# Longitudinal Diffusion Tensor Imaging Study of a Rat Brain Glioma Model

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## Introduction

Diffusion weighted MRI (DWMRI) is being applied to evaluate tumors response to therapy in preclinical studies in animal models and in clinical trials. DWMRI is also being carried out in the attempt to better define the border of tumors, conventionally done using contrast enhanced (CE) and T2-weighted MRI [1]. Recently, diffusion tensor imaging (DTI) has been applied in humans to categorize the varied appearances of tumor-altered brain regions [2]. In such studies, normal values of anisotropy are chosen from the contralateral side of the brain from the tumor, which can be somewhat problematic as this is also often affected by the tumor. Because of its clinical importance, a more thorough understanding of the diffusion of water within and around tumors is warranted. In the present study, longitudinal DTI examinations have been carried out to study the diffusion properties of water within and around C6 gliomas grown in rats.

## Methods

MRI was carried out prior to, and 3-5 times after, surgical implantations of C6 glioma cells in female Wistar rats using a Bruker Biospec 4.7T instrument. C6 cells (~$10^5$ cells in 10 µl) were injected stereotaxically in the right caudate nucleus in the brains of six rats. As controls, two rats were imaged that received stereotactic injections of 10 µl of DMEM cell culture medium without glioma cells and one rat was imaged without any manipulation. All animals were anesthetized by isoflurane gas and placed into a custom rat holder. An 18 mm OD circular surface coil, used as the MR signal receiver, was placed on top of the rat head and rats were placed into a 72 mm volume excitation coil. Body temperature was monitored using a fiber optic rectal probe and maintained using a circulating water bath. Rat brains were imaged in the coronal plane with 1 mm slice thickness (no gap) with a 4×4 $cm^2$ field of view. DTI was carried out using a diffusion-weighted radial spin-echo pulse sequence [3] with the following parameters: TR/TE = 2000/56 ms, $\Delta/\delta$ = 25/9 ms, matrix size = 256×256. Images were obtained without (b = 0 s/$mm^2$) and with (b = 1065 s/$mm^2$) diffusion weighting in six non-colinear gradient directions. Maps of diffusion anisotropy (FA, RA, CP, CL, ADC) were generated for each slice (6 slices) using standard algorithms written in IDL. After the final imaging time point, the rats were euthanized and their brains were placed in formalin in preparation for histopathology. Hematoxylin-eosin (H&E) and luxol fast blue staining were performed on 5-µm-thick paraffin sections.

## Results and Discussion

Fig.1 shows T2-weighted images and fractional anisotropy (FA) maps of a rat brain obtained before implantation of tumor cells (day 0) and 3, 6, 8, and 10 days post implantation. Disruption of white matter (WM) tracts in the cingulum of the right hemisphere is visible in the anisotropy map on day 6. After day 8 this WM track is destroyed. The existence of high anisotropy surrounding the tumor is observed in the peritumoral tissue (days 8 and 10). Similar results were also observed around smaller tumors. This anisotropy likely comes from pressure generated by the tumor forcing normally spherical cells into more planar geometries, which is evident in the histology slides and in anisotropy shape analysis.

Significant anisotropy was also observed within the tumors which changed as a function of growth. This can be seen in the anisotropy maps in Fig.1 where higher intensity values were observed within the tumor border. Histological analysis indicated some macroscopic organization of the cells within the tumor.

Values of FA in the cingulum and corpus callosum (CC) were also measured as a function of the tumor growth and are plotted in Fig. 2. A decrease in anisotropy in the cingulum, adjacent to tumor, is observed in day 6, which may be caused by WM deterioration due to tumor infiltration. Conversely, a significant increase in FA is observed on day 10 in the right side of the CC, which can be due to compression of CC from the tumor. Thus, tumor infiltration through WM tracks may be hard to observe with DTI since tumor invasion compresses WM and changes FA values.

The results of this study indicate that diffusion experiments in brain tumors need to account for anisotropy changes within and surrounding tumors. Furthermore, evaluating borders of tumor via DTI can be complicated by the fact that the presence of tumor may cause increases or decreases in the observed anisotropy. These findings have implications for many experiments using DWMRI to study tumor growth, infiltration and response to chemotherapy.

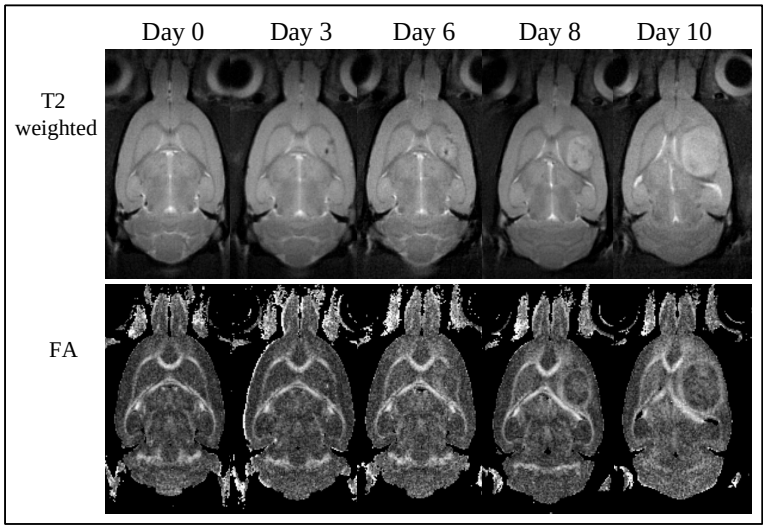


Fig. 1. T2 weighted images and FA maps of a rat brain obtained before glioma cells were injected (Day 0) and 4 times following the growth of the tumor (Days 3-10).

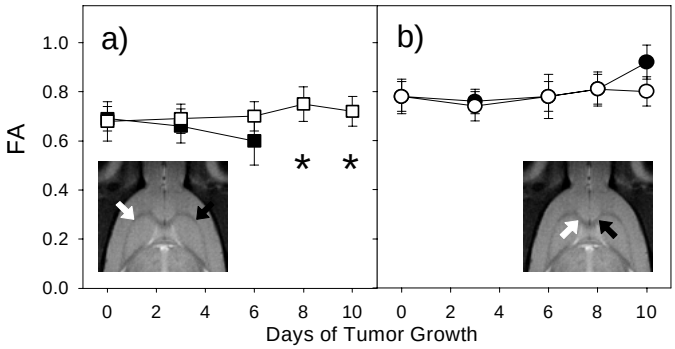


Fig. 2. FA as a function of the tumor growth. FA values from ROIs taken in the WM tract in the cingulum (a) and in the CC (b) from the tumor side (filled) and the contralateral side (blank). * No values available at these days due to significant tumor infiltration.

## References

[1] Price, *et al*., *Clinical Radiology*, 58:455 (2003), [2] Provenzale, *et al*., *Radiology*, 232:451 (2004), [3] Trouard, *et al*., *MRM*., 42:11 (1999).