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Review

Bystander effects and radiotherapy

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ABSTRACT

Radiation-induced bystander effects are defined as biological effects expressed after irradiation by cells whose nuclei have not been directly irradiated. These effects include DNA damage, chromosomal instability, mutation, and apoptosis. There is considerable evidence that ionizing radiation affects cells located near the site of irradiation, which respond individually and collectively as part of a large interconnected web. These bystander signals can alter the dynamic equilibrium between proliferation, apoptosis, quiescence or differentiation. The aim of this review is to examine the most important biological effects of this phenomenon with regard to areas of major interest in radiotherapy. Such aspects include radiation-induced bystander effects during the cell cycle under hypoxic conditions when administering fractionated modalities or combined radio-chemotherapy. Other relevant aspects include individual variation and genetics in toxicity of bystander factors and normal tissue collateral damage. In advanced radiotherapy techniques, such as intensity-modulated radiation therapy (IMRT), the high degree of dose conformity to the target volume reduces the dose and, therefore, the risk of complications, to normal tissues. However, significant doses can accumulate out-of-field due to photon scattering and this may impact cellular response in these regions. Protons may offer a solution to reduce out-of-field doses. The bystander effect has numerous associated phenomena, including adaptive response, genomic instability, and abscopal effects. Also, the bystander effect can influence radiation protection and oxidative stress. It is essential that we understand the mechanisms underlying the bystander effect in order to more accurately assess radiation risk and to evaluate protocols for cancer radiotherapy.

1. Introduction

Ionizing radiation affects not only the cells that are directly irradiated but also their non-irradiated neighbours. When non-irradiated cells respond to radiation, the response is known as the bystander effect. In general, the bystander effect mimics the direct effects of radiation including an increased frequency of apoptosis, micronucleation, DNA strand breaks and mutations, altered levels or activity of regulatory proteins

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Table 1 – Lesions producing radiation injury.

- Cytocidal effects
- Indirect effects
- Functional effects

and enzymes, reduced clonogenic efficiency, and oncogenic transformation. These responses have been attributed to signals transmitted directly through gap junctions and by factors released into the growth medium.

Results suggest that the genetic damage in cells exposed to scattered radiation is caused by factors released by irradiated cells into the medium rather than by DNA damage induced directly by X-rays. It seems that bystander effects may have important clinical implications for health risk after low-level radiation exposure of cells lying outside the radiation field during clinical treatment.[1]

In the present review, we highlight the issues and problems associated with radiation-induced bystander effects from a clinical perspective.

2. Types of radiation injuries

Radiation injuries are typically classified as either "early" or "late" injuries, depending on the time interval between exposure and clinical expression, and this simple system has served us well. This classification is based on two mechanistic models of injury, the "target cell" and "vascular injury" models. These two models support quantitative biomathematical modelling, using the very simple parameters of the linear quadratic model.

Incorporating bystander effects into the science underpinning clinical radiotherapy will involve moving beyond simple mechanistic models and towards a more system-based approach. The increasing knowledge of molecular mechanisms of radiation injury has provided us with opportunities to understand their genesis at a more basic level. The new formalism holds that lesions producing radiation injury fall into one of three categories: cytocidal effects, indirect effects, and functional effects (Table 1).

Cytocidal effects relate to the phenomena characterized by the "target cell" model. The time interval between irradiation and manifestation of injury depends on target cell characteristics (radiation sensitivity, repair capacity, proliferation rate, *etc.*) and tissue organization. Indirect effects refer to reactive phenomena that occur in response to radiation-induced injury in other cells or tissues (*i.e.*, parenchymal cell depletion secondary to vascular damage). Indirect effects also include such phenomena as the bystander effect. Functional effects result from nonlethal effects on different intra- and extra-cellular molecules and changes in gene expression in irradiated cells. In most tissues, injury occurs through interactions that involve all three types of effects.

Assessment of radiation-induced bystander effects has not been limited exclusively to tissue culture analyses. *In vivo* experiments, performed as early as 1974, have also demonstrated the existence of such effects. Brooks et al.[2] showed that when α-particle emitters are concentrated in the liver of Chinese hamsters, all cells in the liver are at the same risk for induction of chromosome damage, even though only a small fraction of the total liver cell population was actually exposed to α-particles. In addition, investigation of genetic effects in partial organ irradiation experiments has demonstrated out-of-field effects.

When irradiated and non-irradiated male mouse bone marrow cells (distinguishable by specific cytogenetic markers) were transplanted into female recipients, chromosomal instability was observed in the descendants of the non-irradiated cells. With relevance to radiotherapy, a cytotoxic bystander effect produced by tumour cells labelled with 5-^{125}iodo-2′-deoxyuridine (^{125}IudR) was recently demonstrated.[3]

3. Bystander effects in areas of major interest in radiotherapy

Tissue responses may not relate directly to the cytotoxic effects of radiation. For example, although local control in tumours requires elimination of tumour clonogens, in some circumstances the vascular damage could be extensive, especially when irradiation is combined with biologics or chemotherapeutic drugs. Also, irradiation modifies the tumour–host relationship, including interactions with infiltrating cells, such as macrophages and lymphocytes, which have been shown to be able to both promote and inhibit tumour growth. Such bystander effects may influence the biological basis of radiation on tumours and normal tissue.

3.1. *Cell cycle effects*

The importance of the cell cycle in radiosensitivity is well known. The advantage of radiotherapy is that cycling cells in the tumour are more radiosensitive than normal cells.[4] Cells in the G2 and M phases are more sensitive to radiation. Some radiosensitizers act by blocking cells in the sensitive phases of the cell cycle, thus optimizing cell killing. The cell cycle phase can affect the ability of cells to produce or respond to bystander factors. Results reported in related fields suggest that it is likely that the G2 phase may be a candidate for involvement in bystander factor production or response (Fig. 1). Low-dose hypersensitivity is known to involve G2 cells. In contrast, P53, which acts in the G1 checkpoint, is not involved in bystander factor production, although it may be involved in apoptotic responses to the receipt of bystander signals. On the basis of the existing published data, it is tempting to suggest that bystander signal production is maximal in G2 phase.[5]

3.2. *Hypoxic conditions*

There are reasons to suspect that hypoxic cells or those with compromised oxidative metabolism will have either reduced or absent cytotoxic bystander effects. A persistent state of oxidative stress is known to be induced in recipients of bystander medium and it has also been linked to the induction of genomic instability in both directly irradiated and bystander cells.

Many tumour cells lines that respire anaerobically do not display cytotoxic bystander effects. Many experiments using cell lines with a mitochondrial malfunction