Metabolite profiling of glioblastoma stem-like cells with ^{1}H NMR identifies α-aminoadipic acid, product of the activity of aldehyde dehydrogenase ALDH7A1, as putative biomarker of tumor aggressiveness

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INTRODUCTION

Glioblastoma multiforme (GBM) is very difficult to treat and patients face a poor prognosis, with a median survival of about 14 months. High recurrence rate and failure of conventional treatments is attributed to the presence of cancer cells with stem-like properties. Extensive efforts have been made to identify markers of stemness, including cell surface, enzymatic, gene expression profile, and functional markers. Among these, the expression of the members of aldehyde dehydrogenase (ALDH) gene superfamily[1] have been considered as a possible marker of stemness and particularly of tumor stemness[2]. ALDHs are implicated in the lysine catabolism in humans that proceeds in a sequence of seven reactions, ending into the pathway for fatty acid metabolism. As first steps, lysine is combined with alpha-ketoglutarate to form saccharopine, which is then oxidized to yield α-aminoadipic semialdehyde (αASA). A second pathway of lysine catabolism, prevalent in brain, through the formation of pipecolic acid leads always to αASA formation The latter molecule is further oxidized to α-aminoadipate (αAAD). ALDH7A1, known also as antiquitin, is the enzyme that facilitates the conversion of αASA to αAAD. In the present study, a dramatic accumulation of αAAD has been detected in a subset of glioblastoma cancer stem-like cell (GSC) lines derived from primary GBM grade IV (WHO classification) by means of ^{1}H NMR and it was related to tumor aggressiveness.

MATERIALS AND METHODS

Cancer stem cells deriving from primary glioblastoma were isolated and kept in culture as exponentially growing neurospheres according to [3]. 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, according to[4], 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 tumors upon orthotopic (intracerebral) transplantation in immunodeficient mice; v) maintenance of the chromosomal aberrations of the parental tumor. ^{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. Perchloric Acid (PCA) extracts from the same cells were obtained according to ref [5].

RESULTS

A triplet at 2.25 ppm, labeled αAAD in Figure 1a, appeared in 1D ^{1}H NMR spectra of some intact GSC neurospheres together with multiplets at 1.65 and 1.85 ppm. Their intensities were high, medium or absent in different GSC lines. In parallel, 2D COSY ^{1}H NMR spectra showed crossing of triplet with the signal at 1.65 ppm (figure 1a') crossing in turn with the peak at 1.85 (arrows in figure 1 a'); this last signal was crossing with a peak at 3.77 ppm. 1D and 2D spectra of αAAD in solution showed analogous signal patterns (Figure 1bb'), in agreement with current literature[6]. Similarly in PCA extracts of GSCs, higher signals resemble the same signal intensities in corresponding cell spectra of different GSC lines.

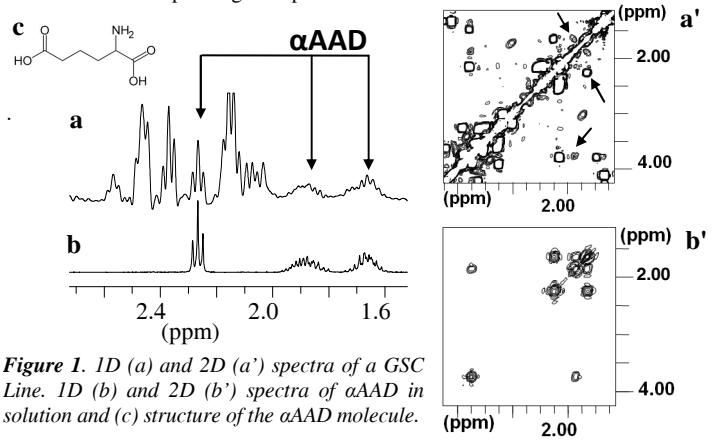

***Figure 1**. 1D (a) and 2D (a') spectra of a GSC Line. 1D (b) and 2D (b') spectra of αAAD in solution and (c) structure of the αAAD molecule.*

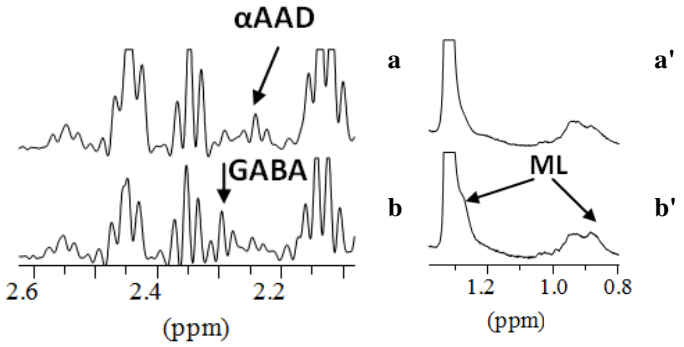

***Figure 2**.* 1D spectra of a GSC line at day 7 (aa') and at day 10 (bb') after seeding.

The peak patterns of Figures 1aa' are therefore indicative of αAAD and the cross peak at 2.25-1.65 ppm is diagnostic for this molecule allowing to discriminate it from other molecules with similar structures. For example, a triplet is present at 2.17 ppm in glutaric acid 1D spectra with a cross peak is at 2.17-1.77 ppm, a multiplet is present at 2.25 ppm in hydroxyglutaric acid 1D spectra, but the cross peak is at 2.25-1.90. Furthermore, the triplet at 2.25 could not be attributed to GABA, triplet of this latter molecule resonating at 2.30 ppm and cross peaks at 2.30-1.92 and 3.00-1.92. Differently from other metabolite signals, the αAAD signal intensities were not constant during cell growth, signals showing similar intensity in the range 4-8 days in culture, while declining afterwards, in parallel with the intensity increase of mobile lipid (ML) signals and of GABA signals when present (Figure 2aa'bb'). Out of a total of 27 GSC lines, we have examined αAAD signal intensity in spectra of 17 GSC lines that according to a clustering based on metabolic ^{1}H NMR signals showed spectra characterized by low ML and high intensity signals of neural origin[7]. By considering that αAAD is a product of Lys catabolism, we have considered the intensity ratio R between αAAD and the sum (Lys+ αAAD) as a function of patients' progression free survival (PFS) (Figure 3). An inverse correlation between R and PFS was shown by the Spearman's coefficient ρ=-0.946 with P<0.0001. Approximately the same statistical result was shown when considering the correlation between R and overall survival OS (ρ= -0.92, P<0.0001).

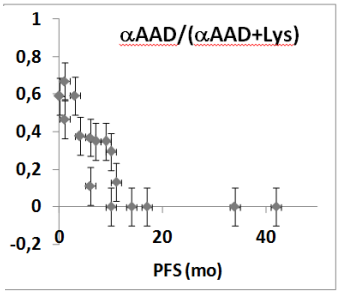

Figure 3. Ratio R as a function of PFS

DISCUSSION AND CONCLUSIONS

Expression and high activity of ALDH7A1 in some GSCs could be envisaged on the basis of the high level of αAAD found by ^{1}H NMR. The study confirms a role of αAAD as biomarker of cancer suggested by previous studies[8]. Finally, the indication that, similarly to prostate cancer[9], ALDH7A1 activity in glioblastoma may correlate with tumour invasiveness, is of potential diagnostic importance.

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