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Comparative *In Vitro* Microdosimetric Study of Murine- and Human-Derived Cancer Cells Exposed to Alpha Particles

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Diffusing alpha-emitter radiation therapy (DaRT) is a proposed new form of brachytherapy using α particles to treat solid tumors. The method relies on implantable ^{224}Ra-loaded sources that continually release short-lived α-particle-emitting atoms that spread inside the tumor over a few millimeters. This treatment was demonstrated to have a significant effect on tumor growth in murine and human-derived models, but the degree of tumor response varied across cell lines. Tumor response was found to correlate with the degree of radionuclide spread inside the tumor. In this work we examined the radiosensitivity of individual cells to determine its relationship to tumor response. Cells were irradiated *in vitro* by α particles using a ^{228}Th irradiator, with the mean lethal dose, D_0, estimated from survival curves generated by standard methods. The results were further analyzed by microdosimetric tools to calculate z_0, the specific energy resulting in a survival probability of 1/e for a single cell, which is considered to better represent the intrinsic radiosensitivity of individual cells. The results of the study demonstrate that, as a rule, tumors that respond more favorably to the DaRT treatment are also characterized by higher intrinsic cellular radiosensitivities, with D_0 ranging from 0.7 Gy to 1.5 Gy for the extreme cases and z_0 following the same trend.

INTRODUCTION

The potential benefits of using α particles for the treatment of cancer are widely recognized (*1–9*). Due to their short range in tissue (<100 μm), the use of α particles may allow the delivery of therapeutic doses to the targeted cancer cells with minimal irradiation of surrounding normal tissue. Their high linear energy transfer (LET) further makes their biological effect largely insensitive to the oxygenation state of the irradiated cells, and – compared to low-LET radiation – much less sensitive to the cell position in the cell cycle (*10*).

Cell survival studies have shown that only a few α-particle tracks across the cell nucleus are typically required for reproductive cell death (*11–15*). The mean number of hits in the cell nucleus, required to induce a lethal lesion, is minimum for α particles having LET of 100–200 keV/μm (*11*). The survival probability of irradiated cells decreases exponentially as a function of the number of nuclear traversals (or the specific energy deposited in the nucleus), whereas particle hits in the cytoplasm do not significantly affect cell survival (*13, 15*).

In previous reports we have suggested a new form of α-particle-based radiation therapy modality for solid tumors using intratumoral sources impregnated with ^{224}Ra atoms. The treatment is termed DaRT*:* Diffusing Alpha-emitters Radiation Therapy (*16–21*). As ^{224}Ra decays, it releases a series of short-lived daughter atoms into the tumor (by recoil) that spread inside the tumor, producing a lethal-dose region through their subsequent α-particle decays. *In vivo* studies showed that this process leads to extensive tissue damage over a region measuring several millimeters in diameter around each source, resulting in significant tumor growth retardation and prolongation of survival for animals bearing squamous cell, lung (*16–20*), pancreatic (*21*), colon and prostate (unpublished results)-derived tumors.

Even though all of the examined tumor types responded to the DaRT treatment, the degree of response varied across cell lines. Squamous cell carcinoma (SCC) tumors (derived from the mouse SQ2 and human FaDu cell lines) as well as lung carcinomas (derived from the human A427 cell line) showed dramatic responses to the insertion of one or two DaRT sources into 6–7-mm-diameter tumors, including complete regression with no recurrence in a considerable number of

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cases (*18, 20*). Lung carcinomas derived from the mouse LL2 cell line showed significant tumor growth arrest when treated at a similar size, with a reduction of more than 40% in tumor volume (21 days post-treatment with a single DaRT wire). On the other hand, lung carcinomas derived from the human NCIH520 cell line were less sensitive to the treatment than tumors derived from the LL2 and A427 cell lines (*20*). DaRT wires were also less effective against pancreatic tumors (derived from the mouse Panc02 cell line) and colon carcinoma (derived from the mouse CT26 cell line). For these cell lines only small tumors of about 4 mm in diameter were significantly affected by one radioactive wire (*21*).

Tumor response to the treatment was found to correlate with the degree of radionuclide spread inside the tumors. Autoradiography of treated tumors demonstrated that for mouse SCC tumors, the typical size of the high-dose (>10 Gy) region is 5–6 mm (*16*), whereas in lung carcinoma the same dose level is obtained over a region of 4–5 mm (*20*) and in mouse pancreatic tumors over a region measuring 2–4 mm (*21*).

In this work we addressed the question whether tumor response also correlates with the radiosensitivity of the individual cells themselves. The sensitivity of different cancer cell lines (mouse and human) to α particles was studied after an *in vitro* exposure to a ^{228}Th irradiator, determining the value of the mean lethal dose, D_0, from cell survival curves generated using a standard clonogenic assay. The data were further analyzed in microdosimetric terms, calculating, in particular, the cell sensitivity parameter z_0 (the specific energy resulting in a survival probability of 1/e for a single cell).

MATERIALS AND METHODS

Tumor Cell Lines

The murine colon carcinoma (CT26) and the human lung squamous cell carcinoma (NCIH520) cells (ATCC, Manassas, VA) were grown in RPMI-1640 medium (Gibco, Israel). The human pharynx squamous cell carcinoma (SCC) (FaDu, ATCC) cells were grown in EMEM (Biological Industries, Israel). Murine squamous cell carcinoma (SQ2, kindly provided by Dr. Gad Lavie from the Sheba medical center, Tel-Hashomer, Israel), human pancreatic carcinoma (Panc1, ATCC), and murine pancreatic carcinoma (Panc02, kindly provided by Dr. M. A. Hollingsworth, UNMC Omaha) were grown in DMEM (Gibco, Israel). All media were supplemented with 10% fetal calf serum, sodium pyruvate (1 m*M*), nonessential amino acids (1%), L-glutamine (2 m*M*), penicillin (100 U/ml), and streptomycin (100 μg/ml) (all obtained from Biological Industries, Israel). The cell lines were grown at 37°C in a 95% air/5% CO_2 humidified incubator.

Alpha-Particle In Vitro Survival Assay

The *in vitro* effect of α particles on the various cell lines was studied in a clonogenic assay, testing the ability of a single cell to grow into a colony (*22*). The irradiator used in this work (Fig. 1) consisted of a small deposit of ^{228}Th (half-life 1.91 years) in secular equilibrium with its daughters (^{224}Ra, ^{220}Rn, ^{216}Po, ^{212}Pb, ^{212}Bi, ^{212}Po, ^{208}Tl) on a silicon substrate. The surface was coated with a 4.0-μm layer of silver to prevent the release of the recoiling daughters of ^{228}Th. The irradiation setup comprised a 7.5-μm-thick Kapton (polyimide) foil (Dupont, Luxembourg) held between two cylindrical stainless steel parts, forming a well with an inner diameter of 9 mm (*19*). Cells were seeded on the Kapton foil at a density of 2×10^4 to 5×10^4 cells per well, depending on the cell line under study, and subsequently incubated for 24 h to reach a confluent monolayer. The Kapton wells were then positioned 10 mm above the ^{228}Th irradiator and exposed to α particles (0–3.5 Gy) at an average dose rate of approximately 0.5 Gy/min. In a given experiment, exposure at each dose level was repeated in 4–5 wells. The dose rate was estimated by a measurement of the total flux of α particles crossing the Kapton foil using a solid state α-particle detector and a Monte Carlo simulation of the passage of α particles through the foil using the SRIM code (*23*). The energy of the α particles crossing the foil was found to lie in a broad range, with 87% of the particles having energy from 0.1 to 3.8 MeV and the rest concentrated in a peak at 6.2 ± 0.1 MeV. Accordingly, 87% of the particles crossing the foil had LET in the range 110–240 keV/μm and the rest were concentrated around a peak at 78 ± 2 keV/μm. The mean LET for the entire α-particle flux was 155 keV/μm and the standard deviation was 48 keV/μm. Dose-rate variations across the foil were of the order of 10%, dropping gradually with radial distance from the center.

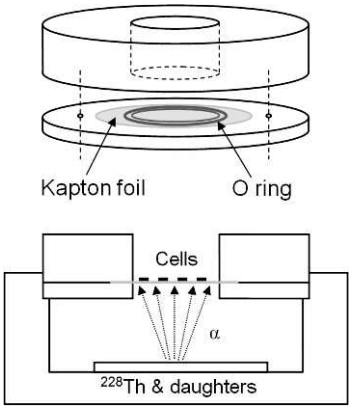

FIG. 1. Schematic drawing of the α-particle *in vitro* irradiation setup. The setup consists of a stainless steel well (top) in which cells are seeded on a thin Kapton foil. The well is then positioned on top of the α-particle irradiator (bottom). Alpha particles emitted from a sealed ^{228}Th surface source pass through the Kapton foil, irradiating the cells.

Immediately after irradiation, the cells were harvested and sparsely seeded in 10-cm culture dishes (Corning, Corning, NY), to allow the formation of well-separated colonies. After a 6- to 13-day incubation period, the colonies were fixed in methanol and stained with Hemacolor (Merck, Darmstadt, Germany) (*24*). For each cell line, the incubation period was chosen to allow for a clear distinction between sustainable and abortive colonies (which usually do not grow beyond 30 to 40 cells) while avoiding overlapping of adjacent colonies. The colonies were counted manually and were considered viable when they contained more than 50 cells. To generate cell survival curves, the surviving fraction was calculated as the ratio between the number of viable colonies in a given petri dish (containing irradiated cells) and the average number of colonies in the control dishes. As is commonly done for high-LET radiation over the dose range we studied (0–3.5 Gy), the data in each experiment were fitted with the function $S(D) = \exp(-D/D_0)$ in a weighted least-squares procedure to estimate the D_0.

In Vitro Survival Assay of FaDu and Panc1 Cells Exposed to X Rays

FaDu and Panc1 cell were also irradiated with X rays. The choice of these two particular cell lines was driven by the following considerations: (1) both are human-derived cells, which would make the results more relevant for possible clinical use, and (2) SCC and

pancreatic cells represent opposite extremes in terms of their *in vivo* response to DaRT. As before, the cells were seeded in the Kapton wells and were incubated for 24 h. However, to avoid secondary emission of X rays from the stainless steel parts of the Kapton wells, the wells were disassembled before the irradiation and each Kapton foil was kept in a 35-mm-diameter petri dish (Corning) containing 1 ml of medium. The dishes were then positioned inside an X-ray system (Philips MG165, Philips Industrial X-Ray GmbH, Hamburg, Germany) for exposures of 0–7 Gy at a dose rate of 0.72 Gy/min. Each exposure level was repeated using three different Kapton foils. The following steps were similar to those for α-particle irradiation.

Confocal Measurement of Nuclear Size

Confocal microscopy was used to determine the cross-sectional area and thickness of the nuclei of the cell lines under study to allow for microdosimetric analysis (the thickness measurement served to verify that the cells are indeed disc-shaped). Cells ($2.5–5 \times 10^4$) were seeded on cover slides in 12-well microplates (Guangzhou Jet Bio-Filtration Products, Co., Ltd. GETDD, Guangzhou, China) and incubated at 37°C in a 95% air/5% CO_2 humidified incubator. After 24 h of incubation, cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) for 15 min at room temperature. The cells were then washed three times with PBS and incubated with 0.2% Triton X-100 in PBS for 10 min, followed by three washes with PBS. Whole cell labeling was carried out with 5 mg/ml FITC (fluorescein isothiocyanate, Sigma, St. Louis, MO) in 0.1 *M* sodium bicarbonate buffer, pH 9, for 1 h at room temperature followed by three washes with PBS. Nuclear labeling was carried out with 5 μg/ml DAPI (4′,6-diamidino-2-phenylindole, Sigma) for 10 min at room temperature, followed by three washes with PBS and mounting (Golden Bridge International, Inc., Mulkiteok, WA). Slides were analyzed with a Leica sp5 confocal microscope. For each cell line, the cross-sectional area of at least 75 cells was measured using ImageJ (*25*).

Microdosimetric Analysis

1. z_0 estimates. Cells exposed to α particles are subject to a stochastic number of α-particle traversals through their nuclei. Individual cells are subject to nuclear doses that may be considerably larger or smaller than the average macroscopic dose, including, in particular, cells that receive no dose at all. The stochastic nature of dose deposition in cells exposed to α particles and the resulting survival probability for the entire cell population can be addressed by considering the probability distribution for the specific energy z deposited in cell nuclei and the intrinsic sensitivity z_0 of the irradiated cells (*26–31*). The survival probability of a cell subject to a nuclear dose z is generally assumed to be $\exp(-z/z_0)$. Thus z_0 is the specific energy required to reduce the survival probability of an individual cell to 1/e.

Estimates for z_0 were obtained based on the measured value of the mean lethal dose D_0, the measured nuclear cross section, and the known properties of the particular α-particle irradiation setup used in our experiments. We began with a simplified approach, in which spatial variations in the average number of α-particle traversals and in the dose deposited in the nucleus by individual passages across the irradiated surface were neglected. We then checked the validity of this approximation using a complete Monte Carlo calculation that accounted for these local variations.

Our approximate calculation follows Charlton and Sephton's approach for microdosimetry of cell monolayers (*26*), with slightly different nomenclature. The average specific energy deposited in the nucleus per passage, $\bar{z}_1$ (Gy), to a disc-shaped cell nucleus (of uniform thickness and a nuclear density of 1 g/cm^3) was calculated by

$$\bar{z}_1 = \frac{0.16}{A}\left\langle \frac{\mathrm{LET}}{\cos\theta} \right\rangle, \tag{1}$$

where A is the average cross-sectional area of the cell nucleus (μm^2), LET is the linear energy transfer (keV/μm), θ is the angle between the particle momentum and the normal to the surface, and 0.16 is the appropriate conversion to Gy. The average $\langle \mathrm{LET}/\cos\theta \rangle$ was taken from a Monte Carlo calculation of the irradiation setup. Note that in our case variations in cosθ are limited to ~0.87–1.0 and thus do not play a major role in the calculation.

The number of passages through the nucleus is assumed to be Poisson distributed (as the typical number of α-particle traversals in a given nucleus is small and the arrival times of consecutive hits are uncorrelated). The total population of cells N is the sum of subpopulations N_k, where N_k is the number of cells whose nuclei experience exactly k α-particle passages. On average, a nucleus subject to k passages receives a specific energy $z = k\bar{z}_1$. The average number of passages through the nucleus is $\bar{n} = D/\bar{z}_1$, where D is the average dose (i.e., the macroscopic absorbed dose – the average energy deposited per unit mass in the medium, regardless of its cellular structure). Assuming that the survival of an individual cell, subject to a specific dose z to the nucleus, is $\exp(-z/z_0)$, the total number of surviving cells [out of an initial population of $N(0)$] is

$$\begin{aligned} N(D) &= \sum_{k=0}^{\infty} N_k e^{-k\bar{z}_1/z_0} = N(0)\sum_{k=0}^{\infty}\frac{\bar{n}^k e^{-\bar{n}}}{k!}e^{-k\bar{z}_1/z_0} \\ &= N(0)e^{-\bar{n}}\sum_{k=0}^{\infty}\frac{1}{k!}\left(\bar{n}e^{-\bar{z}_1/z_0}\right)^k = N(0)e^{-\bar{n}}\exp\left(\bar{n}e^{-\bar{z}_1/z}\right) \\ &= N(0)\exp\left(-\bar{n}\cdot\left(1-e^{-\bar{z}_1/z}\right)\right). \end{aligned} \tag{2}$$

Comparing this to the observed survival, $N(D) = N(0)\exp(-D/D_0)$ (where D_0 is the mean lethal dose), one obtains

$$z_0 = -\frac{\bar{z}_1}{\ln(1-\bar{z}_1/D_0)}. \tag{3}$$

Note that the above derivation is identical to that of Charlton and Sephton (*26*), with the replacement of the dose per passage d in their work by the average specific energy per passage $\bar{z}_1$, which takes into account the distribution of values for the LET and incidence angle (Charlton and Sephton assumed a perpendicular flux of monoenergetic particles). Note further that if $\bar{z}_1/D_0 << D_0$, i.e., the dose deposited by a single α particle in the nucleus is small compared to the mean lethal dose and many traversals are required to inactivate the cell, the discreteness of the process disappears and $z_0 \approx D_0$. For intermediate (small) values of $\bar{z}_1/D_0$, Taylor expansion of Eq. (3) to first order gives the period $z_0 \approx D_0 - \bar{z}_1/2 \approx D_0 - 0.08\langle \mathrm{LET}/\cos\theta \rangle/A$.

The above calculation implicitly assumes that variations in $\bar{n}$ and $\bar{z}_1$ across the irradiated surface are sufficiently small to allow for using spatially lumped averages for both. To check the validity of this assumption, a refined Monte Carlo calculation was employed that incorporated spatial variations in the average number of α-particle traversals, LET spectrum and incidence angle across the irradiated surface. The SRIM code was used to follow the trajectories of a large number of α particles emitted isotropically from the ^{228}Th source (assumed to be point-like) in a cone with a sufficiently large opening angle to generously cover the entire area of the seeded Kapton foil. The particles were followed through all layers composing the irradiator setup: 4 μm of silver, 10 mm of air and 7.5 μm of Kapton. The particles crossing the Kapton surface were then divided into 10 groups of equal size, based on the radial distance of their crossing point from the irradiator axis. This then defined a series of 10 concentric rings, each assigned an equal number of crossing particles. For each ring, a histogram of LET/cosθ was built and normalized to yield the corresponding probability distribution function. The number of α particles crossing each ring was then normalized such that the average macroscopic dose across the entire Kapton surface was D_0. The average number of particles crossing a nucleus, $\bar{n}$, in a given ring was calculated by multiplying the total number of particles crossing the ring by the ratio of nuclear and ring areas. Each ring was assigned

10^4 cells, which were divided into subpopulations defined by the number of α-particle hits to the nucleus – assumed to be Poisson distributed with a separate value of $\bar{n}$ for each ring. A cell subject to k hits was assigned a specific energy z consisting of k random values drawn from the ring-dependent probability distribution function for LET/cosθ. Scanning a suitable range of values for z_0 (based on the results obtained in the simplified approach of Eqs. 1–3), the survival probability was calculated for each cell as $\exp(-z/z_0)$ and the total survival probability for the entire population was calculated as the average of individual cell survival. Since the macroscopic dose was assumed to be D_0, the corresponding value of z_0 was the one for which the total surviving fraction equaled 1/e.

2. In vivo LET estimates. Cells irradiated *in vitro* are subjected to α particles, which follow a statistical distribution of LET values characteristic of the particular irradiation setup in use. As noted above, in this study the ^{228}Th irradiator delivered a broad spectrum of α particles with an LET of 155 ± 48 keV/μm. A separate simple microdosimetry calculation was performed to estimate the relevance of this to *in vivo* irradiation of cells subjected to an actual DaRT treatment. The calculation was done for spherical nuclei, assuming for simplicity that they are subjected to an isotropic flux of α particles emitted by atoms that are evenly distributed in the vicinity of the cell. The α-particle emitters considered were the progeny atoms of ^{224}Ra, namely ^{220}Rn (with an α-particle energy of 6.29 MeV), ^{216}Po (6.78 MeV), ^{212}Bi (mostly 6.05 and 6.09 MeV), and ^{212}Po (8.79 MeV), but not by ^{224}Ra itself (as radium stays fixed to the source). Using tabulated data for dE/dX values for α particles in water, the distribution of dE/dX values for particles hitting the nucleus was calculated (assuming the particles follow straight lines with no straggling).

RESULTS

Survival Curves of Cells Exposed to α Particles

The results of irradiation experiments performed on SQ2, FaDu, Panc02 Panc1, CT26 and NCIH520[1] cells using the ^{228}Th irradiator are shown in Fig. 2. In each experiment, the dose rate was recalculated to account for the radioactive decay of the ^{228}Th source. Each data point on the survival curves represents an average value of three or four replicates (i.e., three or four independent Kapton wells that were irradiated in the same experiment). For all cell lines except CT26, two complete irradiation experiments were performed on different dates. The resulting values for D_0 are shown in the legend of each figure (where D_0 is calculated separately for each experiment). D_0 values derived from the entire data set for each cell line are summarized in Table 1.

Among the human cell lines, FaDu cells were found to be the most radiosensitive (lowest $D_0 = 0.69 \pm 0.09$ Gy) whereas the NCIH520 cells were the least radiosensitive (highest $D_0 = 1.50 \pm 0.14$ Gy). The human pancreatic cancer cells tested, Panc1, had an intermediate sensitivity ($D_0 = 0.95 \pm 0.06$ Gy).

Tests of mouse-derived cells revealed that SQ2 cells were the most sensitive ($D_0 = 0.85 \pm 0.02$ Gy), Panc02 cells were more resistant ($D_0 = 1.09 \pm 0.04$ Gy), and CT26 cells showed the least sensitivity ($D_0 = 1.25 \pm 0.34$ Gy). Note that since the CT26 cells tend to grow without forming well-defined colonies, the quantification of colonies was less straightforward (though possible) and only one such experiment was performed with this cell line.

The surviving fractions obtained for the FaDu cells at high dose (~3.5 Gy) are higher than expected, lying above the exponential survival curve (whose slope is dominated by the data at lower doses). A possible explanation may be related to the observation that when FaDu cells were exposed to very high doses (15 to 40 Gy) in this setup, survival of about 0.5% was obtained. That is, not all the cells were hit even for very long exposures. This is likely because of a geometrical flaw that may allow cells that attach to the foil at its outmost periphery to be shielded from the incoming α particles. When the cells are seeded, increasing numbers of cells are seeded with increasing dose (about 2.5% of the irradiated cells are seeded in the 1-Gy dish compared to 25% in the 3-Gy dish). Thus, as larger numbers of cells are seeded, there is a higher probability that some of them have avoided being irradiated due to the presumed geometrical flaw. An alternative explanation is that the increased surviving fractions may be a result of statistical fluctuations, which are more pronounced for higher doses, because the number of colonies in a dish is small. However, since this behavior was not observed for the other cell lines, we cannot completely rule out the possibility that this effect is cell line-specific.

Survival Curves of Cells Exposed to X Rays

The survival curves of Panc1 and FaDu cells after exposure to X rays are shown in Fig. 3, fitted by the linear-quadratic model. Each curve gathers data obtained in three separate experiments. Three repetitions were performed for every dose in each experiment. The survival levels of the two cell lines for the different X-ray doses are quite similar, with FaDu cells somewhat more sensitive at higher doses.

Shown in Fig. 4 are the survival curves of Panc1 and FaDu cells after X irradiation compared to α-particle irradiation (emitted by the ^{228}Th source). Each survival curve represents an average of all experiments performed with these cell lines. The data were fitted with the function $S(D) = \exp(-\alpha D - \beta D^2)$ for X rays and with $S(D) = \exp(-D/D_0)$ for α particles. Interestingly, FaDu cells, which have a higher probability of being inactivated by α particles than Panc1 cells, have a similar probability of being inactivated by X rays. Therefore, higher values of RBE (relative biological effectiveness) are obtained for the FaDu cell line. For 37% survival, the RBE for FaDu cells is 3.0 ± 1.1 compared to 2.1 ± 1.1 for the Panc1 cells. For 10% survival, the RBE is 2.4 ± 0.9 for FaDu cells and 1.8 ± 1.2 for Panc1 cells.

Microdosimetry Calculations

Using the average measured values of the nuclear area A and mean lethal dose D_0, the value z_0 was calculated for all cell lines using both the simple ''lumped averages'' approach (Eqs. 1–3) and the refined Monte Carlo simulation. The values obtained by both methods agreed to within ~2%, confirming that variations attributed to the angular spread of α-particle momenta can indeed be neglected. In addition, we

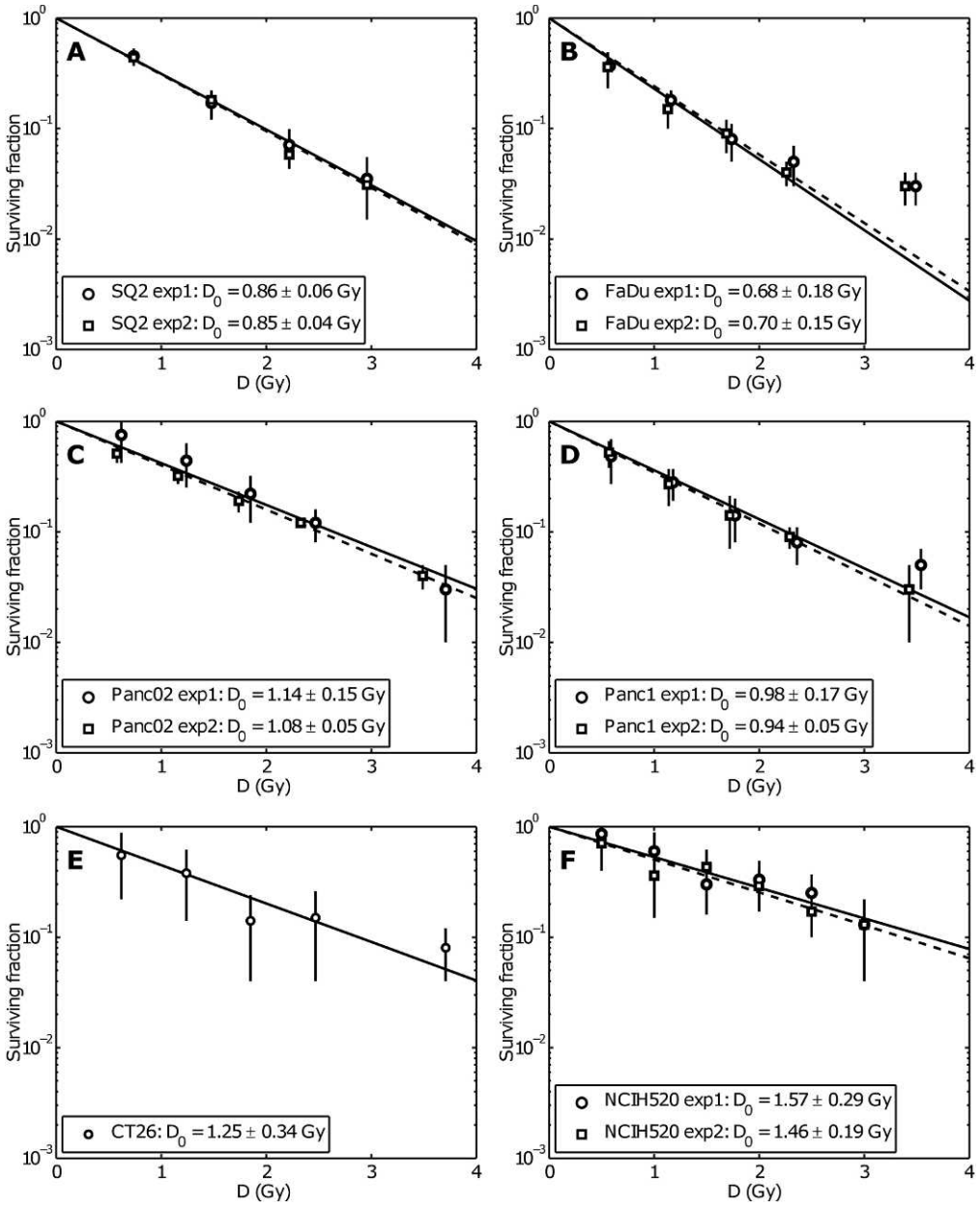

FIG. 2. Survival curves of SQ2 (panel A), FaDu (panel B), Panc02 (panel C), Panc1 (panel D), CT26 (panel E) and NCIH520 (panel F) cells exposed to α particles emitted from a sealed ^{228}Th source. Each curve represents data from one experiment (three or four repetitions at each dose level). The data were fitted with the function $S(D) = \exp(-D/D_0)$, and the resulting values for D_0 are shown in the legend. The error bars represent standard deviations. The uncertainties in D_0 represent the 95% confidence intervals.

TABLE 1
Summary of the Measured and Calculated Data for the Nuclear Area, D_0, z_0, the Average Lethal Number of Hits in a Nucleus, and the Single-Hit Survival Probability for All Cell Lines Studied

Cell line	Nuclear area (μm²)	D_0 (Gy)	z_0 (Gy)	Lethal number of hits in nucleus	Single-hit survival probability
FaDu	113 ± 37	0.69 ± 0.09	0.56 ± 0.10	3.0 ± 1.1	0.66 ± 0.10
SQ2	122 ± 46	0.85 ± 0.02	0.74 ± 0.05	4.0 ± 1.5	0.74 ± 0.08
Panc1	160 ± 48	0.95 ± 0.06	0.86 ± 0.07	5.8 ± 1.8	0.83 ± 0.05
Panc02	89 ± 28	1.09 ± 0.04	0.92 ± 0.07	3.7 ± 1.2	0.73 ± 0.07
CT26	61 ± 17	1.25 ± 0.34	1.00 ± 0.35	2.9 ± 1.1	0.65 ± 0.12
NCIH520	82 ± 31	1.50 ± 0.14	1.32 ± 0.16	4.7 ± 1.8	0.79 ± 0.08

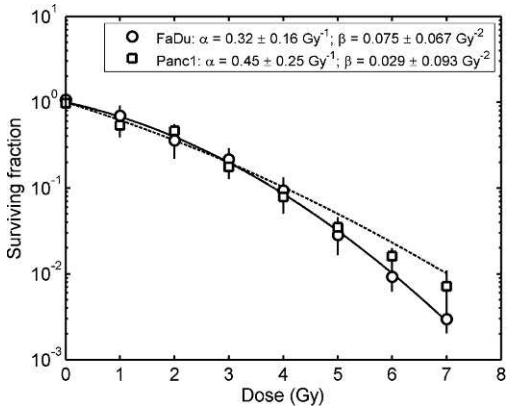

FIG. 3. Survival curves of Panc1 and FaDu cells after exposure to X rays. Each curve represents data from three experiments (three repetitions at each dose level in each experiment). The data were fitted with the function $S(D) = \exp(-\alpha D - \beta D^2)$. The resulting values for α and β are shown in the legend. The error bars represent standard deviations.

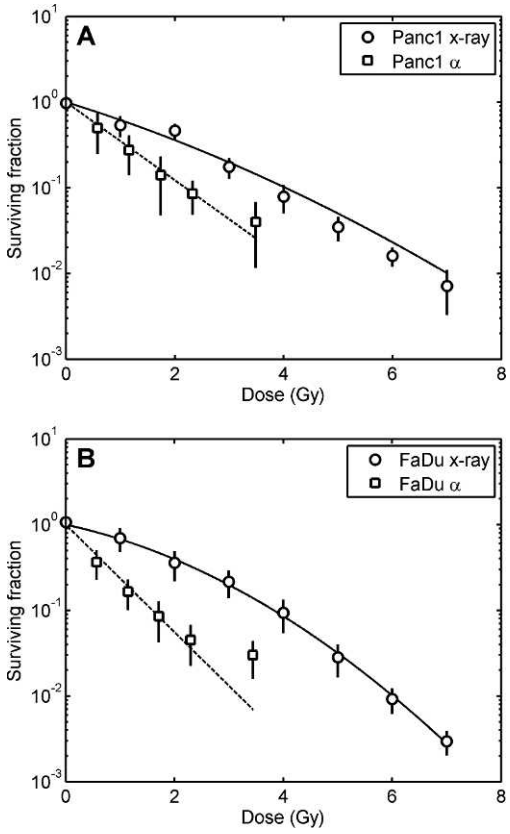

FIG. 4. Survival curves of Panc1 (panel A) and FaDu (panel B) cells after exposure to X rays or ^{228}Th α particles.

calculated the lethal number of hits in a nucleus (i.e., the number of hits producing an average dose equal to D_0) and the single-hit survival probability [evaluated as $\exp(-\bar{z}_1/z_0)$]. The results are summarized in Table 1 and Fig. 5 and show a strong correlation between z_0 and D_0 in spite of considerable differences in the nuclear area. Also shown in Fig. 5 is the approximate expression relating z_0 and D_0 (Eqs. 2 and 3), for an average nuclear area of 104.5 μm^2 (the average nuclear area of all cell lines under study). The number of hits required to induce a lethal dose is highest for Panc1 cells due to their large cross-sectional nuclear area. Therefore, Panc1 cells have the highest probability of surviving a single α-particle hit in the nucleus. The smallest lethal number of hits is obtained for CT26 and FaDu cells, which, accordingly, have the lowest probability of surviving a single hit in the nucleus. Note that for these two cell lines the single-hit survival probability is essentially the same, even though z_0 for CT26 cells is larger by 80%. This comes about because of the small size of the CT26 cell nucleus: the specific energy deposited by a single α particle traversing this nucleus is ~80% higher than that deposited in a FaDu cell nucleus.

The microdosimetry calculation of the *in vivo* LET distribution found it to be rather flat, with a mean of about 150 keV/μm and a standard deviation of 50 keV/μm. These values were largely independent of the initial energy of the emitted α particles and the nuclear radius (taken in the range 2–20 μm). Thus the characteristic LET values of the ^{228}Th irradiation setup (155 ± 48 keV/μm) are indeed representative of those found in *in vivo* DaRT treatments.

DISCUSSION

The experimental results obtained in this work show a considerable variance in the sensitivity of individual cell lines to α-particle radiation. When combined with previous *in vivo* observations, these findings show that, as a rule, cell lines whose tumors respond more favorably to the DaRT treatment are generally more sensitive to α-particle radiation on the cellular level. Namely, the SCC tumor cells, FaDu and SQ2, whose tumors show the most pronounced response *in vivo*, are also the most sensitive of the cells studied *in vitro* ($z_0 = 0.56$ Gy and 0.74 Gy respectively). The less responsive tumors – NCIH520 lung carcinoma, Panc1 and Panc02 pancreatic carcinomas and CT26 colon carcinoma – are characterized by higher z_0 values (0.86–1.32 Gy). The results of similar clonogenic assays for other cell lines, in which the same experimental setup was used, showed a similar trend: the LL2 cell line (murine lung carcinoma), which shows good *in vivo* response, was also sensitive *in vitro* ($D_0 = 0.8$ Gy) (*20*), whereas the less responsive human colorectal adenocarcinoma HCT15 tumors, displayed lower *in vitro* sensitivity ($D_0 = 1.1$ Gy). The values of z_0 obtained in this work were found to be 10–20% lower than the corresponding values of D_0 and could be estimated accurately using measured values for the nuclear cross section and the known characteristics of the irradiation setup.

As noted in the Introduction, the *in vivo* tumor response generally correlates with the degree of radionuclide spread inside the treated tumors. This may indicate that the process of radionuclide spread might be affected by local cellular response inside the tumor. A possible mechanism might be

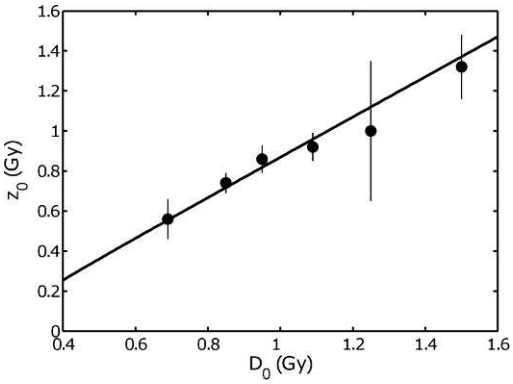

FIG. 5. Calculated values for z_0 as a function of the mean lethal dose D_0 for the cell lines under study. The curve represents the approximate dependence of z_0 on D_0 (Eqs. 2 and 3) for an average nuclear area of 104.5 μm^2.

that the more sensitive cells tend to readily disintegrate after their inactivation, leading to the formation of local necrosis, which in turn facilitates further dispersion of the diffusing α-particle emitters.

The apparent correlation between tumor response and cellular sensitivity to α-particle radiation may in principle serve as a guide for choosing the clinical indications for future DaRT treatments. Furthermore, D_0 and z_0 values estimated using the methods described in this work may serve as input for future treatment planning algorithms and tumor control probability calculations. In particular, microdosimetry calculations that take into account the geometry of the tumor cells, the location of α-particle emitters relative to them, and the intrinsic cellular sensitivity (essentially z_0) can shed light on the statistics of cellular survival and can thus help in establishing the prescription dose for a given tumor type. If the α-particle-emitting atoms do not penetrate the cell membranes, the probability that a nucleus is hit will be much smaller than in the case of a homogeneous distribution of α-particle emitters throughout the tissue. In this case, the macroscopic dose (averaged over distances that are much larger than the size of individual cells) can seriously overestimate the dose to the cell nuclei and one must turn to microdosimetry calculations, which rely on z_0 rather than D_0.

The observation that the FaDu and Panc1 cell lines show similar *in vitro* responses to X rays but considerably different responses to α particles is intriguing. It may be indicative of significant differences either in the rate formation of lethal DNA lesions for a given dose level or in the cellular mechanisms employed by the two cell lines in response to DNA damage inflicted by α particles. Further studies to answer this question are presently under way in our group.

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