Targeting the endothelial cell surface: Novel transgenic mice for molecular imaging of vascular development

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Introduction

Paramagnetic contrast agents targeted to cell membrane receptors or other surface proteins are currently of great interest for molecular imaging with MRI [1]. A potential problem with current targeting methods is the limited targeting efficiency, which combined with the low sensitivity of many paramagnetic agents can severely compromise the application of these approaches for *in vivo* imaging. One way to circumvent problems in targeting contrast agents to surface receptors is to increase the binding affinity of the ligand to its target. An intriguing possibility is to take advantage of the high binding affinity of avidin and biotin ($kd=10^{-15}$). Other groups have exploited this system by labeling cell surface proteins using biotin ligase *in vitro* [2]. In the current study, transgenic mice expressing an engineered biotin ligase (BirA) and a cluster of biotinylation substrate sequences (Biotags) fused to a transmembrane protein domain were generated. Expression was driven by a minimal Tie2 promoter-enhancer [3], providing high transgene levels during angiogenesis in developing mouse embryos. Targeting was tested in embryos by means of intracardiac injections of an Avidin-Gd based T1-agent and high resolution 3D T1-weighted imaging.

Methods

For this study we developed transgenic mice expressing an engineered biotin ligase (BirA) and a cluster of biotinylation substrate sequences (Biotags) fused to a transmembrane protein domain from a minimal element of a Tie2 endothelial cell specific promoter [3].

As an initial experiment to test targeting efficiency in vivo, intracardiac injections of an Avidin-FITC probe were performed on E11.5 transgenic and wild type embryos, using ultrasound biomicroscopy (UBM)-guidance [4]. The fluorescent probe was allowed to circulate in the embryo bloodstream for approximately 1hr. Subsequently, the embryos were surgically extracted from the uterus, blood was flushed with a PBS heparin solution and the tissues were fixed for in ice cold 4% PFA in preparation for histological analysis. We performed similar injections with an Avidin-DTPA-Gd T1 contrast agent. Following surgical extraction from the uterus and flushing of blood with a PBS-heparin solution embryos were fixed in 4% PFA and maintained at 4° until imaging with micro-MRI.

Embryos were mounted in a syringe phantom surrounded by Fomblin perfluoroether (Solvay Solexis) for micro-MRI. 3D T1-weighted gradient echo (TE=6.3ms; TR=50ms; Flip Angle=40°; FOV=$(25.6mm)^3$; Matrix=512^3; Isotropic resolution=50-µm; Total imaging time=7hr, 17mins) images were acquired on a 7T Bruker Biospec system, imaging multiple embryos simultaneously in overnight scans. 3D image analyses were performed using Amira (Mercury systems) and Analyze software (Mayo Clinic), including segmentation and maximum intensity projection (MIP) to examine the 3D vascular structures and contrast agent targeting [5].

Results and Conclusions

Differential binding of the targeted Avidin-FITC was observed in Tie2-Biotag transgenic compared to wild type embryos (Fig.1). Expression of the transgene was higher in the smaller blood vessels and microvasculature that was undergoing active angiogenesis, but the transgene was also expressed in larger blood vessels within the transgenic embryos. Furthermore 3D-T1 weighted images of Tie2-Biotag transgenic embryos injected with Avidin-DTPA-Gd demonstrated higher contrast enhancement when compared to wildtype littermates (Fig. 2). Taken together, these results show great potential for the use of Tie2-Biotag transgenic mice for vascular development studies. In future, we plan to utilize motion-gated MRI acquisition [6,7] to investigate the extension of these approaches for *in vivo* imaging.

Acknowledgements

This work was supported in part by NIH grant R01 HL078665.

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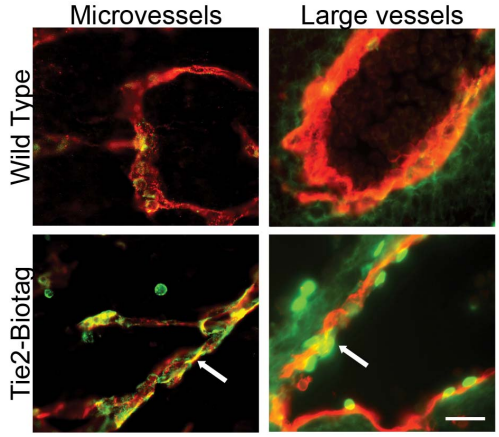

Fig 1: Fluorescence microscopy of E11.5 wildtype and Biotag transgenic histological sections of embryos injected with Avidin-FITC (green). Note the higher binding of an Avidin-FITC probe in the vasculature of transgenic embryos (arrows) when compared to wild type littermates. Red=PECAM endothelial cell marker. Scale bar = 25µm

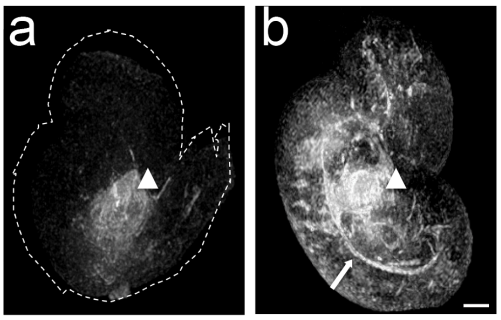

Fig 2: 3D T1-weighted images of E11.5 wild type (a) and Biotag transgenic (b) embryos injected with Avidin-DTPA-Gd contrast agent. Note the higher contrast enhancement in the dorsal aorta (arrow), heart (arrowhead) as well as sub-resolution vasculature in transgenic embryos when compared to wild type. Scale bar = 750µm