In Vivo Targeting of αβ3-specific Dual-modality Micellar Nanoprobes

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Introduction

Multimodality tumor-specific nanoprobes that incorporate two or more imaging functionalities are becoming an integral part of early detection, molecular diagnosis and post-therapy assessment of cancer (1,2). Magnetic resonance imaging (MRI) and optical imaging are two complementary imaging modalities that provide superb anatomical and functional imaging information, respectively. Here we report the development of a dual-modality fluorescent superparamagnetic polymeric micelles (FSPPM, Fig. 1A) and demonstrate specific tumor targeting in vivo. During tumor growth, one essential requirement is its ability to acquire an adequate blood supply; therefore, tumor vasculature is in a perpetual state of angiogenesis. Integrin αβ3 is a well-established biomarker for angiogenesis with overexpression in tumor vasculatures and low to no expressions in resting endothelial cells. Here we demonstrate the use of dynamic contrast enhancement (DCE) MRI and fluorescent imaging to image αβ3 biomarkers in a human lung A549 xenograft in mice.

Experimental Methods

FSPPM were prepared by loading SPIO nanoparticles (9 nm dia.) into the core of tetramethylrhodamine (TMR) conjugated polymeric micelles. FSPPM were split equally into two batches and underwent conjugation with cyclic Arg-Gly-Asp (cRGD) or cyclic Arg-Ala-Asp (cRAD, a control peptide that does not bind to αβ3) (3). FSPPM from 0.1 to 100 µM Fe concentrations in PBS buffer were analyzed to determine optimal acquisition conditions with T2*-weighted method. Athymic nude mice bearing A549 non-small cell lung cancer xenografts were injected with cRGD- or cRAD-FSPPM. All MRI experiments were performed on a 7T Varian small animal imager. Temporal resolution was 1.3 s. For histological analysis, mice were injected with Hoechst 33342 i.v. and the dye was allowed to circulate for one minute. Tissue sections (10 µm) were imaged by a fluorescence microscope.

Results and Discussion

The optimal repetition time (TR), echo time (TE) and flip angle from FSPPM phantom studies were 10 ms, 3 ms and 45° using a gradient echo sequence, respectively. To analyze the αβ3-targeting specificity of cRGD-FSPPM, an imaging array of T2*-w images were collected for 1 hr. Baseline images (0-60 s) before FSPPM injection showed constant image contrast. After FSPPM injection, leg blood vessels (systemic circulation) showed a maximum MR signal enhancement (SE) of 12.5 and 15.6 for cRGD- and cRAD-FSPPM treated mice, respectively, indicating higher systemic concentration of cRAD-FSPPM. In contrast, hot-spot ROIs in tumors for cRGD- and cRAD-FSPPM treated mice, respectively, indicating higher systemic concentration of cRAD-FSPPM. In contrast, hot-spot ROIs in tumors for cRGD-FSPPM (green, Fig.1B) showed a significant increase of image contrast over cRAD-FSPPM (pink, Fig.1B). cRGD-FSPPM reached a maximum SE of 6.4 at 160s, while cRAD-FSPPM showed a maximum of 0.3-0.8 SE. The difference in SE between αβ3-targeted cRGD-FSPPM and non-targeted, cRAD-FSPPM, was 6.8±1.8 fold (n=2). Fluorescent imaging of tumor tissue sections confirmed the higher accumulation and close association of cRGD-FSPPM (red color, Fig. 1C) with perfused vessels (stained blue by Hoechst) whereas RAD-FSPPM (Fig. 1C) showed a diffusive pattern in the tumor parenchyma.

Conclusion

αβ3-specific FSPPM showed increase in tumor accumulation compared to non-specific cRAD-FSPPM nanoprobes. The combination of MR and fluorescent imaging with a cancer-targeted, dual modality FSPPM may offer new insights on molecular imaging of tumor angiogenesis.

References


Figure 1. In vivo αβ3 targeting using dual-modality micellar nanoprobes. (A) Scheme of a dual-functional FSPPM nanoprobe; PEG-PLA= poly(ethylene glycol)-b-poly(D,L-lactide), MeO=methoxy, Mal=maleimide. (B) Quantification of cRGD- and cRAD-FSPPM signal enhancement by DCE-MRI in leg blood vessels (blue, red) and tumor vessels (green, purple) using a gradient echo sequence. MR parameters: TR/TE=10/3 ms, NA=1, FOV: 40mm x 40mm, matrix=128 x 128. (C, D) Corresponding tissue sections from cRGD- and cRAD-FSPPM, respectively. Red: FSPPM, blue: Hoechst dye, scale bar = 50µm.