In vivo imaging of $\alpha_v\beta_3$ - targeted micellar nanoparticles in an NSCLC tumor xenograft model by off-resonance saturation method.

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Introduction

The increasing use of superparamagnetic iron oxide (SPIO) particles as MRI contrast agents in molecular imaging and cell labeling and tracking has augmented the development of novel imaging acquisition methods and SPIO formulations.[1] Vital to SPIO imaging is the ability to accurately identify and quantify SPIO particles *in vivo*. Conventional, T_2-w and T_2*-w methods are used for imaging SPIO. The negative contrast produced by SPIO particles, under these parameters, can limit the accuracy and specificity of SPIO detection by causing image voids at high local SPIO concentrations and the necessity of a pre-contrast scan. Here we combine an ultra-sensitive design of $\alpha_v\beta_3$ - targeted superparamagnetic polymeric micelles (SPPM) and an off-resonance saturation (ORS) method[2,3] to allow for accurate *in vivo* contrast imaging of tumor neovasculature in mouse xenografts.

Experimental Methods

SPPM were fabricated by loading SPIO nanoparticles (9 nm dia.) into the core of polymeric micelles. SPPM underwent surface functionalization, via malemide chemistry, with cyclic Arg-Gly-Asp (cRGD) or were quenched with Cys to produce two populations of SPPM, cRGD-encoded and cRGD-free.[4] MR phantom studies were performed by suspending SPPM in physiological buffers. Nude athymic mice were used with NSCLC tumor xenografts. ORS imaging was performed using a spin-echo pulse sequence modified by the addition of a frequency-selective Gaussian-shaped pre-saturation pulse. RF irradiation was applied using a B_1 of 104 Hz and frequency offsets of ±200 Hz – 4kHz (relative to water). Reference images were collected using identical parameters except without the pre-saturation pulse, along with conventional T_2-w images. All images were collected at 4.7T (Varian, Small Animal Imager)

Results and Discussion

The ORS contrast was represented as the ratio of Mz^o/Mz, where Mz^o and Mz are the magnitudes of water z-magnetization without and with a pre-saturation pulse, respectively. SPIO phantom studies showed that (1) at the same offset frequency, higher SPPM concentrations yield greater ORS contrast; (2) at equivalent SPPM concentrations, greater ORS contrast was observed with a decrease in offset frequency. Moreover, the ORS method was able to detect SPPM concentrations as low as 22pM, demonstrating the ultra-sensitive nature of SPPM probes; and finally, ORS was sensitive even at the highest SPPM concentration (data not shown).

To demonstrate the ORS method in vivo, mice with human NSCLC tumor xenografts were injected intravenously with either cRGD-encoded SPPM (Fig. 1, A-D) or cRGD-free SPPM (Fig. 1, E-H). Prior to SPPM injection, ORS images were obtained and neither cRGD-encoded nor cRGD-free SPPM showed observable ORS contrast (data not shown). After SPPM injection, ORS images showed a clear identification of tumor by cRGD-encoded SPPM (Fig. 1C). Histological analysis confirmed the targeting of cRGD-encoded SPPM in tumor vasculature cells (Fig. 1D). In comparison, ORS images showed a weaker tumor contract with cRGD-free SPPM (Fig. 1G), and showed a diffusive pattern of SPPM in the tumor parenchyma (Fig. 1H). This result agrees with previously published results of $\alpha_v\beta_3$ targeting with cRGD-encoded micelles.[5]

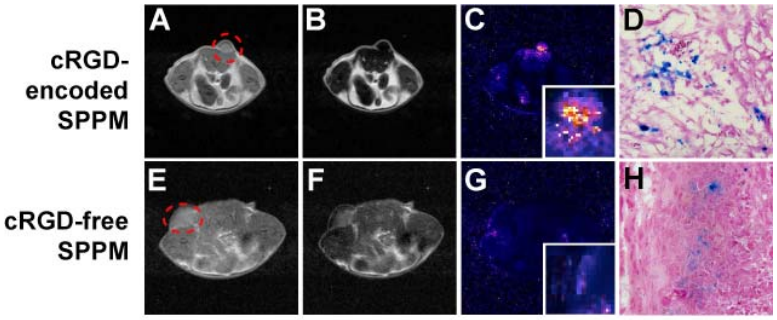

Figure 1. *In vivo* ORS imaging of intravenously administered cRGD-encoded and cRGD-free SPPM. (A,E) Post-injection MR image without the pre-saturation pulse; (B,F) post-injection MR image with the pre-saturation pulse; (C,G) the ORS Mz/Mz^o ratio image obtained by dividing image (B,F) by image (A,E), respectively. (D,H) Prussian Blue staining of related tissue sections, depicts SPIO in blue and tumor tissue in pink, scale bar = 10μm. Tumor circled in red, insets are magnifications of tumor ROI (C,G). Other imaging parameters: TR=2s, TE=9ms, FOV 40x40mm; data matrix=128x128, slice thickness = 3mm; B_1=104Hz and t=500ms.

Conclusion

ORS method greatly improved the tumor detection accuracy and sensitivity over the conventional T_2-*w* method by its ability to turn "ON" and "OFF" the SPPM contrast. This combination of ORS imaging with cancer-targeted, ultra-sensitive SPPM design offers new opportunities in molecular imaging of cancer.

References

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