Evaluation of chemoresistance on human GBM by amide proton transfer (APT) imaging in mice

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Target audience: Researchers and clinicians interested in molecular MR imaging of brain tumor.

Introduction: The prognosis and management in patients with glioma is vastly different depending on whether one detects tumor progression or treatment effects. Currently, management decisions during all phases of diagnosis, treatment and follow up rely on MRI [1]. However, current standard MRI methods are not satisfactory to determine if therapeutic intervention has resulted in growth arrest of tumor cells and to accurately predict a patients' clinical course. In addition, with a plethora of new molecularly targeted therapies poised to enter early phase clinical testing, it will be critically important to identify drugs that stop and/or reverse tumor growth. Therefore, there is an urgent need for the development of novel imaging techniques that can distinguish between tumor progression and treatment effects in glioblastoma patients at these critical decision points as early and accurate as possible. We demonstrated that the amide proton transfer (APT) imaging which is one subset of the chemical exchange transfer (CEST) imaging methods could provide an imaging biomarker to determine the treatment effect on the glioblastoma multiforme (GBM) in chemotherapy with temozolomide (TMZ) [2]. In this study, we tested whether the APT signal could differentiate two different GBM cell lines that were responsive or resistant to TMZ treatment in a human orthotopic tumor (HOT) mouse models generated from human GBM tumor [3].

Materials and Methods: We chose two HOT models generated with two tumor lines derived from the same patient. The first tumor line was harvested at the time of initial resection and was subsequently shown to be responsive to adjuvant course of concurrent radiation and TMZ (initial) in the patient. The second tumor line was harvested from the second resection (18 months after the first resection) at the time of recurrence (recurrent). As anticipated, the initial and recurrent tumor HOT models were TMZ sensitive and resistant, respectively. Since genomic profiles of these initial and recurrent tumors from the same patient were remarkably similar, this *in vivo* model offers a unique and powerful system to compare directly the APT signal in the setting of TMZ sensitivity and resistance.

Animal Protocol: MRI screening was performed from 4 weeks after implantation. Mice were subjected to the APT imaging study and divided into two groups when the tumor was detected at the size of 3-5 mm. Both Initial and Recurrent groups (n = 6), mice underwent a course of chemotherapy (TMZ 80mg/kg i.p. for 3 days and then rest for 4 days; 7 days total) after the baseline imaging. The same MRI session was repeated 7 days after the baseline. The brains were harvested after the final MRI session for histology.

MRI: MR imaging was conducted in a 7-T small animal MR system (Varian, Inc, Palo Alto, CA) with a 40 mm (I.D.) radiofrequency (RF) coil. All animals were anesthetized with 1%-2% isoflurane (AERRANE, Baxter Healthcare Corporation, IL) mixed in 100% oxygen. First, low-resolution localizer imaging was performed to confirm reproducible positioning. High-resolution axial multislice T1-weighted images (T1WI) and T2-weighted images (T2WI) were obtained on entire brain using a fast spin sequence (TR/TE = 500/10.3 msec for T1WI, 2500/60 msec for T2WI; FOV = 25.6×25.6 mm; matrix size = 256×256; slice thickness = 1 mm; gapless; NEX = 4). On a single 1-mm-slice delineating the maximum area of each tumor, the APT imaging was performed as follows: Gradient echo images were obtained following a presaturation pulse (continuous-wave block pulse, B1 = 2.3 μT, duration = 5 s) which was applied at 29 frequency offsets from 7 to -7 ppm with an interval of 0.5 ppm. Other imaging parameters were: TR/TE = 6.52/3.16 ms, flip angle = 20°, FOV = 25.6 × 25.6 mm, matrix = 128 × 64 (reconstructed to 256 × 256), NEX = 8. A control image was obtained with the presaturation pulse at 300 pm. Total acquisition time for each animal was approximately 40 min.

Image Analysis: Tumor volumes were measured on the T2WI. The Z-spectra were fitted through all offsets on a pixel-by-pixel basis according to the procedure using the 12th-order polynomial fitting followed by the correction for B_0 inhomogeneity as Salhotra et al. reported [4]. MTR asymmetry (MTR_{asym}) was defined as: $MTR_{asym} = [S_{sat}(-offset)/S_0 - S_{sat}(+offset)]/S_0$, where S_{sat} and S_0 are signal intensities on the images with presaturation pulse at 7 to -7 ppm and control (300 ppm), respectively. The calculated MTR_{asym} map at the offset of 3.5 ppm is called the APT-weighted image (APTWI). Region-of-interests (ROIs) were carefully placed around the edge of the tumors on APTWI to measure APT ratio (APTR, %). APTR was also measured in contralateral normal appearing brain for a reference in each mouse. Corrected APTR was calculated as the difference between these two APTRs (tumor – normal).

Histopathology: Hematoxylin/eosine (HE) staining, caspase3 assay (apoptosis) and Ki67 (proliferation) staining were performed for microscopic examination.

Results and Discussion: The mean tumor volume that was measured on multislice T2WI was not significantly different between the initial and recurrent tumor at baseline (44.88 ± 12.84 mm^3 vs. 32.55 ± 7.52 mm^3, P = 0.10) while the volume was significantly higher in the recurrent tumors at 1 week (15.47 ± 7.90 mm^3 vs. 77.53 ± 14.64 mm^3, P < 0.001). In parallel, the enhanced area on APT map (APTR: ≥ -0.5%) increased significantly at 1 week relative to baseline (5.37 ± 1.56 mm2 vs. 11.56 ± 2.97 mm2, P < 0.01) in the recurrent group whereas it became smaller in the initial group (5.60 ± 3.51 mm2 vs. 2.30 ± 1.03 mm2, P = 0.14) although this change was not significant. Despite the same treatment with TMZ, the corrected APTR increased (2.50 ± 0.21% vs. 3.45 ± 0.32%, P < 0.01, Fig. 1A) in the recurrent group whereas it decreased after chemotherapy (2.18 ± 0.07% vs. 1.62 ± 0.12%, P < 0.05) from baseline (Fig. 1A). As a consequence, the differences in % change from baseline in the corrected APTR between initial and recurrent tumors were significant (-25.76 ± 6.91% vs. 39.52 ± 21.82%, P < 0.01, Fig. 1B). The cell density was significantly higher in the recurrent tumor than that in the primary tumor (2084 ± 224.0 /mm^2 vs. 1395 ± 137.8 /mm^2, P < 0.05). In addition, the percentage of apoptotic cells was lower in the recurrent tumor than that in the primary tumor (3.02 ± 0.19% vs. 1.95 ± 0.26%, P < 0.01). The Ki67LI in the recurrent tumor was substantially higher in spite of chemotherapy (84.76 ± 5.51%, P < 0.0001) compared with the primary tumor (14.25 ± 3.08%, Fig. 2). This study demonstrates early detection of a therapeutic response in GBM and chemoresistence during chemotherapy, which was validated in the immunohistology. The APT imaging could serve useful biomarker to accurately determine treatment response or tumor progression in GBM, which is of great interest and make a great impact to the neuro-oncology clinical community.

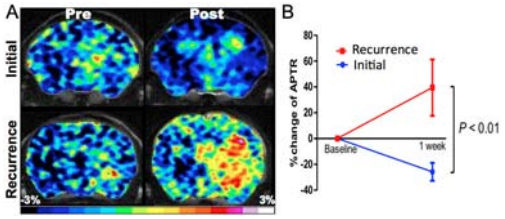

Fig 1. Temporal change in the corrected MTR_{asym} after short-term treatment of temozolomide in the initial and recurrent groups. The corrected APTR decreased to show the treatment effect in the initial tumor. By contrast, it increased in the recurrent tumor, indicating chemoresistance. P < 0.01 by t-test at 3.5 ppm.

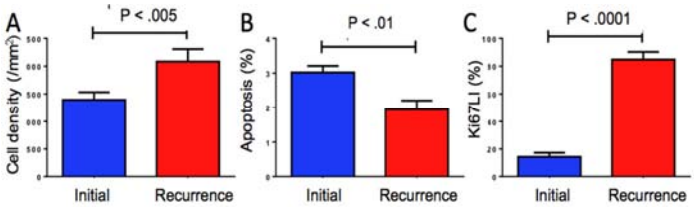

Fig 2. Histological evaluation of the initial and recurrent glioblastoma in the orthotopic mouse model with short-term treatment of temozolomide. (*A*) Cell density, (*B*) The percent of cells showing apoptosis in the caspase-3 assay, (*C*) The Ki67 labeling index (Ki67LI: percent of cells that shows Ki67 positive).

References: 1. Wen, et al. New Eng J Med 2008;359(5):492 (2008). 2. Sagiyama, et al. ISMRM (2012), 3. Marin-Valencia, et al. NMR Biomed 25:1177 (2012). 4. Salhotra, et al. NMR Biomed 21:489 (2008).