Sodium-diffusion MRI of emerging drug resistance in rat glioma model

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Introduction

It is well known that during tumor progression, especially after therapeutic interventions, the tumor may become more resistant to therapies; consequently, it needs a much higher concentration of a chemotherapeutic drug to achieve the same level of response, or the chemotherapy may not be effective at all. Energy metabolism in tumors is different from normal cells and it is a promising target in our fight with cancer (1-6). Tumors generally have higher sodium concentration than normal tissue, which can be detected by sodium MRI. Sodium concentration in glioma is not the same and its variation shows a remarkable correlation with glioma drug resistance (7). The hypothesis of this study is that increased tumor resistance is determined by the energy metabolism shift which can be detected by a corresponding shift in sodium homeostasis. It is noteworthy that diffusion can also follow the corresponding changes in glioma and has the potential to convey alterations in tumor resistance using the strong MR signal from protons. Thus, MRI has the potential to reflect changes in tumor drug resistance noninvasively.

Materials and Methods

Six 9L gliosarcoma cell lines with a range of resistance to 1,3 bis(2-chloroethyl)-1-nitrosurea (carmustine, BCNU) were created. In four lines, the naïve glioma cells were subjected to a range of BCNU concentration up to 150 μM during their cultivation. The two most resistant cell lines were prepared the same way but started from glioma cells extracted from the tumor after the animal was subjected to BCNU therapy. Glioma cell line resistance to BCNU was determined shortly before intracranial implantation by growing the cells for 72 hours in media having an array of BCNU concentrations and assessing the number of viable cells through protein detection by sulforhodamine blue (using the *In Vitro* Toxicology Assay Kit Sigma-Aldrich TOX-6). Six groups of male Fisher 344 rats (n=4-6 in each group, weight ~ 150 g) were implanted with the cells and after ~11 days tumor sodium and diffusion were evaluated. The experiments were performed on a 21.1 T MRI scanner (Bruker Avance III console equipped with 64 mm gradient coil (RR Inc) and operated by Paravison V5.1 software) using proton (900 MHz) and sodium (237 MHz) signals. 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 was selected to minimize the partial volume effect from bi-exponential FID of sodium signals. Sodium MRI scans had TR of 100 ms to reduce MR saturation and resolution of 0.5x0.5x0.5 mm (scan time = 27 min). The diffusion SE pulse sequence had flow/motion compensated diffusion gradients with two b values of 100 and 1000 (s/mm^2), TE=34 ms and 15 slices. Here, the back-projection acquisition mode allowed for additional motion compensation. Sodium and diffusion MRI scans were performed without repositioning on animals by using the double tuned sodium/proton RF probe. All animal experiments were conducted according to the protocols approved by the Florida State University ACUC.

Results and Discussion

The naïve and resistant 9L cells yielded different brain tumors. The difference is very noticeable from sodium MRI without additional processing (Fig.1). Tumors from naïve 9L cells have a high tumor sodium concentration relative to the normal brain, while tumors from resistant glioma cells show a very low sodium contrast pattern. Sodium concentration in glioma has a strong correlation (R=0.99) with diffusion in glioma for a wide range of the glioma resistance (Fig. 2). Resistance of the naïve glioma to carmustine was 21.8 ± 1.3 μM, while the most resistant glioma cell line had resistance of 163 ± 23 μM. Sodium has an almost doubled sensitivity to the changes in resistance relative to diffusion (Fig. 2). The most resistant glioma has the lowest tumor sodium concentration and the lowest diffusion values. The question arises whether sodium in tumors can be related to energy metabolism in cancer cells. The Na/K pump is the major extrusion mechanism of sodium out of the intracellular space, and it may consume up to ~ 60% of total ATP to perform this task. It is also known that increased intracellular Na content leads to an additional activation of the Na/K pump and to a higher consumption rate of ATP. Thus, increased sodium can be an indicator of energy deficit in cancer cells and be detectable by MRI. Naïve cells (less resistant) have the largest deficit of energy and such cells are the most vulnerable to therapeutic interventions. The absence of an energy deficit in resistant tumor cells is an advantage to such cells in their fight against drug interventions.

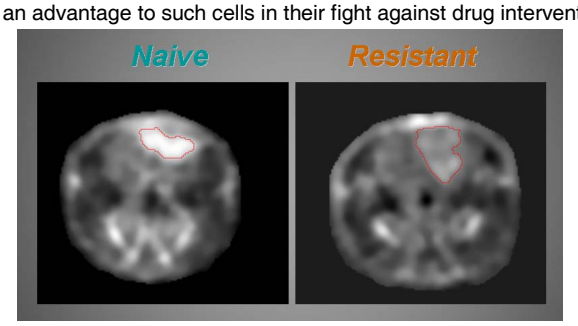

Fig. 1. Sodium MRI of non-treated rat glioma originated from 9L cells having different carmustine resistances. Resolution is 0.5x0.5x0.5 mm.

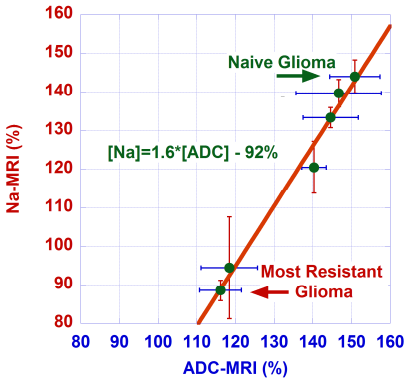

Fig. 2. Correlation of sodium concentration and diffusion in non-treated rat glioma for a range of glioma resistance to carmustine. Sodium and diffusion are presented in percent relative to the normal brain values of 45 mM (8) and 0.78 *10^{-3} mm^2/s (9) respectively.

Conclusion

The results of this study demonstrate that emerging tumor resistance can be detected by sodium and diffusion MRI. The evaluation can be done noninvasively and prior to therapy. It is important to note here that we are speaking not only about resistance to carmustine. It is expected that an energy-based MRI indicator of tumor resistance can be predictive for a range of different therapeutic interventions. The prompt evaluation of tumor resistance may help to formulate individual treatment and avoid unsuccessful therapies.

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