Multimodal Molecular Imaging of Angiogenesis in a Mouse Model of Melanoma

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Introduction: *In vivo i*maging provides a powerful approach for non-invasive, longitudinal characterization of preclinical small animal cancer models. Moreover, *targeted imaging* has the potential to address the specific molecular and functional abnormalities underlying the process of tumor development. Angiogenesis, the formation of new blood vessels from preexisting mature vasculature, is a critical feature of tumor growth and metastasis, and endothelial cell activation represents one of its main biomarkers [1, 2]. In this study, we utilized novel transgenic mice that genetically biotinylate developing vascular endothelial cells and selectively targeted these cells with multiple avidinated probes to achieve multimodal contrast enhancement of vessels involved in angiogenesis.

Methods: *Ts-Biotag* transgenic mice were generated to coexpress an engineered bacterial biotin ligase (BirA) and a cluster of BirA substrate sequences (Biotags) under the expression of a minimal/short *Tie2* promoter (*Ts*) [3] to effectively biotinylate endothelial cell membranes during angiogenesis [4]. To demonstrate the potential of this system for analysis of tumor angiogenesis, we subcutaneously inoculated 1.10x6 B16-BL6 melanoma cells in the lower flank of wildtype (WT) and *Ts-Biotag* mice, and performed targeted imaging studies 12-14 days post inoculation when tumors maximum diameter ranged at 5-10 mm and angiogenesis was expected to be active. To assess targeting to biotinylated vasculature, we obtained images after tail vein injection of streptavidin conjugated to Alexafluor-680 for near infrared (NIR) imaging (data not shown), Avidin-fluorescene (Av-FITC) for fluorescence microscopy, avidinated microbubbles (Av-Microbubbles) (Micromarker™ Target-Ready, VisualSonics) for ultrasound (US), and avidin-DTPA-Gd (Av-DTPA-Gd) for MRI. MRI experiments were performed on a 7T micro-MRI (Bruker Biospec), using a volume coil transmit / surface coil receive system (Bruker). Pre and post contrast T1-weighted images were obtained with a 3D gradient echo sequence (TE/TR=3.27/20ms, FA=90°, FOV=2.0 cm^3, resolution= 200^3, NEX=6, imaging time ~27mins). 3D analysis of the MR images were performed using AMIRA (Visage Imaging) including segmentation, 3D surface rendering and maximun intensity projection (MIP) to examine increase in signal intensity due to effective contrast binding. Quantification of differential contrast enhancement in MRI images was performed by ROI analysis of the entire tumor volume using Image J software. After imaging, mice were cardio-perfused and tumors extracted for histological and immunohistochemical analysis.

Results and Discussion: *Ts-Biotag* transgenic mouse melanomas showed, with high specificity and sensitivity, *in vivo* binding of avidinated probes using multiple imaging modalities (Fig. 1). This system provided selective labeling of neovasculature in melanoma tumors by targeting angiogenic endothelial cells expressing *Tie2* as confirmed by histology (Fig. 2). Our results demonstrated that the *Ts-Biotag* mouse is a novel multimodal targeted imaging system with the potential to provide spatiotemporal information about tumor angiogenesis and its relationship to specific disease stages. In future, this imaging approach should allow early assessment of the response and efficacy of different anti-angiogenic therapies.

References: [1] Carmeliet P et al. Nature (2000). 407:249–57; [2] Kerbel, R, Folkman J. Nat. Rev. Cancer (2002). 2:727-739; [3] Minami T et al (2003). Arterioscl Thromb vasc Biol 23(11):2041-7; [4] Berrios CA, Bartelle, B et al Proc. Intl. Soc. Mag. Reson. Med. 18 (2010); [5] Willmann J et al.Radiology(2008). 246(2):508-518.

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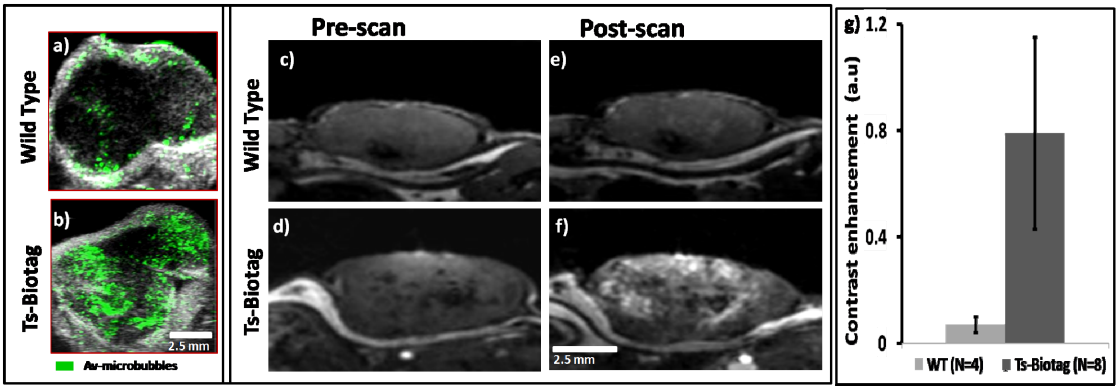

Fig. 1. Multimodal *in vivo* imaging of tumor angiogenesis. US images were acquired 4 minutes after injection of Av-Microbubbles in WT (a) and *Ts-Biotag* melanomas (b), using an acquisition sequence for nonlinear detection of microbubbles (green) [5]. MRI images were obtained pre (c,d) and 1h post (e,f) injection of Av-DTPA-Gd, showing contrast enhancement in the *Ts–Biotag* (f), but not in WT tumor (e). Contrast enhancement from MRI images was quantified in both WT and *Ts-Biotag* tumors (g)

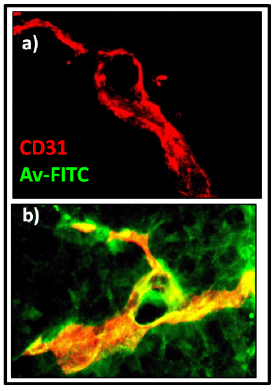

Fig.2. Histological Validation. Sections of extracted tumors 1hr after Av-FITC injection showed colocalization (yellow) of FITC signal (green) with immunostaining for endothelial cells (red) in *Ts-Biotag* (b) compared to WT tumors (a).