In vivo T2 Measurements of Glioma Growth in Mouse Brain at 9.4T

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Introduction

Gliomas are primary brain neoplasms with high-grade gliomas being the most common CNS tumors in adults. Despite considerable efforts over the past several decades, mean survival remains less than one year. This can largely be attributed to late tumor detection, its heterogeneity and variable response to conventional therapies. Angiogenesis plays an important role in high-grade glioma growth. Hypervascularization contributes to malignant glioma phenotype by: i) providing oxygenation and nutritional supply needed for tumor growth, ii) supporting the invasion of tumor cells into the surrounding intact brain tissue [1] and iii) acquiring a multi-drug resistance phenotype by upregulating membrane efflux pumps on the endothelial cell surface [2]. Grade IV glioma (glioblastoma multiforme) vessels have a characteristic phenotype; they are tortuous and leaky, lose some of the blood-brain barrier properties. They show microvascular hyperplasia, with micro-aggregates of proliferating endothelial cells at the edge of parental blood vessels. Chronic overproduction of angiogenic factors in tumors leads to uncontrolled development of new blood vessels, increased vascular permeability and a unique phenotype. In glioblastoma, microvessel density is an indicator of invasiveness and, as a result, a prognostic indicator.

Conventional MRI, including T_1 and T_2-weighted images, has been used in gliomas diagnosis [e.g. 4,5]. However, although the early diagnosis is crucial for successful treatment, the changes in T_2 at the very early stage of the tumor development *in vivo* remain poorly understood. The possibility of the tumor detection at its early stage of the tumor growth would enable earlier and more accurate diagnosis and treatment. Furthermore, as we still rely on tumor size to determine a patient's response to therapy, the investigation of the tumor detection at the early stage is very important.

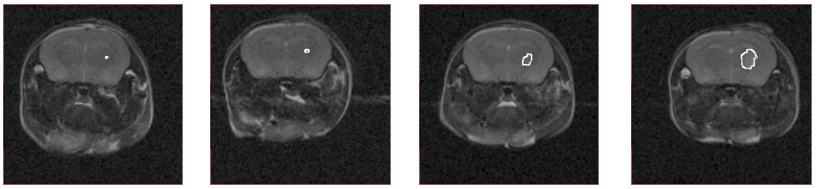
Materials and Methods

Cell and animal preparation: The U87MG cell line used in the study is highly malignant, derived from a human tumor known to express high levels of VEGF and EGFR. The U87MG implants grow as solid, nonencapsulated spheroidal tumor. The tumor displays a vascular network, with many characteristics of brain endothelium [4]. U87MG cells were cultured in DMEM solution supplemented with 10% fetal calf serum and maintained in a humidified 5% CO_2 atmosphere at 37°C. Cells were harvested by trypsinization in ethylenediaminetetraacetic acid/trypsin, washed in phosphate-buffered saline (PBS), and centrifuged at 200 G three times. After cell density was determined, cells were brought into suspension at a final concentration of 5×10^4/2.5 µl and mixed with 2.5 µl of matrigel for a total volume of 5 µl. Cells were kept on ice until injection. Six CD-1 nude mice (female, 6 weeks old, Charles River, Canada) were anesthetized by intraperitoneal injection of a mixture of ketamine (8—120 mg/kg) and xylzine (6 mg/kg) and placed in a stereotactic head frame. Tumor cells were inoculated using procedures described previously [3]. Briefly, the scalp was shaved and swabbed with iodine and alcohol. The skin was incised and a 0.18-mm-diameter hole was drilled in the skull. Approximately 5×10^4 U87 glioma cells, suspended in a total volume of 5µl, were injected intracerebrally into the frontal lobe of each mouse with a chromatography syringe at a depth of 2.5-3 mm, 1mm anertior and 1.8mm lateral to the bregma) using a Kopf stereotactic apparatus. Subsequently, the bony calvarium was sealed by a droplet of bone wax to prevent reflux and the skin was sutured. After the surgery animlas were allowed to recover from the anesthesia and were placed in the cages. Following the completion of the last imaging session, 14 days after the injection, when the tumor occupied about 1/3 of the brian, rats were immediately euthanasied with pentobarbital (120mg/kg, i.v.). The animal procedures were approved by the local Animal Care Committees.

Experimental setup: The MR imaging sessions started 10 days after the cell injection. At his time tumor size was about 2mm^3. A 9.4T/21cm horizontal bore magnet (Magnex, UK) with a Biospec console (Bruker, Germany) was used. Data acquisition was gated with the respiratory cycle. The volume (3 cm diam × 3 cm) rf coil was placed over the animal's head covering the ROI. T2 images were acquired from the tumor region. Axial slices were positioned within the tumor. A multislice, multi-echo sequence was used (TR = 5000ms, 16 echoes, 10 ms apart each; first echo was at 10 ms, FOV = 3 × 3 cm, matrix size 256 × 256 and slice thickness of 1mm. T_2 values of the tumor tissues were measured at each time point using a single exponential fitting of the echo train from ROIs. The volume of the tumor was calculated by measuring the area of the each slice and multiplied by its thickness and adding the results over the entire tumor volume.

Results and Conclusions:

We have successfully obtained MR images of glioma beginning 10 days after the cell injection (Fig.1). We have observed a linear increase of the tumor volume in days 10 to 13 (Fig. 1,2). We have noticed, that T_2 of the tumor tissue at its early stage of the growth (before day 12, when the tumor volume was less than 2.5 mm^3) was about 10% higher than T_2 of normal tissue (~40 ms). The difference in T_2 increased to about 20% (~ 48ms) when its size excided 2.5 mm^3, in day 12 and 13 after the injection (Fig. 3). We concluded, that the detection of a small tumor with MRI, at its earlier stage of the growth is very difficult due to the both small size of the tumor as well as small difference in the relaxation times causing low contrast to noise ratio. The results are important in the early diagnosis of the glioma. They also indicated a need for molecular imaging using targeted contrast agents to enhance the detection of a relatively small number of glioma cells.



A B C D

Fig. 1. An example of glioma growth. MRIs were obtained 10 (A), 11 (B), 12 (C) and 13 (D) after the cell injection. Pulse sequence: MSME, TR/TE= 5s/60ms, FOV 3 × 3 cm, 256 × 256.

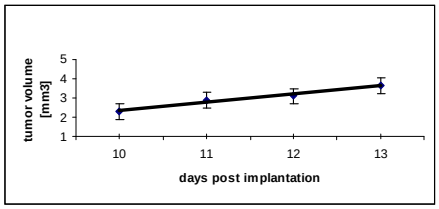


Fig. 2. The changes in the tumor size with time after the cell injection.

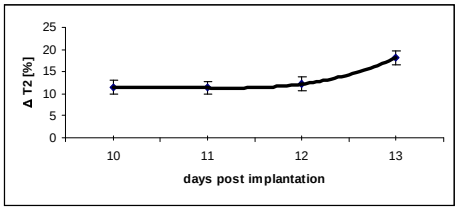


Fig. 3. The changes in the T_2 difference in normal brain and tumor after the cell injection.

References

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Acknowledgment: This work was supported by a CIHR Team Grant.