Bimodal Targeted Near Infrared/Positron Emission Tomography Contrast Agent to Image Liver Fibrogenesis

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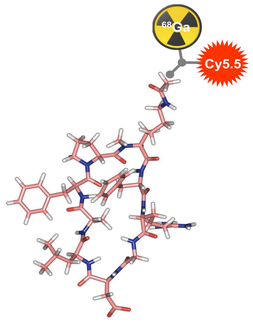
Introduction

There is an urgent need for quantitative imaging of liver fibrogenesis. The integrin αvβ6 is highly upregulated on cholangiocytes that drive fibrogenesis in advanced liver fibrosis of various etiology. αvβ6 binds to fibronectin and tenascin C, and activates latent TGFβ1. Here, we report on a bimodal αvβ6-targeted contrast agent for near infrared (NIR) and positron emission tomography (PET) imaging of liver fibrogenesis.

Methods

We developed a dual-labeled cyclic peptide specifically recognizing integrin αvβ6 based on a 9-mer cyclic RGD peptide (Maltsev et al., 2016), with sulfo-Cy5.5 for NIR fluorescence imaging and a Ga68-chelator for PET imaging. In vitro binding specificity was tested on αvβ6-positive and αvβ6-negative cell lines. In vivo uptake, biodistribution and clearance studies were performed in mouse models of biliary (Mdr2 KO) and parenchymal (CCl_4-induced) fibrosis compared to nonfibrotic controls. For NIR imaging a low and high dose (20 and 100 µg; 5.84 and 29.2 nM, resp.) of construct were injected intravenously. IVIS NIR in vivo whole body imaging was performed at 0.4, 2, 4, 6 and 12 h after contrast agent administration. For micro-PET imaging the Ga68-labeled construct (5 MBq/mouse) was injected and followed by mPET; organ specific signals were also quantified via gamma-counting. Fibrosis, fibrogenesis and αvβ6 expression were assessed by hydroxyproline quantification, ELISA and qRT-PCR.

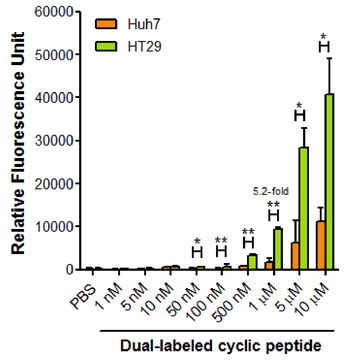


Results

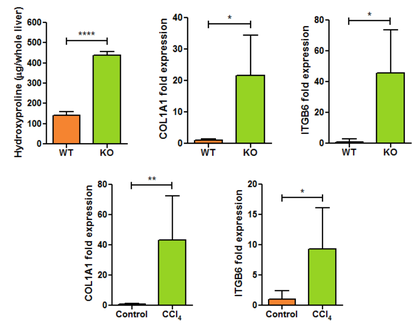
The NIR-dye-labeled cyclic peptide dose-dependently bound to αvβ6 expressing, but not to αvβ6 deficient cells ($p<0.01$). Mdr2 KO mice displayed a 3-fold elevated hepatic collagen deposition, and a 21.6-fold increased COL1A1 and 47.9-fold increased αvβ6 mRNA expression compared to wildtype mice. CCl_4-treatment induced a 43-fold COL1A1 and a 9.3-fold αvβ6 expression. NIR imaging with the bimodal cyclic peptide revealed a liver-specific 3.7-fold and a 3.2-fold enhanced uptake in biliary and CCl4 fibrotic livers, respectively, 6 h post injection, compared to the nonfibrotic controls ($p>0.01$). Comparable results were obtained with the mPET imaging using the Ga68-radiolabeled bimodal cyclopeptide.

Results

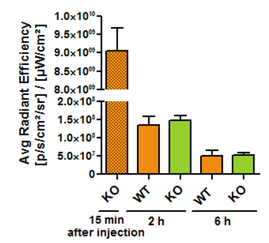
Binding affinities on HT-29 and Huh7 cells



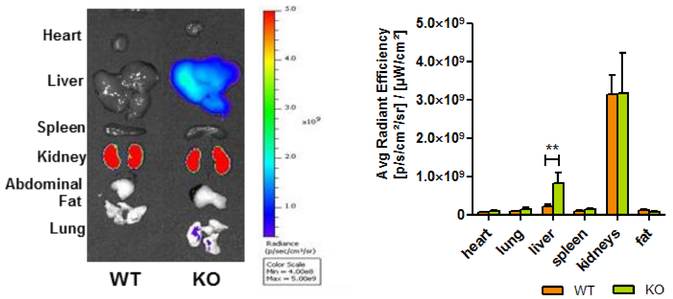
Hepatic collagen content & mRNA expression



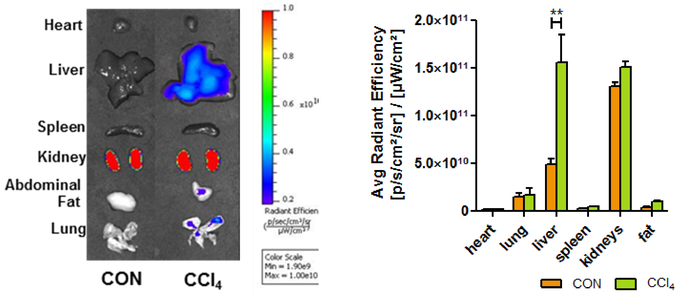
Time-course blood clearance



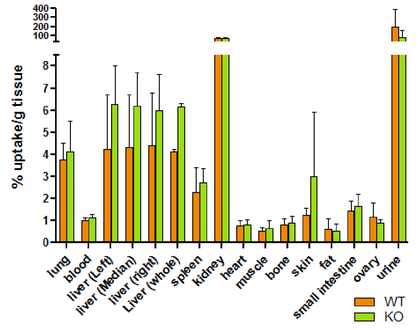
Organ NIR signals at 6 h post-injection in Mdr2 KO mice and WT controls



Organ NIR signals at 6 h post-injection in CCL4-induced fibrosis mice



Biodistribution of [^{68}Ga]cyclopeptide at 2 h post-injection in Mdr2 KO and WT mice



Conclusion

We designed a bimodal integrin αvβ6-specific liver fibrogenesis imaging agent based on a cyclic RGD peptide that can be used for NIR mouse imaging and for PET imaging in patients. To our knowledge, this is the first report on targeted molecular imaging to quantify liver fibrogenesis in vivo that may be used for early quantification of antifibrotic drug effects in vivo.

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