Biodegradable polymeric nanoparticles targeted by a novel biomimetic peptide to human breast cancer

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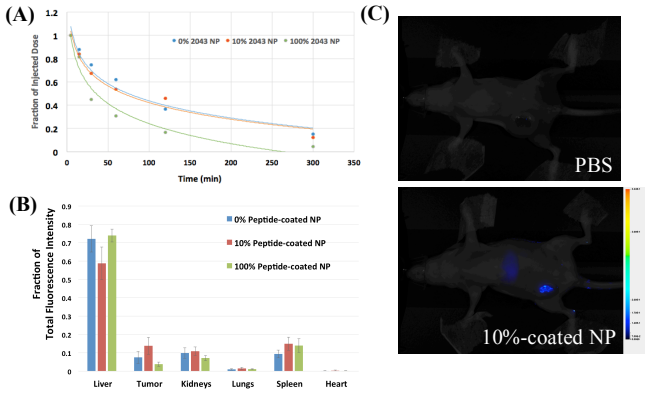
Statement of Purpose: Progression of tumor requires angiogenesis in order to achieve the increased need for oxygen and nutrients. Anti-angiogenesis is a widely investigated approach to prevent tumor growth (Bhise NS. Expert Opin Drug Deliv 2011; 8(4): 485-504). We have discovered a biomimetic peptide that shows potent anti-angiogenic activity *in vitro* and *in vivo* (Karagiannis ED. PNAS 2008; 105(37): 13775-13780). Nanoparticle (NP) formulations can be used for efficient systemic delivery and controlled release of such peptides at the target site to significantly enhance bioavailability while minimizing side effects. However, unmodified or poly(ethylene glycol) (PEG)-coated polymeric NPs can suffer from a high rate of clearance and a low level of tumor accumulation (Wilhelm S. Nat. Rev. Mater. 2016; 1: 1-12). To overcome these limitations, we have designed a targeted poly(lactic-co-glycolic acid) (PLGA) NP with a biomimetic peptide as a novel targeting agent. In this study, we test PLGA NPs coated and loaded with peptide for the dual functionality of actively targeting human breast cancer and inducing anti-angiogenesis.

Methods: Biomimetic peptide was conjugated to poly(lactide-co-glycolide) (PLGA)-PEG polymer (Akina, West Lafayette, IN) by an NHS-amine coupling reaction, and confirmed by HPLC. Varying mass ratios of PLGA-PEG and PLGA-PEG-peptide in DMF were mixed with the peptide in DMSO at 5% (w/w) and allowed to form NPs by nanoprecipitation in water. A Malvern Zetasizer was used to determine physical characteristics including size and surface charge of the resulting NPs. For *in vitro* adhesion and proliferation assays, naked peptide at different concentrations and nanoparticles with different surface densities of peptide were incubated with human triple-negative breast cancer cells (MDA-MB 231) and primary microvascular endothelial cells (MEC) prior to detection with Calcein AM and MTS assays. For *in vivo* pharmacokinetics and biodistribution studies, MDA-MB 231 cells were inoculated into flank of athymic nude mice. NPs at 0%, 10%, or 100% surface density of peptide (determined by the weight % of PLGA-PEG-peptide to total polymer) with 1% by weight of PLGA-PEG functionalized with infrared dye were injected intravenously. PBS vehicle and naked peptide were used as controls. Collected blood at different timepoints, whole animal, and harvested organs were imaged with LiCOR PEARL instrument and fluorescence intensity was quantified.

Results: The size and surface charge of targeted PLGA NPs were not significantly affected by surface functionalization. Average hydrodynamic diameter of resulting NPs was 60-80 (nm) with -20 (mV) zeta potential. Increased binding of targeted NPs compared to non-targeted NPs was observed following incubation with human triple-negative breast cancer cells (MDA-MB 231) and microvascular endothelial cells (MEC), validating the peptide's function as a targeting ligand. These targeted NPs also showed dose-dependent inhibition of adhesion of MDA-MB-231 and MEC and proliferation of MEC *in vitro*, also confirming the peptide's effect in anti-angiogenesis. 100% surface-coated NPs inhibited adhesion of both cell types by 85% and proliferation of MEC by 75%, compared to 90% and 55% respectively by 100 (μM) naked peptide. Further analysis by ForteBio Octet showed a strong binding of the peptide to its cell surface target. When injected via tail-vein in mice with MDA-MB 231 xenografts, 10% and 100% surface-coated, targeted NPs showed longer half-life in circulation of 130 mins compared to non-targeted control NPs (Fig. 1A). Interestingly, 100% surface-coated NPs resulted in the least accumulation at tumor site (Fig. 1B). More than 10% of the total 10% surface-coated NPs distributed in analyzed organs accumulated in the tumors (Fig. 1C). Additionally, 10% NPs showed an approximately 13% decrease in liver accumulation in comparison to both non-targeted and 100% targeted NPs.



Conclusions: Peptide-functionalized PLGA NPs bind to and inhibit proliferation of MDA-MD-231 and MEC cells *in vitro*. Combined with greater *in vivo* tumor accumulation compared to non-targeted NPs in a murine biodistribution model, 10% surface-coated NPs suggest their potential in suppressing breast tumor growth *in vivo*.

References:

1. Bhise NS. Expert Opin Drug Deliv 2011; 8(4): 485-504
2. Karagiannis ED. PNAS 2008; 105(37): 13775-13780
3. Wilhelm S. Nat. Rev. Mater. 2016; 1: 1-12