Specific Targeting of EGF Receptor Expression with Monoclonal Antibody Conjugates in Human Gliomas Using MRI

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Introduction: The goal of this study was to image EGFR- and EGFRvIII-overexpressing aggressive orthotopic human glioma tumors using local retention of peroxidase-generated products of a paramagnetic molecular substrate di(tyramido)-DTPA(Gd) (diTyr-DTPA(Gd), Fig. 1). EGF receptor variants were targeted by using specific humanized anti-EGFR EMD72000 and non-specific anti-EpCAM mAb monoclonal antibody (mAb) conjugates. The conjugates were synthesized using peroxidase (HRP) and glucose oxidase (GO) as a self-complementing enzymatic signal amplification system [1] as depicted in Fig. 1. MR signal was generated at the EGFR expression sites due to co-localization of targeted conjugates which resulted in specific binding of reactive intermediate products of diTyr-DTPA(Gd) oxidation by HRP-mAb conjugate.

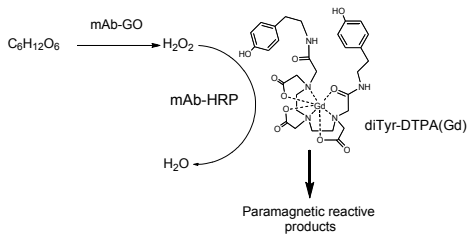

Fig. 1 – Reaction of peroxidase substrate diTyr-DTPA(Gd) with the enzyme pair (glucose oxidase/peroxidase) conjugated to either anti-EGFR or anti-EpCAM mAb.

Methods: The paramagnetic substrate diTyr-DTPA(Gd) was synthesized as described in [2]; mAb conjugates were synthesized by linking HRP or GO to mAb via bisaromatic hydrazone bonds. Size-exclusion HPLC purified conjugates were characterized in human glioma Gli36ΔEGFR cell culture and the ratios of HRP and GO conjugates were selected to provide the maximum signal with low cytotoxicity. Gli36ΔEGFR tumor xenografts were stereotaxically implanted in the brains of athymic rats. MR images were acquired at 3T. T1-weighted (T1-WT) spin-echo MRI was performed with the following parameters: TR/TE = 700ms/8.2ms, FOV = 2.56 cm X 2.56 cm, matrix = 256x256, NEX = 4. Two weeks after tumor implantation, each animal was anesthetized with isofluorane and imaged on two occasions. 1) Day 1 – a pre-contrast image was acquired followed by IV injection of 0.1 mmol/kg diTyr-DTPA(Gd). Twenty T1-WT images were then acquired over a 2-h period. 2) Day 2 – either specific anti-EGFR or non-specific EpCAM conjugates (100 µg mAb/animal) were injected IV. Four hours later, a pre-contrast image was acquired followed by IV injection of 0.1 mmol/kg diTyr-DTPA(Gd). Thirty T1-WT images were then acquired over a 3-h period. Animals were sacrificed and frozen brain sections were stained for peroxidase activity and EGFR expression.

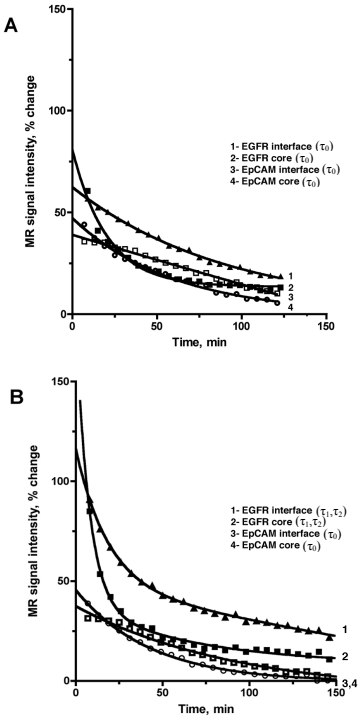

Fig. 3 – Normalized T1-WT signal intensities (derived from the same tumors shown in Fig. 2) measured in the interface or core regions of Gli36ΔEGFR tumors prior to injection of conjugates (**A**), and after the pre-injection of either specific anti-EGFR ("EGFR") or non-specific EpCAM ("EpCAM") conjugates (**B**) as a function of time post-diTyr-DTPA(Gd) injection.

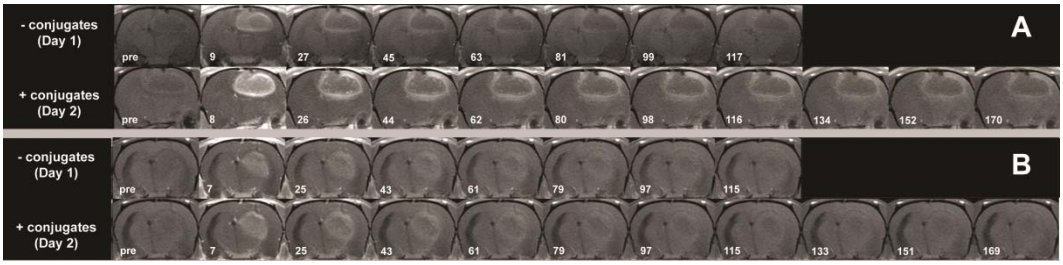

Fig. 2 A – T1-WT sequential rat brain images depicting enhancement of human glioma xenografts as a function of time post injection of diTyr-GdDTPA; Top row - temporal washout of diTyr-GdDTPA with no conjugate pre-injection (Day 1); Bottom row - washout of diTyr-GdDTPA following IV injection with anti-EGFR conjugates (Day 2) in the same animal slice (time shown in mins); **B** – human glioma xenografts without and with pre-injection of EpCAM-targeted conjugates. The images correspond to the same pattern as shown in Panel A.

Results and Discussion: T1-WT images showed enhancement of the tumor within minutes after IV contrast injection – either without (Day 1) or with (Day 2) the preinjection of either mAb conjugates (Fig. 2). However, the initial enhancement of the tumor with pre-injected anti-EGFR conjugates (Fig. 2A, Day 2 – 8 min) was significantly higher than that observed on Day 1 in the same animal (Fig. 2A, Day 1 – 9 min) and on both days in a different animal without and with pre-injected anti-EpCAM conjugates (Fig. 2B, – 7 min). Furthermore, contrast agent retention was also higher in the tumor pre-injected with anti-EGFR conjugates (Fig. 2A – Day 2) compared to all the other cases – particularly in the tumor interface region over the same time period. Spatial deconvolution of the tumor signal showed different rates of contrast washout for the interface and the core regions of the tumor.

$$S_1 = a_0 \cdot e^{-t/\tau_0} + offset \quad \text{[Eq. 1]} \qquad S_2 = a_1 \cdot e^{-t/\tau_1} + a_2 \cdot e^{-t/\tau_2} \quad \text{[Eq. 2]}$$

Bioelimination of diTyr-DTPA(Gd) was quantified by fitting the temporal signal-intensity decay (SID) for each tumor region. On Day 1, without any anti-EGFR or anti-EpCAM conjugate pre-treatment, a monoexponential [Eq. 1] best modeled the data for both the tumor interface and core regions (Fig. 3A, curves 1-4). Animals pre-injected with anti-EGFR conjugates (Fig. 3B, curves 1,2) exhibited biexponential signal decay both in the tumor interface and core regions; however, after the pre-injection of non-specific anti-EpCAM conjugates (Fig. 3B, curves 3,4), the SID showed only a single decay component. The single component of monoexponential decay – decay time constant (DTC) τ_0 – was attributed to unbound contrast agent. The two components of the biexponential model yielded a long and a short DTC (τ_1 and τ_2, respectively). The component with the short DTC (τ_2) was attributed to unbound contrast agent while the component with the long DTC (τ_1) was attributed to contrast agent bound to the cells.

Conclusions: Following anti-EpCAM conjugate pre-treatment or no conjugate administration, a monoexponential SID of the contrast agent indicates the absence of specific mAb conjugates binding to the tumor. This is expected since anti-EpCAM conjugates do not bind to glioma cells and diTyr-DTPA(Gd) does not bind to tumor cells in the absence of conjugates. With anti-EGFR conjugate administration, a biexponential mode of contrast SID indicates specific binding of paramagnetic products to cells, which led to long-term enhancement of MR signal (Fig. 2A – Day 2). This long-lasting MR signal component is consistent with enzyme-mediated coupling of the paramagnetic agent to EGFR-overexpressing cells in the tumor allowing effective MRI visualization of conjugate co-localization at the specific targeted site.

References: [1] Bogdanov, A., et al. (2007). Bioconjug Chem **18**: 1123-30; [2] Querol, M., et al. (2007). Chembiochem **8**: 1637-41.