Biological response of glioblastoma multiforme cancer stem cells to carbon ions irradiation

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Several experimental evidences have shown the advantages of heavy ions for localized tumour treatment. Carbon ions' high relative biological effectiveness in the Bragg peak region is related to the quality of the DNA damage they induce. The biological effects of densely ionising radiations are qualitatively and quantitatively different from those caused by photon irradiation, directly inducing complex clustered DNA lesions that make cellular enzymatic repair particularly difficult. These data suggest a potential advantage in terms of efficacy of Hadrontherapy with respect to conventional treatment plans. The effectiveness of the use of charged particles consists in enabling an excellent localization of the energy released and in allowing a greater saving of healthy tissues. Currently, the main targets of Hadrontherapy are those cancers that are incurable with conventional radiotherapy and/or surgery for their radio-resistance or proximity to critical organs. For this reason it seems suitable to study its effects on Glioblastoma multiforme (GBM, World Health Organization grade IV glioma), which is marked by a highly aggressive tumorigenic phenotype, and this analysis aims at making GBM treatment more efficient. This primary brain tumour is significantly resistant to radiation and chemotherapy thus being characterized by the lack of efficacy of the conventional treatments and, therefore, by a high recurrence rate, resulting in patients' extremely poor prognosis. According to the cancer stem cells (CSCs) hypothesis, a small fraction of CSCs residing inside the tumour mass is able to self-renew and differentiate into different lineages, maintaining the ability of the tumour to grow and overcome radiation-induced damages by their better capability to repair DNA. Therefore cancer treatment should be aimed not only to eliminate the bulk of cycling and differentiated cancer cells but especially to the eradication of the cancer stem cells reservoir.
In order to investigate the differential mechanisms involved in the molecular and cellular response of GBM CSCs to C-ions and photons, we irradiated a primary GBM CSC line with C-ions at the CNAO Hadrontherapy facility using a 2 cm SOBP at 43 mm depth (average LET: 92,2 keV/µm; dose range: 5-40 Gy). Photons were used as reference and ^{137}Cs gamma irradiation was performed at the ISS, Rome, at a dose rate of about 0.8 Gy/min.
Radiation induced cell killing was evaluated after microtiter plating and scoring for survivors. The dose-response curves showed a biphasic trend (with an initial decrease followed by a plateau) for both radiation types. The fit parameters of the experimental data showed a higher effectiveness of C-ions compared to photons in inducing cell killing at low doses and a faster entrance in the plateau phase. This finding is consistent with the presence of two cell populations with different radiation sensitivity. Cell growth data showed a dose and time dependent cytostatic effect lasting more than 1 month. A G2/M cell cycle arrest and an increase of the Chk2 checkpoint kinase expression were also observed. Interestingly, if arrested cells were harvested and reseeded in fresh medium, cells slowly started growing again, testifying their ability to overcome even the severe damages induced by high doses.
The data obtained so far suggest the existence of cellular heterogeneity within the GBM CSC line and the presence of different mechanisms of radiation damage response, possibly related to cell death capability and/or triggering a quiescent-like state. A deeper understanding of these mechanisms can provide important insights for novel and cell line specific therapeutic approaches.

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