# Amide proton transfer imaging and magnetization transfer imaging for characterizing rat brain tumors

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**INTRODUCTION:** Conventional MRI has limited diagnostic specificity with respect to its ability to distinguish the core of the tumor from regions of tumor-infiltrating edema. Proteins perform most cellular activities and have distinct levels of activity in various forms of intracranial tissues including tumor and edematous normal brain. Thus, a non-invasive approach to imaging cellular events at the protein level through high-resolution in vivo MRI could be useful for additional tumor discrimination. Two broad types of proteins can be distinguished with MRI: bound proteins, which possess solid-like properties and have protons with very short $T_2$ (~10 μs), and mobile proteins and peptides, which rotate rapidly. The protons of mobile proteins have relatively long $T_2$. The solid-like component can be assessed via conventional magnetization transfer contrast (MTC)[1,2]. However, endogenous mobile protein molecules do not provide intense MR signals, which has prohibited their clinical use. Recently, we proposed a new chemical exchange saturation transfer (CEST[3])-based MRI technique, called amide proton transfer (APT) imaging[4,5], in which endogenous mobile proteins and peptides can be detected via the water signal. This enhances the detection of mobile proteins dramatically. The purpose of this abstract is to quantify the APTR and MTR values at 4.7T for two brain tumor models, and to compare these two MRI contrasts that are, respectively, related to mobile proteins and peptides and semi-solid macromolecules in tissue.

**MATERIALS & METHODS:** Eleven Fischer 344 rats and six nude rats received 9L gliosarcoma cells (25,000 cells/2 μl) or primary human glioblastoma cells (randomly called primary-22, 25,000 cells/2 μl), respectively, by stereotactic injection to right caudate/putamen. On each day of the MRI measurements, isoflurane anesthetized rats were scanned using a Bruker 4.7T animal imager. Single-shot SE EPI was used for data acquisition (matrix 64×64, FOV 28×28 mm$^2$, slice thickness 2 mm). Conventional MT and APT are incorporated in a pulse sequence with several different offsets. We acquired three offsets of ±3.5ppm (for APT) and 10ppm (for conventional MT) with 16 averages ($S_{sat}$). One unsaturated image (no saturation pulses added) was acquired for control ($S_0$). The MR ratio (MTR) is defined as: $MTR = 1 - S_{sat}/S_0$. APT can be separated out through asymmetry analysis of the total MT data with respect to the water frequency, i.e., through an MTR asymmetry parameter: $MTR_{asym}(3.5ppm) = (S_{sat}/S_0)(-3.5ppm) - (S_{sat}/S_0)(+3.5ppm)$. The $MTR_{asym}(3.5ppm)$-based images were referred to as APT-weighted images. Three other MRI scans are: (i) $T_2$ map (spin echo, TR 3 s, TE 30-90 ms, NA 4); (ii) $T_1$ map (inversion recovery, TR 3 s, TE 30 ms, TI 0.05-3.5 s, NA 4); and (iii) $ADC_{av}$ map (single-shot trace diffusion weighting, TR 3 s, TE 80 ms, b-value 0-1000 s/mm$^2$, NA 8).

**RESULTS & DISCUSSION:** Fig. 1 shows the APT-weighted image and the conventional MT image along with several other MRI types acquired on a primary-22 tumor (7 weeks post-implantation). It can be seen that $T_1$, $T_2$, and $ADC_{av}$ are all increased in the tumor region. The MTR maps at offsets 3.5 and 10ppm are both dark (hypointensity) in the tumor region due to increased water content, while the $MTR_{asym}(3.5ppm)$ images are bright (hyperintensity), presumably due to higher protein and peptide concentration in gliomas[5,6]. The $MTR_{asym}(3.5ppm)$ and conventional MT images display different MRI contrasts between tumor and contralateral normal tissue. It is important to notice that the hyperintense ($T_2$, $T_1$, $ADC_{av}$) or hypointense (MTR) regions on several conventional MR images are generally larger than that on the APT-weighted image. Similar to the previous work[5], we assigned regions as being peritumoral when exhibiting increased $ADC_{av}$ and approximately normal $MTR_{asym}(3.5ppm)$. The peritumoral regions also showed markedly increased $T_2$ and $T_1$, and decreased MTR with respect to the normal brain. These regions may be associated with white matter tract edema. Based on increased APT and increased $ADC_{av}$, we could easily find a well-contrasted area of tumor for all rats. Table 1 shows the comparison of the MRI parameters measured for 9L tumors (n = 8) and for primary-22 tumors (n = 6). In particular, we should notice increased $MTR_{asym}(3.5ppm)$, but decreased MTR(10ppm) in the tumor with respect to the contralateral brain tissue. According to Table 1, except the MTR values at 10ppm, the other MRI parameters (including $T_2$ and $T_1$, data not shown) are all smaller in the primary-22 gliomas than in the 9L gliomas ($T_1$, $P \le 0.01$; $T_2$, $ADC_{av}$, and $MTR_{asym}(3.5ppm)$, $P \le 0.001$). The MRI contrasts between tumor and normal tissue are also smaller for the primary tumor model than for the 9L tumor model. These observations may be attributed to the fact that a primary brain tumor consists of tumor tissue and normal brain tissue (invasive growth) but a 9L gliosarcoma includes pure tumor cells only.

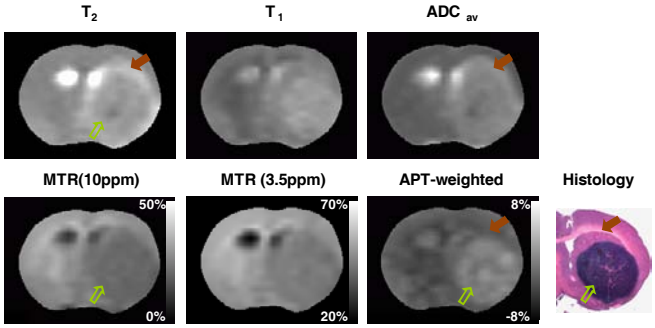


**Fig. 1.** MR images and histology for a primary-22 brain tumor (7 weeks post-implantation). The hyperintense ($T_2$, $T_1$, ADC) or hypointense (MTR) regions on conventional MR images are larger than that on the APT-weighted image. The APT-weighted image is able to separate the likely edematous part (orange arrow) from tumor, and its hyperintensity profile is in good correspondence with histology.

**Table 1.** MRI parameters (value and STD) measured for the 9L brain tumors (12 or 13 days post-implantation; n = 8) and for the primary brain tumors (5-7 weeks post-implantation; n = 6).

| | In vivo $ADC_{av}$ ($10^{-9}$ m$^2$/s) | | $MTR_{asym}$ at 3.5ppm | | MTR at 10ppm | |
|---|---|---|---|---|---|---|
| | 9L | Primary-22 | 9L | Primary-22 | 9L | Primary-22 |
| Contralateral | 0.72 (0.03) | 0.68 (0.02) | -2.36% (0.19%) | -2.77% (0.42%) | 31.7% (0.9%) | 31.4% (0.8%) |
| Tumor | 1.16 (0.05) | 0.92 (0.03) | 1.49% (0.66%) | -1.18% (0.60%) | 19.2% (0.7%) | 22.0% (2.1%) |

**CONCLUSIONS:** APT provides a unique MRI contrast that may identify tumor regions and map tumor distribution more accurately. This non-invasive approach to image cellular events at the protein level through high-resolution in vivo MRI may have clinical applications in the future.

1) Wolff & Balaban MRM 1989;10:135. 2) Henkelman et al. NMR Biomed. 2001;14:57. 3) Ward et al. JMR 2000;143:79. 4) Zhou et al. Nature Med. 2003; 9:1085. 5) Zhou et al. MRM 2003;50:1120. 6) Howe et al. MRM 2003;49,223. Thank C. David James (Mayo Clinic) for providing the primary glioma xenograft model used in this study.