Initial metabolomic analysis of glioblastoma multiforme subtypes by HRMAS

D. Valverde-Saubí[1,2], A. P. Candiota[1,2], M. A. Molins[3], M. Feliz[3], Ó. Godino[4], J. Martino[4], J. J. Acebes[2,4], and C. Arús[1,2]

[1]Bioquímica i Biologia molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Barcelona, Spain, [2]Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Cerdanyola del Vallès, Barcelona, Spain, [3]Servei RMN Parc Científic de Barcelona, Universitat de Barcelona, Barcelona, Barcelona, Spain, [4]Servei de Neurocirurgia, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain

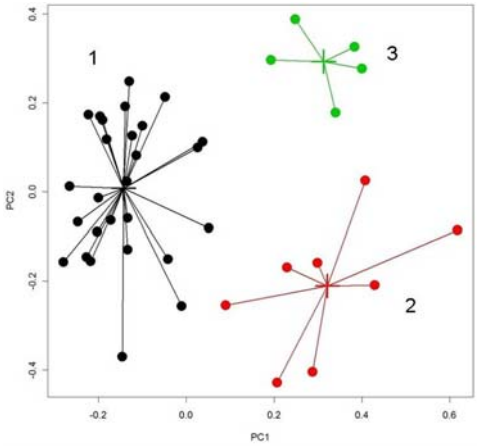

Figure 1. Latent space obtained by displaying the two first principal components (PC) showing the clustering resulting from the K-means analysis on the pulse and acquire 0ºC glioblastoma multiforme HRMAS spectra (n=42). Each dot represents a GBM case. Crosses label group centroids. Numbers (1: black (n=29), 2: red (n=8), 3: green (n=5)) refer to potential GBM subtypes

Purpose/Introduction

The existence of molecular subtypes of glioblastoma multiforme (GBM) [1,2] according to genomic and transcriptomic data is nowadays accepted. The purpose of this study was to search for possible GBM subtypes based in their HRMAS detected metabolomic profile.

Samples and Methods

42 GBM biopsies were analyzed. HRMAS spectra were obtained at 0ºC using a pulse and acquire sequence with water presaturation. Spectra were processed with 0.5Hz line broadening, phased and baseline corrected. Chemical shift calibration was carried out by setting the lactate doublet central position at 1.33ppm. If lactate was too low, the central position of the alanine doublet (1.45ppm) or the creatine methyl singlet (3.03ppm) was used. Unit length normalization (L2 norm) was applied. All statistical procedures were carried out using R. Principal Component Analysis (PCA) was applied to the whole set of spectral vectors. Hierarchical Clustering Analysis (HCA) followed by K-means analysis was carried out along with Goodman-Kruskal index (GKi) calculation to validate the optimal number of clusters within the analyzed GBM population. This number was also validated by developing an LDA-based classifier using the clustering vector as the vector class and testing it with LOO-CV[3]. Mean spectra were calculated for the different clusters found. Change in major spectral pattern differences between clusters was calculated using the intensity of the relevant metabolite maximum peak height of cluster 1 as reference and providing the variation encountered as a percentage of cluster 1 resonances initial values. A One-Way ANOVA test was used to search for statistically significant differences between major spectral pattern changes. Twenty five out of 42 biopsies were analyzed by post-HRMAS histopathology to control sample integrity and correlation with the biopsy used for initial histopathology.

Results

HCA and GKi showed that the optimal number of GBM clusters for the k-means analysis was 3 (Figure 1). An LDA-based classifier produced 100% accuracy when validated with LOO-CV. Mean spectra (Figure 2) and PCA loadings (results not shown) showed differences between groups in glycine (Gly), myo-inositol (mI), phosphocholine (PC), choline (Cho), glutamine (Gln) and mobile lipids (ML). These differences are quantified in Table 1. No samples were discarded from the analysis as all the post-HRMAS characterization agreed with the original diagnoses.

Metabolite	Group 2	Group 3
Glx	74.0	71.8
Gly	97.6	90.0
mI*$#	69.0	46.4
PC*$#	63.6	43.6
Cho	60.5	67.7
PC/Cho$#	99.2	64.6
Gln$#	89.3	31.6
ML*$#	206.2	389.2
PC/Cr*$#	58.9	142.1
ML/Cr*$#	226.1	962.0
mI/Gly*$	67.8	55.3

*Table 1. Changes in the resonances with most important visual variation among clusters. Values are tabulated as a percentage of group 1 (100% means no change). * label statistically significant changes between group 1 and 2. $ label statistically significant changes between group 1 and 3. # labels statistically significant changes between group 2 and 3.*

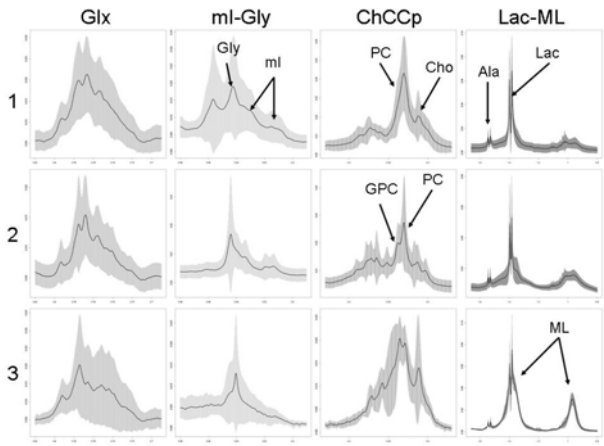

Figure 2. Glx, mI-Gly, Choline Containing Compounds(ChCCp) and ML regions of the mean spectra of each cluster shown in Figure 1. Grey area corresponds to mean±SD

Discussion/conclusion

K-means analysis of HRMAS spectra shows that it is possible to establish three different GBM clusters. Mean spectra of each cluster shows clear differences in their pattern. These differences correlate with those seen when proliferation rate changes either *in vitro* [4,5] or *in vivo* [6-9]. Thus, it is possible to hypothesize the existence of three different metabolic glioblastoma multiforme subtypes. However, mI or mI/Gly variations would not agree with previous cell model data[4]. Various reasons may explain this discrepancy, like differential Gly visibility *in vivo* vs. HRMAS or different mI concentration in culture media vs. the *in vivo* situation, although further work will be needed to clarify this point. Finally, correlation of these metabolomic clusters with clinical (survival, Karnofsky index) and transcriptomic data will be necessary to further validate the HRMAS detected GBM subtypes.

References

[1] Korshunov *et al*, Acta Neuropathol 2006;111:467–474, [2] Beier *et al*, Cancer Res 2007;67:4010–4016, [3] Golub *et al*, Science, 1999;286:531–537, [4] Valverde *et al*, NMR Biomed 2006;19:223–230, [5] Barba *et al*, Cancer Res 1999;59:1861–1869, [6] Usenius *et al*, Comput AssistTomogr 1994;18:705–713, [7] Gillies *et al*, Magn Res Med 1994;32:310–318, [8] Aiken *et al*, Anticancer Res 1996;16:1393–1397, [9] Herminghaus *et al*, NMR Biomed 2002; 15:185–192