MR Imaging of U87EGFR Human Glioma Tumor Xenografts Using Targeted Signal-Amplifying Enzymatic System

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Introduction: A truncated and constitutively active form of the EGF receptor variant III (EGFRvIII) is a major determinant of tumor growth and poor prognosis in glioblastoma multiforme (GBM) [1]. To test a system for targeted imaging of EGFRvIII we investigated the retention of peroxidase-generated products of a paramagnetic molecular substrate di(tyramido)-DTPA(Gd) (diTyr-DTPA(Gd), Fig. 1) in U87EGFR human glioma xenografts. F(ab’); fragments of humanized anti-EGFRvIII monoclonal antibody (mAb) EMD7200 were conjugated to deglycosylated horseradish peroxidase (HRP) and glucose oxidase (GOX) and used as a self-complementing enzymatic signal amplification system [2] for EGFRvIII targeted imaging. We anticipated that receptor expression sites will exhibit a prolonged MR signal enhancement due to the formation of polymerized products of diTyr-DTPA(Gd) oxidation by mAb conjugates [2].

Methods: The paramagnetic substrate diTyr-DTPA(Gd) was synthesized as described in [3]; F(ab’); fragments of mAb were linked to deglycosylated and hydroxylamine-capped HRP or GOX via bisaromatic hydrazine bonds and purified by size-exclusion HPLC. The purified conjugates were characterized in human glioma U87EGFR cell culture and optimal ratios of HRP and GOX conjugates were determined to provide the maximum signal with low cytotoxicity. F(ab’);–GOX and F(ab’);–HRP were also modified with NHS-MAG3 for radiolabeling with 99mTc to study the binding and uptake of the conjugates in vitro and in vivo. For in vivo studies, 5×106 U87EGFR cells were stereotaxically implanted in the brains of RNU rats. T1-weighted (T1-WT) spin-echo MRI at 3T was performed with the following parameters: TR/TE=700ms/8.2ms, FOV=2.56cm×2.56cm, matrix = 256×256, NEX=4. Ten days after tumor implantation, each animal was imaged on two occasions under isoflurane anesthesia. 1) Day 1 – a pre-contrast image was acquired followed by IV injection of 0.1 mmol/kg diTyr-DTPA(Gd). T1-WT images were then acquired over a 1.5-h period. 2) Day 2 – anti-EGFRvIII conjugates (100 µg mAb/animal) were injected IV. Both the DTCs (time in minutes) were determined and the duration of contrast agent enhancement measured.

Results and Discussion: Both 99mTc-labeled antibody conjugates showed specific binding to U87EGFR cells in vitro (Fig. 2A) and in vivo (Fig. 2BC). The binding was inhibited by the excess of cold mAb conjugates (Fig. 2A). Cell-binding and internalization studies showed that 85% of total cell-bound conjugates were retained on the surface at 4°C, whereas ~80% conjugates were internalized at 37°C (Fig. 3). T1-WT images showed significantly higher initial enhancement of the tumor pre-injected with conjugates (Fig. 4 (+)) over the same time period compared to Day 1. The washout of the contrast agent was best modeled using a biexponential decay equation: \( P(t) = s_1 \exp(-t/t_1) + s_2 \exp(-t/t_2) \). Bioelimination of diTyr-DTPA(Gd) was quantified by fitting the temporal signal-intensity decay for each day in the absence and presence of anti-EGFRvIII conjugates. In the current study, a short (\( t_1 \)) and long (\( t_2 \)) decay time constant (DTC) was observed as compared to Gil36aEGFR-bearing rats reported by us previously [4].

Conclusion: Administration of EGFRvIII-targeted mAb conjugates resulted in specific binding to U87EGFR cells of which at least ~20% remained on the surface enabling the reaction with the contrast agent. Following conjugate administration in vivo (Day 2), the retention of contrast agent was attributed to the longer DTCs compared to that of Day 1. These longer DTCs are consistent with enzyme-mediated retention of the paramagnetic products in the EGFRvIII-overexpressing cells in the tumor.