Targeted Radiotherapy: Is the "Holy Grail" in Sight?

The "Holy Grail" of radiotherapy is to find a treatment or technique that can maximize tumor cell sterilization, minimize normal tissue damage, and be refractive to selection for resistance. It is too early to tell whether targeted radiotherapy using radiopharmaceuticals will fulfill its promises but the work of Boyd et al. (*1*), which is so technically elegant and biologically sound, leads one to hope. Their article shows that potent toxins are produced by the tumor cells that have concentrated radiohalogenated metaiodobenzylguanidine (MIBG). The toxins appear to be distinct from those elicited by conventional radiotherapy, and toxicity increases with increasing dose. They describe this as a bystander effect of targeted radiotherapy.

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"Bystander effects" mean different things to different people; the term was commonly associated with gene therapy, where it meant the amplification of cell kill resulting from the spread of toxic metabolites through gap junctions, from cells transfected with suicide genes, to cells in the vicinity (*2*). In the old literature, bystander effects were reported as early as 1922 (*3*), where it was reported that serum from irradiated animals stimulated growth of lymphoid cells in vitro, whereas serum from controls caused rapid disintegration. Several articles in the 1950s and 1960s reported a variety of stimulatory and inhibitory effects of serum-irradiated patients, bomb survivors, or accident victims as well as experimental animals (*4*). These effects were variously referred to as "indirect," "clastogenic," or, occasionally "abscopal" effects, but were generally regarded as oddities. A useful modern review of abscopal effects can be found in the report by Kaminski et al. (*5*).

More recently, bystander effects have excited considerable interest in the field of low-dose radiobiology, where they are suspected of amplifying effects of radiation at doses where not every cell could receive a radiation hit. Here bystander effects refer to the detection of radiation-like effects in unhit cells. Effects include induction of mutations, gene expression, neoplastic transformation, chromosomal instability, apoptosis, and delayed cell death (*6*). Doses needed to induce bystander effects are very low (anything above 3 mGy). As these doses are environmentally relevant doses and also doses of concern in diagnostic imaging and intensity-modulated radiotherapy, the mechanisms underlying bystander effects are the subject of intense investigation. Very recently, several articles have appeared exploiting bystander effects to increase tumor cell kill. These studies have shown that indirect effects of ionizing radiation may contribute significantly to the effectiveness of radiotherapy by sterilizing malignant cells that are not directly hit by the radiation. Reports by Kassis (*7*), Bodei et al. (*8*), Marples et al. (*9*), Mothersill et al. (*10*), Mothersill and Seymour (*11*), and Trott (*12*) define or discuss the importance of bystander effects in vivo in clinical situations; however, there have been few investigations of the importance of indirect effects in targeted radionuclide treatment. This makes the article by Boyd et al. (*1*) both a landmark and a unifying study as it brings together the original gene therapy bystander concept and the radiobiologic concept. Because induction of bystander effects is prevalent at low radiation dose and low dose rate, the technique is especially interesting as these are features of targeted radionuclide treatment of cancer. The key previous papers in this area include several by the authors (*13–18*).

They have optimized several aspects of targeted radiotherapy/gene therapy strategies to achieve tumor-specific transcriptional regulation of therapeutic genes and to maximize collateral cell damage via cross-fire irradiation, thereby overcoming the problem of heterogeneity of transgene expression. The efficacy of these ploys has been demonstrated in their unique transfectant mosaic spheroid model. Recognizing that, in addition to the physical bystander effect (cross-fire), there is a more subtle biologic bystander effect associated with targeted radionuclide therapy, they embarked on a study of the characterization of this phenomenon.

The current article (*1*) employs an adaptation of the media transfer procedure developed by Mothersill and Seymour (*19*) to compare the induction of bystander effects by external beam γ-radiation with those generated by MIBG labeled with radionuclides emitting β-particles, α-particles, or Auger electrons. This is a good example of the use of gene transfection to construct a therapy model, inasmuch as it allowed the creation of an excellent control—that is, non-(noradrenaline transporter [NAT] gene) transfected

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cells that were incapable of active uptake of I-radiolabeled MIBG.

In this article, Boyd et al. (*1*) refrained from commenting on the relationship between absorbed dose to the cell and radioactivity concentration. To do so, they would need more complete information concerning uptake and washout dynamics and transfer constants between media, cell surface, and intracellular and nuclear compartments. Instead, the investigators estimated effective dose by comparing the clonogenic cell kill achieved by external beam radiation or radiopharmaceutical treatment. This indicated that intracellularly accumulated radionuclides powerfully stimulated the production of bystander effects. Active cellular accumulation was necessary for the induction of bystander effects. Those cells that had not been transfected with the NAT gene produced no toxin.

Cells exposed to media derived from external beam–irradiated cells produced a dose-dependent reduction in survival fraction, at low dosage, followed by a plateau with respect to clonogenic cell kill at levels of >2 Gy. In contrast, cells receiving media from cultures treated with meta-^{211}At-astatobenzylguanidine (^{211}At-MABG) or ^{123}I-MIBG exhibited dose-dependent toxicity at low dose but elimination of cytotoxicity with increasing radiation dose. Cells treated with media from ^{131}I-MIBG demonstrated a dose–response relationship with respect to cell death and no annihilation of this effect at high radiopharmaceutical dosage. These findings suggested that bystander effect mechanisms after radiopharmaceutical administration may be dependent on linear energy transfer and distinct from those elicited by conventional radiotherapy.

One of the major questions remaining in the bystander field is the nature of the "factor" molecules. Much concerted research has failed to identify these, suggesting they may be complex or that multiple steps may be involved. The identification of bystander factors will stimulate the design of strategies to maximize damage to tumor cells while minimizing damage to normal cells and should provide a whole new range of targets for novel drugs, including radiopharmaceuticals. However, from the practical point of view, as this article demonstrates so effectively, it is not necessary to understand why, what, or how to exploit bystander effects for therapy.

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