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 with immune cell therapies. 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* ^{19}F MRI technique for intracellular oximetry of glioma cells located in mouse striatum. This method uses intracellular perfluorocarbon (PFC) labeling of glioma cells, combined with ^{19}F T_1 measurements to measure the partial pressure of oxygen (pO_2) (2). We show that intracellular oximetry can be used to sensitively detect the influx of CD8+ T cells from circulation into brain glioma in mouse. These methods 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, 20×10^6 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 pO_2:** The linear $1/T_1$ versus pO_2 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* ^{19}F MRI:** Anesthetized mice were initially scanned on day 3 before T cell infusion and longitudinally thereafter using ^{19}F/^{1}H MRI at 11.7 T. Anatomical images of the brain were acquired via a ^{1}H spin-echo sequence with parameters: TR/TE=700/12 ms, NA=4, FOV= 4×4 cm^2, number of slices=7, slice thickness = 2 mm, and matrix size=256×256. To image the tumor location, ^{19}F MRI was acquired using a RARE sequence with parameters: TR/TE=1200/12 ms, RARE factor=8, NA=128, FOV= 4×4 cm^2, number of slices=7, slice thickness = 2 mm, and matrix size=64×64. The ^{19}F T_1 was measured using a PRESS sequence and a single voxel encompassing the entire tumor, as seen in the ^{19}F MRI scan. Twelve different TR times were used ranging between 0.15 and 10 seconds to map the T_1 relaxation curve. From the linear relationship between $1/T_1$ and pO_2, 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.

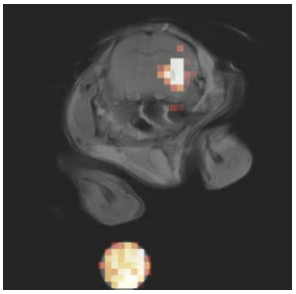

Fig. 1 *In vivo* ^{19}F/^{1}H MRI of mouse GL261 glioma showing ^{19}F labeled tumor cells implanted in the right striatum at day 5 post tumor inoculation. Nonspecific cells were injected into the contralateral striatum without ^{19}F labels. The diluted PCE emulsion was used as ^{19}F position reference.

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 T_1 relaxation rate (R_1) versus pO_2 was determined to be $R_1 = 0.0027[pO_2]+0.84$ using linear least-square analysis (R^2 >0.99). Fig. 1 shows a representative ^{19}F/^{1}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 ^{19}F signal. On day 5, two days after antigen-specific cytotoxic T cell injection, the tumor cells exhibited a significant reduction in the T_1 (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 T_1 shortening revealed a pronouncedly elevated pO_2 spike inside the treated glioma cells when compared to 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 pO_2 was absent in the non-specific T cell control group (p=NS). The improvement in tumor pO_2 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).

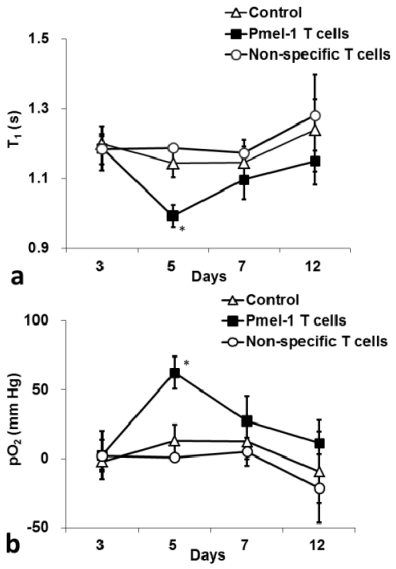

Fig. 2 Longitudinal changes of *in vivo* T_1 and pO_2 of GL261 tumor after T cell infusion. **a**: T_1 relaxation time; **b**: intracellular pO_2. Antigen-specific CD8+ T cells or non-specific T cells were injected at day 3 into the corresponding groups, the control group received no cell therapy at the end of day 3. (*) denotes $p<0.05$ with control and non-specific T cells.

Conclusions

In this study, we developed an *in vivo* ^{19}F MRI method to measure intracellular pO_2 noninvasively. We show that this method can be used to monitor T cell therapy. The oxygen pressure elevation was caused by the infiltration of cytotoxic T cells to the GL261 brain tumor which induces tumor apoptosis. Our results indicate that intracellular oximetry measured by ^{19}F MRI serves as a sensitive biomarker of *in vivo* delivery of therapeutic cells and it provides valuable feedback to the action of immunotherapy.

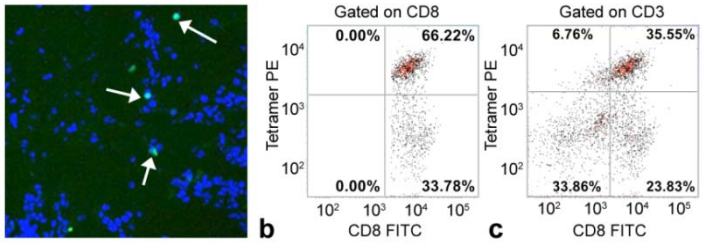

Fig. 3 *Ex vivo* analysis shows the infiltration of CD8+ T cells to glioma. **a**: immunohistochemistry of fluorescently labeled antigen-specific CD8+ T cells. Animals were infused with T cells labeled with CFSE on day 3 and sacrificed for IHC staining two days after cell infusion. **b&c**: flow cytometry of T cells in glioma that are CD8+ (**b**) and CD3+ (**c**). Animals were infused with T cells on day 12 and sacrificed for flow cytometry two days after cell infusion.

Reference

1. Tatum, *Int J Radiat Biol*, 2006; 82:699-757. 2. Kadayakkara, *Magn Reson Med,* 2010; 64:1252-9.
3. Srinivas, *Magn Reson Med*, 2007; 58; 725-34.