

A Novel Targeted MRI Contrast for Glioma Using Interleukin-13 Receptor Conjugated Liposome

Xiaoli Liu¹, AB. Madhankumar¹, Patti A. Miller², Becky Webb¹, Kari A Duck¹, James R. Connor¹, and Qing X. Yang^{1,2}

¹Department of Neurosurgery, The Pennsylvania State University College of Medicine, Hershey, PA, United States, ²Department of Radiology, The Pennsylvania State University College of Medicine, Hershey, PA, United States

Introduction: Current contrast agent enhanced MRI is non-specific for brain tumor detection. The entire clinical MRI contrast agent such as Gd-DTPA (Magnevist[®]) requires a compromised blood brain barrier (BBB) for uptake by brain tissue. On the other hand, GBMs aggressively infiltrate into brain tissue where, in most cases, may not be enhanced by the MRI contrast. These undetectable infiltrating tumors are the major reason for relapses because it is difficult to resect these tumors completely, posing serious challenge for treating glioma. Thus, there is a critical need for a new MRI contrast agent with abilities to specifically highlight infiltrating glioma tumor cells. Previous work demonstrated that interleukin-13 receptors alpha 2 (IL-13R α 2) are highly expressed in glioma cells, but not in the normal cells. Thus, the IL-13R α 2 receptor can be a novel target for delivering imaging contrast agent specifically to gliomas. In this work, we demonstrated that our targeted liposomes captured infiltrating tumor that were totally absent in Magnevist-enhanced MRI.

Methods: Preparation of IL-13-Liposomes-Gd-FITC: IL-13-Liposomes-Gd-FITC were prepared using the lipids refer to [2] except the green fluorescent, lipophilic carbocyanine DiOC₁₈(3) was mixed with the lipids and the film was reconstituted in a saturated solution of Gd-DTPA with poly-L-lysine to form multilamellar vesicles. The average particle size was 100-150 nm. The Gd concentration was quantified in the range of 4.0-10.0 mg/L using ICPAES. The uptake of IL-13-Lip-Gd was tested in glioma cells. The relaxivity of Liposome-Gd was 0.7mmol/L when measured at 7T.

Glioma Tumor Model: Female athymic nude mice weighing 20-30g (5-8-weeks old) were anesthetized by intraperitoneal injection of ketamine-xylazine 10mg/kg-100 mg/kg body weight. Intracranial glioma was induced by implanting 50,000 human glioma stem cells (T3691) in the caudate putamen region. The tumor formation and volume will be routinely monitored using MRI with Magnevist enhanced T1-weighted MRI at 2 weeks post tumor induction. When tumor reaches to 5-10 mm in diameter within 3-4 weeks, it is ready for experiment.

Imaging: Adult mice with intracranial tumor were anesthetized with 2% isoflurane/oxygen. Axial T1-weighted images were acquired on a 7T MRI system (Bruker Biospin 7/20a, Ettlingen, Germany) with 540/11ms TR/TE, 8 NEX, 192x192, 3.2cm FOV, 0.5mm thickness with 0.5mm gap at following time-points: a pre-dose and post-dose scans at 10, 60, 120-minutes and 24-hours following a tail vein (IV) injection of either Gd-DTPA or the IL-13 Liposome-gadolinium. Then the brain tissue was in sect for making the slides with the thickness of 12 micron, which was stained by H&E or DAPI and then viewed by microscopy.

Results and Discussion: Fig. 1a and 1b demonstrated the results of MRI from an intracranial tumor enhanced by Magnevist and IL-13-Lip-Gd-FITC of the same mouse 24 hr apart. Our IL-13-Lip-Gd-FITC produced a similar contrast enhancement to Magnevist. Most excitingly, however, the MRI enhanced with our targeted liposome revealed several additional smaller tumor masses shown in Fig. 1b that are not visible in Magnevist enhanced image shown in Fig. 1a. These tumors (arrow) infiltrated away from primary tumor mass (not shown) were validated with histology of the same brain slice in Fig. 1c and 1e. The targeted liposomes appeared to distribute around the tumor tissues along white matter tracks, typically seen in human GBM. It is likely that these tumors had not caused a significant BBB damage since Magnevist enhanced MRI did not show these tumors. Our result from H&E staining (Fig. 1c and Fig. 1e) and fluorescent image in Fig. 1d demonstrated the sensitivity and specificity of our liposome targeting infiltrating gliomas.

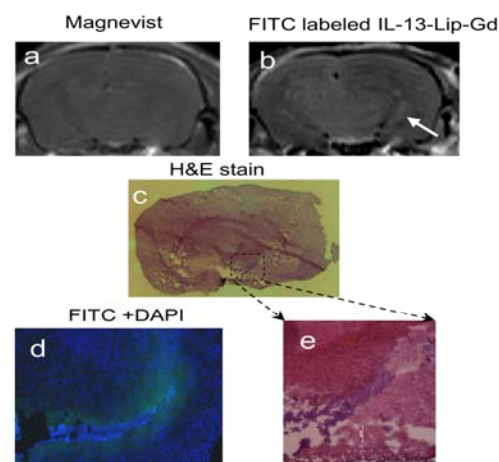


Fig. 1. MRI images enhanced with IL-13-Lip-Gd (b) identified infiltrating tumor masses (arrow) that are absent in the Magnivist enhanced MRI (a) of the same brain slice. The tumor tissues in the same brain area appear to be blue in H&E stain image (c and e), validated MRI finding. The green fluorescence of FITC+DAPI image of an adjacent slice (d) indicates targeted liposome distribution coincided with infiltrating tumors along the white matter track.

1. Debinski, W. et al., Mol Med, 2000. 6(5): p. 440-9.

2. Madhankumar, A.B., et al., Mol Cancer Ther, 2006. 5(12): p. 3162-9.