A NIR830-Bevacizumab-Conjugated Iron Oxide Nanoparticle Probe for Vascular Endothelial Growth Factor (VGEF) Targeted MRI

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Propose: Vascular endothelial growth factor (VEGF) plays a pivotal role in the cascade of development and progression of cancers by promoting angiogenesis [1]. Targeting this biomarker would be a logical strategy for imaging based cancer detection and anti-angiogenesis treatment [2]. Bevacizumab (Avastin®) is a recombinant humanized monoclonal antibody directly against VEGF and is currently used in clinic as angiogenesis inhibitor for treating various cancers with VEGF overexpression [3]. In this study, a VEGF targeted molecular imaging probe was developed by conjugating near infrared dye (NIR830) labeled bevacizumab to magnetic iron oxide nanoparticles (IONP) for optical and magnetic resonance (MR) imaging of cancers overexpressing VEGF. It will be benefit potentially for monitoring tumor treatment response.

Methods: NIR830-NHS [4] or fluorescein isothiocyanate (FITC) labeled bevacizumab was conjugated on water soluble IONP (a core size of 10 nm in diameter). The ultraviolet-visible (UV) absorbance spectra of NIR830-NHS, bevacizumab, bevacizumab-NIR830 were measured. The characterizations of NIR830-Bevacizumab-IONP were measured by transmission electron microscopy (TEM) and dynamic light scattering (DLS). Bovine serum albumin (BSA) was employed as a control and the preparation of BSA-NIR830-IONP was similar to that of bevacizumab-NIR830-IONP. The targeting effect of NIR830-bevacizumab-IONP to VEGF over expressed cells was investigated by the cell uptake experiment and blocking assay using 4T1 breast cancer cells overexpressing VGEF. A breast cancer mouse model was established by subcutaneous injection of 1.0×10^6 4T1 cells to mouse mammary fat pad in 6- to 8-week old female Balb/c mice. NIR830-Bevacizumab-IONP or NIR830-BSA-IONP were injected into tumor bearing mice (n = 3/group) at a dosage of 20 mg Fe/kg of body weight, respectively. Optical and MR imaging were performed before, 48 hours and 96 hours after administration. For MRI done at 3T, T2-weighted fast spin echo imaging (T2WI) and T2 mapping sequences were included using a volumetric coil with 120 mm × 96 mm field of view, flip angle of 150°, and slice thickness of 1.0 mm. The changes in MRI signal intensity and T2 values were calculated from four slices selected from images of each tumor using the region of interest (ROI) approach. Results are presented as mean ± standard deviations (SD).

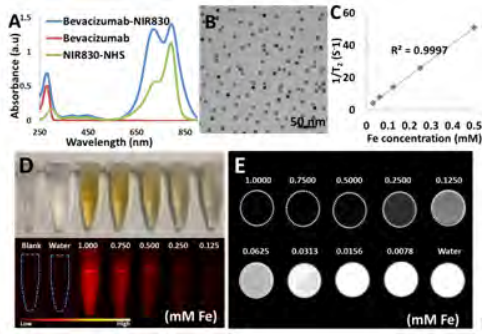

Figure 1. (A) UV spectra of bevacizumab, NIR830-NHS and bevacizumab-NIR830; (B) TEM of NIR830-bevacizumab-IONP; (C) the r_2 relaxivity of NIR830-bevacizumab-IONP; (D) optical and (E) T2 contrast effect of NIR830-bevacizumab-IONP at different conxentrations.

Results: The successful conjugation of NIR830 with bevacizumab was confirmed by the UV-vis spectra, where bevacizumab-NIR830 exhibited characteristic peaks for bevacizumab at 280 nm and NIR830 at 790 nm (**Figure 1A**). The NIR830-bevacizumab-IONP was monodisperse in aqueous as observed in TEM and with hydrodynamics size of 21.3 ± 4.1 nm and zeta potential of -26.6 ± 1.3 mV (**Figure 1B**). The optical and MRI contrast effect of NIR830-bevacizumab-IONP was concentration dependent and the r_2 relaxivity of the probe is 99.2 $s^{-1}mM^{-1}$ at 3T (**Figure 1C-1E**). The cell uptake experiments and blocking assay demonstrated the targeting effect of bevacizumab conjugated IONP to VEGF as 4T1 cells showed remarkable uptake of the probe and then significantly reduced blocked when VEGF receptor was inhibited (**Figure 2**).

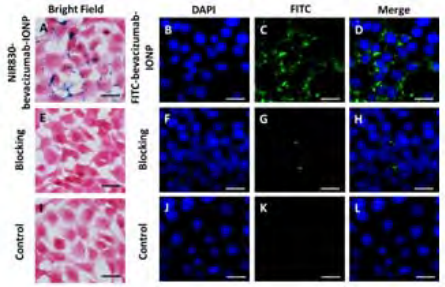

Figure 2. Microscopic images of Prussian blue stained 4T1 cells (A) incubated with NIR830-bevacizumab-IONP, (E) blocking assay and (I) control. Fluorescent images of 4T1 cells incubated with FICT-bevacizumab-IONP: (B) DAPI, (C) FITC, (D) merge; blocking assay: (F) DAPI, (G) FITC, (H) merge; and control: (J) DAPI, (K) FITC, (L) merge.

Before administration of the VEGF targeted probe and controls, the tumors were hyperintense on the T_2WI images with MRI signal intensity of 848.3 ± 51.0 for the group receiving bevacizumab-NIR830-IONP and 801.132 ± 76.3 for the group receiving NIR830-BSA-IONP. The tumors exhibited strong T2w "darkening" contrast at both 48 and 96 hours post injection in tumors treated with targeting NIR830-bevacizumab-IONP compared to the control indicating accumulation of IONPs (**Figure 3A-3F**). Quantitative analysis revealed that the extent of hypointense in MRI signal was more significant in mice received NIR830-bevacizumab-IONP in comparison to those injected with BSA-NIR830-IONP as the percentage of MRI signal intensity decrease in NIR830-bevacizumab-IONP group and NIR830-BSA-IONP group were 52.4 ± 11.0% versus 26.9 ± 12.4% at 48 hours post injection, n = 3, $p < 0.05$; and 51.3 ± 14.6% versus 26.2 ± 13.6% at 96 hours post injection, n = 3, $p < 0.05$, respectively (**Figure 3 G**). For optical imaging, tumors revealed no optical signal before receiving contrast agents. At 48 hours and 96 hours post-contrast, tumors received NIR830-bevacizumab-IONP presented strong optical signal whereas those injected with BSA-NIR830-IONP showed much lower optical signal (**Figure 3H-3M**).

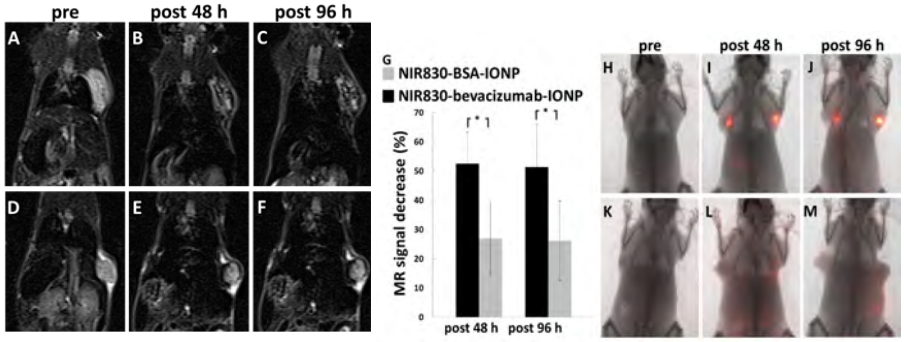

Figure 3. MR images of mice treated with NIR830-bevacizumab-IONP: (A) pre, (B) 48 h and (C) 96 h post injection; and mice treated with NIR830-BSA-IONP: (D) pre, (E) 48 h post injection and (F) 96 h post injection. (G) MR signal decrease post injection (%), * $p < 0.05$. Optical images of mice treated with NIR830-bevacizumab-IONP: (H) pre, (I) 48 h post and (J) 96 h post injection; and mice treated with NIR830-BSA-IONP: (K) pre, (L) 48 h and (M) 96 h post injection.

Conclusion: The results demonstrated the feasibility and efficacy of NIR830-bevacizumab-IONP probe as a VEGF targeting molecular imaging probe. This optical and MRI dual-modality imaging probe can potentially be used for imaging of cancers with VEGF overexpression and delivery of bevacizumab for imaging guided anti-cancer treatment.

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