Metabolic profiling of RG2 glioma using in vivo 1H MRS and ex vivo HRMAS 1H MRS
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Introduction.

1\textsuperscript{H} MRS is a powerful analytical method to investigate the complex metabolic consequences of cancer disease. In vivo 1\textsuperscript{H} MRS can provide information regarding glioma growth and response to treatment. A wider range of metabolites can be obtained ex vivo in biopsies using 1\textsuperscript{H} High Resolution Magic Angle Spinning (HRMAS) 1\textsuperscript{H} MRS. Besides, the metabolic data can also be interpreted and classified using multivariate pattern recognition methods, such as Projection to Latent Structure-Discriminant Analysis (PLS-DA) [1]. Comparison of metabolic profiles between 1\textsuperscript{H} MRS and HRMAS 1\textsuperscript{H} MRS is essential [2] and the ability of both methods to discriminate tumoral from normal tissue with statistical tools such as PLS-DA might be of great help for diagnosis. Here, we have used this approach to analyse in vivo and ex vivo spectra in the rat RG2 model of glioma.

Materials and methods.

Experimental design: Fischer rats (n = 7, 8 weeks old) were orthotopically implanted with RG2 glioma (5.10\textsuperscript{5} cells) at day 0 under 2.5\% isoflurane anesthesia. In vivo HRMAS 1\textsuperscript{H} MRS was performed on well established tumor at day 14. At day 15, animal were deeply anesthetized with 5\% isoflurane and quickly decapitated. The tumor bulk and the contralateral striatum were rapidly isolated (in less than 3 minutes), subsequently frozen and kept in liquid nitrogen. For HRMAS 1\textsuperscript{H} MRS experiments, about 15 mg of the frozen biopsies were rapidly introduced in a 4 mm ZrO\textsubscript{2} rotor, and a cold 1 mM D\textsubscript{2}O solution of 3-(trimethylsilyl) propionic-2,2,3,3-d\textsubscript{4} acid (TSP) was added (δ\textsubscript{TSP} = 0 ppm).

Data acquisition.

In-vivo: 1\textsuperscript{H} MRS experiments were performed on a 7 Tesla Bruker BioSpec AVANCE III (Ettingen, Germany) MRI system. Tumor and contralateral striata were acquired with short TE PRESS sequence in a 3x3x3 mm\textsuperscript{3} voxel with TE/TR=20/2500 ms, 4006 Hz bandwidth, 2048 data points, 400 averages resulting in a total acquisition time of 16m40s. The PRESS localization was preceded by water suppression and outer volume suppression modules. The localization of the VOI was based on T2-weighted RARE images (TE/TR=33/4000 ms, 20 slices 1mm thick, 117x117 mm\textsuperscript{2} in plane resolution, Fig. 1). Ex-vivo: the HRMAS 1\textsuperscript{H} MRS experiments were performed at 9.4 Tesla on a Bruker Avance III spectrometer using a 4 mm 1\textsuperscript{H}-2\textsuperscript{C}-1\textsuperscript{P} probehead. Samples were spun at 4 KHz and the temperature maintained at 4°C. 1D spectra were all acquired with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence to attenuate macromolecule and lipid resonances, synchronized with the spinning rate (interpulse delay 250 µs, total spin echo time 30 ms) [3]. The residual water signal was saturated during the 2s relaxation delay. Total acquisition of one spectrum with 256 scans lasted 16 min. Resonance assignment was performed as previously described [4].

Metabolic quantification: the signals were processed using the "substract-QUEST" algorithm [5] of the jMROI-software (\url{http://www.mrui.uab.es/mrui/}). The procedure involves a simulated metabolite data basis set. For HRMAS 1\textsuperscript{H} MRS spectra 20 metabolites were included in the basis set: acetate (Ace), alanine (Ala), aspartate (Asp), creatine and phosphocreatine (tCr), choline (Cho), ethanolamine (Eth), γ-amino-butyric acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (Gsh), glycerophosphocholine (GPC), glycine (Gly), hypotaurine (Hyp), lactate (Lac), myo-inositol (m-Ins), N-acetylaspartate (NAA), phosphoethanolamine (PE), phosphorylcholine (PC), scyllo-inositol (Syll), taurine (Tau). For MRS spectra 10 metabolites were included in the basis set: Asp, tCr, tCho (Cho+PC), GABA, Glu, Gln, Lac, m-Ins, NAA, Tau. For both analysis the total spectrum intensity was used for normalization. Multivariate statistical analysis: Quantified data were loaded in the SIMCA-P software version 12 (Umetrics, Umeå, Sweden) as variables and scaled to unit variance before PLS-DA analysis. All PLS-DA models were cross-validated, to allow evaluation of the statistical significance of the model. Cross validation is a procedure during which the model is iteratively rebuilt using only 6/7 of the data as training set. The model is then used to predict the class of the remaining 1/7 data which served as a test set. Cross-validation led to the calculation of the Q\textsuperscript{2} and R\textsuperscript{2Y} factors. R\textsuperscript{2Y} is a quality factor while Q\textsuperscript{2} is a predictive factor, i.e. a good Q\textsuperscript{2} (>0.5) allows the model to be used for prediction. The results were visualized by plotting the first two principal components of the analysis against each other in a scatter plot. Each point in a scatter plot represents one individual animal.

Results. In vivo and ex vivo spectra gave similar results in tumor with an increase of tCho and a decrease of NAA, tCr (Fig. 2). Interestingly the increase of Lac observed ex vivo confirmed the Lac increase observed in vivo at 1.3 ppm that is usually controversial. Ex vivo HRMAS 1\textsuperscript{H} MRS provided additional information in tumor metabolism: an increase of Ala and Gly, a decrease of GABA, the identification of choline components (Cho, PC, GPC) and the emergence of Hyp. Both analyses yielded robust statistical models with a clear separation between tumor and contralateral striatum (Fig. 3).

Conclusion: Quantification with jMROI is reliable since metabolic profiles from contralateral striatum are well grouped. The PLS-DA models are robust despite inter-individual variability. These models are highly predictive (high Q\textsuperscript{2} values) and could be a powerful tool for evaluation of treatment efficiency of glioma in preclinical research. Further studies are needed to validate this approach.