Brain metabolism in rat model of human glioma initiating cells

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Introduction:

Human gliomas are diffusely growing in the brain showing various grades and aggressiveness. Tumorigenesis and its effect on cerebral metabolism can be studied *in vivo* following the implantation of human glioma cells into rodents brain. *In vivo* ^{1}H MRS allows to non-invasively characterize brain metabolism in a wide range of disease models. Glioma initiation cells (GIC), a subpopulation of stem-cell-like cancer cells, can be cultured as spheroïds in serum-free condition, maintaining their undifferentiated state. It was shown that stereotactic injection of GIC into mice brain leads to the development of brain tumor[1,2]. So far very few studies have been carried out to evaluate brain metabolism in tumors developed from GIC[2,3]. In this context, we show that stereotactic injection of human GIC into immunodeficient rat results in the development of glioma tumors. The biochemical profile of these human GIC was studied *in vivo* by ^{1}H MRS.

Methods:

GIC were obtained from human glioma biopsy as described in ref(1). Cells were then cultured (passage 14) and injected (10^6 cells) stereotactly into the striatum in the right hemisphere of imunodeficient 7 weeks old nude female rats (200 gr). 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 length of the experiments. Fast spin echo multi slice (fsems) T_2 weighted images were acquired (TR = 4000 ms, effective TE = 52 ms, 6 scans). Fsems T_1-weighed images were recorded following the infusion of Gd contrast agent (TR = 350 ms, TE = 10 ms,10 scan). Localized single voxel ^{1}H MRS measurements were acquired using the SPECIAL sequence (TR = 4000 ms, TE = 2.8 ms, 200 ms aquisition in 10 blocks of 16 scans)[4]. The metabolites concentrations were deduced using a LCModel-based fitting routine[5].

Results and discussion:

Typical T_2-weighted (T_2W) images showed the appearance of diffusible tumor resulting from the stereotactic injection of GIC (Fig 1). Lack of contrast enhancement in the T_1-weighted (T_1W) images indicates the very little neovascularization in this type of tumors[2,3](Fig. 1). ^{1}H spectra measured in the tumorous site and the contra lateral hemisphere exhibited excellent signal-to-noise ratio and notable differences in metabolites signals were discernible (Fig. 2). The neurochemical profile (mean ± SD) measured on 3 different rats is presented in Fig. 3 in absolute concentrations. Glu and tNAA concentrations were lower in tumors whereas a significant increase in Ins, Lac, Asp, Tau, tCho, Gly and Gln was observed compared to the contra lateral hemisphere. The decrease in tNAA and Glu is most likely associated with the neuronal loss[3,6] and the increase in the astrocyte marker, Ins, reflects the glial nature of the tumor[3,6]. The increase in tCho, a characteristic biomarker in tumor, is related to cellular membrane turnover and cell proliferation[3,6]. Finally, the larger Lac concentration indicates an increase in aerobic-glycolysis (Warburg-effect)[3,6]. In conclusion, high-field MR spectroscopy using high-order shimming allows for the *in vivo* quantification of 15 cerebral metabolites in brain tumor derived from implanted human glioma initiating cells. These can be are compared to the concentrations in healthy brain tissue of immunodeficient nude rat. The trends of change in cerebral metabolites concentrations observed are in agreement with those reported for low-generation tumors described by Thorsen[3]. The improved signal-to-noise ratio and resolution at high-field will allow us to perform longitudinal studies to investigate tumor growth.

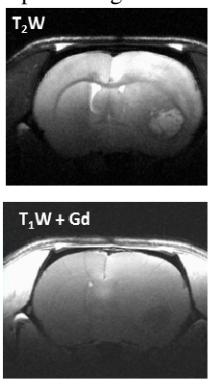

Figure1. Typical T_2W image and T_1W+Gd contrast image.

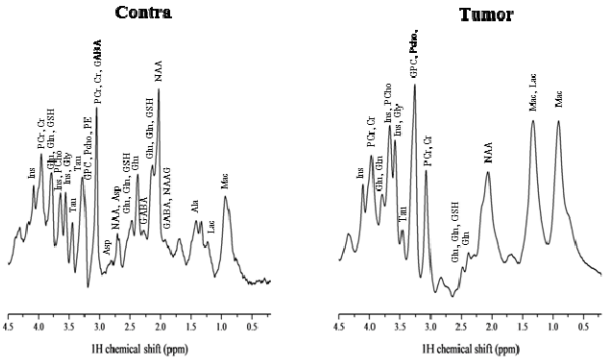

Figure 2. SPECIAL ^{1}H spectra acquired in a voxel located in the tumor area and in the contra lateral hemisphere.

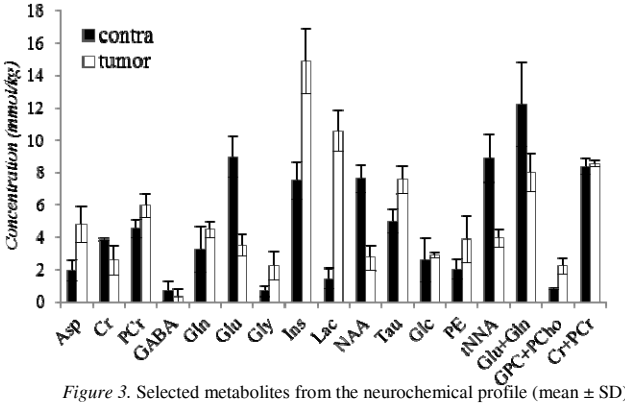

Figure 3. Selected metabolites from the neurochemical profile (mean ± SD) in the tumor and contra lateral tissue

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References: [1] Clément V. *et al.* Nat. Methods 2010; 7: 224-228. [2] Mlynárik V. *et al*. NMR biomed. 2012; 25: 506-13. [3] Thorsen F. *et al.* NMR biomed. 2008; 21: 830-8. [4] Mlynárik V. *et al.* Magn. Reson. Med.2006; 56, 965-970.[5] Provencher, S.W. *et al* Magn. Reson. Med.1993; 30:672-679. [6] Bernsen *et. al.* J. Neuroncol. 1992; 13:119-30.