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Ionizing radiation increases systemic nanoparticle tumor accumulation

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Abstract

Nanoparticle-based therapies are currently being explored for both the imaging and treatment of primary and metastatic cancers. Effective nanoparticle cancer therapy requires significant accumulations of nanoparticles within the tumor environment. Various techniques have been used to improve tumor nanoparticle uptake and biodistribution. Most notable of these techniques are the use of tumor-specific-peptide-conjugated nanoparticles and chemical modification of the nanoparticles with immune-evading polymers. Another strategy for improving the tumor uptake of the nanoparticles is modification of the tumor microenvironment with a goal of enhancing the enhanced permeability and retention effect inherent to solid tumors. We demonstrate a two-fold increase in the tumor accumulation of systemically delivered iron oxide nanoparticles following a single, 15 Gy radiation dose in a syngeneic mouse breast tumor model. This increase in nanoparticle tumor accumulation correlates with a radiation-induced decrease in tumor interstitial pressure and a subsequent increase in vascular permeability.

Keywords

ionizing radiation; nanoparticle; tumor; biodistribution; interstitial pressure

1. Background

A variety of hyperthermia-based techniques[1], including intratumoral magnetic nanoparticle (mNP) hyperthermia[2], have been used to treat tumors. The difference in mNP hyperthermia, as compared to conventional hyperthermia, is the ability tofocally heat according to mNP uptake and biodistribution in both primary and metastatic tumors. One of the most significant challenges is delivering an effective concentration ofnanoparticles to tumor cells. Investigators have relied on increasing nanoparticle circulation time through evasion of the reticuloendothelial system (RES)[2] by modifying nanoparticle surface

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coating[3], the enhanced permeability and retention (EPR) effect[4] and tumor-specific peptide conjugates to mNP[5] to increase nanoparticle accumulation in tumors.

High interstitial tumor pressure (ITP) has been shown to hinder diffusion of macromolecules into tumors[6]. Ionizing radiation has been shown lower ITP at doses above 10 GyI[7].

2. Materials Methods

2.1. Cell Culture

MTG-B mouse mammary adenocarcinoma cells were cultured in 150 cm^2 cell culture flasks (Corning Inc., Lowell, MA) in Alpha MEM medium (10% fetal bovine serum, 1% penicillin-streptomycin, 1% L-glutamine; all from Thermo Fisher Scientific Inc., Waltham, MA., USA). Cells were then trypsinized (0.25% trypsin in EDTA, Mediatech, Inc., Manassas, Va) and resuspended in serum-free Alpha MEM at 10^7 cells/ml.

2.2. Murine tumor model

One hundred μL (10^6 cells) was implanted bilaterally in the flanks 6-8 week old female C3H mice (Charles River Laboratories, Wilmington, MA, USA). Mice were treated once tumors reached 150±40 mm^3. All animal experimentation was approved by the Dartmouth Institutional Animal Care and Use Committee, in accordance with all federal, institutional and AAALAC guidelines.

2.3. Interstitial pressure measurements

Interstitial tumor pressure measurements in ten tumors were accomplished by placing a fiber optic pressure sensor (0.5 mm diameter FPI-HR, FISO Technologies Inc., Quebec, Canada) in the centers of thetumors, using a techniquesimilar to others[8].

2.4. Ionizing radiation

Within one hour of establishing a baseline ITP, half of the tumors received a single 15 Gy, 6 MeV electron radiationdose (surfaced based homogeneous dose,100 cm source-skin distance (SSD), 1.7 cm diameter circular lead cut-out, average tumor depth 0.5 cm and width 0.7 cm) using a Varian Clinac 2100C linear accelerator (Varian Medical Systems, Inc., Palo Alto, CA, USA). ITP measurements were repeated daily, for five days.

2.5. Tumor vascular permeability assessment

Three days following irradiation, a vascular permeability assay[9] was performed using Evans blue (Sigma Aldrich, St. Louis, MO, USA) suspended in phosphate buffered saline (PBS, Mediatech) at 1.25 mg/ml. The solution was injected into the left jugular vein to 10 mg/kg mouse. Two hours later the mouse was perfused with 20 ml PBS and the tumors removed, weighed and digested in 10 ml formamide (Sigma Aldrich) per gram of tissue. Three days following tumor removal, tumor dye concentration was determined using a Perkin Elmer MBA 2000 spectrophotometer.

2.6. Nanoparticle composition and quantification

Iron oxide nanoparticles (70 and 120 nm diameter, Micromod Partikeltechnologie GmbH, Rostock, Germany) with various coatings (Table 1) were purchasedsuspended in deionized water. NaCl (0.9%) was added to ensure an appropriate isotonic balance.

When an individual tumor reached treatment size, it was irradiated with the contralateral tumor serving as a non-irradiated control. MNPs or PBS (control) were injected into the left jugular vein at a concentration of 0.2 mg Fe/g mouse three days following irradiation. Six

days following irradiation, the mice were perfused with 20 ml PBS and tumors removed. Half of each tumor was fixed in 10% buffered formalin and processed for microscopic assessment of iron (Prussian blue/non-heme iron) anda subset of these for vascular endothelium (anti-CD-31). The other half of each tumor was weighed, digested in 3:1 trace element grade Nitric Acid:HCl (Thermo Fisher Scientific) and analyzed for iron content via ICP-MS (Agilent 7500cx inductively coupled plasma mass spectrometer) .

2.7. Statistical analysis

Statistical significance was assessed with a two-tailed, two-sample t-test with Matlab software (The MathWorks, Inc. Natick, MA, USA).

3. Results

Interstitial tumor pressure (ITP) was decreased 3, 4 and 5 days post-15 Gy irradiation (Figure 1) by up to 40% as compared with controls. Three days post tumor irradiation, vascular permeability, as assessed by higher Evans blue absorbance, was increased by approximately 60% (Figure 1).

Three days following mNP administration the concentration of both 70 and 120 nm NPs was doubled in the irradiated tumors, as compared to controls (Figure 2). The median concentrations of systemically delivered PEG 200 coated BNF mNPs increased 2.5 fold in mouse flank tumors, as compared to non-coated mNPs (0.1 mg Fe/g vs 0.25 mg Fe/g). Prussian blue histology and iron quantification results were confirmed with ICP-MS (Figure 3). Mice receiving PBS instead of mNPs had ICP-MS iron levels less than 5% of the mNP treatment mice and no histologic iron staining.

4. Discussion

Our results demonstrate tumor irradiation, PEG coating and small size (70 vs. 120 nm) increase mNP accumulation in a murine flank breast tumor model.This increased deposition of mNP within tumors due to addition of PEG to the surface is likely due to the increased circulation time and evasion of the RES which PEG confers to the mNP[10].

These results demonstrate that it is possible to increase accumulation of nanoparticles within tumors by modification of the tumor microenvironment with clinically relevant ionizing radiation. While modification of nanoparticles with targeting agents and RES-evading polymers also assists nanoparticle accumulation in tumors, these effects can be enhanced through modification of interstitial tumor pressure and tumor vascular permeability with the use of ionizing radiation.

Acknowledgments

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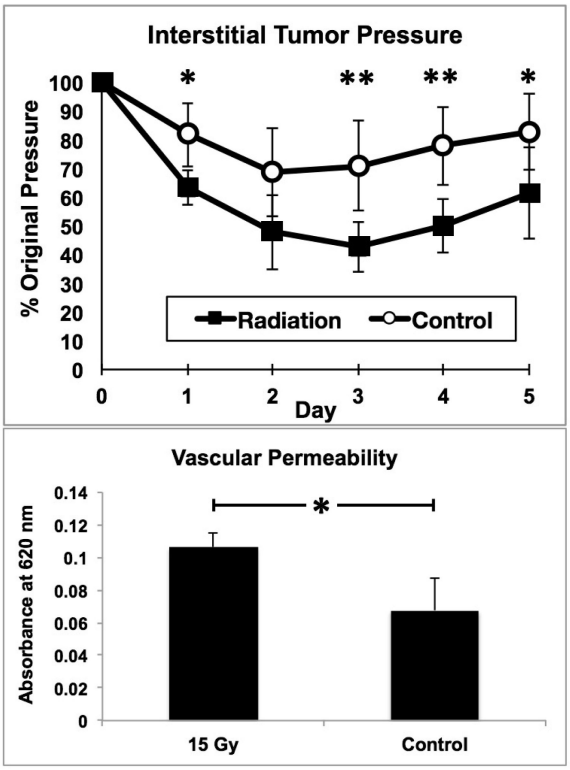

Figure 1.

Top:A single 15 Gy fraction of 6 MeV electron radiation significantly decreases interstitial tumor pressure as compared to non-irradiated controls. Bottom: Three days following irradiation vascular permeability is correspondingly increased (Evans blue spectrophotometry assessment). * = $p < 0.05$ and ** = $p < 0.01$. Error bars show standard deviations.

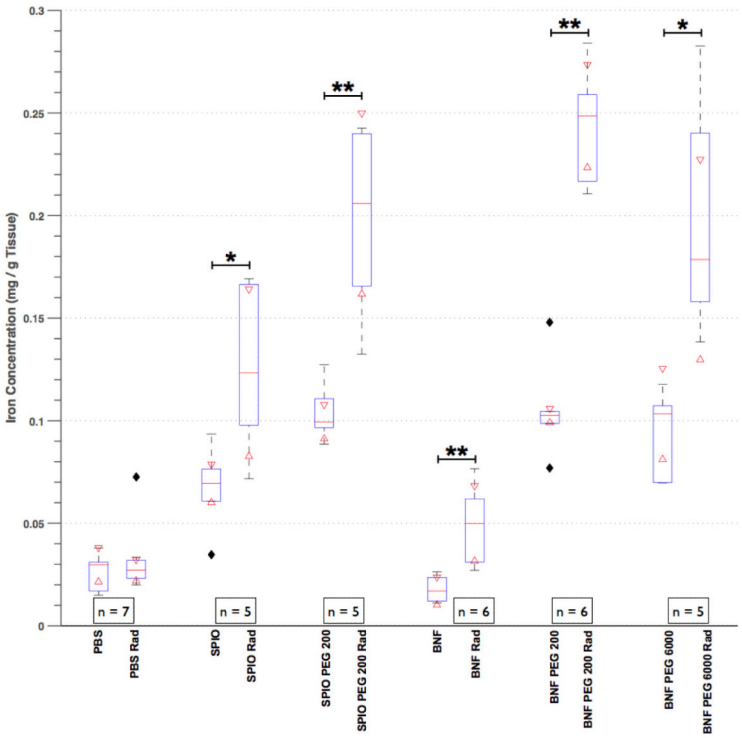

Figure 2.

This boxplot of ICP-MS-based tumor iron quantification demonstrates that PEG coating increases nanoparticle accumulation in irradiated tumors. Nanoparticle size was irrelevant with respect to tumor accumulation when PEG-coated. When not PEG coated, smaller nanoparticles (SPIO) accumulated in tumors more than larger nanoparticles (BNF). The numbers in the figure legends (i.e. n=7) refers to number of animals in each group; each animal had anirradiated (Rad) and non-treated (control) tumor.The top of each box is the 75^{th} percentile, the middle line the median and the bottom of the box the 25^{th} percentile for each group. The errors bars show the range of data in each group and the filled diamonds show outliers. The 95% confidence interval for each group is the range between the two triangles overlaying each box. * = $p < 0.05$ and ** = $p < 0.01$.

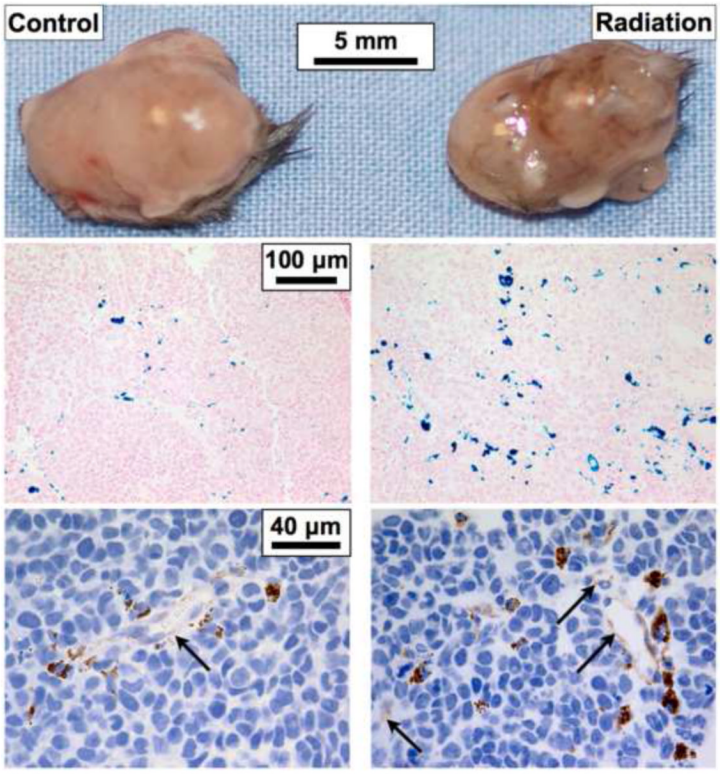

Figure 3.
Bilateral flank tumors were excised from the same mouse three days after systemic administration of BNF PEG200 nanoparticles. Gross and histological analyses demonstrate an increased level of mNPs (brown in topand bottom, blue in middle) in irradiated tumors. IHC-stained endothelial cells (anti-CD31) demonstrate the close spatial relationship of tumor vessels (light brown, arrows) and nanoparticles (dark brown).

Table 1

Nanoparticle Characteristics

Iron oxide nanoparticles and zeta potential measurements acquired from Micromod. Hydrodynamicdiameters (core plus coating)were acquired using a Malvern Instruments Ltd. (Westborough, MA) SPIO = superparamagnetic iron oxide; BNF = bionized nanoferrite ferromagnetic nanoparticles; PEG 200 = polyethylene glycol polymer(200 Da molecular weight); PEG 6000 = polyethylene glycol polymer (6000 Da); HES = hydroxyethyl starch.

Nanoparticle Designation	Average Particle Hydrodynamic Diameter (nm)	Coating	Zeta Potential (mV) at pH 7
SPIO	74	HES	−2.6
SPIO PEG 200	72	HES + PEG 200	−7.9
BNF	116	HES	−18
BNF PEG 200	125	HES + PEG 200	−6.5
BNF PEG 6000	132	HES + PEG 6000	−3.5