Correlations with gene expression profile may generate new MR spectroscopy markers for glioblastoma prognosis and treatment planning

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Introduction

Gliomas account for 42% of all central nervous system tumours [1] and in their most malignant form, the glioblastomas (GBMs), cause the death of most patients within a year of diagnosis. Glioblastomas can either be primary (*de novo*) malignant neoplasm or secondary (transformed) low grade glioma [2]. These two types of GBM are equivalent histopathologically but develop with different genetic pathways and may differ in their response to treatment [3]. Microarray technology can determine tumour gene expression patterns and, therefore, GBM subtype. *In vivo* Magnetic Resonance Spectroscopy has been shown to be a potential diagnostic aid for brain tumours [4] with some lipid resonances implicated as markers for glioma grade [5]. *Ex vivo* HR-MAS MRS of excised tumour tissue has much higher resolution than *in vivo* spectroscopy and can be used to assign and quantify the resonances of more brain tumour metabolites [6]. We hypothesise that quantitative *ex vivo* MRS can establish patterns of metabolite concentrations that relate to the different genetic pathways underlying glioma malignancy.

Methods

Surgically resected brain tumour samples were frozen and split for microarray and HR-MAS analysis. Total mRNA gene expression was measured using the whole genome HG-U133 gene chip array (Affymetrix). Thirteen samples (10 GBM, three grade II and III astrocytomas) provided suitable quality data and were background corrected, normalised and summarised using Genechip software. An unsupervised hierarchical clustering was performed using only genes specified by KEGG pathway hsa05214, a subset of genes that are involved in either (or both) the *de novo* and transformation pathways of GBM genesis. ^{1}H MRS HR-MAS and subsequent quantitation with LCModel was performed as described previously [6]. Significant differences in metabolite concentrations between two tumour clusters were taken at $p<0.05$ from pair wise-independent T-tests after Bonferroni correction for multiple comparisons.

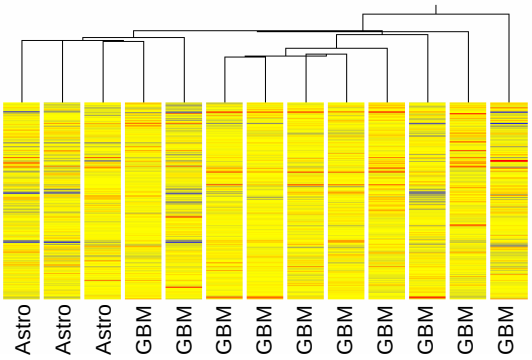

Figure 1. **Heirarchical clustering of microarray data**

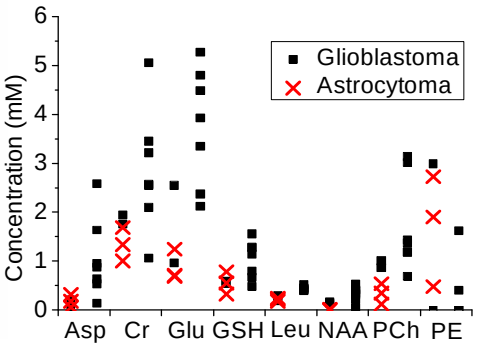

Figure 2. **Individual metabolite concentrations**

Results and Discussion

Hierarchical clustering of microarray data from the 13 samples was performed using a subset of genes that are involved in either (or both) the *de novo* and transformation pathways of GBM genesis. Apart from one GBM the samples fell into two distinct clusters (Figure 1), one containing the three lower-grade astrocytomas and two GBMs the other containing another 7 GBMs. The largest average-expression level difference between the two clusters was the EGF receptor gene with greatest expression in cluster 2. Over-expression of this gene is associated with primary GBM [3]. Conversely the highest average expression of a gene in cluster 1 relative to cluster 2 is the PDGF receptor which is associated with low-grade astrocytomas and transformed glioblastomas [3]. This clustering suggests that cluster 2 consists of *de novo* glioblastomas whereas cluster 1 is the lower grade tumours and two transformation GBMs that share similar patterns of expression. When average metabolite concentrations of cluster 1 and cluster 2 are compared for each metabolite, some significant differences between the groups emerge for aspartate (Asp), creatine (Cr), glutamate (Glu), glutathione (GSH), leucine (Leu), N-acetyl-aspartate (NAA), phosphorylcholine (PCh) and phosphoethanolamine (PE). The individual metabolite concentrations for these metabolites in each sample are shown in Figure 2 with cluster 1 to the left and cluster 2 to the right. The GBMs in cluster 1 have similar metabolite concentrations to the lower grade tumours for aspartate, glutamate, glutathione and leucine, which are lower than those concentrations in cluster 2. This suggests that higher concentrations of these metabolites may be markers for primary GBM. Phosphorylcholine concentrations in lower-grade tumours are less than in all the GBMs which suggests that PCh may give information on glioma grade. Detailed genetic characterisation of gliomas may reveal important markers for treatment planning or prognosis and if *ex vivo* HR-MAS spectroscopy can determine metabolites correlated to gene expression then perhaps *in vivo* spectroscopy techniques can be developed that will provide similar information to help plan patient care.

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