MINI REVIEW

Effects Of Radiation: The Paradigm Shifts, Adaptive Response And Bystander Models.

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Abstract
Evidence accumulated over the last 50 years shows that radiation also has indirect 'non-target' actions in which effects of radiation on cells or tissues are transported to cells or tissues that were not 'hit' by radiation, leading to changes in their function. Radiation- induced cytotoxic and genotoxic effects by the bystander effect is in contrast to the observations of the adaptive responses, which are generally induced following exposure to low dose, low linear energy transfer radiation and which tend to attenuate radiation – induced damage. In this paper the evidence of the radiation induced bystander effect and adaptive response are discussed. The implications of these non-targeted effects to radiotherapy and radiation protection practices are also highlighted.

Keywords: bystander effects, adaptive response, radiation protection.

Introduction
Los Alamos National Laboratory biologist Bruce Behnert's study of the effects of extra cellular mediators, including proteins, from irradiated human cells on non-irradiated cells has confirmed the existence of bystander effect. Treatment of cells with low doses of radiation results in the release of specific factors outside the cell that seem to be responsible for biological changes in cells not directly exposed to radiation. These are called "bystander effects" (1). The classical dogma of radiation biology, as narrowly interpreted from target theory (2) asserts that genetic damage occurs only during or very shortly after deposition of energy in nuclear DNA (targeted effects), is either due to the direct action of the irradiation or from very short lived oxy-radicals generated by it, and that the course of biological consequences is fixed

within one or two cell generations (3). The major feature of non-targeted effects is that direct nuclear (DNA) exposure is not required for their expression. Much evidence has accumulated that cannot be explained by this dogma. Among these heretical results are 'bystander effects' (BSE), defined as effects elicited in cells that are not directly 'hit' by radiation (4). There are other non-targeted phenomena, including radiation – induced adaptive response and long-lasting alterations in gene expression, transmissible genomic instability (TGI), low-dose radio hypersensitivity (HRS), delayed reproductive death, and radiation-induced long lived radicals (5-7). This present review is on adaptive response and radiation bystander effects.

PARADIGM SHIFT

Non-targeted phenomena have sometimes been referred to as paradigm shifting". As defined by Kuhn (8) a 'paradigm shift' is an intellectually violent revolution in which one conceptual world view is replaced by another, as for example, the shift from Ptolemaic to the Copernican view of the universe. So, while these findings need careful consideration, especially as they may come to affect estimates of risk, they do not constitute a true paradigm shift, especially since much of the heretical evidence existed for a long time (9), so that even this relatively minor shift in the world view has been via evolution rather than revolution (8). Thus, characterization of these results as 'paradigm shifting' is dramatic but seems unwarranted (4).

ADAPTIVE – RESPONSE MODEL

In addition to threshold and non-threshold models of radiation injury, adaptive- response model also exists. This model postulates that certain doses of low-dose radiation may even be beneficial. Typically the adaptive response is induced with **1 – 100mGy of gamma-rays**, doses **100-10,000** times larger than the natural background radiation dose of approximately 0.01 msv/day. This model was first proposed in 1984 to explain the finding that cultures of human lymphocytes growing in low concentrations of radioactive thymidine developed fewer chromosomal aberrations than cultures of no radioactive lymphocytes when both were challenged with high –dose radiation (10).

BYSTANDER EFFECT MODEL AND OCCURRENCE

This postulates that low dose radiation may even be more damaging than that predicted by the linear non-threshold model (which postulates that low-dose radiation is just as harmful per gray as high-dose radiation). Springer (1) noted that subjecting cells to stresses, such as oxidative damage and radiation, induces the release of cell surface proteins by a process of regulated "shedding", or proteolysis. Proteolysis is responsible for the generation of numerous biologically active molecules, such as growth factors and cytokines.

The effect induces a response that could hold the key to the causes of gene instability that underlie cancer, as well as other phenomena such as increases in cell growth that have been observed with low doses of ionizing radiation. Radio-adaptive responses to low dose of radiation provide protection against the cidal effects of subsequent high-dose exposure (11). According to Lehnert, (11) mounting evidence suggests that many important effects of radiation can occur in the absence of direct irradiation of cell nuclei. Results from recent experiments show that at least some cancer-associated effects of ionizing radiation, including the induction of genetic mutations, can occur in cells that have not been directly exposed to radiation. These results have profound implications for assessing cancer risk and other collateral effects of environmental, diagnostic or therapeutic exposure to ionizing radiation.

Recent advances in charged-particle micro-beam technology have provided a means to directly assess the consequences of irradiating cell nuclei as opposed to irradiating extra nuclear regions (11). With these approaches, the nucleus and the cell's body, or cytoplasm, are differentially stained with compounds that fluoresce with different emission spectra when illuminated by ultraviolet light. This allows visualization of the subcellular regions so that sub-

compartments can be preferentially targeted for irradiation by charged particles and the results observed. Such studies have confirmed that the irradiation of parts of cells aside from their nuclei can cause numerous effects. Lehnert and his group obtained evidence that alpha particles like those emitted by radon, radon progeny and plutonium 238 can cause increases in sister chromatid exchanges – an indicator of DNA damage that involves symmetrical transfers of DNA fragments between two chromatids of the same chromosome- in normal human cells without direct nuclear transversals. They also found that these increases were maximally induced over a low-dose range in an "all or none" manner. They concluded that the excessive chromatid exchange response could have been induced by an effect of alpha particles in some region outside the nucleus and theoretically even outside the cell itself.

There is good evidence, at least *in vitro*, that bystander signals can be transferred through medium (12-17) or by physical cell – cell contact, usually via gap junctions (18-26). It seems clear that both modes of transmission exist, at least in vitro and probably *in vivo*. Some evidence indicates that communication via gap junctions may be more common for signals induced by high linear energy transfer (LET) radiation. Radiation induced bystander effects occur *in vivo*. It is known for example, that normal cells can influence growth of neighboring tumour cells, and that tumour cells can in turn, further distort the micro-environment (27-33) to promote growth of other tumour cells. Radiation has been demonstrated to affect these processes both *in vitro* and in vivo (34-40). It seems clear that some signaling occurs with direct cell contact.

BETWEEN BYSTANDER EFFECTS AND ADAPTIVE RESPONSE

Some mechanisms (e.g. Oxidative metabolism) that underlie the bystander effect have also been implicated in the adaptive response to ionizing radiation (IR). In the adaptive response protocol, cells are pre-exposed to a small dose prior to a high dose of ionizing radiation. While the same factors may modulate cell death in both phenomena, the occurrence of pro-survival rather than cytotoxic effect may reflect changes in concentration of the inducing factors (41). However, studies have indicated that the bystander effect and adaptive response are likely to be mediated by distinct mechanisms/ mediating factors. Induction of an adaptive response to low LET Ionizing Radiation protected against bystander damage induced by alpha particles (42). While DNA damage was shown to be unequivocally induced in bystander cells, the adaptive response implicates the involvement of DNA repair and up-regulation of antioxidation resulting in reduced residual DNA damage (41).

IMPACT OF IN VIVO BYSTANDER EFFECTS ON RADIOTHERAPY

Brooks et al (43) have shown that when alpha particle emitters are concentrated in the liver of Chinese hamsters, all cells in the liver are at the same risk for the induction of chromosome damage, even though a small fraction of the total liver cell population were actually exposed to alpha particles. In addition, investigation of genetic effects in partial organ irradiation experiments has demonstrated out-of–field effects(44). With relevance to radiotherapy, a cytotoxic bystander effect produced by tumour cells labeled with 5- (^{125}) iodo – 2^{1} – deoxy – uridine (^{125} IUDR) was recently demonstrated (45).

It was suggested that IR induces the release of cytokines into the circulation, which in turn mediate a systematic anti-tumour effect that may involve up-regulation of immune activity (41). Interestingly, recent *in vivo* mouse experiments have shown that the p53 protein is a mediator of radiation–induced abscopal effect. The secretion of factors capable of inhibitory abscopal/bystander effects when P53 wild–type tumours are irradiated would potentate that effect of radiation on eradicating tumours. The importance of bystander effects to fractionated radiotherapy has been emphasized (47). Growth medium harvested from cultured cells receiving fractionated irradiation resulted in greater cytotoxicity when added to bystander cells than growth medium harvested from cultures receiving a single dose irradiation. If bystander factors were produced *in vivo*, they

may reduce the sparing effect observed in dose fractionation regimen. However, the existence of such factors is likely to be patient, tissue and lifestyle specific (47).

THE BYSTANDER EFFECT AND RADIATION PROTECTION

Distance remains one of the cardinal principles of radiation protection, a principle based on the classical radiation biology dogma; the targeted effect of radiation. The occurrence of a bystander effect in cell population exposed to low fluences of high LET radiation, such as alpha particles, could have an impact on the estimation of risks of such exposure. It suggests that cell populations or tissues respond as a whole to radiation exposure and the response is not restricted to that of the individual traversed cells but involves the non-traversed cells. This would imply that the modeling of dose- response relationships at low mean dose, based on the number of cells hit or even on the type of DNA damage they receive, may not be a valid approach. In light of this, the authors hereby suggest non-targeted studies, including elucidation of the relationship between the bystander effect and propagation of genomic instability. This should contribute to the establishment of adequate environment and occupational radiation protection standards.

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