Tumor drug resistance and sodium-diffusion MRI in rat glioma model

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Introduction

During cancer progression, many tumors develop evasive mechanisms permitting them to thwart chemotherapeutic interventions. Consequently, it is crucial to formulate individualized effective treatments promptly to provide a definitive and noninvasive assessment of tumor resistance. Mitochondria play a central role in energy metabolism, in apoptosis and ion gradients; their function is closely associated with changes in tumor resistance (1-6). We hypothesize that the energy metabolism shift due to increased tumor resistance can affect sodium homeostasis, and MRI has the potential to reflect changes in tumor drug resistance (7). The study was performed using the ultra-short echo time for sodium MRI to detect total sodium concentration in tumor. High resolution sodium (Fig.1) and diffusion MRI were achieved using the ultra high magnetic field of 21.1T created at the NHMFL (Tallahassee).

Materials and Methods

Three sub-clones of 9L gliosarcoma cells with different resistance to 1,3 bis(2-chloroethyl)-1-nitrosurea (BCNU) were selected by using the *In Vitro* Toxicology Assay Kit (Sigma-Aldrich TOX-6) based on the sulforhodamine blue (SRB) method. Cell viabilities were determined after cells were exposed for 72 hours to media having a range of BCNU concentration (Fig.2). The naïve and resistant cells were implanted intra-cranially in three groups of male Fisher 344 rats (n=5 in each group, weight ~ 150 g). At ~11 days after tumor implantation tumor sodium, diffusion and tumor volume were detected. The experiments were performed on a 21.1T MRI scanner using proton (900 MHz) and sodium (237 MHz) signals, Bruker Avance III console equipped with 64 mm gradient coil (RR Inc), GREAT60 amplifiers and operated by Paravison V5.1 software. Sodium was detected by 3D back-projection MRI with ultra-short echo time of TE =0.1 ms. The short readout time of ~ 2 ms minimized the partial volume effect of bi-exponential FID of sodium signals. To avoid saturation, a long repetition time TR was selected (100 ms). Sodium MRI scans had a duration period of 27 min and resolution of 0.5x0.5x0.5 mm. Diffusion SE pulse sequence had flow/motion compensated diffusion gradients, two b values of 100 and 1000 (sec/mm2), TE=34 ms and 15 slices. The back-projection acquisition used here allowed for additional motion compensation. The double tuned sodium/proton RF probe was developed by the authors for *in vivo* rat MR imaging at 21.1 T. All animal experiments were conducted according to the protocols approved by The Florida State University ACUC.

Results and Discussion

Naïve 9L cells were much more susceptible to BCNU presence than resistant cells (Fig.2) and displayed a population decrease of 2.7 times during cultivation with 7.8 μM of BCNU. For the resistant sub-cultures, the corresponding BCNU resistances were 24.7 μM (R1) and 161.7 μM (R2). All cell lines initiate gliomas *in vivo* with doubling times of approximately 2.5 days. Eleven days after tumor implantation, sodium concentration in tumor from naïve cells was 173% relative to a normal contra-lateral brain (Fig.3). The glioma formed from the resistant cell lines had lesser sodium concentrations of 127% (R1) and 99% (R2). The corresponding values of diffusion were – 151% in naïve glioma and 140% (R1) and 121% (R2). The values are also given relative to a normal brain. All observed differences for sodium and diffusion are statistically significant ($p < 0.05$).

Tumor sodium concentration is mainly determined by the efficiency of the Na/K pump and in this way can reflect the available ATP cellular level. The results of this study support the mechanism that resistant tumor cells may have an increased glucose transport and glycolysis leading to increased ATP production which is capable to compensate the often observed ATP deficit experienced by naïve tumors. The experiments demonstrate the enhanced sensitivity of sodium MRI to detect small variations in glioma cell drug resistance to therapy.

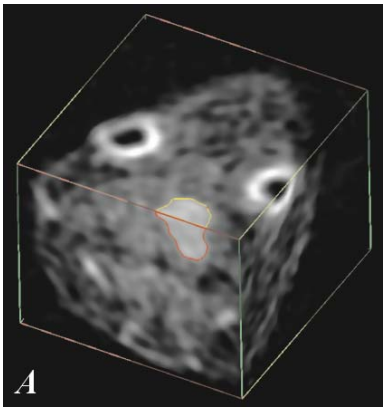

Fig. 1. Sodium MRI of rat glioma. Resolution is 0.5x0.5x0.5 mm.

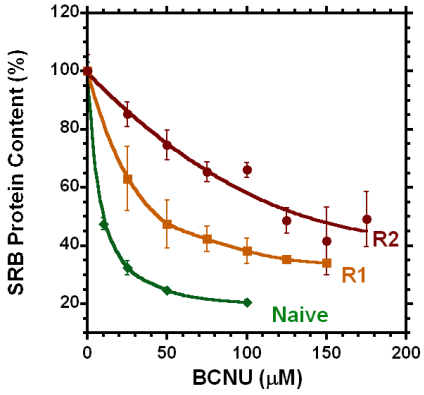

Fig. 2. Sulfarodamine blue assays of 9L cell resistance to carmustine (BCNU). The cell lines R1, R2 have increased resistance to BCNU.

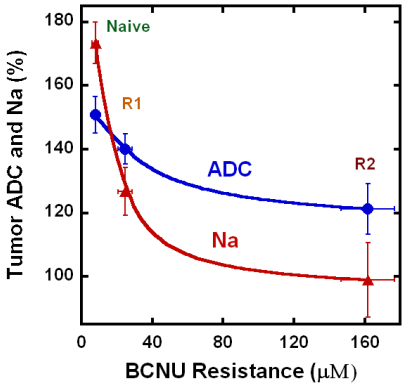

Fig. 3. *In vivo* sodium concentration and diffusion in non-treated rat glioma relative to its tumor cell carmustine resistance. Note an enhanced sensitivity of sodium to the changes in tumor resistance.

Conclusion

The results of this study demonstrate that changes in tumor resistance can be detected by sodium and diffusion MRI. The relationship between resistance and MRI correlate with the shift in energy metabolism often observed in tumors (Warburg effect) which could be further intensified in resistant tumors. The result demonstrates that alterations in tumor resistance can be assessed prior to treatment, allowing individualized adjustments of the therapy to prevent ineffective treatments. The finding warrants further investigation and confirmation for other tumor types.

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