Targeted MRI Contrast Agent Using Bioengineered scFv Fragments with Gadolinium Labelled Metal Binding Domain

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Introduction: The development of receptor targeted contrast agents for MRI using monoclonal antibody (MAb) technology has been a longstanding goal. However, previous attempts have been limited due to steric hindrance in accessing the antigens, non-specific binding, low sensitivity of relaxation agents, limited numbers of available receptors, random incorporation of chelates, and reduced affinity of the gadolinium labeled antibody toward the targeted receptor. We postulate that MAb composed of single chain variable fragments (scFv) bioengineered with Gadolinium metal coordination sites as imaging reporting domains would circumvent many of these limitations. The objective of this work is to bioengineer high affinity scFv-Gd complexes, determine relaxivity of the agents in solution, and demonstrate signal changes in cell culture at 1.5T.

Materials/Methods: The scFv-Gd may be targeted towards any antigen, but we chose to use anti-transferrin scFv for our antibody, because many tumors are known to over-express this receptor. We used C6 glioma cells as our target, because of their known high concentration of transferrin antigens. Fibroblasts were used as our controls because of their relative paucity of transferring receptors.

The anti-TfR antibodies were bioengineered using two complementary routes: (1) natural immunization, affinity purification, and generation of fragments followed by conjugation of chelates and (2) preparation of the libraries with expression in yeast Pichia Pastoris and bacteria Escherichia Coli, followed by selection through bio-panning, and affinity purification. The antibodies were subsequently labeled with Gadolinium ions. Affinity testing was performed using Western blotting, surface plasmon resonance, ELISA, and immuno-fluorescence. Incorporation of Gd was verified using energy dispersive x-ray spectral imaging. Sub cellular localization of scFv-Gd derivatives was determined with atomic resolution by means of electron energy loss spectral imaging (EEL-SI).

Results/Discussion: We created anti-TfR antibodies capable of binding up to 100 Gd ions per molecule. The scFv-Gd complexes retained affinity towards TfR even at high levels of Gd labeling. Molecular relaxivity of water protons was ~200 mM ^-1 s^-1 at 9.4T. The high relaxivity resulted in MRI contrast changes at antibody concentrations as little as 0.1 μM, which is sufficient for imaging of receptors and ligands in vivo. The scFv-Gd fragments have much smaller size (29kDa) than natural IgG (155kDa), which improves their hydrodynamic diameter volumetric density and penetration of the target tissue. Absence of Fc fragments eliminates non-specific binding. Qualitative and quantitative differences in image contrast were demonstrated between the C6 glioma and fibroblast cells on inversion-recovery T1 weighted images following labeling. Measurements of T1 during MRI at 1.5T of cells labeled with our scFv antibodies indicated significant enhancement of proton relaxation times in TfR over-expressing gliomas (1443ms) relative to normal fibroblasts (1587 ms), and unlabelled cells (1750ms). EEL-SI confirmed localization of the scFv-Gd complex to the extracellular membrane of the C6 cells. The EEL-SI provided us the capability for definitive sub-cellular localization of the of the same scFv-Gd complex that we used for in-vitro MRI

Conclusions: We pioneered a technology of recombinant chimeric proteins consisting of scFv antibodies as targeting domains with metal coordination sites as MR imaging reporter domains. We then demonstrated that the scFv antibodies bioengineered with Gd domains are capable of labeling glioma cells in vitro, and that these contrast agents create a clinically relevant change in relaxivity by MRI at 1.5T.

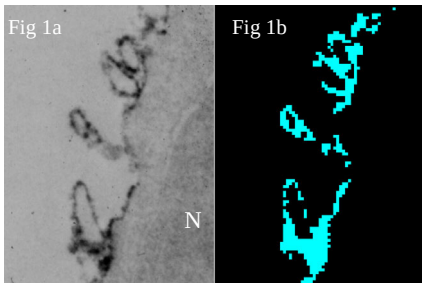
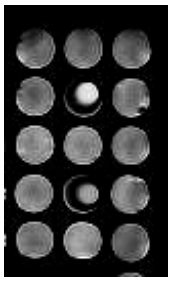


Fig 2

LabelledC6

Labelled Fibroblast



With electron energy loss spectroscopic imaging, we demonstrated sub-cellular structure at zero loss (Fig.1a) and specificity of scFv*Gd labeling at Gd peak (Fig. 1b).

Figure 2 shows an image of cell culture samples at 1.5T obtained using a T1-FLAIR sequence demonstrating a 7% contrast difference between labeled C6 cells and labeled fibroblast control.

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