Synthesis and in vitro Characterization of a Combined MR/NIRF Imaging Probe for Cancer Detection

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Synopsis

This study describes the synthesis and in vitro characterization of a multimodal MR/NIRF imaging probe, CLIO-EPPT, which exploits cancer-associated upregulation and underglycosylation of the epithelial mucin antigen, MUC-1. The specificity of CLIO-EPPT for tumor cells was evaluated by a quantitative cell binding assay, flow cytometry, and fluorescence microscopy. MUC-1 negative cancer cell lines as well as noncancerous cell lines were used as controls. CLIO-EPPT was shown to specifically accumulate in MUC-1 expressing tumor cells. This investigation substantiates the plausibility of targeting underglycosylated MUC-1 in order to specifically recognize cells derived from MUC-1 expressing tumors.

Background

An early manifestation of carcinogenesis in a number of human cancers, including the majority of epithelial adenocarcinomas, hematological malignancies, as well as melanomas, astrocytomas, and neuroblastomas is the deregulated expression of the transmembrane mucin antigen, MUC-1. Aberrant MUC-1 expression by human tumors is characterized by two essential events: upregulated transcription and underglycosylation of the antigen's protein core. As a result, peptide epitopes cryptic in the nontransformed state, become available, making MUC-1 a legitimate tumor specific antigen which lends itself to targeting for imaging and therapy. This study describes the synthesis and *in vitro* characterization of a multimodal MR/NIRF imaging probe, CLIO-EPPT, which exploits the unique properties of cancer associated MUC-1.

Materials and Methods

CLIO-EPPT was synthesized as a nanoparticle consisting of a dextran cross-linked superparamagnetic iron oxide core for MR imaging labeled with Cy5.5 for NIRF imaging and polyvalently linked to a FITC-conjugated fifteen-residue peptide, EPPT, for flow cytometry and fluorescence microscopy (Fig.1). EPPT is derived from a monoclonal antibody specific for cancer-associated MUC-1 and shown to retain significant affinity for the antigen.

The specificity of CLIO-EPPT for tumor cells was evaluated by a quantitative cell binding assay, flow cytometry, and fluorescence microscopy. MUC-1 negative cancer cell lines (U87 and 293) as well as a cell line derived from normal breast epithelium (MCF10A) were used as controls.

Results

CLIO-EPPT had approximately the same size and R1 and R2 relaxivities as the parental compound CLIO-NH_2. Analysis of the absorption spectrum of CLIO-EPPT demonstrates three peaks associated with the absorption of Cy5.5 dye at 675 nm, FITC at 494 nm, and iron oxide absorption in the ultraviolet part of the spectrum. These peaks coincided with the corresponding peaks of the mixture of free compounds at the same concentration; therefore, we concluded that the obtained nanoparticles consisted of Cy5.5-labeled CLIO, conjugated to FITC-labeled EPPT peptides (data not shown).

In a cell binding assay which quantified the amount of iron associated with a panel of cell lines, MUC-1 positive cancer cell lines accumulated significantly higher levels of CLIO-EPPT ($p<0.05$) compared to nonneoplastic and MUC-1 negative cancer cell lines (Fig.2). The specificity of CLIO-EPPT for cancer associated MUC-1 was confirmed by fluorescence microscopy (Fig.3a). Signal from the FITC and Cy5.5 channels co-localized in the MUC-1 expressing cancer cell lines, whereas no signal was detected from either channel in the control line. Flow cytometric analysis in the FITC (FL1) and Cy5.5 (FL4) channels demonstrated variable binding of CLIO-EPPT by all MUC-1 positive cell lines and no binding by MUC-1 negative cells (Fig.3b).

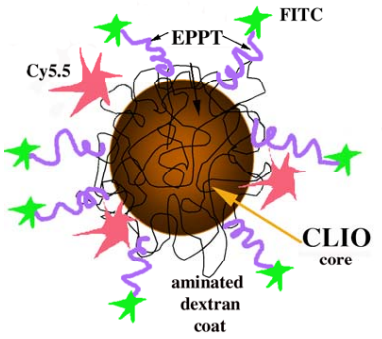

Figure 1. Design of CLIO-EPPT, a triple labeled probe for the detection of cancer associated MUC-1.

Summary

The current study describes the synthesis of a novel multimodal imaging probe for cancer detection. In addition, this investigation substantiates the plausibility of targeting underglycosylated MUC-1 in order to specifically recognize cells derived from MUC-1 expressing tumors. We believe that the target chosen for this study is highly relevant because in 1999 alone (latest data available), cancers that express the underglycosylated MUC-1 protein accounted for 72% of new cases and for 66% of deaths. The *in vitro* experiments described here set the stage for *in vivo* studies aimed at validating the potential of CLIO-EPPT for cancer imaging.

References

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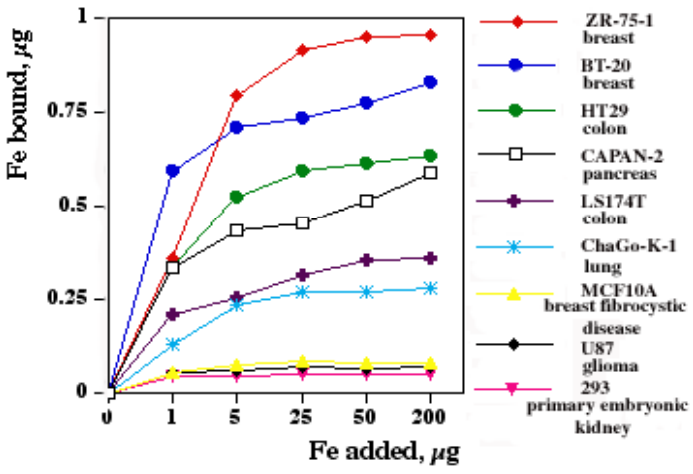

Figure 2. Differential accumulation of CLIO-EPPT in MUC-1 positive cancer cell lines versus MUC-1 negative cancer cell lines (U87, 293) and a nontransformed cancer cell line (MCF10A).

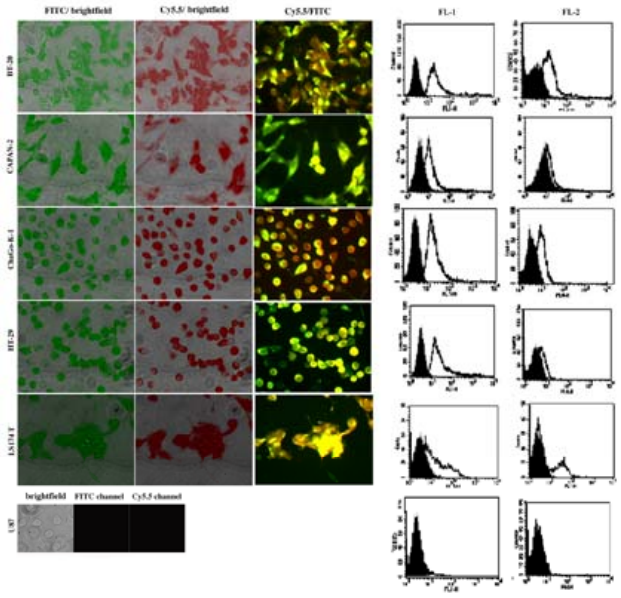

Figure 3. Specificity of CLIO-EPPT for MUC-1 positive cancer cell lines compared to a MUC-1 negative cancer cell line (U87) determined by a) fluorescent microscopy and b) flow cytometry. Signal from both the FITC (FL1) and Cy5.5 (FL4) channels was assessed