



# Bystander Effect Produced by Radiolabeled Tumor Cells *in vivo*

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## ABSTRACT

The bystander effect, originating from cells irradiated *in vitro*, describes the biologic response(s) of surrounding cells not directly targeted by the radiation insult. To overcome the limitations of *in-vitro* tissue culture models and determine whether a bystander effect that is initiated by radionuclide decay can be demonstrated *in vivo*, the ability of 5-[<sup>125</sup>I]iodo-2'-deoxyuridine (<sup>125</sup>IUdR)-labeled tumor cells to exert a damaging effect on neighboring unlabeled tumor cells growing subcutaneously in nude mice has been investigated. When mice are injected with a mixture of human colon LS174T adenocarcinoma cells and LS174T cells prelabeled with lethal doses of DNA-incorporated <sup>125</sup>I, a distinct inhibitory effect on the growth of subcutaneous tumor (derived from unlabeled cells) is observed. Since (i) the <sup>125</sup>I present within the cells is DNA-bound, (ii) ~99% of the electrons emitted by the decaying <sup>125</sup>I atoms have a subcellular range (<0.5 μm), and (iii) the overall radiation dose deposited by radiolabeled cells in the unlabeled cells within the growing tumor is <10 cGy, we conclude that the results obtained are a consequence of a bystander effect that seems to be generated *in vivo* by factor(s) present within and/or released from the <sup>125</sup>IUdR-labeled cells. These *in-vivo* findings significantly impact the current dogma for assessing the therapeutic potential of internally administered radionuclides. They also call for a re-evaluation of the approaches currently used for estimating the risks to populations inadvertently exposed internally to radioactivity as well as to patients undergoing routine diagnostic nuclear medical procedures.

## INTRODUCTION

It has long been accepted that radiation-induced biologic effects, such as decreased cell survival, increased levels of sister chromatid exchanges, mutations, and micronuclei formation, changes in gene expression, and oncogenic transformation, occur only as a consequence of direct/indirect ionization and damage to the intranuclear DNA of mammalian cells. However, recent *in-vitro* studies have provided strong evidence that unirradiated cells react to signals produced by irradiated cells. If such a phenomenon were to occur *in vivo*, it would greatly impact our current understanding of (i) the therapeutic potential of radionuclides used in targeted therapy, and (ii) risk assessment following the administration of radiopharmaceuticals to patients and the inadvertent exposure of the population as a whole to radioactivity. This presentation describes an approach demonstrating that (i) the radiation-induced bystander effect is a phenomenon that can be demonstrated *in vivo*, and (ii) the decay of the DNA-incorporated Auger electron emitter <sup>125</sup>I leads to substantial bystander effect.

## MATERIALS AND METHODS

### *In-Vitro and In-Vivo Studies*

**Cell Labeling with <sup>125</sup>I and Tumor Growth Determinations.** LS174T cells, an adenocarcinoma developed from human colon cancer, were radiolabeled with <sup>125</sup>I by incubating logarithmically growing cells with the thymidine analog <sup>125</sup>IUdR (3–3.5 μCi/ml, 48 h, 37°C). The cells were washed, counted, their radioactive DNA-incorporated <sup>125</sup>I activity determined, mixed with unlabeled cells, and injected subcutaneously (s.c.) into male NCr nude mice. Tumor growth was assessed using calipers.

**Externally Irradiated Cell Preparations.** Cells were exposed to various doses of external gamma radiation with a Gammacell 220 cobalt irradiator (15 cGy/sec) and injected s.c. into mice in the presence/absence of unirradiated tumor cells.

### DOSIMETRY

**Calculation of Cumulated Dose from a Uniformly Distributed Radionuclide in the LS174T Growing Tumor.** The calculation of the average dose (cGy) to a mass of tissue in which a gamma-emitting radiopharmaceutical resides for a period of time is generally carried out using the MIRD formalism (1). In this approach, we need to know only two parameters: (i) the absorbed dose per unit cumulated activity (cGy/μCi•h) for the radionuclide in question, known as the S value, which has been calculated and tabulated for various tissue masses (1, 2), and (ii) the experimentally determined radioactivity-time curve from which the cumulative dose (μCi•h) is calculated. In MIRD, the volume of the organ/tissue is assumed to be constant over time. In the current work, however, LS174T tumor cells injected subcutaneously into mice grew into sizeable tumors. Under these circumstances, the radioactivity and the S value both change due to an additional factor, the time-dependent change of tumor size. Consequently, dose calculations for a growing tumor must take into account the effective half-life(s) with which the intratumoral radioactivity (TR) and the S value (TS) decline over the observation period. It is important to note that we had previously shown that when the ratio of radiolabeled cells to unlabeled cells in a cluster is <1:10, i.e., as in the current experiments, the *same* absorbed dose is obtained whether classical MIRD or microdosimetric methods are used (3-6).

Using the equation below, we have calculated the doses absorbed by the tumors shown in Figure 1 where 1.0 x 10<sup>6</sup>, 0.2 x 10<sup>6</sup>, and 0.1 x 10<sup>6</sup> <sup>125</sup>IUdR-labeled cells containing 0.19 pCi/cell were mixed with 1.0 x 10<sup>6</sup> unlabeled cells. The diameter of the LS174T cell, measured in histology sections from 14-d tumors, is 28 μm. Briefly, S values for calculating self-absorbed doses to spheres of varying diameters that contain uniformly distributed <sup>125</sup>I radioactivity (2) were plotted versus tumor diameter, the data points were fitted (linear regression), and the parameters of the equation were used to determine the S values (cGy/decay) that correlate with the tumor diameters after each doubling in volume. We then plotted these S values as a function of the doubling times (d.t.) for each tumor growth curve (Fig. 1), i.e., after 1 x d.t., 2 x d.t., 3 x d.t., etc., fitted the data points (linear regression), and used the equation parameters to obtain the T1/2 of the S values (i.e. ts).

$$D_T = \text{total dose in rad} = D_{d0-1} + D_{d1-15}$$

$$\left[ \frac{[S_{d0} \cdot R_{d0}]}{0.693 \cdot \left( \frac{1}{T_{(d0-1)}} \right)} \right] \cdot \left[ 1 - e^{-0.693 \cdot t \cdot \left( \frac{1}{T_{(d0-1)}} \right)} \right] + \left[ \frac{[S_{d1} \cdot R_{d1}]}{0.693 \cdot \left( \frac{1}{T_{(d1-15)}} \right)} \right] \cdot \left[ 1 - e^{-0.693 \cdot t \cdot \left( \frac{1}{T_{(d1-15)}} \right)} \right]$$

where D<sub>d0-1</sub> is the tumor dose cumulated in the first day and D<sub>d1-15</sub> is the tumor dose cumulated from day 1 to day 15, S<sub>d0</sub> is the S value on day zero (i.e. when the tumor is composed of the injected tumor cells), S<sub>d1</sub> is the S value on day 1, R<sub>d0</sub> is the initial radioactive content of the tumor at day zero, R<sub>d1</sub> is the activity remaining in the tumor after the first day (Fig. 4), and T<sub>(d0-1)</sub> and T<sub>(d1-15)</sub>, which are the overall half-lives for days 0-1 and days 1-14, respectively, are defined as:

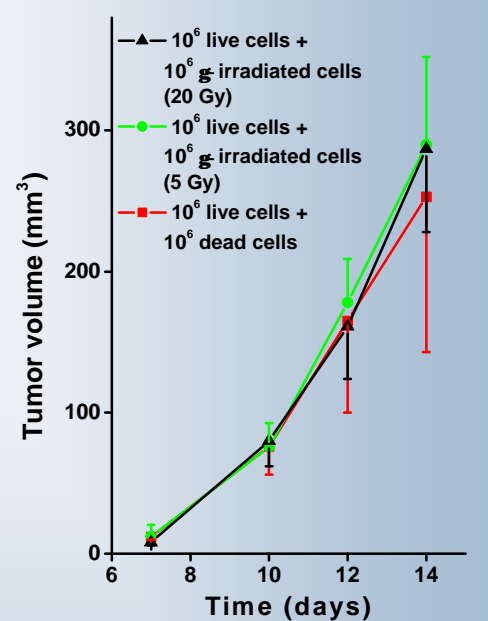
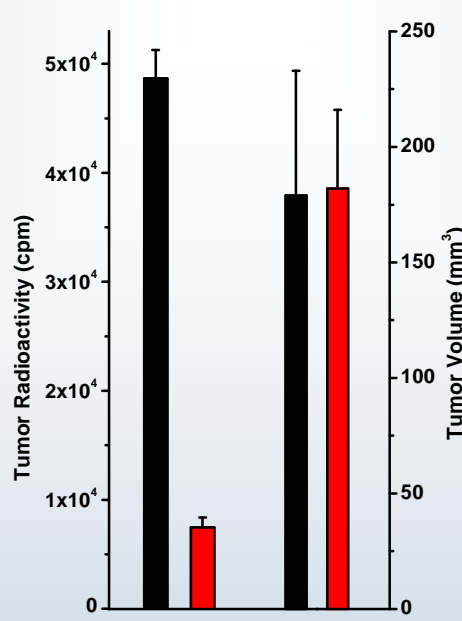
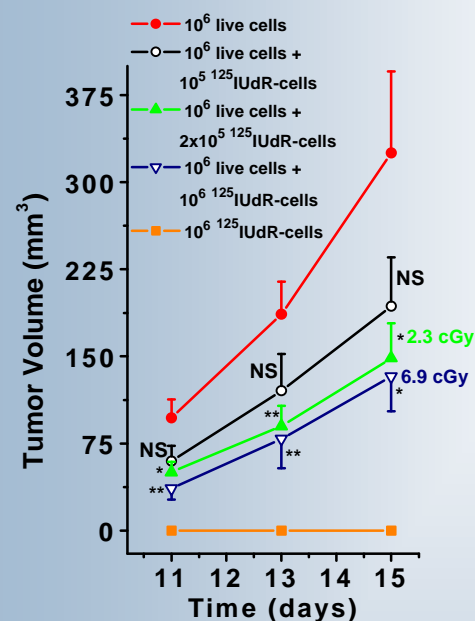
$$\frac{1}{T_{(d0-1)}} = \frac{1}{T_{S(d0-1)}} + \frac{1}{T_{R(d0-1)}}$$

and

$$\frac{1}{T_{(d1-15)}} = \frac{1}{T_{S(d1-15)}} + \frac{1}{T_{R(d1-15)}}$$

where TS<sub>(d0-1)</sub> and TS<sub>(d1-15)</sub> are the effective half-lives with which the S value declines over day 0-1 and day 1-15 (derived from a fit as described above), and TR<sub>(d0-1)</sub> and TR<sub>(d1-15)</sub> are the effective half-lives derived from the  $\lambda$  and  $\lambda$  fits of the data in Fig. 4 (0.65 d and 3.85 d, respectively).

## RESULTS



**Patterns of *in-vivo* <sup>125</sup>IUdR-labeled-cell-induced bystander effect.** Five groups of nude mice were injected subcutaneously with 1 x 10<sup>6</sup> live LS174T cells only (●), 0.1 x 10<sup>6</sup> <sup>125</sup>IUdR-labeled cells + 1.0 x 10<sup>6</sup> live cells (○), 0.2 x 10<sup>6</sup> <sup>125</sup>IUdR-labeled cells + 1.0 x 10<sup>6</sup> live cells (▲), 1.0 x 10<sup>6</sup> <sup>125</sup>IUdR-labeled cells + 1.0 x 10<sup>6</sup> live cells (▽), or 1.0 x 10<sup>6</sup> <sup>125</sup>IUdR-labeled cells only (■). Tumor volumes were assessed and the data plotted as mean ± s.e.m (n = 10/group). At each time point, the differences between the mean tumor volumes for each experimental group and those for the controls were determined using the Student's t-test. NS, not significant; \*, P<0.05; \*\*, P<0.01

**Relationship of tumor-associated radioactivity and tumor size in bystander study.** Two groups of nude mice were injected subcutaneously with 1.0 x 10<sup>6</sup> <sup>125</sup>IUdR-labeled cells + 1.0 x 10<sup>6</sup> live LS174T cells (■) or 0.2 x 10<sup>6</sup> <sup>125</sup>IUdR-labeled cells + 1.0 x 10<sup>6</sup> live cells (●). Tumor radioactivity and tumor volume were determined 15 days after the injection and the data were plotted as mean ± s.e.m. (n = 5/group). Left bars represent radioactivity and right bars time values.

***In-vivo* growth rate of LS174T tumor cells exposed to low-dose gamma radiation.** Tumor-cell suspensions of 1.0 x 10<sup>6</sup> cells/0.1 ml were exposed to 0 (■), 50 (▲), 100 (●), or 200 (▽) cGy of gamma radiation and immediately injected subcutaneously into nude mice. Tumor volumes were assessed and the data were plotted as mean ± s.e.m. (n = 10/group).

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## CONCLUSIONS

1. The decay of the Auger electron emitter <sup>125</sup>I within DNA
  - kills/retards the *in-vivo* growth of subcutaneous tumors
  - kills/retards the *in-vivo* growth of adjacent tumor cells
  - leads to a bystander effect *in vivo*
2. The <sup>125</sup>I-induced bystander effect seems to be generated *in vivo* by factor(s) present within and/or released from the <sup>125</sup>IUdR-labeled cells
3. There is a need to re-examine the current dogma for assessing the therapeutic potential of the Auger electron emitter <sup>125</sup>I and possibly other internally administered radionuclides
4. There is a need to re-evaluate the approaches currently used for estimating the risks to populations inadvertently exposed internally to radioactivity as well as to patients undergoing routine diagnostic nuclear medical procedures.