Tumor Imaging in a Mouse Xenograft Model of Human Adenocarcinoma Using a Novel Multimodal NIRF/MRI Probe

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Background

Two challenges which modern molecular imaging needs to overcome in order to acquire reliable and specific, high fidelity visual information for the purposes of cancer diagnosis are:

1. the development of targeted strategies for tumor detection, based on precise molecular alterations in the process of carcinogenesis, and
2. the utilization of multimodal contrast agents that would allow one to simultaneously extract anatomic, physiological, and molecular information.

This study describes the application of such a targeted multimodal imaging probe, CLIO-EPPT, for tumor detection in a mouse model of human adenocarcinoma. CLIO-EPPT, a triple labeled nanoparticle consisting of an iron oxide core, labeled with Cy5.5, and polyvalently attached to FITC-labeled tumor-antigen specific peptides was developed by our group and shown to have significant specificity for epithelial adenocarcinomas. CLIO-EPPT targets underglycosylated MUC-1 (uMUC-1), a tumor associated antigen, which becomes available early in carcinogenesis due to deregulated expression and glycosylation of normal MUC-1. Furthermore, CLIO-EPPT combines the MR and optical imaging modalities, thus taking advantage of the high spatial resolution of MRI and the relative cost-effectiveness, potential for continuous data acquisition, and minimal tissue-autofluorescence associated with optical imaging.

Materials and Methods

In vivo MR imaging, using a 9.4T scanner, was performed on animals bearing bilaterally-injected uMUC-1-positive (ChaGo-K-1 lung, LS174T and HT-29 colorectal, and CAPAN-2 pancreatic adenocarcinoma) and uMUC-1-negative (U87 glioblastoma) tumors before and 24 hours after i.v. injection of CLIO-EPPT. NIRF imaging on the same animals was performed immediately after each MRI session. The MR and NIRF imaging results were correlated to data on the *in vivo* biodistribution of the probe in tumor-bearing animals. In addition, tumors were imaged *ex vivo* and analyzed by immunohistochemistry.

Results

Analysis of T2-weighted MR images of tumor-bearing animals revealed no significant change in signal intensity in uMUC-1-negative tumors, indicating that there was little or no accumulation of the probe. In contrast, a significant signal reduction was observed in regions of uMUC-1-positive tumors (52% decrease for LS174T tumors (Fig.1), 53% decrease for CAPAN-2 and 43% decrease for ChaGo-K-1 and HT-29 tumors vs 13-18% decrease in control U87 glioblastoma tumors).

The differential accumulation of CLIO-EPPT in uMUC-1 positive versus negative tumors as demonstrated by MR imaging was also confirmed by NIRF imaging. A high intensity NIRF signal was obtained from the uMUC-1-positive tumors (HT-29 shown in Fig. 2a, LS174T, ChaGo-K-1 and CAPAN-2) after injection of the CLIO-EPPT probe, whereas no significant signal was observed from the control U87 tumors.

Correlative dual channel fluorescence microscopy of tumor sections showed co-localization of signal in the green channel (derived from the FITC-labeled EPPT peptide) with fluorescence in the Cy5.5 channel (derived from the Cy5.5-labeled CLIO), indicating accumulation of the probe in uMUC-1-positive tumors after i.v. injection. Sections from MUC-1 negative glioblastoma tumors did not show any signal in either channel (Fig.2b).

CLIO-EPPT demonstrated *in vivo* biodistribution to organs comparable to that of the parental crosslinked iron oxides. Differential tumor accumulation between uMUC-1 positive and negative tumors was consistent with the *in vivo* imaging data. uMUC-1 positive tumors accumulated on average 3.4 times more CLIO-EPPT probe than uMUC-1 negative tumors (1.7±0.2 %ID/g vs 0.5±0.2 %ID/g; $p<0.0001$).

Summary

This study demonstrates the plausibility of using multimodal targeted imaging probes in order to obtain specific and comprehensive information about tumor localization and biology. CLIO-EPPT successfully localized to uMUC-1 expressing tumors and demonstrated favorable *in vivo* biodistribution. Considering the fact that uMUC-1, the tumor associated antigen targeted by CLIO-EPPT, is present on 50% of all human cancers, we believe that the imaging probe described in this abstract shows promise as the basis of a clinically relevant tool.

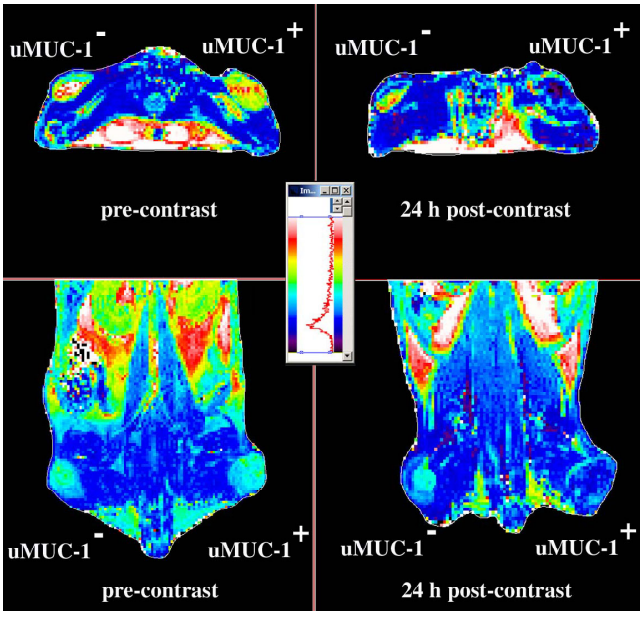

Figure 1. T2-weighted images of a mouse bearing bi-lateral tumors (LS174T uMUC-1 positive colorectal carcinoma and U87 uMUC-1 negative glioblastoma). Note the change in SI on the post-contrast image of the LS174T tumor.

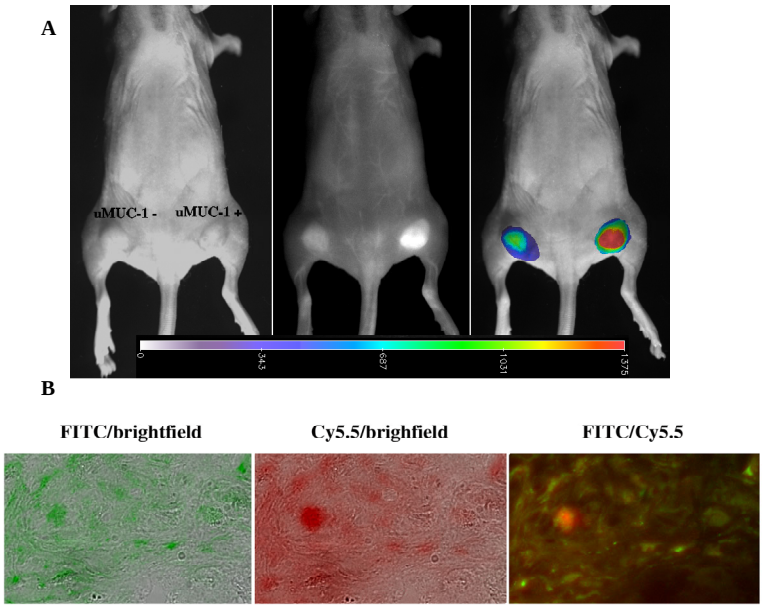

Figure 2. a) White light (left) and NIRF (middle) images, and color-coded map (right) of a mouse bearing bi-lateral HT-29 colorectal carcinoma and uMUC-1 negative U87 glioblastoma tumors. Note the high signal from the uMUC-1 positive HT-29 tumor.
b) Correlative fluorescence microscopy of tumor sections derived from the HT-29 tumor. Note the co-localization of signal from the FITC and Cy5.5 channels.