Enhanced relative biological effectiveness of proton radiotherapy in tumor cells with internalized gold nanoparticles

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The development and use of sensitizing agents to improve the effectiveness of radiotherapy have long been sought to improve our ability to treat cancer. In this letter, we have studied the relative biological effectiveness of proton beam radiotherapy on prostate tumor cells with and without internalized gold nanoparticles. The effectiveness of proton radiotherapy for the killing of prostate tumor cells was increased by approximately 15%–20% for those cells containing internalized gold nanoparticles. © 2011 American Institute of Physics. [doi:10.1063/1.3589914]

The goal of radiotherapy is the delivery of a lethal dose of radiation to a tumor with the concomitant sparing of surrounding healthy tissues. Much of the recent effort to attain this goal has been concentrated in two distinct categories, namely, conforming the delivered dose to the tumor volume, and enhancing the sensitivity of the tumor to therapeutic radiation. The ability to conform radiation dose to the tumor has greatly improved with the use of intensity-modulated radiotherapy, proton radiotherapy, and modulated arc radiotherapy. Additionally, increases in the sensitivity of the tumor to radiation have been demonstrated by treating them with high-Z contrast agents¹ or nanoparticles² prior to radiotherapy. Several recent studies have focused on the use of gold (Au) nanoparticles to increase the sensitivity of tumors irradiated with clinical x-ray beams. Both theoretical and *in vivo* studies with gold nanoparticle treated tumors coupled with x-ray therapy have shown that clinically significant enhancements are achievable.^{3–5}

In this letter, we present a study of changes in the efficacy of proton radiotherapy for tumor cells containing gold nanoparticles. Recent studies have established that treating a tumor with gold nanoparticles leads to an increase in low-energy x-ray emission from the tumor,⁶ and potentially to an increase in the dose absorbed within the tumor cells. These findings support a hypothesis that dose absorption, and thus the relative biological effectiveness⁷ (RBE), of proton radiotherapy can be enhanced by delivering targeted gold nanoparticles to a tumor prior to treatment. In this study, we quantified enhancements of cell death and RBE over a range of clinically relevant doses for human prostate carcinoma cells that contain internalized gold nanoparticles.

For our experiments, DU145 human prostate carcinoma cells were prepared under the following three different conditions: “Au-treated” cells incubated with specifically internalizing phage-nanoscaffolds^{8–10} (internalizing phage plus gold nanoparticles), and two controls, “phage-only” cells with only internalizing phage (no Au nanoparticles), and “untreated” cells which contained no internalizing phage or gold nanoparticles. To prepare the Au-treated samples, we first synthesized phage-nanoscaffolds according to described

techniques,⁹ which included synthesis of gold nanoparticles (diameter = 44 ± 8 nm) by the Au-chloric-citrate reduction technique.⁹ Subsequently, equal volumes of serially diluted phage (dissolved in 1M imidazole) and gold nanoparticle solutions were mixed to allow the phage-nanoscaffolds to self-assemble. Finally, cells were incubated with phage-nanoscaffold solution to produce the Au-treated cells or with a solution containing the phage alone to produce the phage-only cells. Based on quantification of internalized phage DNA (by polymerase chain reaction) and gold/phage ratios in the nanoscaffolds (by inductively coupled plasma atomic emission spectroscopy), the concentration of gold in the Au-treated cells was estimated to be approximately 1 ng/cell, which is consistent with values reported by Kim *et al.*⁶

The three sample types were plated in standard six-well sample holders, placed in a tissue equivalent plastic phantom, and irradiated to doses of 0, 1, 2, 3, 4, and 6 Gy with clinical photon and proton treatment beams (Fig. 1). First, irradiations were performed in a clinical ⁶⁰Co (1.25 MeV average energy) treatment beam at the depth of maximum dose (5 mm) in the phantom for untreated samples to determine the baseline sensitivity of the DU145 cells to standard photon therapy. Next, untreated, phage-only, and Au-treated

FIG. 1. Schematic diagram of cell irradiations in the clinical (a) ⁶⁰Co and (b) proton therapy beams. Six-well Petri-dish sample holders containing the cells were placed within a tissue equivalent phantom at a depth of maximum dose for the ⁶⁰Co beam and at depth within the uniform dose region of the SOBP for the proton irradiations.

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FIG. 2. (Color online) Cell survival as a function of dose for untreated cells irradiated with ^{60}Co (circles) and proton (squares) beams, as well as for phage-only (diamonds) and Au-treated (triangles) cells irradiated with protons. Errors bars represent one-sigma standard deviation of the mean of the six samples irradiated for each dose. Lines represent LQ fits to the data for untreated cell irradiations with ^{60}Co (solid) and untreated (dashed), and Au-treated (dot-dashed) cell irradiations with proton beams.

cell samples were irradiated with a clinical proton radiotherapy beam [160 MeV, 12 cm range in the phantom, and 10 cm spread out Bragg peak (SOBP)] at a depth of 9 cm in the phantom, placing them within the uniform clinical dose region of the SOBP. Additionally, Au-treated, phage-only, and untreated samples were prepared and not irradiated to serve as baseline (0 Gy) samples in the analysis of cell kill efficiency for the ^{60}Co and proton beam irradiations.

After irradiation, the cell samples were prepared for analysis according to a standard *in vitro* clonogenic assay.¹⁰ In brief, the cells were incubated at 37 °C for 2 weeks to allow surviving cells to form colonies, which were ultimately stained and counted. For each clinical dose value, six separate untreated and Au-treated cell samples were irradiated, assayed, and counted with the mean value used for analysis. From these studies, we determined the surviving fraction (SF), as well as *in vitro* RBE for the untreated and Au-treated cells. SF for each delivered dose is presented as a percentage of the cell survival count for the unirradiated (0 Gy) baseline samples. The measured SF data were fit to a line by the linear-quadratic (LQ) cell survival model^{7,11} given by the following equation:

$$\text{SF} = \exp[-\alpha D - \beta D^2], \quad (1)$$

where D is the delivered dose and α and β are fitting constants. From the LQ model fits we determined the ^{60}Co doses ($D_{^{60}\text{Co}}$) and proton doses (D_{Proton}) necessary to produce SF values equal to 50% and 10%, respectively, and we calculated the RBE as the ratio⁷ of these two doses, as given by

$$\text{RBE}(\text{SF}) = D_{^{60}\text{Co}}(\text{SF})/D_{\text{Proton}}(\text{SF}). \quad (2)$$

SF values as a function of delivered dose for the ^{60}Co (untreated cells) and proton beam (untreated, phage-only, and Au-treated cells) irradiations are plotted in Fig. 2. These results show that the untreated DU145 prostate carcinoma cells were more sensitive to clinical proton irradiation than to ^{60}Co irradiation. Phage-only cells showed a response to proton irradiation identical to that of the untreated cells, demonstrating that the targeted phage does not affect the sensitivity of the cells to proton irradiation. However, the Au-treated

TABLE I. Calculated RBE values and RBE enhancement for untreated and Au-treated cells.

	Cell sample		RBE enhancement (%)
	Untreated	Au-treated	
RBE(SF=50%)	1.6	1.9	19
RBE(SF=10%)	1.3	1.5	15

cells showed a further decrease in cell survival, a result indicating an increase in the sensitivity of the cells to proton beam irradiation due to the presence of the gold nanoparticles.

From the curve fits of the data (Fig. 2), we see that the LQ model predicts SF values that approach that of a single exponential function for the proton irradiated cells (untreated, phage-only, and Au-treated). This is evident by the disappearance of the characteristic low-dose curvature or “shoulder” of the SF curve (seen in the data fit for the ^{60}Co irradiated cells), with the proton irradiated cells appearing as nearly straight lines on the log-linear scale. This lack of a low-dose shoulder (i.e., single exponential shape) is characteristic of SF curves for more densely ionizing radiation.⁷ The curvature is seen to be smallest for the Au-treated cells. We interpret this result as an indication of increased ionization density within the cells resulting from interactions between the proton beam and internalized gold nanoparticles. These proton-nanoparticle ionizations lead to increased production of low energy delta-ray electrons, which produce a high degree of lethal damage⁷ within the cells resulting in a lower SF for a given proton dose.

Calculations of the *in vitro* RBE based on the LQ model fitting of the data are shown (Table I). From the results of these experiments, we calculated *in vitro* proton RBE(50%) and RBE(10%) values for the untreated DU145 cells that were higher than the published average *in vitro* proton RBE value¹¹ of 1.2. This result indicates that the cell line used for these experiments has a greater *in vitro* sensitivity to proton irradiation than most cell lines typically used in proton RBE experiments. Additionally from Table I, we see a further increase in RBE(50%) and RBE(10%) values, representing approximately a 15%–20% enhancement for Au-treated cells over untreated cells.

We conclude that the specific targeting and internalization of gold nanoparticles into cancer cells can produce a clinically meaningful increase in the RBE of proton radiotherapy. From the changes in the shape of the cell survival curves, we hypothesize that proton-Au scatter interactions cause an increase in the ionization density within the cells that results in an increased rate of cell death. From a clinical standpoint, for the dose delivered during a standard treatment (1–2 Gy/fraction) and hypofractionated treatment (>4 Gy/fraction), our results indicate a possible increase of ~20% and ~15%, respectively, in the effectiveness (i.e., RBE increase) of proton therapy. We believe these results justify further clinical research and development into the use of gold nanoparticles as a proton radiotherapy sensitizing agent.

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