleus or cytoplasm with a microbeam facility, and their results showed that the fraction of 53BP1 positive bystander cells peaked at 1 or 3 h when the nucleus or cytoplasm were irradiated, respectively [47].

With this method, many research groups have assessed RIBE in *in vitro* and *in vivo* models. Han et al. observed significantly increased 53BP1 foci formation in proliferating bystander Chinese Hamster Ovary (CHO) cells, which were co-cultured with cells

irradiated by α particles [48]. They also used 53BP1 immunofluorescence to assess the protection of low-concentration carbon monoxide on bystander CHO cells [49]. A lack of RIBE in cultured human mesenchymal and embryonic stem cells, which were treated with conditioned medium or co-cultured with irradiated cells, was observed by Sokolov who determined the fraction of 53BP1 positive cells to assess RIBE-induced DNA damages [50]. Yang et al. showed that the bystander response dominated in the entire exposed cell population, when the dose of high-energy protons or iron ions was less than about 5 cGy, through detection with MN formation and 53BP1 foci induction [19]. In an *in vivo* study, Mancuso et al. showed that 53BP1 foci could be induced by abscopal RIBE signals from the partially X-ray exposed bodies of *Ptch1*$^{+/-}$ mice as early as 30 min post irradiation in the external granule layer of shielded cerebellum, despite that the animals' heads were fully protected by suitable lead cylinders [51].

2.3. Phosphorylation of ATM

ATM, which phosphorylates several key proteins participating in DNA damage response, is recruited and activated by DSBs. ATM phosphorylates several key proteins such as p53, CHK2 and H2AX to activate cell cycle checkpoints, DNA repair or apoptosis [52]. Activation of ATM, which was dependent on ataxia-telangiectasia and Rad3-related (ATR) function, was found in bystander cells while phosphorylated ATM foci were found to co-localize with γ-H2AX foci, and deficiency of ATM or ATR function would completely abrogate RIBE [35,53]. With the immunofluorescence technique, Ojima's group found an increase in the number of DSBs (surrogated by ATM foci) in MRC-5 cells after 1.2-5 mGy X-ray irradiation, which was attributed to RIBE [23]. In a further study, they counted the phosphorylated ATM foci to study the repair kinetics of DSBs in bystander MRC-5 cells co-cultured with X-ray irradiated cells, and showed that ATM foci could be observed as early as 0.05 h post irradiation in bystander cells and was almost constant for 48 h on a level of 4–5-folds higher than that of the sham-irradiated control, but on the contrary DSBs induced by direct radiation were repaired relatively quickly [54].

2.4. p21^{Waf1}

The involvement of p21^{Waf1}, which promoted cell cycle arrest in response to many stimuli and which was controlled by the tumor suppressor protein p53 [55], in RIBE was first revealed by Azzam et al. [6,56]. They noticed that RIBE up-regulated the protein expression of p53 and p21^{Waf1} with western blot analysis. With *in situ* immunofluorescence, they observed up-regulation of p21^{Waf1} expression in clusters of AG1522 cells in close proximity to each other after exposure to 0.3 cGy α-particle irradiation [6]. However, no quantitative results of p21^{Waf1} expression in bystander cells were provided in their study. Burdak-Rothkamm et al. observed that the foci of p21^{Waf1} co-localized with other proteins associated with DSBs such as 53BP1 and BRAC1 [53]. Yang et al. quantified the p21^{Waf1} expression by counting the fraction of positive cells, and remarked that about onefold increase in the fraction of p21^{Waf1} positive cells could be detected in the bystander cells which had shared the medium with X-ray irradiated cells in a co-culture insert system [34]. With western blot analysis, Mitra and Krishna noticed a strong increase in p21^{Waf1} protein expression levels in bystander human erythroleukemia K562 cells with a peak, nearly threefolds that of the control, at 4 h after receiving the conditioned medium harvested from a sample of the same kind of cells 20 min after 2 Gy X-rays irradiation [57]. Gaillard et al. combined the techniques of p21^{Waf1} imunofluorescence staining and α-particle track revelation to elucidate the distribution of RIBE signal propagation in a fully confluent normal human skin fibroblast AG 1522 cell population [58]. They proposed that p21^{Waf1} could be induced in bystander cells within a 100-μm radius from an irradiated cell, and that the mean propagation distance ranged from 20 to 40 μm which covered about 30 cells [58].

2.5. PCNA

Proliferating cell nuclear antigen (PCNA) acts as a processivity factor for DNA polymerase δ and ε in eukaryotic cells during DNA replication, and is involved in the RAD6-dependent DNA repair pathway in response to DNA damages [59]. Iyer and Lehnert reported that the levels of PCNA, analyzed with western blot, in bystander HFL1 cells treated with supernatants from 1 cGy α-particle irradiated cells, were increased by 1.25- and 2-fold at 6 and 24 h, respectively [60]. In a cell culture/*ex vivo* rat model of respiratory tissue, Hill et al. detected a maximum PCNA positive response in up to 28% of the cells in sub-confluent cultures, after only 2 mGy α-particle irradiation, where less than 2% of the cell nuclei or 6% of the cells would be traversed by α particles [61]. In an artificial three-dimensional human skin tissue model, substantially lower levels of PCNA positive cells were observed, namely, 1% in AIR-100 and 1.7% in EFT-300 tissues, when compared to those in the control mock-irradiated bystander cells [43].

Despite the advent of these rapid assessment techniques using immunofluorescence of factors related to DNA damage, cell cycle arrest, cell proliferation, etc., no studies have been carried out to compare the sensitivities among these techniques, although the techniques with γ-H2AX or 53 BP1 were employed in most works described above. It was noted that the two markers of DSB, γ-H2AX or 53BP1, were not always related to radiation-induced DSBs but might due to other factors such as oxidative stress from metabolism or DNA replication [26]. Gollapalle et al. also reported that RIBE induced oxidative clustered DNA lesions [62], and subsequent studies further revealed that non-DSBs clusters played a very important role in radiation-induced mutagenesis and genomic instability [63]. More researches are needed to better understand and to assess the risk of radiation and RIBE in the future.

3. Early process of RIBE and mechanism studies

3.1. Existence of early process

Various research groups have studied the earliest time point of RIBE generation using different techniques in different employed models or systems. Evidence showed that RIBE could be induced within 1-2 h, or even as early as 5 min in some cases, after irradiation (shown in Table 1). The existence of early process would be described in the following according to the employed research system or model.

The cell co-culture system has been frequently employed in RIBE research. By using γ-H2AX as a biomarker of DSB, Wu and his colleagues observed a larger fraction of positive cells at 30 min post irradiation than that expected from hit cells in fully confluent AG 1522 fibroblast cell population irradiated with 0.5 or 1 cGy α particles [36]. These authors further studied the temporal variation of RIBE-induced DSBs in the low-dose (1 cGy) α-particle irradiated area and bystander area, formed by shielding half of the area of a fully confluent cell population. They showed that the fraction of γ-H2AX positive cells in both irradiated and bystander areas rapidly increased within as short as 5 min, reached the peak at 30 min after irradiation and then showed a slow decrease afterwards [25]. Hu et al. detected a distinct increase of γ-H2AX positive cells at a distance of 2 mm from the irradiated area at even 2 min post radiation, which reached a maximum 30 min after radiation [24]. In a no-physical-contact co-culture system, in which the

Table 1
Brief summary of contributions to investigate the early process of RIBE.

Assessing technique	Research system	Radiation type	Cell type	The earliest time of detecting RIBE	Reference
Immunofluorescence of γ-H2AX	Partial irradiation	α-Particle	Normal human diploid skin fibroblasts AG1522	2 min Post irradiation	Hu et al. [24]
Immunofluorescence of 53 BP1	Co-culture	Microbeam (helium ion)	HeLa	1 h Post irradiation	Tartier et al. [47]
Immunofluorescence of phosphorylated ATM	Co-culture	X-ray	Normal human fibroblast cells (MRC-5)	0.05 h Post irradiation	Ojima et al. [54]
Western blot of $p21^{Waf1}$	Medium transfer	γ-Ray	K562 human erythroleukemia cells	20 min Post irradiation	Mitra and Krishna [57]
MN test	Co-culture	X-ray	Me45 human melanoma cells; normal human dermal fibroblasts (NHDF)	30 min Post irradiation	Widel et al. [64]
ROS production	Medium transfer	α-Particle	A_L human–hamster hybrid cell	10 min Post irradiation	Chen et al. [69]
Gene mutation ($CD59^-$) gene	Medium transfer	α-Particle	A_L human–hamster hybrid cell	10 min Post irradiation	Chen et al. [69]

bystander cells were cultured on a glass slide and co-cultured with irradiated cells in the same container, a threefold increase in the mean phosphorylated ATM foci number per bystander MRC-5 cells was detected at as early as 0.05 h after 20 or 200 mGy X-ray irradiation and most of these foci persisted in the following 50 h [54].

Using the transwell insert co-culture system, an approximately twofold increase in the percentage of γ-H2AX positive cells was detected in the bystander cell population at 2 h after co-culturing with 0.1-10 Gy X-ray irradiated cells immediately after irradiation [34]. A two- to threefold increase in the percentage of γ-H2AX positive bystander cells, which shared the medium with irradiated cells in a transwell insert co-culture system immediately after irradiation, was reported as early as 1 h after irradiation by 1 GeV/nucleon iron ions [19]. MN formation was also observed when bystander cells were co-cultured with irradiated cells for only 1 h after irradiation [19]. Research on the temporal variation of RIBE in the transwell insert system with MN assay showed a twofold increase in the MN yield in bystander cells when they were co-cultured with irradiated cells either immediately, or 1 or 3 h after irradiation, but no RIBE was detected when the cells were co-cultured 6 h or later after irradiation [42]. An earlier time point for RIBE transduction was reported in both Me45 and NHDF neonatal human dermal fibroblast cells co-cultured with irradiated Me45 human melanoma cells, where distinct increases in MN over the control level were observed when the irradiated and bystander cells shared the medium for 30 min immediately after irradiation [64]. Moreover, tk^- mutation induced by RIBE transduction was significantly detected in bystander WTK1 human lymphoblastoid cells, which only shared the irradiated cell conditioned medium for 30 min immediately after irradiation, and the full mutagenesis-inducing level (2.5-fold increase over background) occurred in the bystander cells co-cultured for only 1 h post irradiation [65]. These results which were based on the transwell insert co-culture system indicated that RIBE transduction occurred within 1 h or even shorter (30 min) after irradiation and did not maintain for a long time [42].

With a microbeam facility, specific cells within a cell population or even specific positions on the cells (nucleus or cytoplasm) can be precisely chosen and irradiated. Using a microbeam facility, a distinct increase in 53BP1 positive bystander HeLa cells sharing the medium conditioned by cells with nuclei irradiated with 20 ^3He ions was detected at 1 h, which remained unchanged after 3 h [47]. Although formation of nuclear 53BP1 foci lagged in the bystander cells following cytoplasm targeting, the appearance of RIBE also reached the maximum at 3 h post radiation [47]. In contrast, after partially irradiating some cell nuclei with 2 or 20 α particles, Sokolov et al. detected a significant increase in γ-H2AX positive bystander cells at 18 h post irradiation, but not at 30 min post irradiation [35].

Medium transfer at different time points after irradiation provides valuable information on RIBE signal transduction. Lyng et al. observed rapid signaling activation, calcium influx pathway and mitogen-activated protein kinase (MAPK) pathways in bystander Human keratinocytes cells [66] within 1 min (intracellular calcium) or 30 min (ERK and JNK but not p38) after receiving the irradiated cell conditioned medium harvested at 1 h post irradiation [66]. A similar study with a microbeam facility revealed that calcium fluxes were observed within 30 s in a fraction of recipient cells (70-90% of AG 1522 and 50-80% of T98G) after receiving the conditioned medium harvested at 1 h post radiation [67]. Furthermore, a strongly increased expression of the $p21^{Waf1}$ protein in bystander K562 human erythroleukemia cells, which received the conditioned medium harvested at 20 min after the cells were irradiated with 2–10 Gy γ-ray, at as early as 0.5 h and reached a peak at 2-4 h. These indicated that RIBE signal(s) were released within as early as 20 min after irradiation [57]. In another study, the bystander cells (fully confluent AG 1522 human skin fibroblasts) displayed a distinct increase in DSBs (surrogated by γ-H2AX foci) after receiving the conditioned medium harvested at 23 min after 1 cGy α-particle irradiation, and the yield of RIBE reached the peak after receiving the conditioned medium harvested at 10 min [25]. In addition to DSBs [68], the conditioned medium harvested at 10 min post irradiation from 1 cGy irradiated A_L human-hamster hybrid cells induced reactive oxygen species (ROS) production (increased by 35% at 10 min after treatment), $CD59^-$ gene loci mutation (increased by 45.9%) and delayed cell death in the recipient bystander cells [69].

Considering the rapid occurrence of RIBE after irradiation, studies on the time of activation of the RIBE signaling pathways, the released RIBE extracellular signal(s) and the response of bystander cells to related signaling pathways should be performed within a shorter timeframe after irradiation.

4. Mechanism studies on early process of RIBE

4.1. Role of ROS and GJIC in the early process of RIBE

The importance of ROS in the early process of RIBE has been confirmed by various experiments. With the application of ROS scavengers, such as dimethyl sulfoxide (DMSO) or antioxidative enzymes (Superoxide Dismutase or Catalase), the RIBE rapidly induced after irradiation would be significantly attenuated to the level of sham-irradiated controls. Treatment with ROS

scavengers/antioxidative enzymes efficiently decreased the bystander γ-H2AX [24,25,34,36,42,69], 53BP1 [47], MN [34,42], p21[Waf1] [34], apoptosis [66] or inhibited the activation of signaling pathways, such as MAPK pathway [66,70] or induced calcium fluxes [66,67]. Direct detection of ROS production in bystander cells provided further evidences [67,69]. In irradiated cells, ROS/RNS generation as a result of mitochondrial permeability transition occurred immediately within several seconds and kept increasing in <15 min after irradiation, and the induced ROS/RNS rapidly activated the MAP kinase activity, which was tightly associated with RIBE transduction, in 4 min after irradiation [71]. The importance of NO in the early process of RIBE was also confirmed through the significant decrease in the number of γ-H2AX [68,72] or 53BP1 [47] foci in bystander cells after treatment with NO scavenger or inhibitor of NO synthase.

On the other hand, studies on GJIC are fewer than those on ROS concerning the early process of RIBE. Treatments with GJIC inhibitors were found to distinctly decrease the RIBE-induced p21[Waf1] [58], ATM [23,54] and γ-H2AX [24,36].

4.2. Involvement of calcium influx in early process of RIBE

Calcium (Ca^{2+}) plays a pivotal role in signal transduction pathways of cells. A prompt activation of calcium influx was observed within 1 min in bystander human keratinocytes cells which had received the conditioned medium harvested at 1 h post irradiation, and the calcium signaling modulated the ERK and JNK but not p38 pathway to control apoptosis in the bystander cells [66]. Furthermore, Shao et al. [67] observed calcium fluxes within 30 s in a fraction of recipient cells (70-90% of AG 1522 and 50-80% of T98G) after these cells received the conditioned medium harvested at 1 h post radiation. The rapid production of ROS/RNS, which played a very important role in RIBE signaling, in the irradiated cells also showed a Ca^{2+} dependent manner, in which the ROS/RNS generation was inhibited by overexpressing the Ca^{2+}-binding protein, calbindin 28 K, or by treating cells with an intracellular Ca^{2+} chelator [71].

Furthermore, pretreatment with calmidazolium chloride, an inhibitor of calmodulin binding with NOS, or ruthenium red, an inhibitor of mitochondrial calcium uptake, prevented the irradiated cells from inducing γ-H2AX and NO production in the bystander cells receiving the conditioned medium [68]. These results highlighted the importance of the calcium influx in the generation of RIBE signaling in irradiated cells and the corresponding response in bystander cells.

4.3. Signal(s) released in early process of RIBE

Evidences of the existence of early process of RIBE, especially those from medium transfer experiments, indicated that the irradiated cells could generate and release some extracellular signal factor(s) rapidly after irradiation. For example, RIBE signal(s) which induced DSBs in bystander cells receiving the conditioned medium were released as early as 2-3 min and reached the peak at 10 min after 1 cGy α-particle irradiation in confluent AG 1522 human skin fibroblasts [25] or A_L cells [69]. A further study showed that a 1 cGy α-particle irradiation rapidly elevated the activity of constitutive NO synthase, but not the inducible isoform, to produce and release NO, which peaked at 10 min post irradiation, to act as extracellular RIBE signal(s) and to cause DSBs in the bystander cells. A transient activation of constitutive NO synthase, the activity of which reached the maximum in 5 min, was previously proved by Leach et al. to be an early signaling pathway for ionizing radiation [70]. These authors suggested that ionization events in one cell might activate signal transduction to neighboring cells through the lipophilic and relatively stable properties of NO, which then activated downsteam signal transduction pathways [70].

Elevated releases of four cytokines [IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1) and regulated upon activation normal T cell expressed and presumably secreted (RANTES)] were detected in cultured human dermal fibroblasts at 2 h after 2 Gy X-ray irradiation, and the excessive increase in DSBs (labeled with γ-H2AX) was considered to be related to the released IL-6, IL-8 and RANTES [40]. Furthermore, the released amount of the first three cytokines (IL-6, IL-8 and MCP-1) nearly reaching the maximum at 2 h suggested that the release of these three cytokines should have started even earlier [40].

4.4. Gene expression profiling in early process of RIBE

Numerous studies about the early process of RIBE, as described in Sections 1 and 2 above, have shown that expression of various genes related to DNA damage and repair, cell cycle control, signaling pathways, etc. can be modulated by RIBE signaling. Recently, application of microarray analysis has provided detailed information about gene expression in the early process of RIBE and has provided clues to the involved signaling pathways.

By using whole human genome microarrays and real time quantitative PCR, Amundson's group investigated the early (30 min post irradiation) signaling and gene regulation in bystander IMR-90 fibroblasts, which shared the conditioned medium with 50 cGy irradiated cells [73]. The global gene expression at 30 min and the signaling pathways between 30 min and 4 h post irradiation were determined. A total of 407 genes were differentially expressed at 30 min post irradiation in the bystander cells when compared to the controls, and these genes were in categories related to protein modification, cell surface receptor mediated signal transduction, cell structure and motility, cation transport and ion transport [73]. Moreover, transcriptional expression of NF-κB responsive genes, IL-6, matrix metalloproteinases 1 (MMP1) and chemokine ligands 5 (CXCL5) in bystander cells showed a time-dependent manner, with two peaks appearing at 30 min (6-8-folds of control) and at 6 h (14-18-folds control) [73]. Activation of AKT-GSK3β-β-catenin signaling was also observed in bystander cells with western blot quantification at 1 h post irradiation, which lagged behind the irradiated cells [73]. The authors further examined the time series of gene expression with a clustering method, namely, the Feature Based Partitioning around medoids Algorithm (FBPA), in the same research system, and the results showed in bystander cells that gene clusters were enriched for cell communication/motility, signal transduction and inflammation processes at 30 min after irradiation [74]. Network analysis also confirmed the p53 and NF-κB transcription factor-regulated gene clusters in irradiated and bystander cells [74].

With real time PCR, Furlong et al. analyzed the transcriptional expression of key apoptotic genes, tumor suppressor gene TP53, pro-apoptotic Bax and anti-apoptotic Bcl2, pro-apoptotic JNK and anti-apoptotic ERK, initiator caspase 2 and 9 and effector caspase 3, 6 and 7 in bystander human keratinocyte HaCaT cells at 1 h after receiving the conditioned medium harvested 1 h after 0.05 and 0.5 Gy γ-ray irradiation. They showed that expression of these genes except TP53, Bcl2, caspase 2, 6 and 9 were significantly up-regulated while the genes JNK and caspase 3 were significantly down-regulated at 1 h after treatment with the conditioned medium [75].

Kalanxhi and Dahle employed genome-wide microarray analysis to monitor the transcriptional responses of F11-hTERT human fibroblasts which had received the conditioned medium harvested at 2 h after 2 Gy γ irradiation, and detected transient enrichment in gene sets belonging to ribosome, oxidative phosphorylation and neurodegenerative disease pathways associated with

mitochondrial dysfunctions. The authors also suggested that a set of 14 p53 regulating genes, which were induced early (<2 h) after irradiation, might be important in the generation or propagation of RIBE [76]. However, Kalanxhi and Dahle showed through microarray analysis no significantly altered genes in bystander fibroblasts at any of the time points after receiving the conditioned medium harvested from 0.1 Gy α-particle irradiated human F11, and repression of only 26 genes were significantly modulated at 4 h after irradiation [77]. Microarray analysis of irradiated cells also showed significant changes in expression of 338 genes, about half of the genes primarily related to cell growth and maintenance, and cell communication [78].

4.5. Role of mitochondrial function in early process of RIBE

Mitochondria were recently shown to play a critical role in generation of extracellular RIBE signal(s) in irradiated cells. Mitochondria constitute the most important source of ROS and play important roles in various radiation-induced biological effects [79,80]. For example, irradiation-induced rapid ROS/RNS generation, which occurred within several seconds and kept increasing in <15 min after irradiation, exhibited a mitochondrial-dependent manner [71]. There was clear evidence that normal mitochondrial function was very critical in the fast generation of RIBE signal(s) at 10 min post irradiation, when the DSB-inducing ability reached the peak [68]. The conditioned medium harvested from irradiated mtDNA-depleted (ρ^0) A_L cells could not induce DSBs [68], gene mutation [69] or cell death [69] in human skin fibroblasts receiving the conditioned medium, and treatment with inhibitors of respiratory chain complex I, III, and V prior to irradiation also distinctly attenuated the DSB-inducing capability of conditioned medium harvested from the irradiated (ρ^+) A_L cells with normal mitochondrial function. The mechanism study showed that irradiation could not stimulate (ρ^0) A_L cells to produce superoxide anion and constitutive NO, which were potential extracellular signaling molecule in the early process of RIBE [68]. Tartier et al. also showed that (ρ^0) HeLa cells irradiated with microbeam helium ions were unable to induce excessive production of BP1 foci in the co-cultured cells, when compared to corresponding experiments with normal (ρ^+) HeLa cells [47]. Furthermore, the murine embryonic cells lacking cytochrome c, which resided between the outer and inner membranes of mitochondria and played an important role in keeping normal mitochondrial respiration, did not respond to the released RIBE signal(s), and no excessive MN could be induced in these bystander cells [81].

Although the mechanisms underlying the early process of RIBE are not yet fully understood and are most probably complex, it is likely that the release of NO and some cytokines (IL-6, IL-6, MCP-1, etc.) is initiated by the irradiation within a short time period (Fig. 1). The secreted factor(s) acts as extracellular signal(s) of RIBE to "attack" the neighboring cells through diffusion or GJIC. Multiple pathways, such as MAPK, and NF-κB related or cytokines related pathways described above, are involved in the transduction of the extracellular signals to act on the nuclei of bystander cells. In the end, distinct increases in genetic damages (γ-H2AX, 53 BP1, ATM, etc.), cell cycle arrest ($p21^{Waf1}$), apoptosis or even gene locus

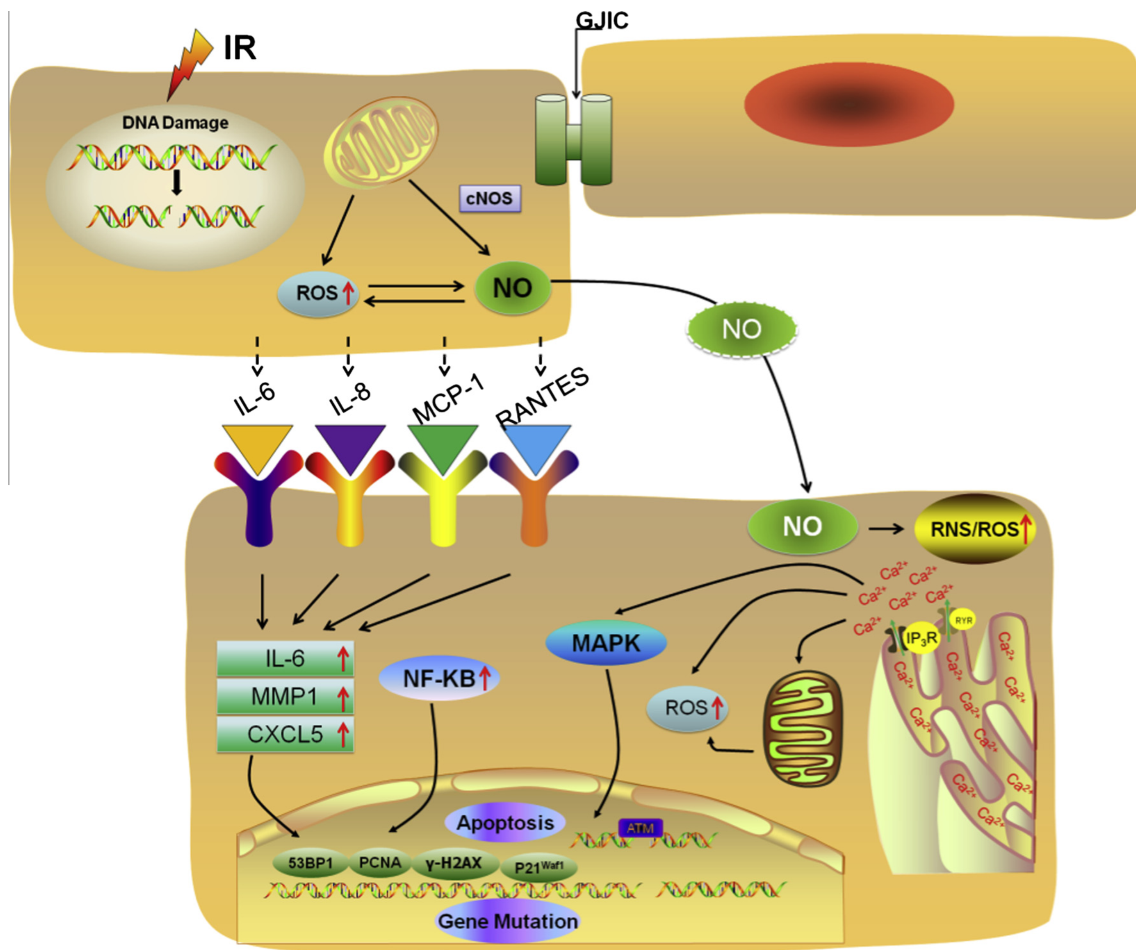

Fig. 1. A schematic model for the early process of radiation-induced bystander effect.

mutation are detected in the bystander cells. Mitochondria also play an important role in the initiation of RIBE signal in the irradiated cells.

5. Conclusion

Rapid detection techniques of RIBE will be helpful for better understanding the cellular response and communication after irradiation. These techniques can also help assess the health risks of unexpected radiation, especially those from low-dose radiation. The early process of RIBE implies fast initiation or activation of signaling cascades within a short period after radiation. Although the precise mechanism involved in the early process of RIBE remains to be elucidated, a clear understanding on the early process and on what happens and how it happens will give valuable information on enhancing the cancer radiotherapy as well as on improving risk assessment for radiation protection purposes.

Conflict of Interest Statement

The authors declare no conflict of interest.

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References

[1] E.J. Hall, Radiation, the two-edged sword: cancer risks at high and low doses, Cancer J. 6 (2000) 343–350.
[2] M.P. Little, Radiation: a dose of the bomb, Nature 424 (2003) 495–496.
[3] H. Nagasawa, J.B. Little, Induction of sister chromatid exchanges by extremely low doses of α-particles, Cancer Res. 52 (1992) 6394–6396.
[4] M.A. Khan, R.P. Hill, J. Van Dyk, Partial volume rat lung irradiation: an evaluation of early DNA damage, Int. J. Radiat. Oncol. Biol. Phys. 40 (1998) 467.
[5] K. Prise, Studies of bystander effects in human fibroblasts using a charged particle microbeam, Int. J. Radiat. Biol. 74 (1998) 793–798.
[6] E.I. Azzam, S.M. De Toledo, J.B. Little, Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from α-particle irradiated to nonirradiated cells, Proc. Natl. Acad. Sci. USA 98 (2001) 473–478.
[7] H. Zhou, M. Suzuki, G. Randers-Pehrson, D. Vannais, G. Chen, J.E. Trosko, C.A. Waldren, T.K. Hei, Radiation risk to low fluences of α particles may be greater than we thought, Proc. Natl. Acad. Sci. USA 98 (2001) 14410–14415.
[8] Z. Goldberg, B.E. Lehnert, Radiation-induced effects in unirradiated cells: a review and implications in cancer, Int. J. Oncol. 21 (2002) 337–350.
[9] K.M. Prise, J.M. O'Sullivan, Radiation-induced bystander signalling in cancer therapy, Nat. Rev. Cancer 9 (2009) 351–360.
[10] F. Ballarini, M. Biaggi, A. Ottolenghi, O. Sapora, Cellular communication and bystander effects: a critical review for modelling low-dose radiation action, Mutat. Res. 501 (2002) 1–12.
[11] J.B. Little, Cellular radiation effects and the bystander response, Mutat. Res. 597 (2006) 113–118.
[12] R. Baskar, Emerging role of radiation induced bystander effects: cell communications and carcinogenesis, Genome Integrity 1 (2010) 13.
[13] W.F. MorganM, B. Sowa, Non-targeted bystander effects induced by ionizing radiation, Mutat. Res. 616 (2007) 159–164.
[14] T.K. Hei, Cyclooxygenase-2 as a signaling molecule in radiation-induced bystander effect, Mol. Carcinogen. 45 (2006) 455–460.
[15] D.J. Brenner, R. Doll, D.T. Goodhead, E.J. Hall, C.E. Land, J.B. Little, J.H. Lubin, D.L. Preston, R.J. Preston, J.S. Puskin, Cancer risks attributable to low doses of ionizing radiation: assessing what we really know, Proc. Natl. Acad. Sci. USA 100 (2003) 13761–13766.
[16] T. Ohnishi, The disaster at Japan's Fukushima-Daiichi nuclear power plant after the March 11, 2011 earthquake and tsunami, and the resulting spread of radioisotope contamination, Radiat. Res. 177 (2012) (2011) 1–14.
[17] W.M. Bonner, Low-dose radiation: thresholds, bystander effects, and adaptive responses, Proc. Natl. Acad. Sci. USA 100 (2003) 4973–4975.
[18] L. Mullenders, M. Atkinson, H. Paretzke, L. Sabatier, S. Bouffler, Assessing cancer risks of low-dose radiation, Nat. Rev. Cancer 9 (2009) 596–604.
[19] H. Yang, N. Magpayo, A. Rusek, I.H. Chiang, M. SivertzK, D. Held, Effects of very low fluences of high-energy protons or iron ions on irradiated and bystander cells, Radiat. Res. 176 (2011) 695–705.
[20] L. Huo, H. Nagasawa, J.B. Little, HPRT mutants induced in bystander cells by very low fluences of alpha particles result primarily from point mutations, Radiat. Res. 156 (2001) 521–525.
[21] H. Nagasawa, A. Cremesti, R. Kolesnick, Z. Fuks, J.B. Little, Involvement of membrane signaling in the bystander effect in irradiated cells, Cancer Res. 62 (2002) 2531–2534.
[22] H. Zhou, G. Randers-Pehrson, C.A. Waldren, D. Vannais, E.J. Hall, T.K. Hei, Induction of a bystander mutagenic effect of alpha particles in mammalian cells, Proc. Natl. Acad. Sci. USA 97 (2000) 2099–2104.
[23] M. Ojima, N. Ban, M. Kai, DNA double-strand breaks induced by very low X-ray doses are largely due to bystander effects, Radiat. Res. (2009) 365–371.
[24] B. Hu, L. Wu, W. Han, L. Zhang, S. Chen, A. Xu, T.K. Hei, Z. Yu, The time and spatial effects of bystander response in mammalian cells induced by low dose radiation, Carcinogenesis 27 (2006) 245–251.
[25] W. Han, L. Wu, B. Hu, L. Zhang, S. Chen, L. Bao, Y. Zhao, A.XuZ. Yu, The early and initiation processes of radiation-induced bystander effects involved in the induction of DNA double strand breaks in non-irradiated cultures, Br. J. Radiol. 80 (2007) S7–S12.
[26] D.C. van Gent, J.H. Hoeijmakers, R. Kanaar, Chromosomal stability and the DNA double-stranded break connection, Nat. Rev. Genet. 2 (2001) 196–206.
[27] J. Fillingham, M.C. Keogh, N.J. Krogan, γ-H2AX and its role in DNA double-strand break repair, Biochem. Cell. Biol. 84 (2006) 568–577.
[28] H. Nagasawa, J.B. Little, Bystander effect for chromosomal aberrations induced in wild-type and repair deficient CHO cells by low fluences of alpha particles, Mutat. Res. 508 (2002) 121–129.
[29] J.B. Little, H. Nagasawa, G.C. Li, D.J. Chen, Involvement of the nonhomologous end joining DNA repair pathway in the bystander effect for chromosomal aberrations, Radiat. Res. 159 (2003) 262–267.
[30] H. Nagasawa, L. Huo, J. Little, Increased bystander mutagenic effect in DNA double-strand break repair-deficient mammalian cells, Int. J. Radiat. Biol. 79 (2003) 35–41.
[31] M. Shrivastav, L.P. De Haro, J.A. Nickoloff, Regulation of DNA double-strand break repair pathway choice, Cell Res. 18 (2007) 134–147.
[32] C. Wyman, R. Kanaar, DNA double-strand break repair: all's well that ends well, Annu. Rev. Genet. 40 (2006) 363–383.
[33] O.A. Sedelnikova, E.P. Rogakou, I.G. Panyutin, W.M. Bonner, Quantitative detection of ^{125}IdU-induced DNA double-strand breaks with γ-H2AX antibody, Radiat. Res. 158 (2002) 486–492.
[34] H. Yang, N. Asaad, K.D. Held, Medium-mediated intercellular communication is involved in bystander responses of X-ray-irradiated normal human fibroblasts, Oncogene 24 (2005) 2096–2103.
[35] M.V. Sokolov, L.B. Smilenov, E.J. Hall, I.G. Panyutin, W.M. Bonner, O.A. Sedelnikova, Ionizing radiation induces DNA double-strand breaks in bystander primary human fibroblasts, Oncogene 24 (2005) 7257–7265.
[36] B. Hu, W. Han, L. Wu, H. Feng, X. Liu, L. Zhang, A. Xu, T. Hei, Z. Yu, *In situ* visualization of DSBs to assess the extranuclear/extracellular effects induced by low-dose α-particle irradiation, Radiat. Res. 164 (2005) 286–291.
[37] M.V. Sokolov, J.S. Dickey, W.M. Bonner, O.A. Martin, γ-H2AX in bystander cells: not just a radiation-triggered event, a cellular response to stress mediated by intercellular communication, Cell Cycle 6 (2007) 2210–2212.
[38] G. Kashino, T. Kondoh, N. Nariyama, K. Umetani, T. Ohigashi, K. Shinohara, A. Kurihara, M. Fukumoto, H. Tanaka, A. Maruhashi, Induction of DNA double-strand breaks and cellular migration through bystander effects in cells irradiated with the slit-type microplanar beam of the SPring-8 synchrotron, Int. J. Radiat. Oncol. Biol. Phys. 74 (2009) 229–236.
[39] S. Horn, S. Barnard, K. Rothkamm, Gamma-H2AX-based dose estimation for whole and partial body radiation exposure, PloS One 6 (2011) e25113.
[40] B. Dieriks, W.H. De Vos, H. Derradji, S. Baatout, P. van Oostveldt, Medium-mediated DNA repair response after ionizing radiation is correlated with the increase of specific cytokines in human fibroblasts, Mutat. Res. 687 (2010) 40–48.
[41] W. Han, L. Zhu, E. Jiang, J. Wang, S. Chen, L. Bao, Y. Zhao, A. Xu, Z. Yu, L. Wu, Elevated sodium chloride concentrations enhance the bystander effects induced by low dose alpha-particle irradiation, Mutat. Res. 624 (2007) 124–131.
[42] H. Yang, V. Anzenberg, K.D. Held, The time dependence of bystander responses induced by iron-ion radiation in normal human skin fibroblasts, Radiat. Res. 168 (2007) 292–298.
[43] O.A. Sedelnikova, A. Nakamura, O. Kovalchuk, I. Koturbash, S.A. Mitchell, S.A. Marino, D.J. Brenner, W.M. Bonner, DNA double-strand breaks form in bystander cells after microbeam irradiation of three-dimensional human tissue models, Cancer Res. 67 (2007) 4295–4302.
[44] I. Koturbash, R. Rugo, C. Hendricks, J. Loree, B. Thibault, K. Kutanzi, I. Pogribny, J. Yanch, B. Engelward, O. Kovalchuk, Irradiation induces DNA damage and modulates epigenetic effectors in distant bystander tissue *in vivo*, Oncogene 25 (2006) 4267–4275.
[45] M. Mancuso, E. Pasquali, S. Leonardi, M. Tanori, S. Rebessi, V. Di Majo, S. Pazzaglia, M.P. Toni, M. Pimpinella, V. Covelli, Oncogenic bystander radiation effects in patched heterozygous mouse cerebellum, Proc. Natl. Acad. Sci. USA 105 (2008) 12445–12450.
[46] B. Wang, S. Matsuoka, P.B. Carpenter, S.J. Elledge, 53BP1, a mediator of the DNA damage checkpoint, Sci. Signal. 298 (2002) 1435.
[47] L. Tartier, S. Gilchrist, S. Burdak-Rothkamm, M. Folkard, K.M. Prise, Cytoplasmic irradiation induces mitochondrial-dependent 53BP1 protein relocalization in irradiated and bystander cells, Cancer Res. 67 (2007) 5872–5879.

[48] W. Han, S. Chen, K. Yu, L. Wu, Nitric oxide mediated DNA double strand breaks induced in proliferating bystander cells after α-particle irradiation, Mutat. Res. 684 (2010) 81–89.
[49] W. Han, L. Wu, S. Chen, K.N. Yu, Exogenous carbon monoxide protects the bystander Chinese hamster ovary cells in mixed coculture system after alpha-particle irradiation, Carcinogenesis 31 (2010) 275–280.
[50] M.V. Sokolov, R.D. Neumann, Radiation-induced bystander effects in cultured human stem cells, PloS One 5 (2010) e14195.
[51] M. Mancuso, P. Giardullo, S. Leonardi, E. Pasquali, A. Casciati, I. De Stefano, M. Tanori, S. Pazzaglia, A. Saran, Dose and spatial effects in long-distance radiation signaling in vivo: implications for abscopal tumorigenesis, Int. J. Radiat. Oncol. Biol. Phys. 85 (2012) 813–819.
[52] M.F. Lavin, Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer, Nat. Rev. Mol. Cell. Biol. 9 (2008) 759–769.
[53] S. Burdak-Rothkamm, K. Rothkamm, K.M. Prise, ATM acts downstream of ATR in the DNA damage response signaling of bystander cells, Cancer Res. 68 (2008) 7059–7065.
[54] M. Ojima, A. Furutani, N. Ban, M. Kai, Persistence of DNA double-strand breaks in normal human cells induced by radiation-induced bystander effect, Radiat. Res. 175 (2010) 90–96.
[55] T. Abbas, A. Dutta, P21 in cancer: intricate networks and multiple activities, Nat. Rev. Cancer 9 (2009) 400–414.
[56] E.I. Azzam, S.M. De Toledo, D.R. Spitz, J.B. Little, Oxidative metabolism modulates signal transduction and micronucleus formation in bystander cells from α-particle-irradiated normal human fibroblast cultures, Cancer Res. 62 (2002) 5436–5442.
[57] A.K. Mitra, M. Krishna, Radiation-induced bystander effect: activation of signaling molecules in K562 erythroleukemia cells, J. Cell Biochem. 100 (2007) 991–997.
[58] S. Gaillard, D. Pusset, S.M. de Toledo, M. Fromm, E.I. Azzam, Propagation distance of the α-particle-induced bystander effect: the role of nuclear traversal and gap junction communication, Radiat. Res. 171 (2009) 513–520.
[59] Z. Kelman, PCNA: structure, functions and interactions, Oncogene 14 (1997) 629–640.
[60] R. Iyer, B.E. Lehnert, Factors underlying the cell growth-related bystander responses to α particles, Cancer Res. 60 (2000) 1290–1298.
[61] M. Hill, J. Ford, P. Clapham, S. Marsden, D. Stevens, K.S. Townsend, D. Goodhead, Bound PCNA in nuclei of primary rat tracheal epithelial cells after exposure to very low doses of plutonium-238 α particles, Radiat. Res. 163 (2005) 36–44.
[62] E. Gollapalle, R. Wang, R. Adetolu, D. Tsao, D. Francisco, G. Sigounas, A.G. Georgakilas, Detection of oxidative clustered DNA lesions in X-irradiated mouse skin tissues and human MCF-7 breast cancer cells, Radiat. Res. 167 (2007) 207–216.
[63] A.G. Georgakilas, P. O'Neill, R.D. Stewart, Induction and repair of clustered DNA lesions: what do we know so far?, Radiat Res. 180 (2013) 100–109.
[64] M. Widel, W.M. Przybyszewski, A. Cieslar-Pobuda, Y.V. Saenko, J. Rzeszowska-Wolny, Bystander normal human fibroblasts reduce damage response in radiation targeted cancer cells through intercellular ROS level modulation, Mutat. Res. 731 (2012) 117–124.
[65] Y. Zhang, J. Zhou, J. Baldwin, K.D. Held, K.M. Prise, R.W. Redmond, H.L. Liber, Ionizing radiation-induced bystander mutagenesis and adaptation: quantitative and temporal aspects, Mutat. Res. 671 (2009) 20–25.
[66] F.M. Lyng, P. Maguire, B. McClean, C. Seymour, C. Mothersill, The involvement of calcium and MAP kinase signaling pathways in the production of radiation-induced bystander effects, Radiat. Res. 165 (2006) 400–409.
[67] C. Shao, F.M. Lyng, M. Folkard, K.M. Prise, Calcium fluxes modulate the radiation-induced bystander responses in targeted glioma and fibroblast cells, Radiat. Res. 166 (2006) 479–487.
[68] S. Chen, Y. Zhao, W. Han, G. Zhao, L. Zhu, J. Wang, L. Bao, E. Jiang, A. Xu, T. Hei, Mitochondria-dependent signalling pathway are involved in the early process of radiation-induced bystander effects, Br. J. Cancer 98 (2008) 1839–1844.
[69] S. Chen, Y. Zhao, G. Zhao, W. Han, L. Bao, K. Yu, L. Wu, Up-regulation of ROS by mitochondria-dependent bystander signaling contributes to genotoxicity of bystander effects, Mutat. Res. 666 (2009) 68–73.
[70] J.K. Leach, S.M. Black, R.K. Schmidt-Ullrich, R.B. Mikkelsen, Activation of constitutive nitric-oxide synthase activity is an early signaling event induced by ionizing radiation, J. Biol. Chem. 277 (2002) 15400–15406.
[71] J.K. Leach, G. Van Tuyle, P.S. Lin, R. Schmidt-Ullrich, R.B. Mikkelsen, Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen, Cancer Res. 61 (2001) 3894–3901.
[72] W. Han, L. Wu, S. Chen, L. Bao, L. Zhang, E. Jiang, Y. Zhao, A. Xu, T. Hei, Z. Yu, Constitutive nitric oxide acting as a possible intercellular signaling molecule in the initiation of radiation-induced DNA double strand breaks in non-irradiated bystander cells, Oncogene 26 (2006) 2330–2339.
[73] S.A. Ghandhi, L. Ming, V.N. Ivanov, T.K. Hei, S.A. Amundson, Regulation of early signaling and gene expression in the α-particle and bystander response of IMR-90 human fibroblasts, BMC Med. Genomics 3 (2010) 31.
[74] S. Ghandhi, A. Sinha, M. Markatou, S. Amundson, Time-series clustering of gene expression in irradiated and bystander fibroblasts: an application of FBPA clustering, BMC Genomics 12 (2011) 2.
[75] H. Furlong, C. Mothersill, F.M. Lyng, O. Howe, Apoptosis is signalled early by low doses of ionizing radiation in a radiation-induced bystander effect, Mutat. Res. 741–742 (2013) 35–43.
[76] E. Kalanxhi, J. Dahle, Genome-wide microarray analysis of human fibroblasts in response to γ radiation and the radiation-induced bystander effect, Radiat. Res. 177 (2011) 35–43.
[77] E. Kalanxhi, J. Dahle, Transcriptional responses in irradiated and bystander fibroblasts after low dose α-particle radiation, Int. J. Radiat. Biol. 88 (2012) 713–719.
[78] A. Turtoi, I. Brown, M. Schläger, F.H. Schneeweiss, Gene expression profile of human lymphocytes exposed to 211 at α particles, Radiat. Res. 174 (2010) 125–136.
[79] R.S. Balaban, S. Nemoto, T. Finkel, Mitochondria, oxidants, and aging, Cell 120 (2005) 483–495.
[80] R.B. Mikkelsen, P. Wardman, Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms, Oncogene 22 (2003) 5734–5754.
[81] G. Yang, L. Wu, S. Chen, L. Zhu, P. Huang, L. Tong, Y. Zhao, G. Zhao, J. Wang, T. Mei, Mitochondrial dysfunction resulting from loss of cytochrome c impairs radiation-induced bystander effect, Br. J. Cancer 100 (2009) 1912–1916.