NMR spectroscopy identifies subtypes of stem-like cells from glioblastoma multiforme characterized by different metabolic fingerprints

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INTRODUCTION

Glioblastoma multiforme (GBM) is one of the most aggressive brain tumour, GBM patients facing a very poor prognosis. The identification of biomarkers to define GBM subtypes aimed at patient-tailored treatments is a challenging field of study. Recurrence of GBM is attributed to the presence of stem-like cells that are resistant to treatments.
Forty Glioblastoma Stem-like Cell (GSC) lines, derived from newly diagnosed cases of glioblastoma patients (Catholic University, Rome) were profiled via NMR spectroscopy to identify patterns to group tumours characterized by different metabolism. Moreover, GSC spectra were compared with those of human neural progenitor cells from human adult olfactory bulb (OB-NPCs) and of a cultured glioblastoma line, namely T98G. The unsupervised cluster analysis performed on twelve metabolic parameters from spectra of the 42 cell lines is here presented.

MATERIALS AND METHODS

GSCs deriving from primary glioblastoma were isolated and kept in culture as exponentially growing neurospheres according to [1]. Neurospheres are floating structures obtained by the dissociation of cancer tissue into individual cells, growing as aggregates resembling spheroids in serum free medium supplemented with growth factors. The criteria used to check the stem cell phenotype of GSCs, were i) formation of primary spheres in vitro; ii) capacity of self-renewal on clonogenic and population analysis; iii) ability to differentiate under serum stimulation both into GFAP-positive astrocyte-like cells and into neurofilament expressing neuron-like cells; iv) generation of tumours upon orthotopic (intracerebral) transplantation in immunodeficient mice; v) maintenance of the chromosomal aberrations of the parental tumour. 1D and 2D COSY ^{1}H MR spectra of intact neurospheres were run at 400.14 MHz on a digital Avance spectrometer (Bruker, Karlsruhe, Germany) equipped with a 1mm microprobe. Signals were acquired with a 90° RF pulse and a sweep width of 4006.4 Hz. Water suppression was obtained by irradiating water signal.

RESULTS AND DISCUSSION

In a previous work, conducted on 13 lines using 11 NMR parameters [2], two subtypes with different metabolic phenotypes have been identified. The Cluster 1 was characterized by a mixed neural–astrocyte metabolic phenotype, with a strong neuronal fingerprint, while in the cluster 2 a glioma-like metabolism prevailed.
^{1}H NMR spectra of cluster 1 cells show low intensity mobile lipid (ML), intense N-acetylaspartate (NAA) and glutamine (Gln) signals, the presence of NAA and Gln being attributed to a metabolic fingerprint typical of neurons. On the other hand, in spectra of cluster 2 cells, high lipid, low Gln are observable while signals from NAA are absent , thus suggesting a prevalent glioma-like metabolism. An exemplificative spectrum of a line belonging to cluster 2 is shown in Figure 1.
In the present work the additional signal of glycine (Gly) was considered for the cluster analysis. Signals from Gly, an inhibitory neurotransmitter in the central nervous system, were absent in some lines and very intense in others, particularly in the spectra of OB. Overall, the study was carried out on 42 lines as specified above and 12 metabolic parameters, i.e. Gly, NAA, Gln, total Creatine (tCr), γ-amino-butyric acid (GABA), N-acetyl galactosamine (GalNac), myoinositol (Myo-I), glutathione (GSH), glutamate (Glu), UDP-hexosamine (U_5), aspartate (Asp), ML. Among them those present in cluster 2 are showed in Figure 1.
Cluster analysis based on selected signals allowed identification of three subtypes (Figure 2). Cutting the tree at an appropriate level, in addition to previously identified clusters (cluster 1 neural like and cluster 2 glioma like) a third cluster is now present. GSCs of this cluster show a metabolic pattern similar to that of neural cells (cluster 1), but with more intense Myo-I and less intense GABA and Gly signals, both inhibitory neurotransmitters. This suggests an astrocytic like fingerprint for this cluster. Moreover OB-NPCs and T98G cell lines were correctly classified in cluster 1 and 2 respectively.
In conclusion NMR based cluster analysis of GSC metabolic parameters allowed to classify cell lines in three main clusters, each characterized by distinct prevalent metabolic phenotypes, possibly related to patients' survival [2]. Correlation of NMR markers and biological data of GSCs with patients' clinical outcomes may open new prognostic and therapeutic perspectives.
Moreover details on GSC metabolism may help in coping with GSC radioresistance to conventional therapies.

ACKNOWLEDGEMENTS

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REFERENCE

[1] R.Pallini et al., Clin Cancer Res 14 (2008) 8205-8212.
[2] L.Guidoni et al., NMR Biomed. 27 (2014) 129-145.

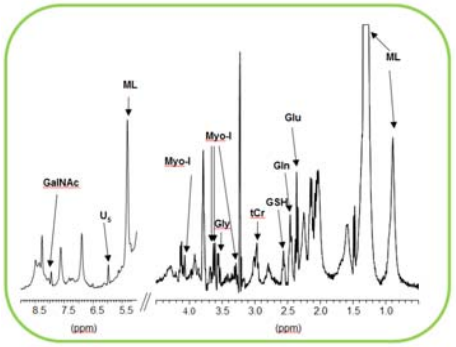

Figure 1. 1D 1H NMR spectrum of line #83 cells, belonging to cluster 2.

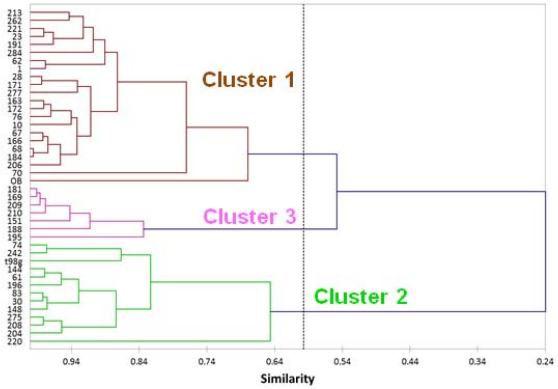

Figure 2. Metabolite analysis of 42 cell lines. Cluster analysis separated GSCs into three groups: cluster 1 neural like, cluster 2 glioma like and cluster 3 astrocytic like.