Correlation of NMR metabolic profile and gene expression profiles in high grade glioma

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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 neoplasms are highly resistant to the different treatments evolving towards patient death in short periods of time. The growing of these tumours is linked to a process of vascular growing, tumor induced angiogenesis, which supplies the needs of the neoplasic cells. Ultimately, these changes produce functional and temporal heterogeneous alterations with important biopathological (hypoxia, thrombosis, necrosis, edema) and clinical (endocraneal hypertension) repercussion. The metabolic consequences of different angiogenesis gene expression profiles in gliomas are still unknown. Gene expression profiling of different metabolic phenotypes of high grade glioma may provide new information for better management of this disease. In this communication, we show high grade glioma NMR molecular profiles correlated with gene expression profiles of 20 high grade glioma biopsies.

Subjects and methods

Samples Thirty samples of human glioma tissue, of which 23 were Glioblastoma Multiforme (GBM) and 7 were Anaplasic Astrocytoma (AA) were analyzed. The amount of human tumor tissue analyzed for each subject ranged from 20 to 40 mg.

NMR spectroscopy 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. All samples were analyzed by post-HRMAS histopathology to assess the tissue integrity and double validate histological diagnosis.

Gene expression profiles Total RNA from twenty samples was extracted using a mirVANA miRNA Isolation Kit (Ambion, Ambion INC•The RNA Company, Austin). The GeneChip Human Gene 1.0. ST Array (Affymetrix, Santa Clara, CA, USA) was used for microarray analysis. The distribution of fluorescent material on the array was obtained using GeneChip Scanner 3000 7G (Affymetrix, Santa Clara, CA, USA).

Statistical analysis 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. GeneChip Operating Software supplied by Affymetrix was used to perform gene expression analysis.

Results

NMR spectra showed narrow line widths and adequate signal-to-noise ratios with well resolved spin-spin multiplicities, as shown in Figure 1. The molecular profile was then analyzed by PCA and cluster analysis to detect potential intrinsic subgroups in the dataset. Two major subgroups (subgroups A and B) were detected including 18 and 10 samples respectively. Two samples were not clustered in any of the two subgroups. Interestingly, all AA were located in the same subgroup. The phospholipids pattern, the glycolytic ratio and the glutamine/glutamate metabolic relatives seem to be the most relevant contribution to this grouping pattern. Microarrays analysis of genome-scale mRNA expression data for these human malignant gliomas revealed a list of >300 probe sets (Figure 2A) that are significantly different between these two metabolic subgroups. These differentially expressed genes include genes relevant to angiogenesis, like VEGF (expression levels shown in Figure 2B).

Discussion

Our results show a correlation between angiogenesis and metabolic profile. This correlation involves metabolites closely related to higher glycolytic rate, to proliferation and to hypoxia. Our approach suggests that combined analysis of existing data sets can reveal new insights and that the large amount of publicly available cancer data sets should be further utilized in a similar manner.
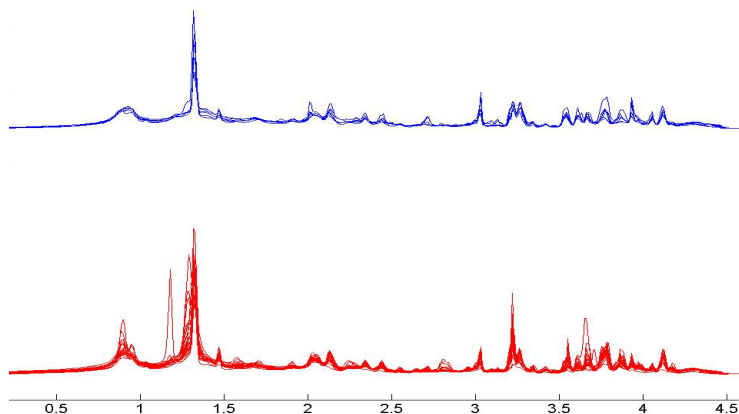
Figura 1. All 1D pre-saturated NMR spectra binned for multivariate analysis for high grade glioma subgroup A (red bottom) and subgroup B (blue top) human biopsies.
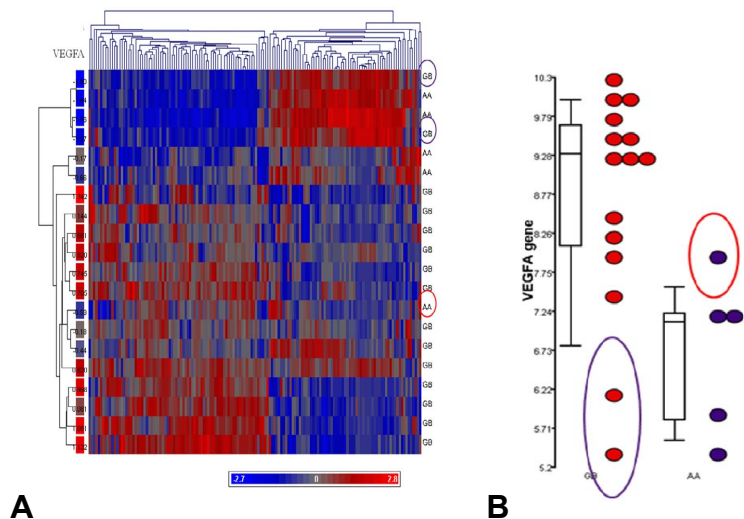

A **B**

Figure 2. A) Hierarchical cluster analysis of metabolic subgroups. The red and blue bars indicate genes demonstrating increased and decreased expression, respectively. Only the top 376 genes are shown in the cluster. B) The fluorescence intensity values in vascular epidermal growth factor A (VEGFA) gene to compare in two metabolic subgroups analyzed.