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Alpha-particle radiotherapy: For large solid tumors diffusion trumps targeting

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ABSTRACT

Diffusion limitations on the penetration of nanocarriers in solid tumors hamper their therapeutic use when labeled with α-particle emitters. This is mostly due to the α-particles' relatively short range (≤100 μm) resulting in partial tumor irradiation and limited killing.

To utilize the high therapeutic potential of α-particles against solid tumors, we designed *non-targeted, non-internalizing* nanometer-sized tunable carriers (pH-tunable liposomes) that are triggered to release, within the slightly acidic tumor interstitium, highly-diffusive forms of the encapsulated α-particle generator Actinium-225 (^{225}Ac) resulting in more homogeneous distributions of the α-particle emitters, improving uniformity in tumor irradiation and increasing killing efficacies.

On large multicellular spheroids (400 μm-in-diameter), used as surrogates of the avascular areas of solid tumors, interstitially-releasing liposomes resulted in best growth control independent of HER2 expression followed in performance by (a) the HER2-targeting radiolabeled antibody or (b) the non-responsive liposomes. In an orthotopic human HER2-negative mouse model, interstitially-releasing ^{225}Ac-loaded liposomes resulted in the longest overall and median survival.

This study demonstrates the therapeutic potential of a general strategy to bypass the diffusion-limited transport of radionuclide carriers in solid tumors enabling interstitial release from *non-internalizing* nanocarriers of highly-diffusing and deeper tumor-penetrating molecular forms of α-particle emitters, independent of cell-targeting.

1. Introduction

In solid tumors the diffusion-limited penetration depths of radionuclide carriers [1–3] combined with the short range of α-particles (40–100 μm) [4] hamper their use mostly due to partial tumor irradiation. Given the high killing efficacy of α-particles (4–5 tracks across the nucleus result in cell kill) [5–11] which is also mostly independent of cell-oxygenation state unlike β-particles [12] this is quite unfortunate. Carrier molecules that diffuse substantially from the vasculature into the viable tumor have been suggested to be necessary for effective irradiation of the viable solid tumor-cell regions [3].

To address the diffusion-limitations on the transport of radionuclide carriers and the partial tumor irradiation, we designed nanometer-sized lipid carriers (liposomes) that are triggered to release in the tumor interstitium *highly-diffusing forms of encapsulated α-particle emitters*. Given that the molecular-size of the released chelated-emitters is significantly smaller than the nanometer-sized carriers, we expect that the corresponding higher diffusivities may result in more uniform distributions and deeper penetration of radionuclides within tumors, resulting in more even

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tumor irradiation. Towards this goal, we designed pH-responsive liposomes encapsulating chelated forms of α-particle emitters. These liposomes are expected to demonstrate enhanced uptake by solid tumors as described by the Enhanced Permeability and Retention (EPR) effect [13]. Additionally, in the slightly acidic tumor interstitium (7.4 > pH > 6.0) [14,15] a pH-responsive mechanism on the liposome membrane results in release of these chelated forms of encapsulated radioactivity [16].

The pH-triggered release mechanism is simply based on the formation of *defective interfaces* - in terms of lipid packing - *that span the lipid bilayer* resulting in high membrane permeability [16,17]. These lipid interfaces originate at the boundaries of domains formed upon lipid phase separation. Liposome membranes are designed to contain two lipid types: one with a non-titratable headgroup (lipid with gray headgroup in cartoon, Fig. 1) and one with a titratable domain-forming headgroup (lipid with red headgroup); the tail lengths are different to enhance lipid packing defects at the interfaces upon phase separation [16,17]. The extent of ionization on the headgroups of the domain-forming lipids is controlled by the pH which is used to shift the balance between electrostatic repulsions and H-bonding attractions [18]. At neutral pH, the headgroups of the domain forming lipids are negatively-charged, opposing close proximity between lipids, resulting in uniform lipid membranes. As the pH is decreased, gradual headgroup protonation minimizes the electrostatic repulsion, and phase separated lipid domains are formed driven mostly by H-bonding [19]. The utility and robustness of this approach has been reported for several agents *in vitro* and *in vivo* [16,20–22].

In this study, pH-responsive (interstitially-releasing) liposomes were loaded with the α-particle generator Actinium-225 (^{225}Ac) and were compared to non-pH-responsive (non-releasing) liposomes and to radiolabeled antibodies (Trastuzumab) targeting HER2. We evaluated the distributions of the carriers, and of the delivered radioactivity, in multicellular spheroids comprised of breast cancer cells with variable radiosensitivities and variable expression levels of HER2. Lastly, the efficacy of all constructs to prolong survival was evaluated on animals with orthotopic MDA-MB-231 triple negative breast cancer (TNBC) xenograft tumors.

2. Materials and methods

2.1. Materials

The lipids 1,2-dihenarachidoyl-sn-glycero-3-phosphocholine (21 PC), 1,2-distearoyl-sn-glycero-3-phosphate (DSPA), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rhd-lipid) were purchased in chloroform from Avanti Polar Lipids (Alabaster, AL). Poly(2-hydroxyethyl methacrylate) (polyHEMA) was obtained from Polysciences, Inc. (Warrington, PA). All materials are described in detail in the Supplemental data. The IgG1κ isotype control antibody was purchased from SouthernBiotech (Birmingham, AL). Actinium-225 (^{225}Ac, actinium chloride) was provided by the Institute for Transuranium Elements, Germany, as described before [23,24].

2.2. Nanocarriers

pH-responsive and non-pH-responsive liposomes were composed of 21 PC:DSPA:cholesterol(chol):DSPE-PEG:Rhd-lipid at a mole ratio of 53.7:23.0:9.5:13.0:0.8 and of 21 PC:chol:DSPE-PEG:Rhd-lipid at a mole ratio of 66.0:28.0:5.0:1.0, respectively, and were prepared using the thin film hydration method [25]. Lipid suspensions were extruded through 100 nm diameter pores, and were characterized for size and zeta potential using a Zetasizer NanoSeries (Malvern Instruments Ltd.). Liposomes encapsulating 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) were loaded using the A23187 ionophore [26]. Loading efficiency was determined by comparing the Bismuth-213 (^{213}Bi) γ-emissions (360–480 keV) at secular equilibrium before and after Sephadex-G-50 separation using a Cobra γ-counter (Packard Instrument Co., Inc.).

To radiolabel antibodies with ^{225}Ac [23,24] or Indium-111 (^{111}In), the isothiocyanate-functionalized metal chelator (DOTA or diethylenetriamine pentaacetic acid (DTPA), respectively) was added in one step as described in Supplemental data [27]. Radiochemical purity was assessed via instant thin layer chromatography (iTLC). The ^{111}In-DTPA-Trastuzumab was used to measure the levels of HER2 receptors per cell as reported before [28,29].

Retention of ^{225}Ac was determined by incubating the ^{225}Ac-loaded carriers in HybriCare media supplemented with 10% fetal bovine serum (FBS) at 37 °C. At pre-determined time points, aliquots were removed, and released radioactivity was separated either with a Sephadex-G-50 (liposomes) or 10-DG (antibodies) column [26].

The extents of released radioactivity from liposomes corresponding to each of the three possible forms (^{225}Ac-DOTA, ^{225}Ac-ionophore complex, ^{225}Ac) were evaluated by separation and quantitation of two of the forms in addition to the overall radioactivity balance (as described in detail in Supplemental data).

2.3. Cell and spheroid culture

The HER2 positive BT474 cell line, the TNBC MDA-MB-231 cell line, and the HER2-negative MCF-7 cell line which was stably

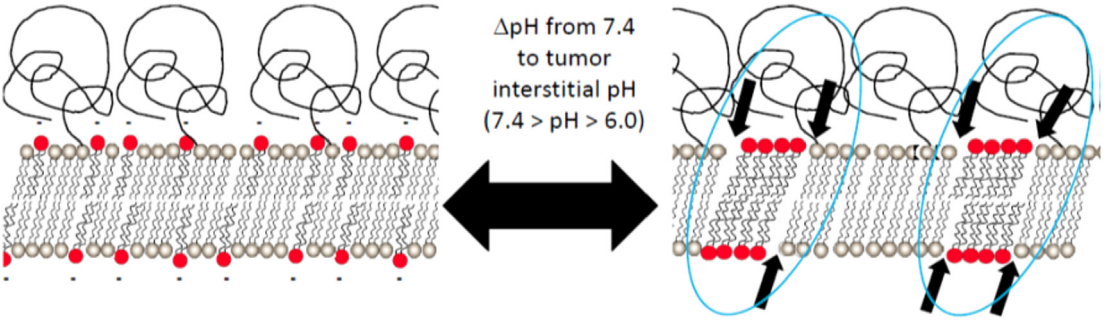

Fig. 1. pH-triggered release of contents. (left) pH = 7.4 during circulation in blood, (right) at tumor interstitial pH. The upper lipid leaflet represents the outer lipid leaflet of liposomes; the lower lipid leaflet represents the inner lipid leaflet of liposomes. Lipid phase separation and domain formation is activated at the tumor interstitial pH (7.4 > pH > 6.0, shown on the right). Black arrows indicate 'leaky' phase boundaries between the different lipid phases.