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PROLIFERATION CAPACITY AND *p53* EXPRESSION OF HTB140 CELLS AFTER PROTON IRRADIATION

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Abstract

Human HTB140 melanoma cells were used to investigate different responses to single irradiation with protons, regarding cell proliferation, induction of apoptosis and expression of *p53*. Exponentially growing cells were irradiated close to the Bragg peak maximum of the unmodulated 62 MeV proton beam. Doses applied ranged from 8 to 24 Gy at the dose rate of 15 Gy/min. Cell proliferation, measured 6 and 48 h post-irradiation, has shown highly significant dose and time dependent decrease. Protons induced apoptosis, 6 and 48 h after irradiation, decreasing with the increase of post-irradiation incubation time. The largest number of apoptotic cells was at 6 h after irradiation with 16 Gy protons. High level of *p53* expression was detected in all irradiated samples, as well as in controls and was independent of dose applied and post-irradiation incubation time.

Introduction

Proton irradiation performs higher relative biological effectiveness (RBE) and provokes better cell elimination even on radioresistant tumors, such as melanoma. This is due to their physical properties [1]. Previous results indicated that proton irradiation induced dose and time dependent inactivation of resistant human HTB140 melanoma cells [2] being more efficient than gamma rays [3]. In this study, we investigated the effects of irradiation within the Bragg peak of an unmodulated 62 MeV proton beam on HTB140 melanoma cell proliferation capacity, induction of apoptosis and expression of *p53* gene.

Results and Discussion

Exponentially growing HTB140 cells were irradiated with single doses, close to those applied in proton therapy, i.e. 8, 12 and 16 Gy as well as with 20 and 24 Gy, at the therapeutic dose rate of 15 Gy/min. Irradiations were carried out at the CATANA treatment facility at INFN, LNS-Catania, Italy. All assays were performed 6 and 48 h after irradiation. Cell proliferation was detected by 5-bromo-2`-deoxyuridine (BrdU) incorporation assay. Irradiation with 8 and 12 Gy protons at 6 h, induced statistically significant ($p < 0.05$) decrease of cell proliferation, compared to non-irradiated controls (Fig. 1). With increase of dose from 16 to 24 Gy cell proliferation decreased ($p < 0.001$) in comparation with non-irradiated controls. Forty eight hours after irradiation almost linear and highly significant ($p < 0.001$) decrease of cell proliferation was observed indicating that the reduced proliferation capacity of HTB140 cells after exposure to protons was dose and time dependent (Fig. 1). As compared to previously re-

ported results [2] considering cell growth inhibition, the effects of proton irradiation on cell proliferation were stronger than the effects on cell viability. Detected decrease of cell proliferation was depended on different signaling pathways involved in cell cycle regulation [4]. The induction of apoptosis was estimated by Annexin-V-FLUOS Kit (Roche, Germany). Apoptotic population was accessed using FACS analysis (Becton Dickinson, Heidelberg, Germany) and calculated using CellQuest computer program (Becton Dickinson, Heidelberg, Germany). Significant percentage of apoptotic cells was detected 6 h after irradiation (having values from 12.98 % ± 0.55 % to 25.15 % ± 1.30 %) as well as after 48 h (ranging from 13.03 % ± 0.24 % to 18.13 % ± 1.00 %). The highest number of apoptotic nuclei was at 6 h post-irradiation with 16 Gy protons (25.15 % ± 1.30 %). With prolonged cell incubation up to 48 h within the same dose level, the number of apoptotic cells slightly decrease. These results clearly showed the ability of protons to eliminate resistant HTB140 human melanoma cells to a certain extent by inducing apoptosis. The level of proton induced apoptosis was in the range reported for resistant melanoma cell lines [5, 6].

To estimate changes of *p53* expression, total cellular RNA was isolated from irradiated HTB140 cells ($1x10^6$) using Qiagen RNeasy Total RNA Preparation Kit. For cDNA synthesis 1μg of total RNA was reverse transcribed using First Strand cDNA Synthesis Kit (Fermentas). Amplification of samples in polymerase chain reaction (PCR) was performed using primers for *p53* and *GAPDH*. Primer sequences for *p53* were: sense 5`AGA TAG CGA TGG TCT GGC 3` and antisense 5`TTG GGC AGT GCT CGC TTA GT 3`, and for *GAPDH* were: sense 5`CGG AGT CAA CGG ATT TGG TCG TAT 3` and antisense 5`AGC CTT CTC CAT GGT GGT GAA GAC 3`. In order to modify method for the detection and quantification *p53* proto-oncogene mRNA, together with *GAPDH* mRNA, various experimental conditions for RT-PCR were tested and optimized. PCR-amplified products were analyzed on 2.7 % agarose gel and quantification was performed using GelDoc1000 (BioRad). The relative mRNA level of *p53* gene was normalized to the corresponding *GAPDH* mRNA level in individual samples and the respective mRNA changes were determined relative to the non-irradiated controls. The obtained results have shown that in HTB140 cells, 6 and 48 h after proton irradiation the level of *p53* expression was high and did not significantly change ($p < 0.05$). Prolonged incubation time did not provoke changes in *p53* expression (Fig. 2). It could be supposed that DNA damages induced by protons lead to an increase in p53 protein synthesis as well as induced changes of their functional activity [7] or even lead to p53-independent apoptosis [8].

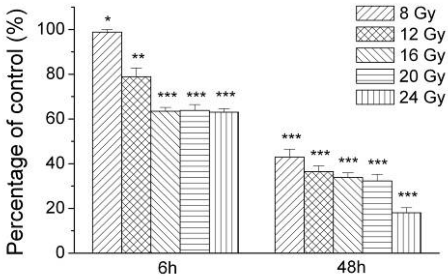

Fig. 1. Proliferation activity of HTB140 cells, 6 and 48 h after proton irradiation. Data presented are means±S.D. of three independent experiments performed in duplicates. *p<0.01, ***p<0.001

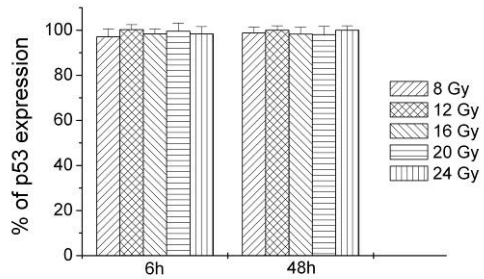

Fig. 2. Level of p53 mRNA expression after proton irradiation. Results are expressed as percent change relative to control levels set at 100%. Data presented are means±S.D. from a minimum three independent assays.

Conclusion

The decrease of HTB140 cell proliferation capacity up to 48 h after proton irradiation was dose and time dependent. The number of apoptotic nuclei at 6 and 48 h was significantly higher than in non-irradiated controls, showing a decrease with the increase of post-irradiation incubation time. The level of *p53* expression remained high and did not change with dose and incubation time. Further studies are required to analyze changes that occurred in "up-" and "down-"regulation of signaling pathways involving *p53* mediated response to DNA damage.

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