Molecular imaging in vivo using signal amplification strategies

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The ability to image specific molecular biomarkers *in vivo* would have important implications for the earliest detection of various diseases, in assessing specific targeted therapies and for monitoring dynamic changes in expression patterns during disease progression. However, many molecular biomarkers are expressed in low numbers necessitating novel imaging signal amplification strategies.

We previously developed long-circulating near-infrared self-quenched probes for optical imaging of proteinases. These probes are based on biocompatible graft copolymers that carry covalently linked self-quenched near-infrared fluorescent dyes. We further hypothesized that by using paramagnetic electron donor compounds that rapidly oxidize and polymerize in the presence of peroxidase one could detect the presence of the enzymes in tissues by measuring T1-weighted magnetic resonance signal of the tissue. The signal of polymerized paramagnetic peroxidase substrate is higher than that of non-polymerized substrate due to the effect of the slower rotation of chelated paramagnetic cations in the polymerized substrates. We synthesized peroxidase-responsive contrast agents consisting of covalent conjugates of DOTA(Gd) and DTPA(Gd) with serotonin (5-hydroxytryptamine). The obtained paramagnetic probes were rapidly oxidized in the presence of peroxidases and human neutrophil myeloperoxidase (MPO) with the resultant 1.8-2 fold increase of gadolinium relaxivity. As a result, the enzymatic reaction could be imaged with MRI. Using a Matrigel™ tissue model system we observed MPO-specific brightening of MR signal suggesting the accumulation of polymerized substrate in Matrigel™ in mice. These experiments were recently expanded into using rabbit models of atherosclerosis and inflammatory cerebrovascular aneurysm.

Furthermore, MRamp technology was tested for targeted imaging of receptor expression in cancer models. Non-invasive assessment of EGFR (170 kD epidermal growth factor receptor) overexpression in cancer is essential in understanding the implication of this receptor in cancer progression and the expression of aggressive phenotype. We report a novel system designed for high-resolution imaging of receptor expression using MRI (magnetic resonance imaging). A therapeutic anti-human EGFR monoclonal antibody (EMD72000, matuzumab, Merck KGaA) was used as a platform for targeted delivery of a binary catalytic system resulting in a specific paramagnetic tagging of EGFR-overexpressing cells *in vivo*. Monoclonal antibody conjugates were obtained by linking horseradish peroxidase (HRP) or glucose oxidase (GO) to the antibody via stable hydrazone bonds. Purified conjugates were characterized using A431 (EGFR+) and SW620 (EGFR-) cell culture followed by in vivo MR imaging in human squamous carcinoma and adenocarcinoma xenograft models. The biodistribution experiments demonstrated preferential binding of both antibody and the conjugates to EGFR-expressing tumors *in vivo*. Receptor expression was detected using T1-weighted pulse sequences after systemic injection of a paramagnetic substrate. The substrate analogue, di-5HT-DTPA[^{111}In], was used in parallel biodistribution studies. In vivo imaging resulted in T1-wieghted signal maps that indicated the presence of specific EFGR+ signatures in tumor xenografts. The prominent MR signal enhancement was associated with EGFR+ tumor margins, and the observed patterns were consistent with the results of immunohistochemistry. The developed imaging approach has a potential in providing "in vivo histology" information reflecting receptor expression patterns and the effects of tumor microenvironment on the receptor expression repertoire.

Therefore, our research suggest that the presence of endogenous proteinases, exogenous peroxidase-linked targeted molecules, as well as endogenous MPO could be visualized *in vivo* using optical imaging or at high resolution using contrast enhanced-MRI.

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