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Mini-review

Non-targeted effects induced by ionizing radiation: Mechanisms and potential impact on radiation induced health effects

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ABSTRACT

Not-targeted effects represent a paradigm shift from the "DNA centric" view that ionizing radiation only elicits biological effects and subsequent health consequences as a result of an energy deposition event in the cell nucleus. While this is likely true at higher radiation doses (>1 Gy), at low doses (<100 mGy) non-targeted effects associated with radiation exposure might play a significant role. Here definitions of non-targeted effects are presented, the potential mechanisms for the communication of signals and signaling networks from irradiated cells/tissues are proposed, and the various effects of this intra- and intercellular signaling are described. We conclude with speculation on how these observations might lead to and impact long-term human health outcomes.

0. Definitions

The following definitions of the primary NTE's are adapted from Kadhim et al. [1] and https://ssl.note-ip.org/index.asp.

0.1. Non-targeted effects (NTE's)

Effects manifesting in non-irradiated cells that received a signal(s) communicated from an irradiated cell. NTE's include a plethora of cellular responses usually associated with radiation exposure, plus a number of other phenotypic responses. Responses to genotoxic stress in a biological system are variable, and NTE's are not observed in all experimental model systems [2]. The degree of response depends on the time of analysis, radiation dose and dose rate and radiation quality.

0.2. Radiation induced bystander effects (RIBE)

Effects observed in non-irradiated cells that responded to signal(s) communicated by an irradiated cell. RIBE induce and/or modulate responses in a non-irradiated cell. These non-irradiated bystander cells may have been in the same physical environment as the irradiated cells using microbeam exposures [3,4], or cultured in medium transferred from cell cultures that had previously been irradiated [5,6]. RIBE have been observed in both *in vitro* cell culture systems [7] and *in vivo* model systems [8], and have been the subject of a recent review [9].

0.3. Radiation induced genomic instability (RIGI)

Effects occurring in the progeny of an irradiated cell generations after the parental cell has been irradiated. While the progeny themselves have not been irradiated they are clonally descended from an irradiated cell, e.g., [10,11].

0.4. Abscopal effects

Abscopal effects are generally associated with clinical exposures to ionizing radiation in a radiotherapy type situation [12]. It should be noted that "abscopal effects" are often included as a non-targeted effects of exposure to ionizing radiation, e.g., [8], however, according to the definitions above, this is not correct. During radiotherapy the tumor receives a very large dose of radiation, e.g., 60–70 Gy, the normal tissue in the field of the beam receives a much smaller but still significant radiation dose, e.g., 4–6 Gy, and the whole body is exposed to a comparatively low dose of radiation via leakage from the head of the therapy unit, scattering at the beam collimators and the flattening filter resulting in incident scatter within the treatment room, and scattering from the directly irradiated region of the patient, i.e., internal scatter [13]. Because the whole body therefore receives some radiation dose, any "out of field" abscopal effects should be considered "low dose radiation effects". For the purpose of this discussion, abscopal effects are not considered NTE's.

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1. Introduction

NTE's are not new and have been the subject of intense research over the last 20 years (http://lowdose.energy.gov and https://ssl.note-ip.org/index.asp). The concept that NTE's might play a role in radiation health effects has been suggested [14,15]. Indeed, many of the phenotypes associated with NTE's have also been associated with cancer and the carcinogenic process and, more recently, with a host of non-cancer effects [16]. Thus, rather than provide a comprehensive review of NTE's research and how they might impact health effects associated with exposure to ionizing radiation, we hypothesize that NTE's do contribute to the health effects caused by exposure to ionizing radiation and focus on the signaling mechanisms that may be involved. This is a hypothesis and hypotheses should be testable. Consequently a challenge for the future is to design testable hypotheses to support or refute the conclusions drawn from observations that suggest a link between observed NTE's and radiation-induced health effects.

2. What are non-targeted effects and are they interrelated?

NTE's include RIBE and RIGI, and reflect a number of endpoints including, but not limited to, the induction of mutation and chromosome rearrangements, gene expression changes and cell killing [17]. There appears to be a link between RIBE and RIGI [18,19]. The overwhelming consensus is that there is a strong link between these manifestations of NTE's and this has been supported experimentally [20]. Indeed, RIGI might be considered under the rubric of a RIBE. NTE's appear to be largely a low radiation dose phenomenon [21], although this is controversial [22]. At high doses the effects observed will likely be largely overwhelmed by direct damage to cells. We should mention that as the dose is decreased, the energy deposition will be heterogeneous on the spatial scale of the cell or tissue [23] and not all cells may have a direct interaction, and non-targeted effects are likely contributors to the observed response. This is particularly the case for low dose exposures of high LET radiation, e.g., iron ions, for discussion see [24].

3. Health effects associated with exposure to ionizing radiation

Ionizing radiation is a carcinogen, albeit at low doses a relatively poor one. The role of radiation in non-cancer effects, i.e., cardiovascular effects, hypertension, stroke and opacities in the lens of the eye [25] as well as central nervous system effects [26], is evolving and subject to intense investigation. At higher doses, radiation induced carcinogenesis is less controversial and estimates of cancer risk suggest a 5% increase in risk per Sv of radiation exposure. The risk for radiation-induced cancer varies as a function of sex, age at exposure, radiation dose and dose rate, the quality of radiation, and a host of genetic, epigenetic and lifestyle factors that characterize the exposed individual [27].

Epidemiological studies suggest a latency period of 4–8 years for leukemia and 15+ years for solid cancers [28]. Obviously many molecular, biochemical, and cellular events occur between the initial radiation exposure and the manifestation of the cancer phenotype. However, much of experimental radiation research has focused on events occurring at much shorter times after exposure; days and weeks, and occasionally months rather than years. These studies include analysis of alterations in gene, protein and metabolome expression, induction of mutations, chromosomal rearrangements, cell cycle alterations, and apoptosis, predominantly in *in vitro* model systems, although there are studies in small and large animal models [29]. Many of these studies have utilized radiation doses greater than 100 mGy. The goals of this review are to discuss how NTE's might impact these well-described initial events associated with radiation exposure and speculate on how they might contribute to long-term health effects.

A central premise is that both targeted and NTE's lead to the activation of inflammatory cytokines [30]. An excellent review on inflammatory cytokines was recently published by Schaue et al. [31]. We hypothesize these cytokines stimulate the innate immune system within the organ/organism, i.e., elicit a stress response, and over time disrupt tissue homeostasis and elicit a plethora of down stream effects, some of which can ultimately result in detrimental health effects. For example, the radiation induced release of cytokines may be the result of the direct action of radiation, or the result of a cascade of reactive radical species induced in targeted and non-targeted cells [32]. Cell populations showing RIGI show persistently elevated levels of reactive oxygen species (ROS) [33,34]. Furthermore, RIGI can be attenuated by ROS scavengers [35]. The persistent elevation in ROS observed in unstable clones appears in part to be mediated by dysfunctional mitochondria [36,37]. Likewise, dysfunctional mitochondria have been implicated in the RIBE [38,39]. This central premise is summarized in Fig. 1.

4. What factors might be involved in communicating non-targeted effects?

As expected from a biological process, there are likely to be multiple pathways to a particular endpoint associated with NTE's. These presumably reflect the experimental system interrogated, the endpoint analyzed and dose and dose rate effects of the radiation exposure. A number of factors are likely to be involved including connexin mediated cell-to-cell gap junction communication [40]; reactive oxygen/nitrogen species [41]; iNOS [42]; and cytokines/chemokines [43]. There are results that suggest that the RIBE and RIGI are at least in part mediated by exosomes, implicating a role for RNA in the propagation of NTE's [44]. Other studies suggest that effects in non-targeted cells might well be modulated by DNA repair capacity [45]; epigenetic factors [46]; and/or mitochondrial dysfunction [36]. All candidate processes have scientific merit and

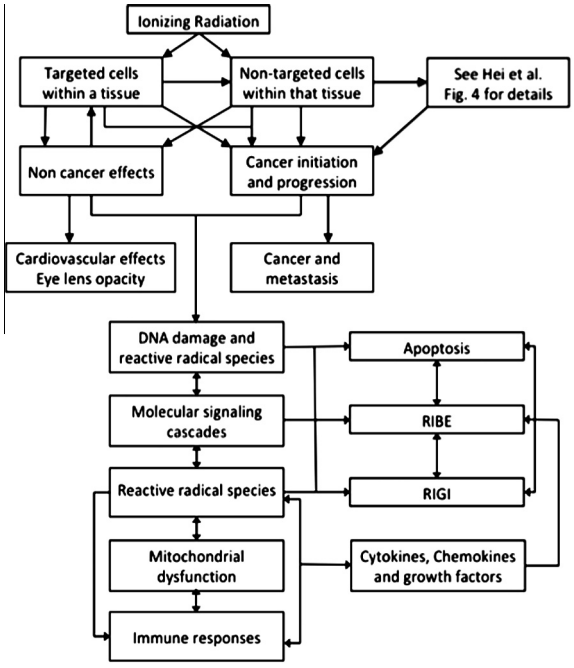

Fig. 1. Schematic proposing the relationship between targeted and NTE's, their potential impact on health outcomes, and the inter- and intra-cellular processes that may drive the manifestation of NTE's in the target tissue.