Charged particles induced metabolic changes in glioblastoma stem cells

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INTRODUCTION

Glioblastoma multiforme (GBM) is a malignant primary brain tumour, with very poor prognosis. The high recurrence rate and failure of conventional treatments are expected to be related to the radioresistance cancer stem cells (CSCs) inside the tumour [1]. On the other hand, recent experimental evidence showed a higher effectiveness of carbon ions respect to photons in inactivating CSCs from colon carcinoma [2]. These results suggest a potential advantage of hadrontherapy with respect to conventional radiotherapy.
In the present work, glioblastoma stem cells (GSCs, namely line #1 and line #83) derived from patients have been irradiated with protons and carbon ions of 62 MeV/u at the Superconducting Cyclotron (CS) radiobiology facility of the LNS-INFN in Catania and monitored by 1H NMR Spectroscopy to investigate effects on GSC metabolism.

MATERIALS AND METHODS

Cell Culture

GSCs isolated from surgical samples of patients with similar tumour location, gender, and age but different clinical outcome were used, namely line #1 and line #83. Specimens of tumour tissues were obtained by surgical resection at the Institute of Neurosurgery, Catholic University School of Medicine, Rome. GSCs were isolated, cultured and stemness evaluated at the Department of Haematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome.

Irradiation conditions

GSCs were irradiated with protons and C-ions at the CS radiobiology facility of the LNS-INFN. Commercial 1 ml polystyrene cuvettes were used as sample holders, positioned into a mask especially designed and realized by the colleagues of the LNS for remote controlled irradiation of up to 5 samples (Figure 1). In these cuvettes, the cell suspensions were centrifuged and irradiated as a pellet, 4 mm thick, with doses of 5, 10, 20, and 40 Gy. The energies at the entrance of the cell suspensions for protons and for C-ions were 61 MeV/u and 52 MeV/u respectively, corresponding to LET values (in water) of 1.1 keV/µm and 43.2 keV/µm. On the contrary for C-ions there is a significant variation of the average LET from 43.2 keV/µm to 59.0 keV/µm. In all the experiments a dose rate of about 2 Gy/min was used.

^{1}H NMR measurements

1D and 2D COSY ^{1}H NMR spectra were run at 400.14 MHz on a Digital AVANCE Bruker (Bruker, Germany) spectrometer equipped by a 1 mm microprobe. The sample volume was 12 µl. Water suppression was obtained by irradiating water signal. Proton and C-ion irradiated line #1 and line #83 cells were monitored by 1H NMR 48, 72 and 96 hrs after irradiation, together with the corresponding control, sham irradiated, samples.

RESULTS AND DISCUSSION

1H NMR spectra of control line #1 cells are characterized by low intensity mobile lipid (ML), intense N-acetylaspartate (NAA) and glutamine (Gln) signals. The presence of NAA and Gln indicates a metabolic fingerprint typical of neurons. On the other hand, in spectra of control line #83 cells, high lipid, low Gln are observable while signals from NAA are absent, suggesting a prevalent astrocyte/glioma-like metabolism, according to [4].
A net increase of ML signals in irradiated line #1 cell spectra, has been observed 96 hrs after both protons and carbon ions irradiation at a dose of 10 Gy, with respect to control samples (Figure 2 a and b).
With respect to Glx pool metabolism (Glx stands for a pool of GABA, Gln, Glu and GSH), related to oxidative stress response, we observed different results. GSH signal intensity decreased after protons and carbon ions irradiation, the signal intensity remaining always lower with respect to the control values. This effect indicates the activation of a detoxification mechanism committed to GSH. GABA signals clearly increased (all doses), with a parallel decrease of Glu and Gln signals after protons and carbon ions irradiation, confirming what observed in experiments performed with gamma rays and carbon ions of different energy at CNAO facility, Pavia.
The increase of ML signals is not visible in both C-ion and proton irradiated line #83 spectra (Figure 2 c and d), at all doses, while some effects on Glx pool signals are evident, similar to those observed in line #1 cells but the size of the effects is smaller.
In conclusion, present data show that a set of metabolic responses/defences is observed in line #1 cells after irradiation. Moreover, the effects on ML signals can be related to a difference in energy metabolism between line #1 and line #83. Indeed, increase of ML has been recently related to mitochondrial impairment [4]; preliminary experiments in progress in our laboratory have shown that line #1 is characterized by an energy metabolism driven by oxidative phosphorylation while in line #83 glycolysis prevails. Finally, the observed results may indicate a modification of the metabolic fingerprint of line #1 cells, suggesting a shift toward a more astrocyte/glioma like metabolism, characterized by higher lipids and lower Gln signal intensities.

ACKNOWLEDGMENTS

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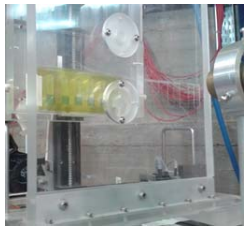

Figure 1. Apparatus for irradiating cell containing cuvettes in sequence.

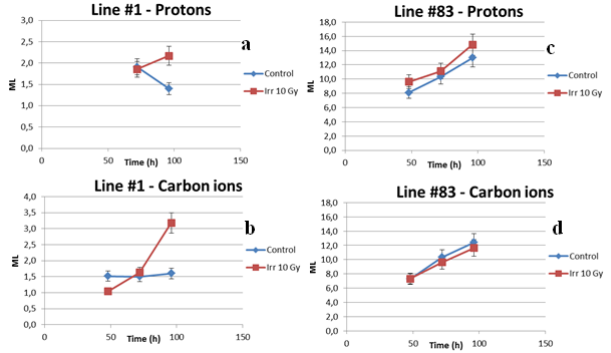

Figure 2. ML intensities (peak A at 1.28-0.98 ppm/Lys) from 2D COSY NMR spectra of control (squares) and irradiated (diamonds) line #1 (a, b) and line #83 cells (c, d).