Tumor invasion visualized by neurochemical profile modification in human GBM induced by cancer stem cells in mice: ^{1}H-MRS longitudinal study

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Target audience: Scientists and radiologists who are interested in studying glioblastoma in animal models.

Introduction: Glioblastoma (GBM) is the most malignant primary brain tumor in adults. GBM show high metabolic activity and are notorious for their resistance to multimodal therapy, with a median survival of only 15 months. *In vivo* ^{1}H MRS is a powerful tool that enables to assess the concentrations of about 20 endogenous metabolites associated with cellular functions [1]. Using this technique, modifications in cerebral metabolite concentrations can be followed longitudinally, allowing early detection of GBM in animal models [2]. The aim of the present study was to examine changes in the neurochemical profile after injection of the highly invasive human glioma derived sphere (GS) lines.

Methods: LN-2669GS was derived from a human GBM as described [3] and cultured under stem cell conditions. Two different subclones of LN-2669GS (LN-2669GS867; LN-2669GS822) were injected (10^5 cells) stereotactically into the striatum at the left hemisphere of imunodeficient male mice (Swiss Nude, n = 9, 8 weeks old). 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. Animals were anesthetized using 1.5% isoflurane and their physiology was monitored during the entire experiments. To determine structural changes associated with implantation of the cells T_2 weighted (T_2W) images were acquired using fast spin echo multi slice (fsems) protocol (FOV 18x18 mm^2, TR = 4000 ms, effective TE = 52 ms, 6 scans). To follow variations in the neurochemical profile single voxel ^{1}H MRS measurements were acquired using the SPECIAL [4] sequence (TR/TE = 4000/2.8 ms, VOI = 2x2x2mm^3 in 10 blocks of 16 scans). Follow-up of both injected and contra lateral hemispheres were performed longitudinally starting 5 weeks post injection. Metabolite concentrations were calculated using LCModel-based fitting routine [5].

Results and Discussion: Typical images and localized proton spectra collected at the designated areas in both hemispheres are shown in Fig 1. Spectral data indicate onset of disease at an earlier stage than when morphological modifications become visible on images. Cerebral metabolite concentrations associated with tumor progression are presented in Fig. 2 for two mice. For both GS-lines continuous increase in myo-Ins and tCho concentrations were observed and decrease in tNAA was detected in the injected hemispheres. These metabolites indicate the glial nature of the disease (myo-Ins), tumor progression (tCho) and neuronal loss (tNAA) [6,7]. Similar modifications of the metabolite concentrations appear in the contralateral hemisphere, approaching similar values at the time when animals became symptomatic and were sacrificed. Histology showed an invasive tumor on the injected side, with dramatic migration to the contralateral side.

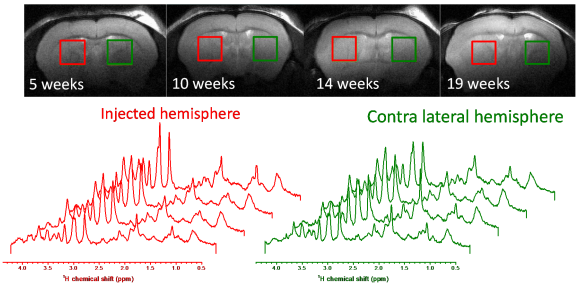

Figure 1: T_2W images measured on one animal at different time points during tumor development and the corresponding spectra measured at the injected (red) and contralateral (green) hemisphere.

Conclusion: In this study we monitored the onset and migration of human GBM derived orthotropic tumors (HOT) via determination of variations in the neurochemical profiles. We show that the metabolic profiles observed in the hemisphere where the cells were injected and the contralateral hemisphere correspond well to the tumor cell density displayed by histology at the end of the experiment. We suggest that longitudinal comparison of modifications in the metabolic profiles of different brain regions allows studying the kinetics of tumor invasion.

References: (1) Duarte JMN et al., NeuroImage 2012; (2) Mlynárik V et al., NMR in Biomed 2012; (3) Sciuscio et al. Clin. Cancer Res. 2011 (4) Mlynárik V. *et al.* Magn. Reson. Med.2006; (5) Provencher, S.W. et al. Magn. Reson. Med.1993 (6) Thorsen F. et al. NMR biomed. 2008; (7) Bernsen et. al. J. Neuroncol. 1992. **Acknowledgments:** Supported by Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL, the Leenards and Louis-Jeantet Foundations

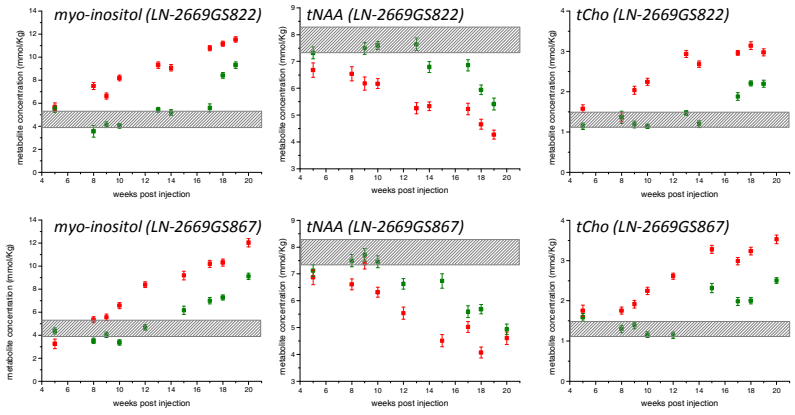

Figure 2: Metabolite concentrations quantified from spectra acquired in tumors derived from the human glioma sphere lines LN-2669GS822 (upper) and LN-2669GS867 (lower). Concentrations are shown for the injected hemisphere (red) and the contra lateral (green) in a similar orientation as designated in Fig 1. Dashed bar reflect the metabolite concentrations at basel-line (mean ± SD)