NMR spectroscopy identifies two subtypes with different metabolic profiles in stem-like cells from Glioblastoma multiforme

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INTRODUCTION

Glioblastoma multiforme (GBM) is a common malignant brain tumour with aggressive and infiltrative characteristics. Treatments include surgical resection, adjuvant radiotherapy and chemotherapy. Only a small percentage of the patients is alive after three years, while many patients face a very short survival. Many studies are presently being conducted to identify biomarkers useful to predict GBM patient survival and, possibly, to offer better patient-tailored treatments.

Recurrence of the tumour after conventional treatments is attributed to the overgrowth of stem-like cancer (CSC) cells that are resistant to treatments causing relapse of the disease. In the frame of the CSC hypothesis, in previous researches some authors[1] found a correlation between clonogenicity and the area of the spectral region where glutamate signals resonate (2.28–2.38 ppm) in NMR spectra from GBM CSCs, and attributed the presence of high lipid signals to the onset of apoptosis. The relationship between this finding and any characteristics of GBM CSCs was not understood. In a previous work[2] we have shown that GBM CSCs grown in neurospheres have ^{1}H NMR spectra testifying for a very active metabolism and are characterized by intense signals common in brain and brain tumours. The unsupervised analysis performed on spectral data of thirteen CSCs from different GBM patients strongly suggested the presence of two subtypes with different metabolic phenotypes. In one group (group 1) a mixed neural–astrocyte metabolic phenotype with a strong neuronal fingerprint prevailed, while in the other cluster (group 2) an astrocytic/glioma-like metabolism was observed.

The analysis of NMR spectra from a larger number of GBM derived cancer stem-like cells by means of cluster analysis is here presented. The presence of different metabolic subtypes is confirmed. Association to different patient survival is also envisaged.

METHODS

CSCs deriving from primary GBM were isolated from adult patients undergoing craniotomy. Informed consent was obtained. Cells were kept in culture as exponentially growing neurospheres according to[3]. The criteria used to check the stem cell phenotype of GBM CSCs are described in[4]. ^{1}H NMR spectra were obtained at 400.14 MHz (Bruker Avance) equipped with a 1mm microprobe. 1D and 2D COSY spectra were acquired under water suppression conditions. Signal intensities were referred to the large macromolecule signal (M) at 0.9 ppm in 1D and to the lysine (Lys) cross peak at 1.7-3.0 ppm in 2D spectra, according to[2]. Agglomerative hierarchical clustering was performed utilizing XLSTAT software. Values of $\log_2$(FC) (FC, fold change) resulting from the comparison between the two main clusters were calculated for each metabolite. Student t test was performed utilizing XLSTAT software. Estimates of survivals were evaluated using the Kaplan–Meier product limit method and compared with Student t test by means of the MedCalc Software.

RESULTS

A total of 27 CSC lines from GBM patients with different clinical outcome were analyzed by 1D and 2D COSY NMR. Individual GBM CSCs showed spectral features bound to the CSC line. The GBM CSC neurospheres, despite the hypoxic environment, host viable and metabolically active cells, as shown in the representative spectra of three different CSCs reported in fig 1. Among other signals, NAA, creatine (Cr), glutamine (Gln) and lipid signals (ML) and the low field signal attributed to N-Acetylgalactosamine (not shown) were detected and quantified to provide a metabolism-based fingerprinting of CSCs[2]. In two CSCs the intense α aminoadipic acid (αAAD) signals were also identified by comparison with pure compound signals (Figure 1 line c).
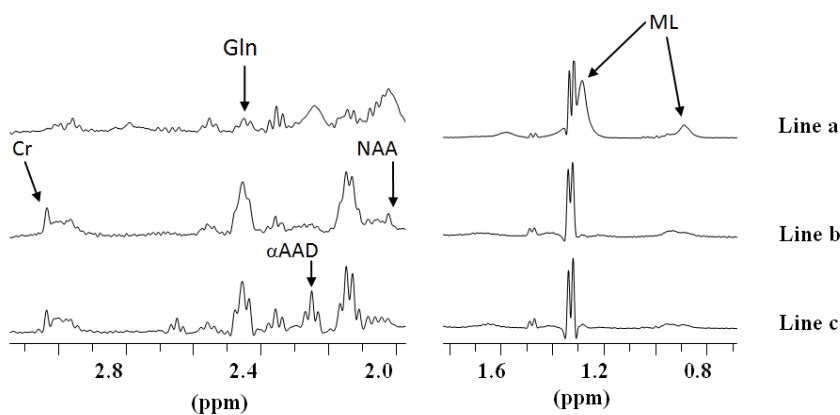

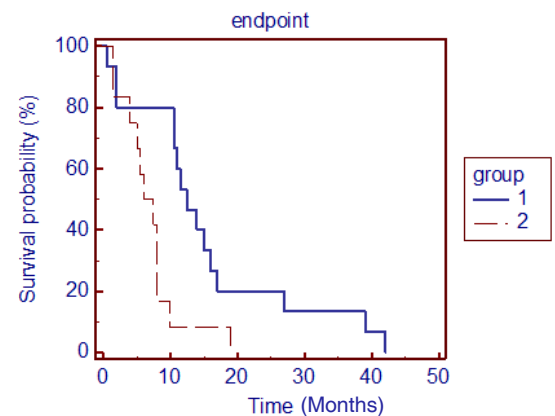**Figure 1** a) Spectra run at day 7 from plating of 3 GBM CSC lines (region 3.5-0.5 ppm).

Figure 2 Kaplan–Meier estimates for overall survival based on clusters identified as indicated in the text (first group n=15, second group n=12).

Cluster analysis based on these signal intensities confirmed the presence of the two subtypes suggested by the preliminary analysis on 13 cell lines[2]. NAA, Cr, Gln and N-Acetylgalactosamine were prevalent in one group (group 1) and lipid signals, mostly from triglycerides, were more relevant in the second group (group 2), irrespective of cells apoptotic fate. The Kaplan–Meier estimates for patients' survival based on this NMR-based subtyping is shown in Fig 2. Identification of metabolism based subtypes points to a prognostic relevance, with cells clustering in group 1 showing a significantly (P = 0,0076) improved median patients' survival of 12,5 months versus 6 months in cells clustering in group 2. Furthermore, the identification of very intense signals from the unusual metabolite α AAD (Fig 1, Line c) in two cell lines of group 1 with very low patients' survivals (lower than 2 months) deserves further attention.

DISCUSSION AND CONCLUSIONS

Present data demonstrate that GBM CSCs host cells with very active metabolism characterized by different metabolic patterns. Neuronal markers and glial markers were detected at different intensities depending on the observed CSC line. Cluster analysis based on selected signals allowed identification of two subtypes in CSCs derived from 27 patients. When survival probability was challenged between the two groups, the second group, characterized by high lipids and lower intensity signals of neuronal markers, had shorter survivals that could be related to a different response to treatments.

A recent study, performed on surgery-derived glioma tissues by different methods based on Mass Spectrometry, demonstrated the presence of metabolic subtypes relevant with respect to patients' survival[5]. The analysis of the metabolomic profiles coupled to gene analysis of the whole cancer tissues suggested the presence of two different subtypes in GBM defined as anabolic metabolism and altered phospholipid metabolism, this latter subtype being related to particularly aggressive disease. In GBM CSCs here observed, the presence of high lipid signals from triglycerides in the second CSCs group was found associated to shorter patient survival. This would be consistent with the altered phospholipid metabolism observed in the most aggressive subtype identified in the study on glioma-derived tissues[5].

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