Studying glioblastoma progression in a rat model of human glioma initiating cells using 1H MRS and DTI

Mor Mishkovsky[1,2], Cristina Cudalbu[3], Emine Can[4], Denis Mario[5], Ivan Radovanovic[5], Arnaud Comment[4], Virginie Clément-Schatlo[5], and Rolf Gruetter[1,6]

[1]Laboratory for Functional and Metabolic Imaging (LIFMET), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [2]Department of radiology, Univesity of Lausanne, Lausanne, Switzerland, [3]CIBM, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [4]Institute of Physics of Biological Systems, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland, [5]Hôpitaux Universitaires de Genève (HUG), Geneva, Switzerland, [6]Department of radiology, Univesity of Lausanne and Geneva, Switzerland

Target audience: Scientists and radiologists who have an interest in studying glioblastoma in animal models.

Purpose: Glioblastoma (GBM) is the most common brain tumor in humans; it shows high metabolic activity and is resistant to multimodal therapy leading to short survival times. Development of animal models bearing the human disease enables to study the metabolic modifications and morphological changes during tumorgenesis which is inaccessible in clinical studies[1-3].In this study we followed the development of glioblastoma tumor in immunodeficient rats, that had been injected with human glioma initiating cells (GIC's). The aim of the study was to characterize the metabolic profile using ^{1}H MRS and at the same time to follow brain structural modification via diffusion tensor imaging (DTI) at different stages during tumor development.

Methods: GIC were harvested from human glioma biopsy[1]. Cells were cultured (passage 14) and injected (10^6) stereotactly into the striatum in the right hemisphere of imunodeficient 7 weeks old nude 200 gr female rats (n = 4). MR measurements were carried out on a 9.4 T/ 31 cm actively shielded animal scanner (Varian/Magnex) using a home-built ^{1}H quadrature probe. Field inhomogeneity was corrected using the FASTMAP protocol. The animals were anesthetized using 1.5% isoflurane and their physiology was monitored during the entire experiments. T_2 weighted (T_2W) images were acquired using fast spin echo multi slice (fsems) protocol (FOV 23x15 mm^2, TR = 4000 ms, effective TE = 52 ms, 6 scans). DIT images were recorded using semi-adiabatic double-spin-echo EPI sequence[4] (FOV 23X15 mm^2, 21 directions with G_{diff} =19.7 G/cm, d=3 ms, D = 20 ms with max b-value 1079.5 s mm^{-1}).Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were derived from the DTI imaging using a Matlab script. ADC and FA were measured in ROIs positioned in: cortex, striatum and hippocampus. Single voxel ^{1}H MRS measurements were acquired using the SPECIAL[5] sequence (TR = 4000 ms, TE = 2.8 ms, in 10 blocks of 16 scans). The metabolites concentrations were deduced using LCModel-based fitting routine[6].

Results and discussion: Anatomical T_2W images indicate the development of diffusible tumor. ADC and FA maps improved the visualization of the tumor. Hyperintensity in ADC maps and hypointensitiy in FA images result from the reduced diffusion directionality in the malignant tissue and allow an earlier detection of the tumor and better visualization of its progression (Fig 1). ^{1}H spectra measured alongside DTI images in the tumorous site and the contra lateral hemisphere exhibited excellent signal-to-noise ratio and showed characteristic changes in the associated metabolic pattern (Fig 2). At an early stage, dissimilarities between the two hemispheres were more evident from the metabolites concentration calculated by spectroscopic data than from the diffusion coefficient deduced with DTI imaging (Fig. 3). During tumor development Glu and tNAA pools constantly decreased and Ins, Lac, Asp, Tau concentrations continuously increased in the injected hemisphere compared to the contra lateral. Only at the late stage of the tumor a major increase in Lac was observed together with elevated levels of Cho and Lipids. The decrease in tNAA and Glu indicates a neuronal loss in the malignant hemisphere[3,7], whereas elevated Ins levels, an astrocyte marker, reflect the glial nature of the tumor[3,7]. The increase in tCho, is related to cellular membrane degradation and cell proliferation[3,7]. The larger Lac concentration indicates an increase in aerobic-glycolysis (Warburg-effect)[3,7] and is particularly pronounced at late stage of the disease.

Conclusion: Glioblastoma tumor development was studied longitudinally *in vivo* in brain tumor derived from implanted human glioma initiating cells. DTI imaging enabled to highlight morphological changes during tumorgenesis prior to T_2W images. High-field MR spectroscopy with high-order shimming was employed to detect and quantify 15 cerebral metabolites. Metabolic profiles are in agreement with expected trends from earlier reports[3,7] and enable to reveal the differences between healthy and tumorus tissue at an earlier stage.

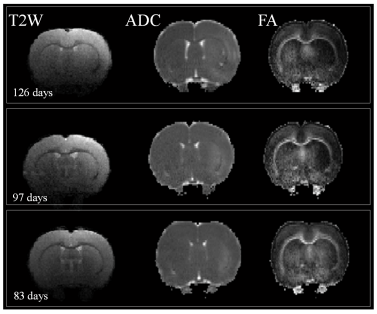

Figure 1: T2W and DTI images measured on one animal at different time points during tumor development. Days indicate the time elapsed from the injection.

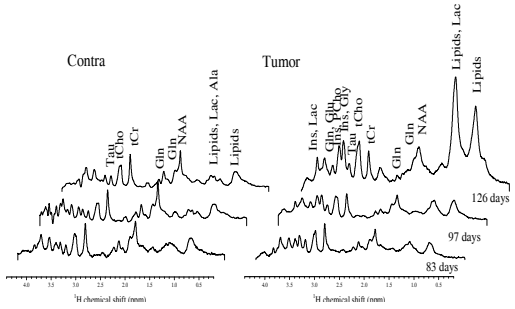

Figure 2: SPECIAL ^{1}H spectra acquired in a voxel located in the tumor area (right) and the contra lateral hemisphere (left) at similar time point as in Fig 1.

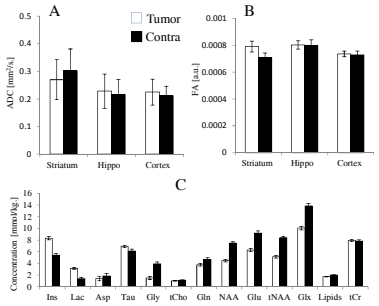

Figure 3: Diffusion parameters ADC (A) FA (B) and metabolites concentration (C) calculated from the data acquired 83 days after injection from the same rat in Fig.1 and Fig. 2.

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