NMR molecular profiling of high grade human glioma reveals distinct metabolic subgroups

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Purpose/Introduction:
Glioblastoma Multiforme (GBM) and Anaplasic Astrocytoma (AA) are astrocytic neoplasic entities of the Central Nervous System that appear in adults, with high biological and clinical aggressiveness. They constitute defined neoplasic forms with grades III (AA) and IV (GBM). These neoplasies are highly resistant to the different treatments evolving towards patient death in short periods of time. Despite the definition of new neoplasms genetic subgroups, the most relevant information in the prognostic of the patient comes still from factors as patient age, localization and size of the tumour, oedema presence and mass shifting. Metabolic phenotyping of high grade glioma may provide new information for better management of this disease. In this communication, we show high grade glioma molecular profiles and metabolic subgroups based on HR-MAS spectra of 31 high grade glioma biopsies.

Subjects and methods
HR-MAS 1H NMR spectra and consequent biochemical profile determination were obtained for 31samples of human glioma tissue, of which 25 were Glioblastoma Multiforme (GBM) and 6 were Anaplasic Astrocytoma (AA). The amount of human tumour tissue analysed for each subject ranged from 20 to 40 mg. The whole HR-MAS study was performed at 4 C. HR-MAS spectra were recorded in a Bruker AVANCE spectrometer at 600 MHz. Samples were spun at 5kHz. Three different types of spectral editing were obtained by recording 1D 1H pre-saturation, 1D 1H NOESY and 1D 1H CPMG (30 ms echo time) experiments. 2D 1H TOCSY and 2D 13C-HSQC experiments were also recorded on selected samples for assignment purposes. 1D spectra were processed with 0.3 Hz line broadening. Central position of Alanine doublet (1.478ppm) was used for spectral referencing purposes. All samples were analyzed by post-HRMAS histopathology to assess the tissue integrity and double validate histological diagnosis.

Statistical analysis was performed using in-house MATLAB scripts and the PLS Toolbox statistical multivariate analysis library. Principal components chosen explained at least 70% of the variance.

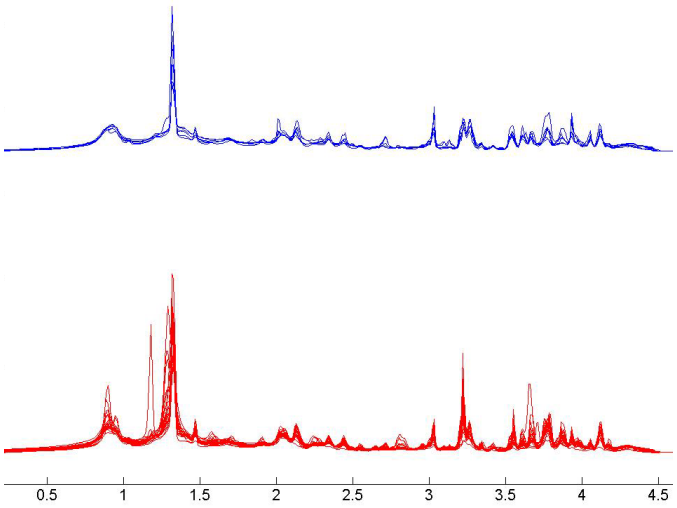

Figura 1. All 1D pre-saturated NMR spectra binned for multivariate analysis for GBM (red bottom) and AA (blue top) human biopsies.

Results
NMR spectra showed narrow line widths and adequate signal-to-noise ratios with well resolved spin-spin multiplicities, as shown in Figure 1. Resonances were assigned by methods described elsewhere [1]. In order to minimize the impact of necrotic tissue content in the biopsy, first a correlation between the different spectral regions and the necrotic content of the tissue analyzed was performed. Regions with correlation higher than 0.6 (some fatty acids and lactate) were removed for subsequent analysis. The remaining molecular profile was then analyzed by PCA and cluster analysis (Figure 2) to detect potential intrinsic groups in the dataset. Two major groups were detected including 16 and 11 samples respectively. Four samples were not included in either of these major groups. Interestingly, most AA were located in the same group. The phospholipids pattern and the glutamine/glutamate metabolic relatives seem to be the most relevant contribution to this grouping pattern.

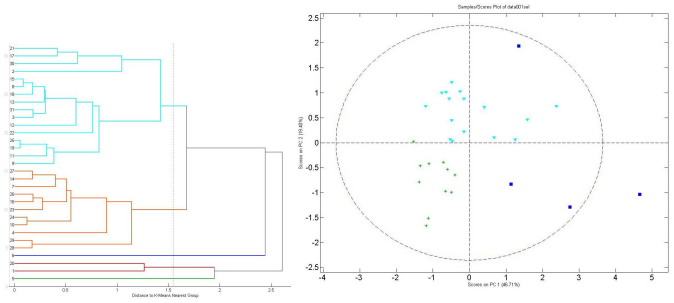
Figura 2. Hierarchical clustering of high grade human glioma samples (left) and PCA scores plot (right) showing the two distinct metabolic subgroups (same coloring).

Discussion
HR-MAS provides high resolution glioma molecular profiles. The removal of the spectral component corresponding to necrosis allows detecting two metabolic patterns which may reflect different molecular groups. Interestingly, one of the groups, which includes all AA samples, seem to reflect a less aggressive type of tumour with lower levels of phosphocholine and higher levels of glutamine. Metabolic discrimination between these subgroups according to the Principal Component Analysis, include the levels of some metabolites which can be seen by MRS 'in vivo'.

References
1. Monleón D, Morales JM, Gonzalez-Darder J, Talamantes F, Cortés O, Gil-Benso R, López-Ginés C, Cerdá-Nicolás M, Celda B. Benign and atypical meningioma metabolic signatures by high-resolution magic-angle spinning molecular profiling. J Proteome Res. 2008 Jul;7(7):2882-8.

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