

# The Yin and Yang of Low-Dose Radiobiology

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**Summary.** Two conflicting phenomena, bystander effect and adaptive response, are important in determining the biological responses at low doses of radiation and have the potential to impact the shape of the dose–response relationship. Using the Columbia University charged-particle microbeam and the highly sensitive human–hamster hybrid (A<sub>L</sub>) cells mutagenic assay, we show here that nonirradiated cells acquire mutagenesis through direct contact with cells whose nuclei have been traversed with a lethal dose of 20 alpha ( $\alpha$ -)particles each. Pretreatment of cells with a low dose of X-rays 4 h before  $\alpha$ -particle irradiation significantly decreased this bystander mutagenic response. Although adaptive response is largely protective in nature and the bystander response, in general, signifies detrimental effects, the two processes share many common characteristics. There is evidence that extracellular signal-related kinase (ERK), nuclear factor- $\kappa$ B, cytokines, and mitochondrial functions play an important role in the bystander effects. However, all these signaling events are applicable to the adaptive response as well. These data suggest a common lineage between these two stress-related phenomena. A better understanding of how these two effects interact at the cellular, tissue, and organ levels will address some of the pressing issues on target size, radiation dose response, and, ultimately, low dose risk assessment.

**Key words** Bystander effects · Adaptive response · Mitochondrial function · Nuclear factor- $\kappa$ B · Signaling events

## Introduction

Radiation is a two-edged sword: on the one hand, it is an effective therapeutic modality for the treatment of many types of human cancers, and on the other hand it is a well-known human carcinogen. The estimated lifetime cancer mortality risk

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from low-dose/low-dose-rate radiation exposure, based on epidemiological data from the Japanese atomic bomb survivors, is estimated to be 0.05 per sievert (Sv) for the whole population [1]. However, direct characterization of risk at low doses is at or beyond the limits of epidemiology. Cancer risk from exposure to ionizing radiation clearly increases at a dose above 10 cGy, and no obvious threshold dose is detectable. At doses below 10 cGy, the radiobiological effects are rather complex and are subjected to modulations by various competing forces, including bystander effects and adaptive response.

Radiation-induced bystander effect is defined as the induction of biological effects in cells that are not directly traversed by a charged particle, but are in close proximity to cells which are. Interest in this effect was sparked by earlier reports demonstrating that, following a low dose of alpha ( $\alpha$ )-particles, a larger proportion of cells showed biological damage than was estimated, based on microdosimetric principle, to have been hit by an  $\alpha$ -particle [2]. To demonstrate the induction of a radiation-induced bystander effect unequivocally, studies were conducted using a microbeam in which a defined proportion of cells in a confluent monolayer were irradiated individually with a defined number of  $\alpha$ -particles [3,4]. These studies provided the first clear-cut indication of a radiation-induced bystander phenomenon.

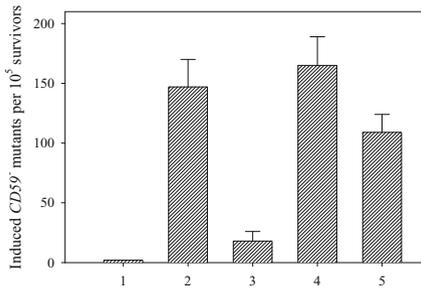
Adaptive response is characterized by a reduction in radiobiological response in cells pretreated with a low dose of ionizing radiation (generally  $\leq 10$  cGy) followed by exposure to a challenging, higher dose. Since the original experiments reported in 1984 [5], numerous data have shown the existence of such a response with a variety of endpoints in various cell types [6]. Although bystander effect and adaptive response are important parameters for low-dose radiation response, there are only limited data available comparing the two effects [7–9].

## Adaptive Response on Bystander Mutagenesis

To define the interaction between adaptive response and bystander effects, human–hamster hybrid ( $A_L$ ) cells were used together with the Columbia University single-particle microbeam. For determining the adaptive response, cells were irradiated with a low dose of X-rays (10 cGy) 4 h before the  $\alpha$ -particle irradiation. To examine the response of bystander cells to the subsequent challenging dose, 10% of the cells were randomly irradiated with a lethal dose of 20  $\alpha$ -particles each directed at the nuclear centroids. Mutant fractions at the *CD59* locus of the  $A_L$  cells were scored using an antibody-complement cytotoxic assay as previously described [10,11]. Western blots were used to identify various signaling proteins, and an electrophoretic mobility shift assay (EMSA) was used to detect the DNA-binding activities of various bystander signaling molecules.

$A_L$  cells irradiated with a 10 cGy dose of X-rays resulted in a low but significant induction of mutations at the *CD59* locus (Fig. 1). The background *CD59* mutant fraction among the population of  $A_L$  cells used in these experiments averaged  $61 \pm 19$ . Consistent with our previously published data, irradiation of 10% of a

Pretreatment with low dose X-rays reduces bystander mutagenesis



Priming dose delivered 4 hours before microbeam irradiation

**Fig. 1.** Induced mutant fraction of human–hamster hybrid (A<sub>1</sub>) cells in which 10% had been irradiated with 20 α-particles through the nucleus with or without pretreatment with a 10-cGy dose of X-rays. 1, Control; 2, 20 α-particles, 10%; 3, X-rays alone, 10 cGy; 4, assuming an additive effect between the two effects; 5, actual mutant fraction of cells pretreated with 10 cGy dose of X-rays followed by targeting 10% of cells with a lethal dose of 20 α-particles. Data are pooled from three independent experiments. Bars ±SD

confluent cell population with a lethal dose of 20 α-particles each through the nuclei resulted in a mutant yield that was about three times higher than the background among the nonirradiated neighboring cells [3]. Pretreatment of cells with a 10-cGy dose of X-rays significantly reduced this bystander mutagenesis ( $P < 0.05$ , Fig. 1) [12]. These results implied that, in the presence of low-dose radiation stress, the bystander mutagenesis is suppressed by the adaptive response, although the mechanism (or mechanisms) is unclear.

### Parameters Affecting the Adaptive Response and the Bystander Effect

Table 1 summarizes the similarity and difference in various parameters that can modulate radiation-induced adaptive response and the bystander effect. In general, there are more similarities than differences between the two phenomena. Both are primarily low-dose phenomena, and neither shows a dose–response induction of effects. Both the adaptive response and the bystander effect have been demonstrated by a range of biological endpoints including cell killing, oncogenic transformation, mutagenesis, chromosomal aberrations, induction of p53 protein, and DNA repair foci. Although bystander effects are not p53 dependent, there are reports that adaptive response, in some studies, is related to p53 function [13]. It should be noted that cancer cells with mutated p53 protein can also demonstrate an adaptive response [14]. Both phenomena involve signals that mediate through either gap junctions or soluble mediators [15,16].

**Table 1.** A comparison of radiation-induced bystander effect versus adaptive response

Parameter	Bystander	Adaptive response
Low-dose phenomenon	+	+
Dose-response relationship	-	-
LET* dependence	-	-
Endpoints examined	Many	Many
Biological consequence	Harmful/protective	Protective
p53 dependence	-	±
Individual variability	-	+
Involves gap junctions	+	+
Involves soluble mediator(s)	+	+
Involves ROS/RNS	+	+
Requires protein synthesis	+	+
Genomic instability	+	+

\*Linear Energy Transfer

**Table 2.** Signaling events that are common to both the adaptive response and the bystander effect

Reactive radical species
Mitochondrial function
NFκB activities
MAPK/ERK kinase activities
Cytokine activities

## Signaling Events Common to Both the Adaptive Response and the Bystander Effect

Table 2 lists several signaling events that are common to both these low-dose phenomena and suggest that the adaptive response and the bystander effect share a common stress-related signaling lineage. These events include reactive radical species such as superoxide anions, hydroxyl radicals, hydrogen peroxide, nitric oxide, and peroxy nitrite anions. The observations that extracellularly applied anti-oxidants, such as superoxide dismutase [17] and catalase [18], can inhibit medium-mediated bystander responses suggest a role of reactive radical species in the bystander signaling scheme. Because mitochondria are the main source of energy production as well as generators of free radicals in cells, it is consistent with the recent observation that mitochondrial DNA-deficient cells exhibit a lower bystander response in the presence of wild-type cells [19]. Similarly, reactive radical species can presumably damage DNA to initiate an adaptive response. The observation that nitric oxide, secreted from irradiated donor cells, can induce an adaptive response in bystander cells [20] provides strong evidence that nitric oxide plays an important role in both these low-dose responses.

## **Activation of Mitogen-Activated Protein Kinase (MAPK)/ Extracellular Signal-Related Kinase (ERK) Signaling Pathways in Bystander and Adaptive Responses**

Previous studies have shown that cyclooxygenase 2 (COX-2) is an important signaling molecule for radiation-induced bystander effects [21]. It is known that insulin growth factor activates the mitogen-activated protein kinase (MAPK) signaling cascade, and activation of extracellular signal-related kinase (ERK) by phosphorylation is a critical upstream event preceding COX-2 expression. Using Western blot analyses, it was evident that a strong upregulation of phospho-ERK levels in both  $\alpha$ -irradiated and bystander normal human fibroblasts occurred 4 h after irradiation [21]. In fact, increased levels of phospho-ERK could even be detected 16 h after treatment, indicating a persistent response to the bystander signaling. In contrast, activity of MAPK p38 kinase was found to be increased 4 h after treatment and was not detectable 16 h after irradiation. It should be noted that, when compared with the controls, the ratio of phosphorylated ERK to native ERK increased from 2 to 13 among the bystander cells. If activation of the MAPK signaling cascade and ERK phosphorylation are essential in mediating the bystander effect, it should be possible to mitigate the later response by using a specific inhibitor of the MEK-ERK signaling cascade. In fact, pretreatment of cells with a non-cytotoxic dose of PD 98059 (50  $\mu$ M), a specific inhibitor of MEK-ERK kinase, completely suppressed bystander toxicity observed in the human fibroblast cultures [21].

The role of MAPK/ERK pathways in radiation-induced adaptive response was previously demonstrated in mammalian cells. A low priming dose of X-rays (2 cGy) induced translocation of protein kinase C- $\alpha$  (PKC- $\alpha$ ) from cytosol to membrane. On subsequent exposure to a challenging dose of X-rays (3 Gy), a lower incidence of chromosomal translocation was observed in preirradiated cultures that could be correlated with activation of p38 MAPK kinase activities [22].

## **Role of Nuclear Factor Kappa B (NF $\kappa$ B) in Bystander and Adaptive Responses**

As NF $\kappa$ B is an important transcription factor for many signaling genes including COX-2, it is likely that NF $\kappa$ B participates in the bystander response. There is clear evidence that  $\alpha$ -particle irradiation upregulates NF $\kappa$ B-binding activity in both directly irradiated and bystander cells, whereas Bay 11-7082, a pharmacological inhibitor of IKK/NF $\kappa$ B, efficiently suppresses this upregulation and also reduces levels below the basal amount [19]. This inhibitor of NF $\kappa$ B activity also efficiently downregulates COX-2 and inducible nitric oxide synthase (iNOS) expression levels in both directly irradiated and bystander fibroblasts. Earlier studies using confluent human skin fibroblasts exposed to low fluences of  $\alpha$ -particles have demonstrated

a rapid upregulation of NF $\kappa$ B, JNK, and ERK in the exposed population [23] and suggest activation of these stress-inducible signaling pathways in bystander cells. As induction of NF $\kappa$ B-binding activity can be found in both directly irradiated and bystander cells, its role in the bystander response in this study is, therefore, equivocal.

In contrast, there is recent evidence that NF $\kappa$ B plays an important function in the adaptive response [24]. Pretreatment of mouse epidermal cells with a 10-cGy dose of X-rays increased survival of the cells to a subsequent, challenging dose of 2 Gy, and the response was abrogated by pretreatment with the NF $\kappa$ B inhibitor, IMD 0354 [25].

## **Effects of Cytokines on Radiation-Induced Bystander and Adaptive Responses**

There is recent evidence that exogenous tumor necrosis factor-alpha (TNF- $\alpha$ ) in concert with interleukin 1-beta (IL-1 $\beta$ ) directly controls COX-2 expression in human lung fibroblast (NHLF) cultures [19]. Both TNF- $\alpha$  and IL-1 $\beta$  were found to be induced following  $\alpha$ -irradiation of NHLF. The inhibitory monoclonal antibody (mAb) against TNF- $\alpha$ , which was introduced into the cell media, substantially decreased levels of NF- $\kappa$ B and JNK, which was accompanied by a well-pronounced decrease in COX-2 expression in both irradiated and, especially, in bystander NHLF [19]. These studies provide a clear link of the binding of cell-surface receptors for the various cytokines with the downstream activation of NF- $\kappa$ B and mitogen-activated protein kinases in bystander effects.

Because NF $\kappa$ B can be activated by several pathways involving TNF- $\alpha$  and IL-1, and NF- $\kappa$ B activities have been linked to radiation-induced adaptive response, it is no surprise that cytokines have a profound effect on the radioadaptive behavior of cells. Earlier studies by Cong et al. have demonstrated that mice pretreated with interferon from extracted human liver RNA showed significant protective effects in bone marrow and germ cells upon exposure to X-rays [26].

## **Conclusion**

Radiation-induced bystander effect and adaptive response are two conflicting phenomena that are stress related and are observed mainly at the low-dose region. At high radiation doses, direct damage to the cells or tissues would dwarf these low-dose effects. Although many of the bystander responses reported thus far have been detrimental in nature, there are reported protective effects as well, for example, induction of apoptosis of potentially damaged cells [27]. On the other hand, there is evidence that bystander cells also show increase in genomic instability, a predisposing factor for carcinogenesis. Hence, the contribution of bystander effects in

radiation risk assessment has to be evaluated in terms of tissue context, the phenotypic behavior of their progeny, and the presence of other competing, low-dose effects that include adaptive response.

Adaptive response generally signifies protective effects. The two phenomena share many common parameters as well as signaling events. A better understanding of the mechanisms involved in the two processes and how they interact will be important in obtaining a better and more accurate assessment of low-dose radiation risk.

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## References

1. International Commission on Radiological Protection (1990) Recommendations of the ICRP. ICRP Publication 60. Pergamon Press, Oxford, England
2. Nagasawa H, Little J (1992) Induction of sister chromatid exchanges by extremely low doses of  $\alpha$ -particles. *Cancer Res* 52:6394–6396
3. Zhou H, Randers-Pehrson G, Waldren CA, et al (2000) Induction of a bystander mutagenic effect of alpha particles in mammalian cells. *Proc Natl Acad Sci U S A* 97:2099–2104
4. Belyakov OV, Malcolmson AM, Folkard M, et al (2001) Direct evidence for a bystander effect of ionizing radiation in primary human fibroblasts. *Br J Cancer* 84:674–679
5. Olivieri G, Bodycote J, Wolff S (1984) Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science* 223:594–597
6. Rigaud O, Moustacchi E (1996) Radioadaptation for gene mutation and the possible molecular mechanisms of the adaptive response (review). *Mutat Res* 358:127–134
7. Sawant SG, Randers-Pehrson G, Metting NF, et al (2001) Adaptive response and the bystander effect induced by radiation in C3H10T1/2 cells in culture. *Radiat Res* 156:177–180
8. Iyer R, Lehnert BE (2002) Low dose, low-LET ionizing radiation-induced radioadaptation and associated early responses in unirradiated cells. *Mutat Res* 503:1–9
9. Iyer R, Lehnert BE (2002) Alpha-particle-induced increases in the radioresistance of normal human bystander cells. *Radiat Res* 157:3–7
10. Waldren CA, Jones C, Puck TT (1979) Measurement of mutagenesis in mammalian cells. *Proc Natl Acad Sci U S A* 76:1358–1362
11. Hei TK, Waldren CA, Hall EJ (1988) Mutation induction and relative biological effectiveness of neutrons in mammalian cells. *Radiat Res* 115:281–291
12. Zhou H, Randers-Pehrson G, Geard CR, et al (2003) Interaction between radiation induced adaptive response and bystander mutagenesis in mammalian cells. *Radiat Res* 160:512–516
13. Wang X, Ohnishi T (1997) p53 dependent signal transduction induced by stress. *J Radiat Res* 38:179–194
14. Boothman DA, Meyers M, Odegaard E, et al (1996) Altered G1 checkpoint control determines adaptive responses to ionizing radiation. *Mutat Res* 358:143–154
15. Kadhim MA, Moore SR, Goodwin EH (2004) Interrelationships amongst radiation induced genomic instability, bystander effects and the adaptive response. *Mutat Res* 568:21–32
16. Matsumoto H, Hamada N, Takahashi A, et al (2007) Vanguard of paradigm shift in radiation biology: radiation induced adaptive and bystander responses. *J Radiat Res* 48:97–106
17. Yang H, Asaad N, Held KD (2005) Medium-mediated intercellular communication is involved in bystander responses of X-ray-irradiated normal human fibroblasts. *Oncogene* 24:2096–2103

18. Lyng FM, Maquire P, McClean B, et al (2006) The involvement of calcium and MAP kinase signaling pathways in the production of radiation induced bystander effects. *Radiat Res* 165:400–409
19. Zhou H, Ivanov HN, Lien YC, et al (2008) Mitochondrial function and NF- $\kappa$ B mediated signaling in radiation induced bystander effects. *Cancer Res* 68:2233–2240
20. Matsumoto H, Hayashi S, Hatashita M, et al (2001) Induction of radio-resistance by a nitric oxide mediated bystander effect. *Radiat Res* 155:387–396
21. Zhou H, Ivanov VN, Gillespie J, et al (2005) Mechanism of radiation-induced bystander effect: role of the cyclooxygenase-2 signaling pathway. *Proc Natl Acad Sci U S A* 41:14641–14646
22. Shimizu T, Kato T Jr, Tachibana A, et al (1999) Coordinated regulation of radioadaptive response by protein kinase C and p38 mitogen activated protein kinase. *Exp Cell Res* 251:424–432
23. Azzam EI, DeToledo SM, Spitz DR, et al (2002) Oxidative metabolism modulates signal transduction and micronucleus formation in bystander cells from alpha particle irradiated normal human fibroblast cultures. *Cancer Res* 62:5426–5442
24. Ahmed KM, Li JJ (2008) NF- $\kappa$ B mediated adaptive resistance to ionizing radiation. *Free Radic Biol Med* 44:1–13
25. Fan M, Ahmed KM, Coleman MC, et al (2007) NF $\kappa$ B and MnSOD mediate adaptive radio-resistance in low dose irradiated mouse skin epithelial cells. *Cancer Res* 67:3220–3228
26. Cong XL, Wang XL, Su Q, et al (1998) Protective effects of extracted human liver RNA, a known interferon inducer, against radiation induced cytogenetic damage in male mice. *Toxicol Lett* 95:189–198
27. Coates PJ, Lorimore SA, Wright EG (2004) Damaging and protective cell signaling in the untargeted effects of ionizing radiation. *Mutat Res* 568:5–20