Metabolic profiling of RG2, F98 and C6 glioma models using $^1$H-MRS and ex vivo $^1$H HRMRS MRS
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Introduction.

$^1$H MRS is a powerful analytical method to investigate the complex metabolic consequences of cancer disease. In vivo $^1$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 $^1$H High Resolution Magic Angle Spinning ($^1$H RMAS) MRS. Comparison of metabolic profiles between in vivo and $^1$H RMAS MRS is essential [1] and the ability of both approaches to discriminate tumor from normal tissue with statistical tools such as PLS-DA [2] might be of great help for diagnosis. Here, we have used this approach to analyze in vivo and ex vivo spectra in the rat RG2, F98 and C6 models of glioma.

Materials and methods.

Fischer rats (RG2 model, n=8; F98 model, n=12) and Wistar rats (C6 model, n=12), 8 weeks old at tumor inoculation, were used. After decapitation under 5 % isoflurane, tumor bulk and contralateral striatum were isolated (<3 minutes) and frozen in liquid N$_2$.

In vivo Data acquisition: $^1$H MRS experiments were performed on a 7 Tesla Bruker BioSpec. A sequence (TE/TR=20/2500 ms) with water suppression and outer volume suppression was used to record two data sets, tumor and contralateral tissue. For each data set the volume of interest was 3x3x3 mm$^3$ and the acquisition time was 16m40s. The localization of the voxels of interest was based on T2-weighted RARE.

Ex vivo Data Acquisition: Approximately 15 mg of biopsy were introduced in 50 µL rotors and spun at 4 kHz. $^1$H HRMRS NMR spectra were acquired on a Bruker Avance III 400 spectrometer (9.4T) at 4°C with a CPMG pulse sequence (TE=30 ms), water presaturation, 3.5 s total repetition time, 256 scan, 15 min total acquisition time.

Quantification: Metabolite quantification was performed with the software package jMRUI (http://www.mrui.uab.es/mrui/) using the "subtract-QUEST" procedure [3] and a simulated metabolite database set. For $^1$H MAS quantification, 16 metabolites were included in the database: acetate (Ace), alanine (Ala), creatine and phosphocholine (Cr), choline (Cho), g-aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (Gsh), glycercophosphocholine (GPC), glycine (Gly), lactate (Lac), myo-inositol (M-ins), N-acetylaspartate (NAA), phosphoethanolamine (PE), phosphocholine (PC) and taurine (Tau). Hypotaurine (Hyp) and betaine (Bet) were detected only in tumor tissue and then were added in the database of tumor. For in vivo spectra, 11 metabolites were quantified: creatine and phosphocholine (tCr), total choline (tCho), GABA, Glu, Gln, Lac, M-ins, NAA, Asppartate (Asp), Hyp, and Tau. Metabolite concentrations are given relative to the sum of all metabolites, both in vivo and ex vivo data.

Statistical analysis: multivariate statistical analysis was performed using the SIMCAP V12 software (Umetrics AB, Umera, Sweden), using quantified data as X variables. PCA (Principal component analysis) allowed to verify the quality of data and to identify outliers. PLS-DA (Partial Least Square-Discriminant analysis) models were then built using the tumor models as Y classes. PLS-DA models are calculated in order to minimize intra-group variance and to maximize inter-group variance. R2Y and Q2 are produced to evaluate respectively the quality of fitting and the predictability of the statistical model. R2Y>0.5 and Q2>0.5 are considered as good models.

Results/Discussion. PCA analysis allows the separation of the 3 glioma models and their respective contralateral hemisphere (Fig.1). The PLS-DA statistical model built with ex vivo data shows a clear separation between the 3 glioma models (Fig.2A) whereas it is less obvious with in vivo data (Fig.2B). Metabolites that are statistically different between glioma models (data not shown) are also the most important for the separation of classes in the PLS-DA model (Fig.3). However, some metabolites such as NAA, lactate and betaine that are modulated in tumor tissue contribute less to the separation of tumor models in the PLS-DA analysis. On the other hand, hypotaurine is only observed in tumor samples [4] and participates to tumor model separation, with a higher level in RG2 and F98 than in C6.
