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Bioconjugation, Labeling, Evaluation and Initial In Vivo Imaging of Peptide Nucleic Acid-Antibody Conjugates

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The use of antibodies against tumor-associated cell surface antigens for the targeted delivery of radionuclides is a commonly used method in preclinical and clinical research. In vivo imaging studies show that maximum tumor concentrations of directly labeled monoclonal antibodies (mAb) are achieved in a day or more, but several days are required for background reduction and sensitive radioimmunoscintigraphy of tumors. Pretargeted delivery of imaging agents may improve imaging efficacy by faster clearance followed by minimization of nontarget tissue background compared to directly labeled antibodies. In this poster, we describe the conjugation, labeling, in vitro and initial in vivo imaging of a functionalized and decorated anti carcinoembryonic antigen (CEA) antibody with peptide nucleic acid (PNA) and fluorescent dye which may be suitable for pretargeted imaging studies. T84.66 mouse monoclonal antibody against human carcino-embryonic antigen was produced by hybridoma cell line and purified by FPLC with protein G column. The thiol-activated 15-mer PNA sequence was conjugated to an antibody by using a water-soluble, non-cleavable and membrane impermeable crosslinker Sulfosuccinimidyl-4- (N-maleimidomethyl) cyclohexane-1-carboxylate with an amine-reactive N-hydroxysuccinimide (NHS ester) and a sulfhydryl-reactive maleimide group. At pH 7.5, NHS esters react with amine groups of lysines present in the antibody, which serve as a good nucleophile to form stable amide bonds. Using the same chemistry principle, the antibodies and antibody-PNA conjugates were directly labeled with the NHS activated esters of Alexa fluor 647 dye. An average of 2 dyes and PNAs were conjugated to each antibody. SDS-PAGE gel, fluorescence imaging and MALDI-MS techniques were used to confirm the fluorescent dye and PNA conjugation. Initial in vivo imaging showed targeting of PNA & dye conjugated-antibodies of a CEA-expressing xenograft tumor model. The fluorescence optical imaging of these bioconjugates enabled us to validate the feasibility of our conjugation chemistry methods that are currently being developed for its in vivo applications in GE Healthcare Molecular Imaging & Medical Diagnostics technology.