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Overview

Bystander Signalling: Exploring Clinical Relevance Through New Approaches and New Models

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Abstract

Classical radiation biology research has centred on nuclear DNA as the main target of radiation-induced damage. Over the past two decades, this has been challenged by a significant amount of scientific evidence clearly showing radiation-induced cell signalling effects to have important roles in mediating overall radiobiological response. These effects, generally termed radiation-induced bystander effects (RIBEs) have challenged the traditional DNA targeted theory in radiation biology and highlighted an important role for cells not directly traversed by radiation. The multiplicity of experimental systems and exposure conditions in which RIBEs have been observed has hindered precise definitions of these effects. However, RIBEs have recently been classified for different relevant human radiation exposure scenarios in an attempt to clarify their role *in vivo*. Despite significant research efforts in this area, there is little direct evidence for their role in clinically relevant exposure scenarios. In this review, we explore the clinical relevance of RIBEs from classical experimental approaches through to novel models that have been used to further determine their potential implications in the clinic.

Key words: Bystander effect; *in vivo*; ionising radiation; microbeam

Statement of Search Strategies Used and Sources of Information

Articles cited in this manuscript were sourced through a literature search on the Medline/Pubmed database using the search terms: 'radiation', bystander' 'in vivo' and 'microbeam'. Full articles were retrieved when the abstract was deemed relevant. The bibliographies of retrieved papers were also searched and relevant articles included.

Introduction

Ionising radiation is an effective cancer therapy due to its ability to induce cell death as a consequence of DNA damage resulting from energy deposition in the cellular environment. Most cellular responses to ionising radiation are mediated through direct energy deposition in the DNA or indirectly through reactive oxygen species and other free radicals formed due to the radiolysis of water [1].

Classically, radiation biology research has focussed on nuclear DNA as the sole target of radiation-induced damage. However, over the last 25 years, a large body of scientific evidence has challenged the view that radiobiological responses occur only in cells directly targeted by radiation as biological effects have been shown to occur outside of the radiation target. These 'non-targeted effects' include genomic instability and several radiation-induced signalling effects [2], generally termed radiation-induced bystander effects (RIBEs). RIBEs were first identified by Nagasawa and Little [3] who observed chromosome damage in the form of sister chromatid exchanges in more than 30% of a cell population under conditions in which only 1% of cell nuclei had been targeted using α particles. Since then, RIBEs have been shown using a range of experimental systems with multiple biological end points. Despite increasing evidence in a growing number of model systems, the implications of RIBEs for radiotherapy and cancer risk remain to be fully determined. In this review, we describe

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RIBEs in the context of current experimental and clinical exposure scenarios and consider potential implications for cancer risk and radiotherapy.

Defining Radiation-induced Bystander Effects

In general terms, RIBEs may be defined as radiobiological responses observed in cellular systems that have not been directly traversed by ionising radiation but are in close proximity to irradiated cells. These effects are cell signal mediated either through direct physical cell contact via gap junction intercellular communication [4] or through secreted, diffusible signalling molecules into the surrounding media [5–7]. A number of candidate signalling molecules have been identified in mediating RIBEs, such as reactive oxygen and nitrogen species, including nitric oxide, and cytokines such as transforming growth factor-β and interleukin-8. These have been shown to initiate multiple downstream signalling pathways, including the mitogen activated protein kinases and nuclear factor-κB pathways [8].

Although RIBEs can be considered primarily as signalling-mediated effects, precise definitions have remained difficult, as effects are often dependent on the experimental system or exposure conditions being measured. The caveats associated with these different effects observed under different experimental and exposure conditions were the subject of a recent review by Blyth and Sykes [9], who stated that 'most reports in the literature are accompanied by the authors' own definition, usually framed in the context of the data presented in that report'. This is an important consideration that the authors addressed by attempting to establish a general framework for the classification of radiation-induced signalling effects based on human radiation exposure scenarios. They define three different categories; bystander effects, cohort effects and abscopal effects.

The most well-established of these classes are abscopal effects. These are defined as radiation-induced effects in unirradiated tissues occurring outside of an irradiated volume. Radiation-induced abscopal effects were observed more than 60 years ago in some patients after radiotherapy and do not seem to be dose dependent, making them particularly relevant to the partial body exposures typically delivered during conformal radiotherapy. Abscopal effects are rarely recognised in the clinic and so their importance in radiotherapy response remains controversial [10].

More recently, bystander effects have been defined for human exposure scenarios as radiation-induced, signal-mediated effects in unirradiated cells within an irradiated volume exposed to a sufficiently low dose that a portion of cells within the exposed volume remain unexposed and survive. These effects are relevant for whole and partial body exposures to very low doses, such as those from background radiation, high altitude flights and ingested radioactive potassium.

A third classification of effects, termed cohort effects, describe the component of overall radiobiological response in irradiated cells that is not a consequence of direct energy deposition in the target cell, but rather due to communication between cells within an irradiated volume. Cohort effects are relevant for any exposures where most of a cell population is exposed to a significant dose and although this interpretation is relatively uncommon in the literature, there is increasing evidence that intercellular signalling plays a role in the overall radiation response.

These classifications of radiation-induced signalling are shown schematically in Figure 1. Distinction between the classifications is difficult in modern radiotherapy as patients are exposed to complex field geometries with steep dose gradients resulting in delivery of differential doses to cells in close proximity which can freely signal to one another. Although these effects are typically classified and studied separately, they have many common characteristics in that they all occur in response to radiation exposure, are mediated by extracellular signalling factors and cause negative radiobiological effects in neighbouring cells.

Consequently, many of these experimentally and clinically observed phenomena, which are often classified as different effects, actually stem from the same or similar cellular signalling origin and may be interpreted as different consequences of the same generalised RIBEs. As a result, there is significant opportunity for novel approaches to investigate these effects in more clinically relevant scenarios.

Classical Experimental Approaches for Studying Radiation-induced Bystander Effects

A diverse range of experimental approaches has been used to investigate RIBEs at the single cell, multicellular and whole organism levels. A selection of these studies in single cells is summarised in Table 1 and the various approaches

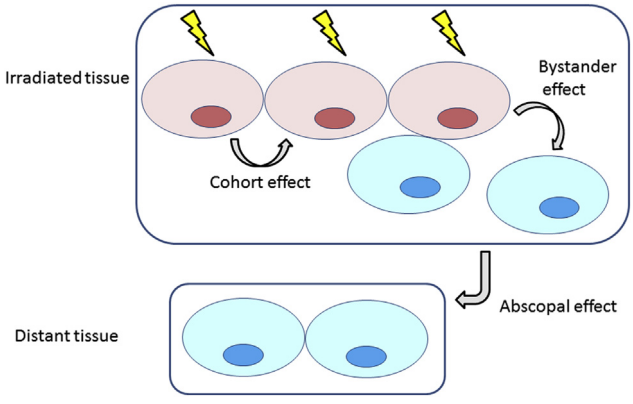

Fig 1. Schematic representation of radiation-induced signalling effects classified by Blyth and Sykes [9]. Irradiated cells are shown in red; unirradiated cells in blue. Bystander effects occur in unirradiated cells within an irradiated volume; within the same volume, radiation-induced signalling contributes to the overall response through cohort effects. Abscopal effects occur in unirradiated tissue at a distant site outside of an irradiated volume.

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