Microscopic 3D-DTI of tumor cell migration, numerical modeling and two- photon microscopic imaging

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Introduction: This study aims to validate diffusion tensor imaging (DTI) parameters in detecting the migration of brain tumor cells along white matter (WM) fibers (1). A microscopic 3D-DTI sequence is developed and applied *ex vivo* on a novel mouse model of glioma using Green Fluorescent Protein (GFP) transfected Glio6 cells (2). Perpendicular diffusivity ($D_\perp$) and fractional anisotropy (FA) parameters are analyzed in WM as well as in grey matter (GM). For validation, two-photon microscopic images of the same brains are obtained. Monte-Carlo simulations of water diffusion in numerical models of cerebral tissue geometry are developed (3) and allow simulation of micro-architecture changes as occurring under pathologic conditions.

Subject and Method: Animal model: Glio6 is an orthotopic tumor model derived from human glioma stem cells with similar growth pattern and invasiveness to clinically encountered glioma. This model is chosen since in a DTI pilot study; thickening of the corpus callosum (cc) was observed, suggesting the migration of tumor cells along the fibers. Nude mice were injected with 500000 GFP labeled Glio6 cells (Glio6-Group, n=6) or with normal saline solution (Sham-Group, n=3) in the caudate-nucleus. The tumor growth was monitored *in vivo* every 15 days with T_2-weighted imaging. At 60 days after tumor implantation, the brains were fixed by transcardiac perfusion (4% paraformaldehyde in phosphate buffered saline containing 6.25mmol/L Gd-DOTA (Guerbet France)). Then, brains were preserved in Flomblin-oil (Solvay-Solexis, Italy). **MRI acquisitions:** Experiments were performed at 7T (Bruker, 600mT). In order to reduce relaxation times, brains were injected and incubated for 11 days (3) with 6.25mmol/L Gd-DOTA. T_1 and T_2 values were respectively 60 and 8 ms uniformly throughout the brain. The DTI sequence was a 3D spin-echo sequence (TE/TR= 16/90ms, NA=34) with diffusion gradients (δ= 3.5ms Δ=8ms) applied in six spatial directions ([1 1 0],[1 -1 0],[0 1 1],[0 1 -1],[1 0 1],[-1 0 1]) with a b-value of 1500s/mm². Field of view (FOV) and spatial resolution were set to 20x8.5x11.5 mm^3 and 100x100x100 mm^3 respectively with a total acquisition time of 59 hours. The diffusion data were analyzed using MedINRIA software (4). Histograms of FA and $D_\perp$ diffusivity were computed in different ROIs. **Two-photon microscopy:** 300 µm thick coronal brain slices were observed with a Two-Photon Laser Scanning Microscope Zeiss LSM 7MP and a water immersion objective Zeiss (x20, N.A. 1.0). Mosaics were obtained with 600x600µm² individual FOV. Whole brain slices were further imaged on a multi-zoom AZ100 Nikon microscope. **DTI Monte Carlo simulations and numerical models of brain geometry:** The NMR signal of water diffusion was simulated using a Monte Carlo method in different models of cerebral tissues to establish the relationship between the DTI parameters, the diffusion signal acquisition and microstructural changes. WM fibres were modelled by longitudinal spaced cylinders and GM cells by spheres with a given diameter distribution (3). To model complex tissues such as a population of cells between fibers, a model composed of a mixture of WM/GM fractions was proposed.

Results*:* In tumor bearing mice, thickening of the cc (Fig.1b) and DTI parameter changes (FA: -12%, and $D_\perp$: +22%) are observed (Fig.1c). Tumor cell invasion, particularly into the cc, is confirmed by two-photon microscopy (Fig.2b). Typical elongated tumor cells distinctly indicate migration along the fibers of the cc (Fig.2c). The maximum FA decrease (-32%) is detected in the ipsilateral cc in which tumor cell density is most elevated as shown in (Fig.2a). Simulations of FA changes as a function of extra/intracellular and WM/GM ratios show the same tendency as detected experimentally.

Discussion/conclusion: DTI parameter changes are demonstrated to be noninvasive biomarkers for tumor cell migration along the fibers. This is confirmed by two-photon microscopy. Our preliminary analysis shows a trend correlation between FA changes and fluorescence. Quantitative analyses are in progress. Simulations reproduce similar FA decrease as a function of extra/intracellular and WM/GM ratios. Then, in the cc of Glio6 bearing mice, the FA decrease should be interpreted as a decrease of the extracellular compartment following the tumor cell invasion, leading to an enlargement of the cc cross section. In conclusion, the Glio6 model and microscopic 3D-DTI together constitute a powerful tool to study tumor cell migration.

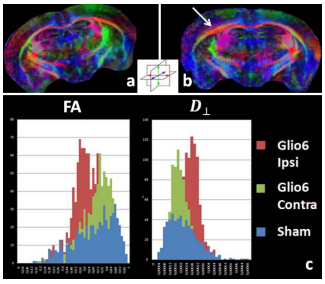

Fig.1: Color maps: (a) sham, (b) Glio6 with thicker cc. (c) FA and $D_\perp$ histograms in the cc.

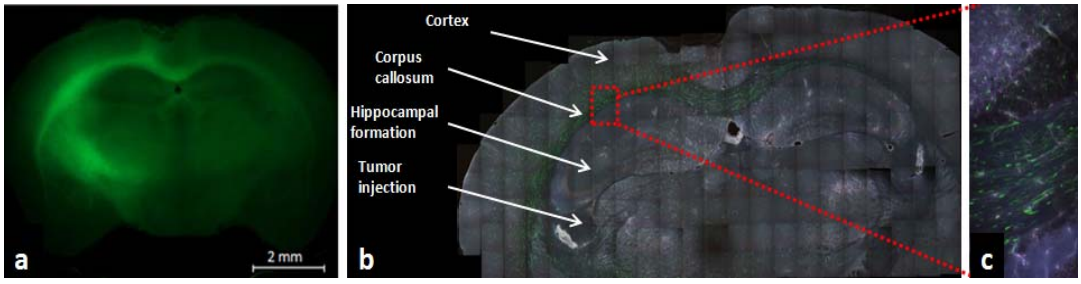

Fig.2: (a) Coronal slice observed at the multizoom microscope. Two-photon microscopy of GFP marked glioma cells: (b) composite image of a coronal slice, (c) ipsilateral cc ROI.

(1) Giese A *et al*. (Neurosurgery,1996); **(2)** Platet N*et al.* (Cancer letters,2007) ; **(3)** Mauconduit F (PhD thesis, UJF–Grenoble, 2011) ; **(4)** (https://gforge.inria.fr/frs/?group_id=727&release_id=4541, INRIA, France).