Intracellular Oximetry Using In Vivo Fluorine-19 MRI as Biomarker of Immunotherapeutic Response of Cytotoxic T Cells in Mouse Glioma

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**Introduction**

Malignant gliomas are the most common type of primary brain tumor and a significant public health problem, with more than 12,000 new cases diagnosed each year in the US. Immunotherapy using live cells is opening up new avenues for brain tumor killing with minimal damage to healthy tissue. Noninvasive biomarkers of therapeutic efficacy is of great importance in the evaluation of emerging immunotherapies. Tumor oxygenation, an important aspect of tumor physiology, has been shown to correlate with tumor angiogenesis, recurrence, and malignant progression (1). It was also reported to be a key factor determining the tumor response to radiation and chemotherapy. However, there has been limited study of tumor oxygenation using immunocompetent immune cells. In the current study, we test the hypothesis that intracellular oxygen level is a sensitive indicator of tumor killing using cytotoxic T cell therapy. To test this hypothesis, we developed a sensitive *in vivo* 19F MRI technique for intracellular oximetry of glioma cells located in mouse striatum. This method uses intracellular perfluorocarbon (PFC) labeling of glioma cells, combined with 19F T1 measurements to measure the partial pressure of oxygen (pO2) (2). We show that intracellular oximetry can be used to sensitively detect the influx of CD8+ T cells from circulation into brain glioma in mice. These data show great promise as a non-invasive biomarker for preclinical studies of anti-cancer therapy.

**Materials and Methods**

**Ex vivo PFC labeling of GL261 cells:** GL261 glioma cells that overexpress hgp100 antigen were labeled *ex vivo* with perfluoro-15-crown-5 ether emulsion (CS-580, Celsense Inc., Pittsburgh, PA) by co-incubation of the emulsion with the cells in conventional medium for 4 hours, followed by a wash step. Animal model of glioma: 6-8 weeks old C57BL/6J mice (n = 16) were anesthetized and 0.5×10^6 PFC-labeled cells in 10 μl PBS were inoculated into the right striatum at day 0. Unlabeled cells were injected into the left striatum. At day 3, 2×10^6 CD8+ T cells from a Pmel-1 transgenic mouse model (Jackson Laboratories, Bar Harbor, ME), which has T cell receptor specific for hgp100 antigen, were injected i.v. (n=7). CD8+ T cells without the hgp100 T receptor were injected as a control group (n=4). An additional control group received no T cell injection (n=5).

**In vitro calibration of pO2:** The linear 1/T1 versus pO2 calibration curve was determined from *in vitro* standards of the PFC emulsion held at four different oxygen tensions. These data were acquired at 37 °C using a 11.7 T microimaging system (Bruker Biospin, Billerica, MA) using a saturation-recovery sequence. In *vivo* 19F MRI: Anesthetized mice were initially scanned on day 3 before T cell infusion and longitudinally thereafter using 19F/H MRI at 11.7 T. Anatomical images of the brain were acquired via a 1H spin-echo sequence with parameters: TR/TE=700/12 ms, NA=4, FOV= 4×4 cm, number of slices=7, slice thickness = 2 mm, and matrix size=256×256. To image the tumor location, 19F MRI was acquired using a RARE sequence with parameters: TR/TE=1200/12 ms, RARE factor=8, NA=128, FOV= 4×4 cm, number of slices=7, slice thickness = 2 mm, and matrix size=64×64. The 19F T1 was measured using a PRESS sequence and a single voxel encompassing the entire tumor, as seen in the 19F MRI scan. Twelve different TR times were used ranging between 0.15 and 10 seconds to map the T1 relaxation curve. From the linear relationship between 1/T1 and pO2, the average oximetry was calculated for the tumor mass. Two days after T cell infusion, additional animals (n=3) were sacrificed and perfused for immunohistochemical (IHC) staining and quantitative analysis of the number of brain infiltrating T cells using flow cytometry.

**Results**

Using NMR analysis (3), the average fluorine content per GL261 cell was 2.5×10^12 fluorine atoms/cell. The *in vitro* calibration curve for T1 relaxation rate (R) versus pO2 was determined to be R1 = 0.0027[pO2]+0.34 using linear least-square analysis (R2 >0.99). Fig. 1 shows a representative 19F/H MRI image of glioma cells at day 5 after implantation. A solid tumor is visible in the right striatum that co-localizes with the 19F signal. On day 5, two days after antigen-specific cytotoxic T cell injection, the tumor cells exhibited a significant reduction in the T1 (0.99±0.03 s) when compared with the control (1.14±0.04 s) and non-specific T cell (1.19±0.01 s) groups (Fig. 2a, p<0.05). The T1 shortening is pronouncedly elevated (0.99±0.03 s) when compared with the control and non-specific T cell groups (62.4±11.6 mm Hg versus 13.0±11.7 mm Hg and 1.0±1.4 mm Hg, p<0.05) (Fig. 2b). This increase in pO2 was absent in the non-specific T cell control group (p=NS). The improvement in tumor pO2 decreased towards day 7 and remained similar among the three groups thereafter (p=NS). *Ex vivo* IHC staining of CFSE-labeled T cells and quantitative flow cytometry showed CD8+ T cell infiltration into the GL261 tumor (Fig. 3). The number of brain infiltrating T cells was small (~1,700 cells for n=3).

**Conclusions**

In this study, we developed an *in vivo* 19F MRI method to measure intracellular pO2 noninvasively. We show that this method can be used to monitor T cell therapy. The oxygen elevation was caused by the delivery of therapeutic cells and it provides valuable feedback to the action of immunotherapy.

**Reference**


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Fig. 1 *In vivo* 19F/H MRI of mouse GL261 glioma showing 19F labeled tumor cells implanted in the right striatum at day 5 post tumor inoculation. Nonspecific cells were injected into the contralateral striatum without 19F labels. The diluted PCE emulsion was used as 19F position reference.

Fig. 2 Longitudinal changes of T1 and pO2 of GL261 tumor after T cell infusion. a: T1 relaxation time; b: intracellular pO2. Antigen-specific CD8+ T cells or non-specific T cells were injected at day 3 into the corresponding groups, the control group received no cell therapy at the end of day 3. (*) denotes p<0.05 with control and non-specific T cells.