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Review Article

Vanguards of Paradigm Shift in Radiation Biology: Radiation-Induced Adaptive and Bystander Responses

Hideki MATSUMOTO[1]*, Nobuyuki HAMADA[2,3], Akihisa TAKAHASHI[4], Yasuhiko KOBAYASHI[2,3] and Takeo OHNISHI[4]

Ionizing radiation/Radioadaptive response/Bystander response/Reactive oxygen species (ROS)/Reactive nitrogen species (RNS)/Nitric Oxide (NO).

The risks of exposure to low dose ionizing radiation (below 100 mSv) are estimated by extrapolating from data obtained after exposure to high dose radiation, using a linear no-threshold model (LNT model). However, the validity of using this dose-response model is controversial because evidence accumulated over the past decade has indicated that living organisms, including humans, respond differently to low dose/low dose-rate radiation than they do to high dose/high dose-rate radiation. In other words, there are accumulated findings which cannot be explained by the classical "target theory" of radiation biology. The radioadaptive response, radiation-induced bystander effects, low-dose radio-hypersensitivity, and genomic instability are specifically observed in response to low dose/low dose-rate radiation, and the mechanisms underlying these responses often involve biochemical/molecular signals that respond to targeted and non-targeted events. Recently, correlations between the radioadaptive and bystander responses have been increasingly reported. The present review focuses on the latter two phenomena by summarizing observations supporting their existence, and discussing the linkage between them from the aspect of production of reactive oxygen and nitrogen species.

INTRODUCTION

In 1984, the term "radioadaptive response" was coined by Wolff and colleagues[1)] who studied chromosomal aberrations in human lymphocytes after irradiation. Their findings, which were subsequently confirmed by others in different types of cells, indicated that the harmful effects of radiation may be attenuated by a priming low radiation dose.[2,3)] In contrast, among reports about the cellular responses to low dose/low fluence radiation, a number of investigations described since the 1990s have shown that the harmful effects of radiation may be amplified due to induced "bystander responses".[4–6)] Radiation-induced bystander responses have been defined as effects observed in cells which were not directly traversed by radiation, and which have resulted from some types of communication or signaling between the irradiated cells (targeted cells) and nearby unirradiated cells (non-targeted or bystander cells).[4)] Such communication is thought to occur through direct physical connections between cells such as gap-junction intercellular communications (GJICs) or through the culture medium.[7)] Hamada *et al*. review in detail the radiation-induced bystander response in this issue of the Journal of Radiation Research, Vol. 48.[8)] Moreover, correlations between the radioadaptive and the radiation-induced bystander responses have been recently discussed in several reports.[9–11)] It appears that the key signaling molecules that constitute a link between the radioadaptive response and the bystander response are reactive oxygen and nitrogen species (ROS and RNS). In the present review, we sum up findings describing radioadaptive and radiation-induced bystander responses, and discuss the contribution of the latter to the former through production of ROS/RNS.

*Corresponding author: Phone: +81-776-61-8333,
Fax: +81-776-61-8146,
E-mail: hidekim@fmsrsa.fukui-med.ac.jp
[1]Division of Oncology, Biomedical Imaging Research Center, University of Fukui, Eiheiji, Fukui 910-1193, Japan; [2]Department of Quantum Biology, Division of Bioregulatory Medicine, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan; [3]Microbeam Radiation Biology Group, Radiation-Applied Biology Division, Quantum Beam Science Directorate, Japan Atomic Energy Agency (JAEA), 1233 Watanuki-machi, Takasaki, Gunma 370-1292, Japan; [4]Department of Biology, Nara Medical University, Kashihara, Nara 634-8521, Japan.
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RADIOADAPTIVE RESPONSE

Wolff and colleagues demonstrated that the observed fre-

quency of chromosomal aberrations after irradiation with X-rays was less than expected if the cells were labeled with low level [^{3}H] thymidine.[1)] This is the first seminal publication regarding the radioadaptive response. Subsequently, adaptive responses were observed *in vitro* and *in vivo* using other types of low linear energy transfer (LET) radiations and various endpoints, such as chromosomal aberrations, mutation induction, and radiosensitivity.[12–15)]

The radioadaptive response is defined by a "window" for a priming dose which is the dose required to induce an effective protective signaling mechanism and an "interval period" between a priming and challenge exposure to radiation.[16)] The range of a window is 0.01–0.2 Gy of low LET radiation in cultured cells. In general, when the priming dose is over 0.2 Gy, adaptive responses are barely induced, and when it is over 0.5 Gy, adaptive responses are almost never induced.[17)] Radiation dose-rate was shown to be an effective modulator of physical and biochemical parameters leading to expression of adaptive responses.[18)] In *in vivo* experiments using mice, Yonezawa *et al*. found that the radioadaptive response was observed during a two week period at 2–2.5 months after irradiation with 0.05–0.1 Gy but not with 0.15–0.2 Gy used as a priming dose.[13)] When mice were primed with 0.3–0.5 Gy, the adaptive response was observed 2 weeks after exposure. This phenomenon in mice after low dose irradiation is the so-called "Yonezawa effect".

Mechanisms of the radioadaptive response

Although mechanisms responsible for the radioadaptive response are not fully known, several notable findings which help to understand them have been reported. Initial experiments in Wolff's laboratory showed that *de novo* syntheses of factors involved in DNA repair and in cell cycle regulation were required for the induction of radioadaptive responses in human lymphocytes.[19)] Subsequent research in several laboratories suggested poly (ADP-ribose) polymerase (PARP),[20)] apurinic/apyrimidinic (AP)-endonuclease,[21,22)] DNA-dependent protein kinase (DNA-PK)[23)] and ERCC5 (XPG)[24)] as effectors for DNA repair, M phase phosphoprotein 10[24)] and p125[24)] as effectors for cell cycle regulation, and ataxia telangiectasia mutated (ATM)[24)] and p53[24–26)] as transducers for both are factors that are required for the induction of radioadaptive responses. Thus, the most important mediators of the radioadaptive response are DNA repair and cell cycle regulation systems governed by the ATM-p53 signal transduction pathway.[27)] In addition, the activation of protein kinase C (PKC) in adapted cells exposed to low doses was also reported in several studies.[28–30)] Sasaki *et al*. showed that adaptive responses were mediated by a rapid and robust feedback in signal transduction pathways involving activation of PKCα and p38 mitogen-activated protein kinase (p38MAPK) with possible feedback via p38MAPK-associated phospholipase δ1 (PLCδ1).[31,32)] In addition to p38MAPK, another member of cell membrane-derived signaling cascades, namely signal transducer and activator of transcription 1 (STAT1), which is a component of the cytokine interferon (IFN) signaling pathway, appears to be a factor required for the induction of radioadaptive responses.[33)] Furthermore, molecular chaperones, which are involved in repair of unfolded, aggregated or damaged proteins, have been also implicated in the radioadaptive response.[34,35)] It has been suggested that radioresistance attributed to some members of the heat shock protein (HSP) family is associated with the activation of PKC and the induction of p27Cip/Kip.[36,37)]

ROLES OF ROS/RNS IN THE RADIOADAPTIVE RESPONSE

The findings mentioned above arouse the possibility that ROS/RNS contribute to expression of radioadaptive responses. Namely, the production of ROS/RNS after exposure to radiation underlies the induction of the factors required for the radioadaptive responses. ROS/RNS may contribute to radioadaptive responses by three modes: (a) ROS/RNS directly induce DNA damage that initiates the radioadaptive response; (b) DNA damage induced by ROS/RNS brings about the transcription/post-transcriptional regulation of certain genes that confer radio-protective properties to cells, or enhance the functions of certain proteins to promote radioadaptive responses; (c) in response to ROS/RNS, certain proteins (e.g. transcription factors) induce the cellular events necessary to conduct radioadaptive responses.

Induction of DNA damage by ROS/RNS

It is well known that ROS, particularly $^{\bullet}O_2^-$, H_2O_2 and $^{\bullet}OH$, can react with DNA through multiple pathways. Although DNA is a biologically important target for reactive oxygen species, free $^{\bullet}O_2^-$ is relatively unreactive with DNA. However, $^{\bullet}O_2^-$ dismutates (via spontaneous or enzyme-catalyzed reactions) to produce H_2O_2 (*reaction 1*). $^{\bullet}O_2^-$ can also reduce and liberate Fe^{3+} from ferritin (*reaction 2*) or liberate Fe^{2+} from iron-sulfur clusters (*reaction 3*); subsequently highly reactive oxygen species can form via the Fenton reaction (*reaction 4*). Thus, the cytotoxic effects of $^{\bullet}O_2^-$ (as well as of iron and H_2O_2) have been linked to DNA damage by way of the Fenton reaction.[38)]

$$2{}^{\bullet}O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \quad (\textit{reaction 1})$$

$$^{\bullet}O_2^- + Fe^{3+} \rightarrow Fe^{2+} + O_2 \quad (\textit{reaction 2})$$

$$[4Fe\text{-}4S]^{2+} + {}^{\bullet}O_2^- + 2H^+ \rightarrow [3Fe\text{-}4S]^+ + H_2O_2 + Fe^{2+} \quad (\textit{reaction 3})$$

$$Fe^{2+} + H^+ + H_2O_2 \rightarrow Fe3 + {}^{\bullet}OH + H_2O \quad (\textit{reaction 4})$$

In addition to the Fenton reaction, the following reactions may occur:

$$^{\bullet}OH + H_2O_2 \rightarrow H_2O + H^+ + {}^{\bullet}O_2^-$$

$$^{\bullet}OH + Fe^{2+} \rightarrow Fe^{3+} + OH^-$$

Generally, the •OH may react by (i) hydrogen abstraction, (ii) electron transfer and (iii) addition reaction. The reaction of •OH with a biomolecule will produce another radical, usually with lower activity. The •OH causes addition to DNA bases leading to generation of a variety of oxidative products. The interaction of •OH with guanine leads to the generation of 8-oxo-7,8-dihydro-20-deoxyguanosine (8-oxo-dG) and 2,6-diamino-5-formamido-4-hydroxy-pyrimidine (FAPy-G). Adenine reacts with •OH in a similar manner to guanine, although oxidative adenine lesions are less prevalent in DNA damage. It has been demonstrated that in the presence of Fe(III) or Fe(III)–EDTA complex, endogenous reductants such as ascorbate, glutathione (GSH), and the reduced form of NADH, cause DNA damage at every type of nucleotide with a slight dominance by guanine. Specifically, NADH in the presence of Fe(III)–EDTA and H_2O_2 generated •OH leads to the formation of 8-oxo-dG. The DNA damage is inhibited by typical •OH scavengers and by catalase, suggesting that these reductants cause DNA damage *via* the Fenton reaction.[39)]

On the other hand, RNS can also react with DNA through multiple pathways. Once produced, subsequent conversion of nitric oxide (NO) into nitrous anhydride (N_2O_3) and/or peroxynitrite ($ONOO^-$) can lead to the nitrosative deamination of DNA bases such as guanine and cytosine. The formation of N_2O_3 can lead to either direct or indirect DNA damage. Direct DNA damage resulting from the nitrosation of primary amines on DNA bases ultimately leads to deamination. The overall reaction rates for amines with N_2O_3 are actually higher at neutral or basic pH than acidic pH because of the increased concentration of free amine under these conditions.[40)] At physiological pH, however, N_2O_3 formation from nitric oxide has been understood to be most important. Direct attack of N_2O_3 on DNA can lead to DNA deamination through diazonium ion formation.[40)] Hydrolysis of the diazonium ion completes the deamination. The end result of this reaction is the net replacement of an amino group by a hydroxyl group. Therefore, adenine, cytosine, 5-methylcytosine and guanine can all be deaminated to form hypoxanthine, uracil, thymine and xanthine, respectively. Although mispairing of xanthine produced by deamination of guanine can cause a G:C→A:T transition, xanthine is labile in DNA and can depurinate readily leaving an abasic site. The abasic site may be cleaved by endonucleases, resulting in the formation of single-strand breaks in DNA.[41)] Exposure to NO can also lead to the formation of single-strand breaks in DNA. The activities of endonucleases are required for this reaction, *i.e.* the reaction with NO results in the formation of xanthine which can depurinate to form abasic site, which are readily cleaved by endonucleases to form single-strand breaks. In addition, if these single-strand breaks were unrepaired, they can be converted to double-strand breaks that may lead to cytotoxicity.[41)]

DNA intrastrand, DNA interstrand, and DNA-protein cross-link formation have been demonstrated upon treatment of DNA with nitrous acid.[42)] In contrast to NO, which is involved primarily in the deamination of DNA, most of the damage impinged on DNA by $ONOO^-$ is oxidative. DNA treatment with $ONOO^-$ generally leads to greater extent damage than treatment with an equivalent dose of NO. In addition, the spectrum of damage also tends to be much more complex, such as 8-oxo-dG, FAPy-G and 5-[hydroxymethyl]-uracil.[43)] The $ONOO^-$ can cause DNA strand breaks much more efficiently than NO. In fact , strand breaks are observed in naked plasmid DNA treated with $ONOO^-$ levels as low as 2–5 μM.[44)]

Induction of gene expression, protein activation and cellular events after formation of DNA damage by ROS/RNS

Accumulating evidence indicates that the *de novo* synthesis of factors is required for the process of radioadaptive responses after initiation by DNA damage. Coleman *et al.* investigated the cytogenetic adaptive response of human lymphoblastoid cells to determine the modification of gene expression in adapted cells and to identify the genes that are associated with reduction of radiation effects by a comprehensive assay using cDNA microarray analyses.[24)] Whereas genes associated with cellular proliferation, signal transduction, apoptosis, ubiquitin-dependent protein degradation, translation, protein modification and DNA double-strand break repair were down-regulated, genes associated with base excision repair (BER), cell cycle control, signal transduction, and stress response were up-regulated.[24)] The results indicating that *XRCC7* was among the down-regulated genes and *ATM* and *ERCC5*/*XPG* were among the up-regulated genes is noticeable and suggest that the major DNA damage in radioadapted cells may be DNA single-strand breaks or base modification rather than double-strand breaks. Therefore, the initiator of radioadaptive responses appears to be single-strand DNA breaks that are likely caused by ROS/RNS. In addition, the activity of p53 has been demonstrated to be remarkably enhanced by RNS, especially NO. NO induces a specific feature of p53 phosphorylation, distinct from the pattern evoked by other inducers, such as ionizing radiation, UV light and adriamycin.[45)] It is suggested that certain specific p53-dependent signaling pathways may be induced in response to NO that is endogenously generated in radioadapted cells. The down-regulation of Mdm2 by NO may contribute to the activation of p53 in these cells.[46)]

The activities of PARPs (PARP-1 and PARP-2) have been shown to be highly stimulated by the presence of DNA lesions such as single-strand breaks and base damage, which are repaired by the single-strand break repair (SSBR) or BER systems, respectively. Particularly, PARP-1 is highly efficient in detecting DNA nicks through two zinc fingers that define a DNA-break-sensing motif, which is also found

in the SSBR/BER enzyme, DNA ligase III.[47] The tight linkage between PARPs and the SSBR/BER systems implies an important role of ROS/RNS in radioadaptive responses.[48]

AP-endonuclease plays a central role in repair of DNA damage induced by ROS/RNS because its DNA 3'-phosphoesterase activity removes 3' blocking groups in DNA that are generated by DNA glycosylase/AP-lyases during removal of oxidized bases and by direct ROS/RNS reaction with DNA. It has been reported that AP-endonulcease is activated selectively by nontoxic levels of a variety of ROS which are produced after exposure to low dose/low dose-rate radiation, and that this transient activation is correlated with increased cellular resistance to oxidizing agents including ionizing radiation.[21,22,49] In addition, the expression and activation of DNA-PKcs has been shown to be significantly induced after exposure of cells to NO.[50]

Not only genes and proteins related to DNA repair but also those related to signaling pathways originating from cell or organelle membranes are recognized to be important for inducing the radioadaptive response. This is supported in that ionizing radiation-induced ROS/RNS can act on cell or organelle membranes, especially mitochondrial membrane, which suggests a cytoplasmic amplification mechanisms of ROS/RNS.[51,52] An imbalance in the cellular oxidative state due to the production of ROS/RNS activates redox signal pathways, leading to the induction of the antioxidant defense systems and the maintenance of redox homeostasis.[53] Manganese superoxide dismutase (MnSOD), catalase, glutathione peroxidase (GPX) and glutathione-S-transferase (GST) activities increased slightly 3 h after a challenge irradiation with 3 Gy, when cultured human lymphoblastoid cells were pre-exposed 6 h earlier, to a priming γ-ray dose of 0.02 Gy.[54] Also the induction and activation of MnSOD and catalase occurred in the spleen of mice irradiated with 0.5 Gy of γ-rays for 23 days.[55] Recently, it has been suggested that RNS, particularly nitric oxide (NO) secreted from irradiated cells may initiate a signaling pathway to induce the radioadaptive response.[10,56]

RADIATION-INDUCED BYSTANDER RESPONSE

A bystander effect induced in cell cultures exposed to radiation was initially described by Nagasawa and Little (1992). An enhanced frequency of sister chromatid exchanges (SCEs) in 20–40% of Chinese hamster ovary cells was observed in cultures exposed to radiation fluences in which only 0.1–1% of the cell nuclei were actually traversed by an α-particle track. Their findings were subsequently confirmed by others using several other endpoints, such as SCE, mutations, genetic instability, formation of micronuclei and apoptosis. In most bystander effect studies using mainly α-particles, the observed non-targeted effects were detrimental to the cells.[57–63] In addition, radiation-induced bystander responses were described in unirradiated cells co-cultivated with cells which had been irradiated with low-LET radiation, such as X- and γ-rays.[64] The cellular communication bringing about the radiation-induced bystander responses is thought to occur through direct physical connections between cells such as gap-junction intercellular communications (GJICs) or through the culture medium[7,65] (please see accompanying article by Hamada *et al.*).[8] Recently, radiation-induced bystander responses were also observed *in vivo*.[66–68] Overall, bystander responses are reminiscent of 'abscopal effects', which were reported in cancer patients receiving radiation treatment. Abscopal effects of ionizing radiation were defined as radiation responses in tissues that are widely separated from the irradiated area.[69] The treatment directed at a tumor at one site can in fact profoundly affect tumors located elsewhere in the body.[70] These data suggest that the occurrence of radiation-induced bystander responses *in vivo* would be of relevance to human health.[71]

Mechanisms of radiation-induced bystander responses

Although the mechanisms for radiation-induced bystander responses have not been fully elucidated, it can easily be speculated that intercellular signal transduction between irradiated cells (target cells) and unirradiated cells (bystander cells) could play a major role in this response. Four possible models for intercellular signaling pathways, capable of producing the radiation-induced bystander response, have been proposed: (a) through gap-junction intercellular communication; (b) through interactions between ligands and their specific receptors; (c) through interaction between the secreted factors and their specific receptors; (d) directly through plasma membranes.[8,10] Models (c) and (d) assume irradiated and unirradiated cells to be non-adjacent, and possibly distant from each other, and that the bystander transmission factors must be soluble elements secreted from the irradiated cells. Mothersill and Seymour demonstrated that the bystander factor(s) become inactive after heating at 70°C, or cooling to 0°C, suggesting that they may be protein containing substances.[64] Possible candidate bystander factor proteins include the redox-modulated tumor necrosis factor-α (TNF-α) or transforming growth factor-β1 (TGF-β1).[72–75] Recent reports indicate that ionizing radiation induces TGF-β1,[76,77] and that exposure of cells to TGF-β1 can elicit ROS production.[74] Recently, it has been reported that nitric oxide (NO) is one of the important factors for radiation-induced bystander responses.[78–80] Thus, to reveal the mechanisms of radiation-induced bystander response, the mode of actions of ROS/RNS induced after irradiation would need to be elucidated.

ROLES OF ROS/RNS IN RADIATION-INDUCED BYSTANDER RESPONSE

Roles of ROS in radiation-induced bystander response

Primarily Lehnert and colleagues reported that a relatively

low dose of α-particles resulted in the generation of extracellular factors which could cause excessive SCEs in unirradiated normal human cells to an extent equivalent to that observed when the cells were directly irradiated with the same irradiation dose.[72] They analyzed the characteristics of the factors and then demonstrated that the factors, which could survive freeze-thawing, were heat labile and could be inhibited by SOD, suggesting that such factors may be ROS. They supported their hypothesis, and measured the intracellular generation of $^{\bullet}O_2^-$ and H_2O_2 in low fluence α-particle exposed (mean dose of 4 mGy) cell cultures using fluorescent dyes, and reported that unirradiated cells harbored excessive SCEs in response to $^{\bullet}O_2^-$ and H_2O_2 excreted from α-irradiated cells.[73] The α-particle-induced ROS response of cells involved mainly the plasma membrane-bound NAD(P)H oxidase complex, because the response was found to be inhibited by diphenyleneiodonium, a selective inhibitor of the enzyme.[81] Recently Azzam *et al*. investigated the role of oxidative metabolism in the up-regulation/activation of stress-inducible signaling pathways as well as induction of micronucleus formation in bystander cells using α-particles.[82] Their findings support the hypothesis that $^{\bullet}O_2^-$ and H_2O_2 produced by flavin-containing oxidase enzymes in the α-irradiated cells mediate radiation-induced bystander responses. They have extensively reviewed the roles of oxidative metabolism in radiation-induced bystander responses,[83] pointing out the possibility that in addition to $^{\bullet}O_2^-$ and H_2O_2, NO may also play important roles in radiation-induced bystander responses.

Biosynthesis of RNS in response to ionizing radiation

NO is endogenously generated as a byproduct in the conversion reaction of L-arginine into L-citrulline catalyzed by NO synthase (NOS) isoenzymes.[84] Among these enzymes, calcium-independent inducible NOS (iNOS or NOS2) is up-regulated and activated in many types of mammalian cells after exposure to numerous inducers.[85–88] The induced iNOS can produce sustained high concentrations of NO. Using cells which were genetically identical except for their *p53* gene status, the induction of iNOS was demonstrated to be strongly attenuated in cells expressing functional p53 after irradiation.[78,79] Several possible mechanisms of *iNOS* suppression by p53 were suggested: (a) prevention of the binding of specific transcriptional factors required for induction[89]; (b) binding and sequestration of transcriptional factors required for *iNOS* gene up-regulation[90]; (c) association with the TATA binding protein (TBP) to disrupt formation of the TFIID complex[91]; (d) transcriptional repression of the *iNOS* promoter via a recently proposed DNA binding sequence for p53.[92] The activity of calcium-dependent constitutive NOS (cNOS or NOS1) is transiently stimulated after exposure to ionizing radiation.[93] High concentrations of NO and its reaction products have been shown to cause DNA damage and to be mutagenic.[94,95] On the other hand, NO is an important modulator involved in immune responses, neurotransmission and vasodilatation.[96] In this way, NO action has the unique property of being concentration-dependent. NO and its reaction products (N_2O_3 and $ONOO^-$) can lead to cell death by causing DNA damage at high concentrations as described above, but may have the opposite effect and protect against apoptosis at lower concentrations.[97] Thus, NO is either cytotoxic or cytoprotective depending on the cell type and the experimental conditions.

Roles of RNS in radiation-induced bystander responses

The radiosensitivity of wild-type (wt) *p53* cells was examined after exposure to conditioned medium from irradiated *p53*-mutated (m*p53*) cells.[78,79] Wt*p53* cells cultured in conditioned medium harvested from m*p53* cells after irradiation with X-rays were more radioresistant than those incubated in fresh growth medium or in conditioned medium from sham-irradiated m*p53* cells. The radioresistance was abolished when an inhibitor of nitric oxide synthase or scavengers of nitric oxide were added to the medium.[78,79] These results indicate that NO excreted from irradiated m*p53* donor cells can induce radioresistance in non-irradiated NO recipient wt*p53* cells through signal transduction mediated by intercellular communication. They are first to describe NO-mediated bystander responses after exposure to ionizing radiation. Subsequently, Shao *et al*. reported the stimulation of cell proliferation and the induction of micronucleus formation elicited by NO-mediated bystander response.[80] Taking a cue from these reports, Prise and colleagues demonstrated a role for NO-mediated events in signaling micronucleus formation in bystander cells in studies using a microbeam for energetic helium ion irradiation.[98] Recently, they demonstrated that calcium fluxes modulate NO-mediated bystander responses, suggesting that calcium signaling may be an early event in expression of the bystander effect.[99] Furthermore, Hei and colleagues demonstrated radiation-induced bystander responses initiated and mediated by NO, which was produced by calcium-dependent cNOS.[100] These observations suggest that RNS, especially NO and its reaction products are critical factors in initiating or mediating radiation-induced bystander response. Significantly, NO is relatively stable and easily excreted from irradiated cells.

CONTRIBUTION OF RADIATION-INDUCED BYSTANDER RESPONSE TO RADIOADAPTIVE RESPONSE

Radiation-induced adaptive responses were assumed to be expressed only when cells are exposed to a priming event such as low-LET irradiation, but not after exposure to high-LET radiation. Also, populations of cells exposed to a low radiation priming dose are usually used as experimental cultures for radiation adaptive response studies. In these exper-

iments, when cell populations are exposed to very low doses of radiation (<1 mGy), it is likely that there are cells which have been traversed by radiation and also cells which have escaped from direct irradiation. On the other hand, most of radiation-induced bystander responses have been demonstrated with a variety of deleterious endpoints for cells, such as SCEs, chromosomal instability, mutations, and apoptosis; in contrast, studies of radiation-induced adaptive response focus on cell survival. Radiation-induced bystander effects have been found after exposure to both low- and high-LET radiation. Thus, it has been thought that the radiation-induced adaptive response and the bystander effect were not related to each other. However, it has been demonstrated in cultured cell lines that acquisition of radioresistance may be induced in a bystander manner, indicating that the radiation-induced bystander response is not only detrimental to non-irradiated cells in a population, but could also be an advantageous phenomenon for cells. Such outcome likely depends on the cell type and experimental conditions used.[78,79)] Consistent with this concept, Iyer and Lehnert demonstrated that compared to directly irradiated cells grown in fresh medium, cells grown in conditioned medium harvested from normal human lung fibroblast cells exposed to low-fluence α-particles, displayed an increased clonogenic survival after subsequent exposures to α-particles.[21)] Similar results were found using γ-rays.[22)] These findings thus clearly demonstrated a link between radiation-induced adaptive and bystander responses. Recently, it was reported that accumulation of iNOS in wild-type *p53* cells was induced by chronic irradiation with γ-rays followed by an acute irradiation with X-rays, but not by either treatment alone, resulting in an increase in nitrite concentrations in the medium.[10)] It has been suggested that the accumulation of iNOS may be due to the depression of acute irradiation-induced p53 function by the chronic pre-challenge exposure.[25)] In addition, it has been reported that the radiosensitivity of wild-type *p53* cells in response to an acute X-irradiation was reduced after chronic irradiation with γ-rays. This reduction in radiosensitivity of wild-type *p53* cells was nearly completely suppressed by the addition of the NO scavenger, carboxy-PTIO to the medium.[10)] Previously, cyclooxygenase-2 (COX-2, also known as prostaglandin (PG) endoperoxide synthase-2) was advocated to be involved in NO-mediated bystander responses that induce cellular radioresistance due to radioprotective effects of PG analogues.[79,101,102)] Hei and colleagues demonstrated that COX-2 signaling cascade plays an essential role in the radiation-induced bystander process. In addition, they indicated that because the critical event of the COX-2 signaling is the activation of the MAPK pathways (the activation of ERK1/2 and p38MAPK), the MAPK pathways also play the important role in this process.[103)] ERK1/2 kinase activity, a cytoprotective response to radiation, was enhanced when NOS1 was activated by ionizing radiation.[93)] These findings suggest a possible link between NO-mediated bystander responses and MAPK pathways via COX-2 signaling pathway. Furthermore, singlet oxygen is produced when the conversion reaction from PGG2 to PGH2 is catalyzed by COX-2. In addition, cAMP, an inducer of COX-2 and EP3A, a receptor of PGE2 are linked in cellular calcium flux.[104)] Therefore, radiation-induced bystander responses appear to be at least partially initiated and mediated by NO, promoted by COX-2, and their cytoprotective mission are likely supported by MAPK pathways. Thus, radiation-induced and NO-mediated bystander responses may actually contribute to the radioadaptive response.

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