Molecular Probes for Targeting and Imaging of Epidermal Growth Factor Receptor on Head and Neck Cancer Cells

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Introduction

A multifuctional molecular probe was investigated for the targeting and imaging of the epidermal growth factor receptors (EGFR) on head and neck (HN) tumor cells by combining a paramagnetic contrast agent, Gadolinium (Gd), with a monoclonal EGFR targeting antibody (mAb). EGFR is known to be over-expressed in more than 80% of HN cancers. The targeted therapy using the EGFR antagonist (Cetuximab, approved by FDA) is currently used for HN cancer treatment with concurrent radiation therapy (RT). Despite some advances in the development of targeted agents in cancer therapy, there is a lack of concurrent diagnostic feedback on targeting specificity of such therapies. Our motivation was that by combining Gd and the EGFR antagonist, we will be able to selectively target and image the tumor at the molecular level and provide diagnostic feedback for drug uptake.

Material and Methods

A chelating agent DiethyleneTriaminePentaacetic Acid (DTPA) anhydride, which reacts to the amine groups on the antibody, was used to bind Gd to mAb in alkaline conditions for molecular probe synthesis. Once the DTPA-mAb was formed, $GdCl_3$ was added to charge the probe for MR imaging. Size exclusion chromatography was performed to remove excess Gd in the solution.

A quantification assay, using methylthymol blue (MTB) as a titration indicator, was performed to determine the Gd content in the probe. The probe was tested on its ability to target various cell lines. Specifically, anti-EGFR antibody (Cetuximab) was used for probe synthesis. The target cells (TU159, Human HN cancer cell) and the control cells (HEK293, human embryonic kidney cell) were cultured to reach a cell density of $5x10^7$ cell/mL. The cells were fixed by 2% of ice cold glutaraldehyde. The probe was mixed with the fixed cells to allow target binding, followed by multiple PBS wash to remove unbound probes. Flow cytometry and MR imaging was applied to verify cell targeting and image contrasting respectively. In flow cytometry, shift in fluorescent intensity (SFI) was used to determine the relative EGFR expression level and the cell targeting ability of the probe. For MR imaging, the sample (cells with the probes attached) was immobilized in 5% alginate with $CaCl_3$. T_1 relaxation times were determined by the least-squares algorithm using a 3.0 Tesla Siemens Tim-Trio system at 25°C with the following settings: TE=12ms, TR= 6000ms, TI =[40, 50, 70, 100, 200, 300, 400, 1400, 2000, 2800ms].

Results and Discussion

MR imaging showed the probe target on TU159 and was able to present an increase in contrast compared with other cell samples (Figure 1). T1 for the TU159 cell with the probe was reduced to ~1400ms, compared to the baseline of 1500-1600ms (Figure 2). MTB assay indicated that an average of 64 Gd molecules was associated to each mAb (Ratio Gd/mAb =64, denoted as R64 probe). When using a R50 probe, T1 of 1470ms was observed. However, R80 probe was not able to show any significant cell affinity on both MR and flow cytometer. The increase in Gd payload was inversely proportional to the *in vitro* binding affinity of the mAb.

Conclusion

Our R64 probe was able to target EGFR on the cancer cell and showed an increase in contrast under MR imaging. This probe may be used to provide the diagniotic feedback of the ongoing targeted therapy with concurrent RT.

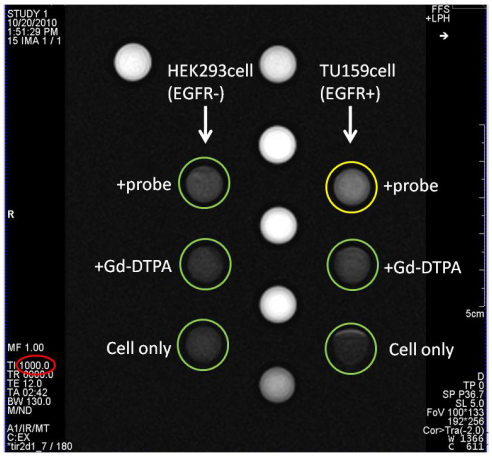

Fig 1. MR image with HEK293 cell (Left, control) and TU159 (Right, target). Cell samples with R64 probe (Gd/mAb ratio =64) and cell sample with Gd-DTPA were also imaged. Only the target cell with the probe (yellow circle) showed moderate contrast.

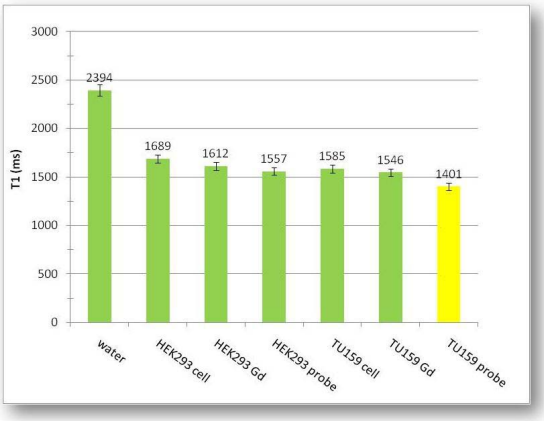

Fig 2. T1 for the samples in Fig 1. While other non-target cell samples has a T1 baseline of ~1600ms, T1 in the target cell line was reduced to ~1400ms (yellow bar)