

Improving Tumor Targeting and MRI of Pancreatic Cancer Using IGF-1R Targeted "Stealth" Iron Oxide Nanoparticles

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INTRODUCTION

Iron oxide nanoparticles (IONPs) has been applied to the therapy delivery and consequent therapeutic response monitoring in the treatment of pancreatic cancer due to their superb MRI contrast enhancing effect as well as the capability of carrying therapeutic agents¹. However, one major challenge in the application of theranostic IONPs *in vivo* is the non-specific uptake of nanoparticles by the mononuclear phagocyte system (e.g. macrophage) and reticuloendothelial system (e.g. liver and spleen), and the non-specific protein adsorption onto the surface that results in the poor delivery of the nanoparticles to the targeted tumor.² In particular, pancreatic cancer has extensive tumor stroma and disordered tumor vasculatures. Based on our previously reported poly ethylene glycol (PEG)-*b*-allyl glycidyl ether (AGE) copolymer coated IONP that showed "stealth" properties with greatly reduction of protein corona and the non-specific cellular uptake³, we have developed highly effective tumor targeted IONPs using a recombinant human insulin-like growth factor 1 (IGF-1, 7kDa) to target insulin-like growth factor 1 receptor (IGF-1R) that is highly expressed in pancreatic cancer cells and stromal cells. Human IGF-1 has a high binding affinity for IGF-1R, leading to targeted delivery to both tumor and stromal cells in the pancreatic cancer mouse model.

MATERIALS AND METHODS

Conjugation of targeting ligands: The PEG-*b*-AGE coated IONP was made solution in PBS at the concentration of 2 mg/mL. The solution was then incubated with the linker Sulfo-SMCC (at the concentration of 2 mg/mL) for one hour. The solution was purified with a PD-10 column followed by incubation with thiolated (using Traut's reagent) IGF-1 and bovine serum albumin (BSA) at the concentration of 1 mg/mL overnight. The solution of ligand conjugated IONP was obtained after purification with a PD-10 column.

Testing cell uptake and targeting of IONPs: MIA PaCa-2 pancreatic cancer cells and MDA-MB-231 breast cancer cells were used for testing the tumor cell targeting of IGF-conjugated IONPs. Cells were seeded into an 8-well chamber slide and incubated overnight. The media was then replaced with that containing IONPs at the concentration of 0.2 mg/mL. Cells were incubated at 37 °C for three hours, and then fixed with 4% paraformaldehyde in PBS solution, followed by Prussian blue staining for iron, and counterstaining using nuclear fast red solution.

Preparation of cell phantoms: PEG-*b*-AGE coated IONP, Tf conjugated PEG-*b*-AGE coated IONP (Tf-IONP), SHP-20, and Tf conjugated SHP-20 (Tf-SHP) were incubated with 8×10^6 D556 medulloblastoma brain tumor cells, A549 lung cancer cells and Raw264.7 macrophage cells, respectively, for three hours at 37 °C at the Fe concentration of 0.2 mg/mL. The cells were washed with PBS three times to remove the free IONPs and then re-suspended in 1 mL of 1.5% agarose gel at 50 °C before the mixture was cooled to room temperature and solidified for MRI scan.

Preparation of orthotopic human pancreatic cancer xenograft model: The pancreatic cancer model was prepared by directly injecting 5×10^6 MIA PaCa-2 cells into the pancreas of 7- to 8-week old nude mice (Harlan Laboratories, Inc., Indianapolis, IN) using a surgical procedure approved by the Emory University Institutional Animal Care and Use Committee. In 3 to 4 weeks, orthotopically xenografted pancreatic tumors typically reached 5 mm in diameter and were ready for use.

Magnetic resonance imaging (MRI) experiment: Cell phantoms and tumor-bearing mice were scanned using a 3-Tesla scanner (Tim/Trio, Siemens, Erlangen, Germany) using a standard head coil with samples placed in the iso-center of the magnet. MRI contrast and signal changes related to the cell binding to IONPs were evaluated using T₂-weighted fast SE sequence and multi-TE SE sequence for T₂ mapping. Image analysis was done using the region of interest (ROI) method. After the mice were injected with IONP (20 mg/kg weight) via tail vein, they were imaged at 48 hours and 96 hours.

RESULTS AND DISCUSSION

Fig 1. R₂ relaxometry maps of cell phantoms containing (A) D556 medulloblastoma cells, A549 lung cancer cells, and RAW264.7 macrophage cells treated with SHP-20 and PEG-*b*-AGE coated IONP; and (B) D556 medulloblastoma cells and A549 lung cancer cells treated with SHP-20, Tf-SHP, PEG-*b*-AGE coated IONP, and Tf-IONP. R₂ values of cell phantoms containing (C) D556 medulloblastoma cells, A549 lung cancer cells, and RAW264.7 macrophage cells treated with SHP and PEG-*b*-AGE coated IONP, and (D) D556 medulloblastoma cells and A549 lung cancer cells treated with Tf-SHP, and Tf-IONP. R₂ values in (C) and (D) are normalized by subtracting the R₂ values of the cells incubated with regular media without nanoparticles.

R₂ relaxometry mapping of the cell phantoms showed substantial increase of R₂ values in cells treated with SHP-20 but no significant R₂ value changes in cells treated with PEG-*b*-AGE polymer coated IONP (Fig 1A,C), suggesting a significant reduction of off-target background with PEG-*b*-AGE coated IONPs. Since the facile conjugation of IONP with Tf has been developed, the Tf-conjugated PEG-*b*-AGE coated IONP (Tf-IONP) was used to demonstrate the improved targeting effect by anti-biofouling property. With Tf as the targeting ligand, Tf-IONP showed expected increase of contrast or "darkening" in T₂-weighted MRI (increased R₂ value) in D556 cells compared to non-targeted IONPs as well as the control A549 cells (Fig 1B). However, The control A546 cells treated with Tf-SHP showed substantially lower signal level comparing to A546 cells treated with Tf-IONP, suggesting the anti-biofouling effect from Tf-IONP led to the reduction of non-specific uptake of targeted IONPs by the cells not presenting targeted biomarkers, therefore, attenuated off-targeted background signal (Fig 1D).

After the conjugation of IONP with IGF-1 (IGF-IONP), MIA PaCa-2 cancer cells (with high expression of IGF-1R) exhibited uptake of the nanoparticles, while MDA-MB-231 breast cancer cells (with low expression of IGF-1R) showed no uptake of the nanoparticles. Non-targeted BSA-conjugated PEG-*b*-AGE coated IONP (BSA-IONP) showed no cellular uptake for both MIA PaCa-2 and MDA-MB-231 cancer cells (Fig 2).

MRI signal intensity of T₂-weighted images decreased 44% and 21% in the pancreatic tumors of mice injected with IGF-IONP (targeted) and BSA-IONP (non-targeted) respectively (Fig 3). The targeted IONP had much improved contrast enhancement than non-targeted one, even though the non-targeted BSA-IONP had comparable contrast enhancing effect with reported result.³ The greatly improved contrast enhancing effect as a result of increased accumulation of IONPs in the tumor is likely due to the combined effect of robust targeting effect of IGF-1R targeting to the tumor and stroma cells and antifouling properties of in reducing the non-specific uptake by mononuclear phagocyte system and reticuloendothelial system.

CONCLUSION

The anti-biofouling poly ethylene glycol-*b*-allyl glycidyl ether coated IONP have demonstrated highly specific targeting effect to MIA PaCa-2 pancreatic cancer cells with IGF-1 as the targeting ligand in the *in vitro* cell experiment. The *in vivo* MRI of pancreatic tumor-bearing mice showed significant signal intensity drop after the systemic administration of the IGF-conjugated anti-biofouling IONP.

REFERENCES

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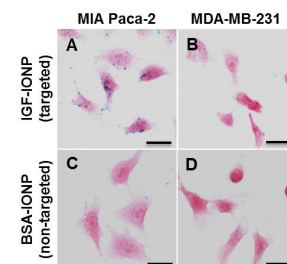


Fig 2. Prussian blue stained images of IGF-IONP incubated with (A) MIA PaCa-2 pancreatic cancer cells, (B) MDA-MB-231 breast cancer cells; and BSA-IONP incubated with (C) MIA PaCa-2 pancreatic cancer cells, (D) MDA-MB-231 breast cancer cells at the Fe concentration of 0.2 mg/mL. Scale bar, 20 μm.

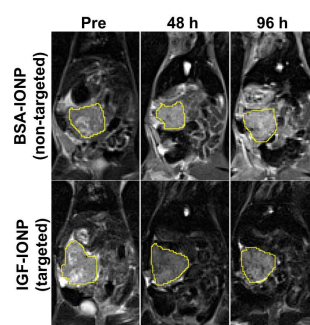


Fig 3. MR images of tumor-bearing mice before (pre) and after injection of cancer cell non-targeted BSA-IONP and cancer cell targeted IGF-IONP.