In Vivo Off Resonance Saturation Magnetic Resonance Imaging of $\alpha_v\beta_3$-Targeted Superparamagnetic Nanoparticles

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Statement of Purpose: Magnetic resonance imaging (MRI) is a powerful clinical imaging technique that allows for non-invasive tomographic visualization of anatomic structures with high spatial resolution and soft tissue contrast. However, its application in molecular imaging of cancer has been limited due to the lack of sensitivity and detection accuracy in identifying the biological processes of this disease. Recently, superparamagnetic nanoparticles have received considerable attention as molecular imaging probes with substantially higher molar relaxivities over small molecular T_1 agents. Conventionally, T_2*-weighted (T_2*-w) method serves as the gold standard for the imaging of superparamagnetic probes. However, this method requires a pre-contrast scan and a post-contrast scan to visually detect contrast changes, and is prone to image artifacts due to B_0 inhomogeneity. Herein, we combine an ultra-sensitive design of cancer-targeted superparamagnetic polymeric micelles (SPPM) and an off-resonance saturation (ORS) method to enhance the imaging efficacy of tumor biomarkers *in vivo*.

Methods: SPPM was prepared by encapsulating Fe_3O_4 nanoparticles inside poly(ethylene glycol)-*block*-poly(D,L-lactic acid) (PEG-PLA) polymeric micelles. A size distribution of SPPM was analyzed using dynamic light scattering (DLS) and TEM. *Cyclic* (Arg-Gly-Asp-D-Phe-Lys) (cRGDfK) peptide was conjugated to SPPM via a thiol-maliemide chemistry and quantified by an amino acid analysis. Radioactive SPPM was prepared by attaching tritium-labeled acetyl chloride ($ClC(O)CT_3$) to the hydroxyl group of the PLA end of the polymer. Three SPPM formulations; cRGD-encoded SPPM, cRGD-free SPPM, and cRGD-encoded SPPM plus free cRGD, were injected *i.v.* (dose 6 mg Fe/kg) into mice bearing A549 lung tumor xenografts (n=4). ORS MR images were acquired prior to and 1 h after the injection. The accumulation of SPPM was validated by Prussian blue staining of tumor slices. Blood half-lives of SPPM were examined in mice (n=3) by scintillation counting of plasma over 24 h. Biodistribution of all SPPM formulations was investigated by injecting tritium-labeled SPPM *i.v.* (n=3 each group). Mice were perfused 1 h after the administration of SPPM. Dissected organs were weighed, homogenized, and treated with scintillation cocktails for scintillation counting. Organ distributions were analyzed as percentage of injected dose per gram of tissue (%ID/g).

Results: TEM analysis revealed that each SPPM contained 45±14 SPIO particles. The average diameter of SPPM was 75±11 nm as obtained from DLS and TEM. The degree of conjugation of cRGD peptide by an amino acid analysis was 18%. One hour after SPPM injection, ORS contrast images showed a clear identification of A549 tumors by cRGD-encoded SPPM probes. The contrast-to-noise ratios of the tumor over background muscle tissue were 10.7 ± 3.0, 5.1 ± 1.6, 5.3 ± 0.7 ($p = 0.02$) for tumors treated with cRGD-encoded SPPM, cRGD-free SPPM, and cRGD SPPM co-injected with free cRGD respectively. Pharmacokinetic studies of the SPPM showed that cRGD-encoded SPPM had blood circulation half-lives of 0.34±0.09 and 3.9±0.8 h for the α- and β- phases, respectively. cRGD-free SPPM had the α-phase half-life of 0.40±0.34 h and the β-phase half-life of 9.2±0.8 h. Moreover, biodistribution of SPPPM showed that tumor uptake of cRGD-encoded SPPM (1.3±0.3% ID/g) was significantly higher than that of cRGD-free SPPM (0.6±0.3% ID/g). The co-injection of the free cRGD peptide decreased the tumor accumulation of cRGD-encoded SPPM (0.6±0.1 % ID/g).

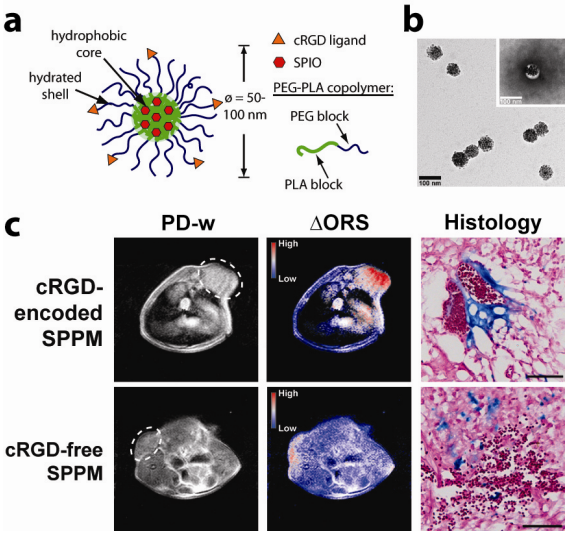

Figure 1. (a) A schematic representation of cRGD-encoded SPPM; (b) a TEM image of SPPM, an inset shows a negative stained image of the same sample; and (c) MRI images of animals 1 h after SPPM administration.

Conclusions: An $\alpha_v\beta_3$-specific cRGD peptide was successfully conjugated onto the surface of monodisperse Fe_3O_4-loaded polymeric micelles. cRGD-encoded SPPM showed an increased accumulation in tumor over cRGD-free SPPM as confirmed by biodistribution studies, ORS MRI, and histology. SPPM showed prolonged blood circulation half-lives in mice. The combination of ORS imaging with a tumor vasculature-targeted, ultra-sensitive SPPM design offers new opportunities in molecular imaging of cancer.

References:

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