Metabolic profiling of RG2, F98 and C6 glioma models using ^{1}H-MRS and ex-vivo ^{1}H HRMAS MRS

Nicolas Coquery[1,2], Vasile Stupar[2,3], Régine Farion[2,3], Séverine Maunoir-Regimbal[4], Emmanuel Luc Barbier[1,2], Chantal Rémy[1,2], and Florence Fauvelle[4]
[1]U836, INSERM, Grenoble, France, [2]Université Joseph Fourier, grenoble, France, [3]Grenoble MRI Facility IRMaGe, Grenoble, France, [4]IRBA-CRSSA, La Tronche, France

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 tumoral 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 3x3x3mm^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 HRMAS 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}HR MAS quantification, 16 metabolites were included in the database : acetate (Ace), alanine (Ala), creatine and phosphocreatine (Cr), choline (Cho), g-amino-butyric acid (GABA), glutamate (Glu), glutamine (Gln), glutathione (Gsh), glycerophosphocholine (GPC), glycine (Gly), lactate (Lac), myo-inositol (M-ins), N-acetylaspartate (NAA), phosphoethanolamine (PE), phosphocholine (PC) and taurine (Tau). Hypotaurine (Hyp) and betaïne (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 phosphocreatine (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.

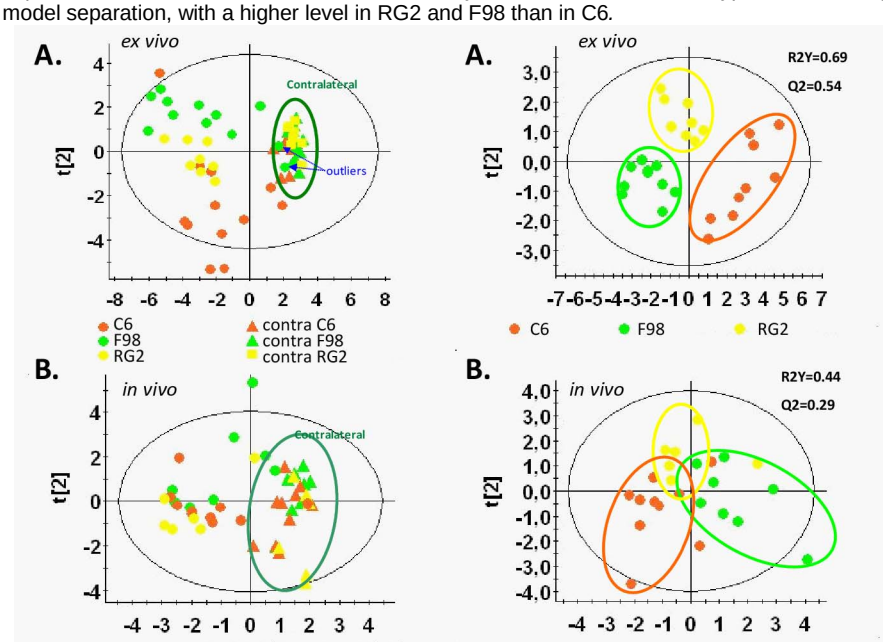

Figure 1: PCA analysis of *ex vivo* ^{1}H RMAS NMR Data (**A.**) show a good separation between metabolic profile of contralateral and tumor biobises. 2 outliers are found in the contralateral region. *In vivo* data (**B.**) are more scattered but contralateral and tumor profiles remain separated.

Figure 2: PLS-DA analysis using the 3 glioma models as Y classes shows a very good separation when using *ex vivo* ^{1}H RMAS data (**A.**). Moreover, the model has a relatively good predictability (Q2>0.5). The *in vivo* PLS-DA model (**B.**) has R2Y and Q2 values <0.5 and is then less reliable.

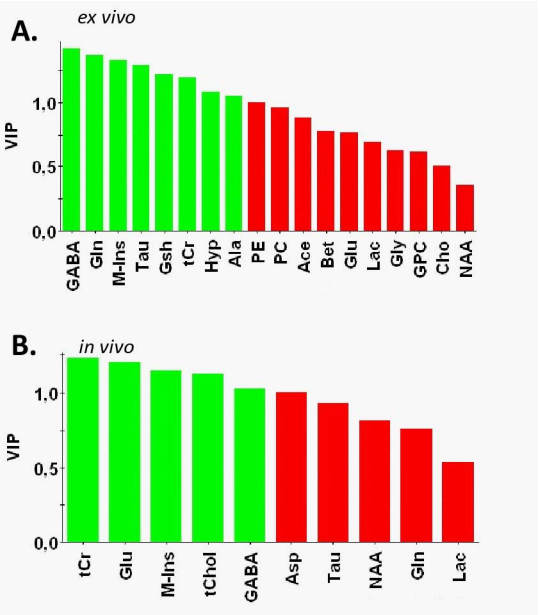

Figure 3: Variable Importance Plot (VIP). GABA, glutamine, myo-inositol, taurine, glutathion, total creatine and hypotaurine have VIP>1 and then are the most important for separation of the 3 tumour models, using *ex vivo* ^{1}H RMAS data (**A.**) and *in vivo* data (**B.**), total creatine, glutamate, myo-inositol, total choline and GABA have the highest VIP.

Conclusion: A robust PLS-DA model (high Q2 values) could be built with ^{1}H RMAS MRS quantitative data despite inter-individual variability.

References: [1] Opstad et al., *J. Mag. Reson. Imaging,* 2010*;* [2] Holmes et al., *NeuroRx*, 2006; [3] Ratiney et al., *NMR biomed.*, 2005; [4] Rémy C. et al., *J Neurochem.* 1994.