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Review

Systemic mechanisms and effects of ionizing radiation: A new 'old' paradigm of how the bystanders and distant can become the players

Zacharenia Nikitaki[a,1], Ifigeneia V. Mavragani[a,1], Danae A. Laskaratou[a], Violeta Gika[a], Vadim P. Moskvin[b], Konstantinos Theofilatos[c], Konstantinos Vougas[d], Robert D. Stewart[e], Alexandros G. Georgakilas[a,*]

[a] Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens (NTUA), Zografou, 15780 Athens, Greece
[b] Department of Radiation Oncology, St. Jude Children's Research Hospital, Memphis, TN, USA
[c] InSyBio Ltd, 109 Uxbridge Road, W5 5TL London, UK
[d] Proteomics Research Unit, Center of Basic Research II, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[e] Department of Radiation Oncology, University of Washington School of Medicine, School of Medicine, 1959 NE Pacific Street, Box 356043, Seattle, WA 98195, USA

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ABSTRACT

Exposure of cells to any form of ionizing radiation (IR) is expected to induce a variety of DNA lesions, including double strand breaks (DSBs), single strand breaks (SSBs) and oxidized bases, as well as loss of bases, i.e., abasic sites. The damaging potential of IR is primarily related to the generation of electrons, which through their interaction with water produce free radicals. In their turn, free radicals attack DNA, proteins and lipids. Damage is induced also through direct deposition of energy. These types of IR interactions with biological materials are collectively called 'targeted effects', since they refer only to the irradiated cells. Earlier and sometimes 'anecdotal' findings were pointing to the possibility of IR actions unrelated to the irradiated cells or area, i.e., a type of systemic response with unknown mechanistic basis. Over the last years, significant experimental evidence has accumulated, showing a variety of radiation effects for 'out-of-field' areas (non-targeted effects-NTE). The NTE involve the release of chemical and biological mediators from the 'in-field' area and thus the communication of the radiation insult via the so called 'danger' signals. The NTE can be separated in two major groups: bystander and distant (systemic). In this review, we have collected a detailed list of proteins implicated in either bystander or systemic effects, including the clinically relevant abscopal phenomenon, using improved text-mining and bioinformatics tools from the literature. We have identified which of these genes belong to the DNA damage response and repair pathway (DDR/R) and made protein–protein interaction (PPi) networks. Our analysis supports that the apoptosis, TLR-like and NOD-like receptor signaling pathways are the main pathways participating in NTE. Based on this analysis, we formulate a biophysical hypothesis for the regulation of NTE, based on DNA damage and apoptosis gradients between the irradiation point and various distances corresponding to bystander (5 mm) or distant effects (5 cm). Last but not least, in order to provide a more realistic support for our model, we calculate the expected DSB and non-DSB clusters along the central axis of a representative 200.6 MeV pencil beam calculated using Monte Carlo DNA damage simulation software (MCDS) based on the actual beam energy-to-depth curves used in therapy.

Abbreviations: DAMP, damage (or danger)-associated molecular patterns; DDR/R, DNA damage response/repair; DSB, double strand break; FWHM, full width at half maximum; GO, gene ontology; IR, ionizing radiation; MCDS, Monte Carlo DNA damage simulation; NO, nitric oxide; NOS, nitric oxide synthase; NTE, non-targeted effects; PDD, percentage dose distribution; PPi, protein–protein interaction; PRR, pattern recognition receptor; RS, reactive species; RBE, relative biological effectiveness; RIBE, radiation-induced bystander effects; ROS/RNS, reactive oxygen/nitrogen species; TE, targeted effects; TLR, toll-like receptor.

* Corresponding author at: DNA Damage Laboratory, Physics Department, School of Applied Mathematical and Physical Sciences, National Technical University of Athens (NTUA), Iroon Polytechniou 9, Zografou, 15780 Athens, Greece. Fax: +30 210 7722932.
E-mail addresses: alexg@mail.ntua.gr, georgakilasa@gmail.com (A.G. Georgakilas).
[1] These authors have equally contributed to the manuscript.

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1. Introduction to the idea of radiation-induced non-targeted effects

Over the last few years a dramatic surge has been recorded in the knowledge of radiation-induced 'out-of-field' effects and the so-called 'non-targeted' effects (NTE). All these phenomena belong to the general thematic area of systemic effects, including the clinically relevant 'abscopal' effects [1]. The cellular response to diverse radiation types and doses has been extensively reported in the literature. According to the classical "target paradigm" of radiobiology, cell nucleus is the critical target of radiation and the resulting DNA damage is responsible for the detrimental biological effects of radiation [2]. An additional assumption is that no radiation-induced effect is to be expected in cells that do not directly interact with radiation. However, strong experimental evidence points to the induction of complex, global cellular responses in cells that are not directly exposed to radiation (NTE). NTE encompass radiation-induced bystander effects (RIBE), genomic instability and radioadaptive responses. RIBE are defined as biological effects elicited in cells that are not directly traversed by or interacting with radiation, but receive damage signals from directly irradiated, neighboring cells. RIBE are expected to play an active role within a maximum distance of 1–5 mm, like in the case of human tissue models [3,4]. The mechanisms underlying these cellular responses are different and multifactorial for each examined system. There is good evidence, at least in vitro, indicating that bystander signals can be transmitted by two different, but not necessarily mutually exclusive mechanisms: either by physical cell–cell contact, usually *via* gap-junction mediated intercellular communication [5–11], or through the excretion of soluble (and potentially clastogenic) factors in the growth medium of irradiated cells [12–16]. Evidence from several systems indicate a plethora of potential mediators of bystander signals secreted by irradiated cells that can stimulate responses in non-irradiated cells. A few examples are reactive oxygen and nitrogen species (ROS/RNS) [17–19], oxidative DNA damage [20], cytokines and chemokines [21], oxidative enzymes [22] and other inflammatory response markers [23]. Nitric oxide (NO) is generated from arginine through the activity of inducible nitric oxide synthase (iNOS) and consists the main source of RNS by its reaction with superoxide ($O_2^{\bullet -}$) to form peroxynitrite ($ONOO^-$). It has been suggested that NO is not only a 'communicator' of bystander signals, but also a regulator of vascular functions and of the inflammatory environment, which undergoes injury or stress [19,24]. In order to identify the most important biological molecules playing a role in RIBE, we performed an analytical literature search using various text mining techniques and keywords, such as "radiation and bystander effects" etc. A complete list of the keywords can be found in the Supplementary information file (SI). The non-exhaustive findings and key genes experimentally confirmed to participate in RIBE in various systems are reported in Table 1 and in Table S1 (Supplementary data). RIBE appear to dominate at low doses of radiation (<1 Gy), but besides that, they have important implications for radiation therapy [25,26]. Non-targeted radiation effects are also observed in vivo [2] and this might be correlated with increased cancer risk.

It has been known for years that radiation can trigger systemic effects outside the radiation field. After localized irradiation, complex systemic tissue responses in non-irradiated areas may be observed. Systemic are the effects or phenomena which occur at a specific site in an organism and can spread throughout the body, affecting distant organs or tissues. A rare clinical, systematic response to IR is tumor regression at sites distant from the locally irradiated volume, the so-called 'abscopal' effect. From a clinician's viewpoint, the term refers to distant tumor regression after localized irradiation and radiation-induced, but immune-mediated, anti-tumor responses [27]. On the other hand, abscopal effects can be considered as a type of non-targeted/systemic mechanism, with the ability to induce DNA damage response (DDR), genomic instability, cell death or senescence and malignant transformation in distant normal tissues [28].

The term 'abscopal', first coined by Mole in 1953, describes a tumor response to radiation therapy that occurs "at a distance from the irradiated volume, but within the same organism" [29]. Proposed mechanisms underlying this phenomenon are the systemic secretion of cytokines, local inflammation leading to a distant effect or a systematic immune response against local tumor antigens. Ionizing radiation (IR) can induce a diversity of responses in the irradiated cells, initiated by the DNA-damage response and repair (DDR/R), apoptosis or inflammation. In some cases, low doses of X- or γ-rays (for example 0.5 Gy) have been shown to induce anti-inflammatory activities with very positive immunosuppressive outcomes in chronic inflammatory diseases [30,31]. In this case, tissue macrophages initiate the resolution of inflammation by the secretion of specific anti-inflammatory cytokines, like the transforming growth factor (TGF)-beta. This mechanistic insight was recently shown using an *ex vivo* model, in which lipopolysaccharide pre-activated peritoneal macrophages (pMPhi) of radiosensitive BALB/c mice were exposed to 0.1–0.5 Gy of X-rays [32].

Key molecules involved in the IR-induced systemic effects are listed in Tables 2 and 3, based on experimental evidence and current status of knowledge. Damage (or Danger)-associated molecular pattern molecules (DAMPs) seem to play an important role in the transmission of a stress system-wide and across the 'tree of life', as nicely illustrated by Heil and Land [33]. The experimental evidence of Table 3 should be considered in the 'grey zone' between bystander and systemic effects but according to our opinion, these medium transfer experiments attempt to simulate the in vivo situation with the release of various (known and unknown) clastogenic factors in the system.

2. Identification of the key players and their association with biological pathways. A mechanistic insight

The interaction of IR with any living material (cells or tissues) results to a variety of biological responses triggered primarily by the induction of DNA, protein or lipid membrane damage. One of the first responses to this exogenous stress is DNA damage response (DDR) and the consequent repair (R). The DDR complex network encompasses a variety of initial sensor proteins and kinases (transducers, mediators, upstream and downstream effectors) that initiate DNA repair pathways corresponding to the type or types of lesions [34,35]. Based on the simple idea that IR differs from endogenous oxidative stress primarily in the high levels of energy deposition per volume, formation of complex (clustered) DNA damage is anticipated even at low doses (<1 Gy) [36]. This complexity of DNA damage and the triggering of a multi-pathway repair mechanism maybe the first 'danger' signal and a way for the cell to label this as a 'special stress', different from the regular endogenous damage above background levels [34]. Accumulating evidence from different sources suggests that this induction of complex and/or unrepaired DNA lesions maybe the crucial link between any type of stress (IR, oxidative or replication stress), innate immune response (ImmR) and possibly systemic effects at the organism level [37]. In our opinion, this damage and repair asymmetry between two sites (irradiated and bystander) may be the surveillance mechanism triggering the initiation of bystander or distant signals, a way for the 'injured' tissue to communicate its problem to the nearby cells and tissues and possibly to the whole organism (systemic). As discussed in a recent review paper [38], a functional asymmetry in the organism exists at many levels, from DNA repair pathways