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Non-targeted bystander effects induced by ionizing radiation

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Abstract

Radiation-induced bystander effects refer to those responses occurring in cells that were not subject to energy deposition events following ionizing radiation. These bystander cells may have been neighbors of irradiated cells, or physically separated but subject to soluble secreted signals from irradiated cells. Bystander effects have been observed *in vitro* and *in vivo* and for various radiation qualities. In tribute to an old friend and colleague, Anthony V. Carrano, who would have said "well what are the critical questions that should be addressed, and so what?", we review the evidence for non-targeted radiation-induced bystander effects with emphasis on prevailing questions in this rapidly developing research field, and the potential significance of bystander effects in evaluating the detrimental health effects of radiation exposure.

Keywords: Bystander effects; Genomic instability; Ionizing radiation; Non-targeted effects

1. Introduction

Ionizing radiation-induced bystander effects are those effects occurring in cells that were not traversed by radiation but were induced by signals from irradiated cells. Bystander effects have been described in numerous *in vitro* cell culture systems [1], and there is also evidence that they can occur *in vivo* in complex model systems and animals [2]. They appear to play an integral role in what are now considered non-targeted effects of ionizing radiation [3]. These non-targeted effects include induced genomic instability, clastogenic factors and abscopal effects. Clastogenic factors are found in blood plasma from some irradiated individuals and can cause chromosome breakage in non-irradiated peripheral blood lymphocytes [2]. Abscopal effects are defined as significant responses occurring in tissues definitively separated from the volume irradiated. The fact that many of the effects associated with cellular exposure to radiation can manifest in non-irradiated bystander cells has implications for any individual who may be exposed to ionizing radiation because they suggest that the target for radiation responses might be greater than the volume actually irradiated. In this paper the current status of radiation-induced bystander effects will be reviewed with emphasis on prevailing questions in this blossoming research area.

2. Radiation-induced bystander effects

Bystander effects can be observed after a variety of different exposure strategies. These include co-culture of irradiated and non-irradiated cells [4], the use of very low fluences of alpha particles where the majority of cells have not been irradiated [5], irradiation of targeted cells within a population of cells using charged-particle microbeams [6–8], and the transfer of

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medium from irradiated cells to non-irradiated cells [9]. The irradiated cell then can send a signal(s) to non-irradiated cells, resulting in a number of bystander responses including increased sister chromatid exchange (SCE) chromosomal rearrangements, micronuclei formation, gene mutations, apoptosis, genomic instability, transformation and a variety of damage-inducible stress responses [1,2]. The majority of studies have focused on endpoints that are associated with genomic damage. However, it would be misleading to imply that all bystander effects have a detrimental impact on the cell. Other non-necessarily detrimental effects have been described in bystander cells including the secretion of growth inhibitory factors [10], increased cell proliferation [11–13], and a radio-protective adaptive response [14,15].

3. What is the mechanism for radiation-induced bystander effects?

The two general strategies for demonstrating that an irradiated cell can produce a signal that elicits an effect in a non-irradiated cell have shed some light on the mechanisms. On one hand, the transfer of medium from irradiated to non-irradiated cells implicates a soluble secreted factor [16]. On the other, the use of low fluences of alpha particles and appropriate cell lines demonstrates a role for intercellular gap junction communication [5,17]. The cell-to-cell gap junction mediated transfer of information appears to require connexin 43 [18,19], indicating that the size of the signaling factor is relatively small [20]. These two mechanisms are not mutually exclusive, and there is likely a role for both processes in communicating the bystander response [21].

Both mechanisms appear to involve enhanced oxidative metabolism, with a role for reactive oxygen and nitrogen species as well as proteins associated with cellular stress responses being the major players [22–28]. Whatever the initiating events, it is clear that some cell types can respond to radiation exposure by producing a signal that can elicit a response in a non-irradiated cell. Not all cell types can produce this signal, and not all cell types are receptive to or will respond to such signals [9,29,30]. Furthermore, it is clear that even in those populations that do elicit a bystander response not all cells within the population respond to the signal and manifest a bystander effect.

4. What is the nature of the bystander signal?

That individual cells in a multi-cellular organism communicate is not unexpected or unusual. They do this in many well defined, and some less well defined ways [31,32]. Understanding bystander effects demands identification of the transmitted signal and how this signal provokes a response in a non-irradiated cell. Is the factor transferred by cell-to-cell gap junction communication the same that is secreted into the culture medium? How long after irradiation is the signal "transmitted" and what is the duration of the signal? While the sphingomylelin membrane-signaling pathway appears to be involved in receiving the signal for the bystander cell [33], how the signal is transported to the nucleus remains to be determined. Understanding the nature of the signal, its time course of expression, and how the signal exerts its effect in the bystander cell is obviously crucial to understanding the molecular mechanism of the bystander effect and ultimately its biological significance.

5. How does radiation elicit a bystander signal?

The majority of the bystander responses described are also observed after targeted exposure to ionizing radiation, i.e., in cells where radiation-induced energy deposition events had occurred. These responses include cytogenetic damage (chromosomal aberrations, SCEs and micronuclei), mutagenesis, transformation, changes in gene expression and cell killing. For many years the DNA double-strand break (DSB) has been considered the primary genotoxic lesion induced by ionizing radiation [34], suggesting that this lesions might also be involved in bystander responses. Formation of DSBs induces phosphorylation of histone H2AX, and this phosphorylated form, γ-H2AX, forms foci at the sites of DNA cleavage. Sokolov et al. [35] reported that irradiation of target cells induces the formation of γ-H2AX foci in bystander cell populations. After 18 h co-culture with cells irradiated with 20 alpha-particles, the fraction of bystander cells showing multiple γ-H2AX foci increased 3.7-fold. Similar changes occurred in bystander populations mixed and cultured with cells irradiated with γ-rays, and in cultures containing media conditioned by γ-irradiated cells. This study indicates that H2AX phosphorylation may well be an early step in the bystander response and that DNA DSBs may be responsible for the observed bystander effects. A similar result was reported by Yang et al. [36], although we urge caution in interpreting a direct association between γ-H2AX foci formation and DSB formation.

It is not immediately obvious how processes involving oxidative metabolism and stress inducible proteins might lead to DNA cleavage in bystander cells, but other lines of evidence also implicate DNA DSBs as playing a role in induced bystander responses. Little and co-workers