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RADIOBIOLOGICAL STUDIES ON THE 62 MeV THERAPEUTIC PROTON BEAM AT LNS CATANIA: I. SURVIVAL OF HTB140 MELANOMA CELLS

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Abstract

The aim of this study was to determine the initial inactivation of cells induced by high-energy proton beam designed for the treatment of eye melanoma. Exponentially growing HTB140 cells were exposed to an unmodulated 62 MeV proton beam delivered over the single dose range from 8 Gy to 24 Gy. Position of samples was in the zone of the Bragg peak, having high LET values. Surviving fractions were evaluated at 6, 24 and 48 h post-irradiation. The survival curves exhibited a well-known shoulder, decreasing for doses higher than 8 Gy. Therefore, a significant dose dependent early cell inactivation after single delivery of 16 Gy to 24 Gy to the cell monolayer was observed. With the increase of the post-irradiation incubation time, a better killing effect, as the consequence of clonogenic survival, was detected.

Introduction

In the past decades, therapeutic proton beams were successfully used in treating several tumour types [1]. The physical properties of protons, especially its Bragg peak, are used to target a large radiation dose precisely in the tumour. When charged particles enter the patient, their specific energy per unit of length deposited along the track (LET, linear energy transfer) increases with decreasing particle velocity. This gives rise to a sharp maximum in ionization near the end of the range at the position of Bragg peak [2, 3]. It is known that the effectiveness of protons on cell survival strongly depends on LET, therefore reaching its maximum in the Bragg peak [4]. Studies on cell inactivation have been performed on different mammalian cells, irradiated with low energy, monoenergetic beams, showing a significant increase in cell killing with LET [2, 5, 6]. Several studies have been reported on the effects of proton beams with energy less than 100 MeV that are used for the treatment of eye melanoma [7].

Materials and Methods

Human melanoma HTB140 cells (5×10^4/ml) were maintained as a monolayer in RPMI 1640 tissue culture medium, 10 % foetal calf serum under standard conditions ($37\ ^0C$, 5 % CO_2). The plating efficiency (PE) for HTB140 cells was approximately 70%, and the doubling time (Td), evaluated from the growth curve, was 24±2,7 h. The thickness of the cell monolyer was between 3-6μm. Irradiations with high energy monoenergetic protons were carried out at the CATANA treatment facility at INFN, LNS - Catania. Exponentially growing cell monolayers were irradiated within the

Bragg peak of the 62 MeV protons obtained by a superconducting cyclotron. Single doses delivered to the cells were 8, 12, 16, 20 and 24 Gy with the dose rate of 15 Gy/min. The viability of cells was measured at different time points by MTT assay (Roche) based on the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide into blue-purple formazan crystals. The absorbance was measured using a microplate reader (Wallac, VICTOR2 1420 Multilabel counter, LKB) at a test wavelength of 550nm and a reference wavelength of 690nm. For cell survival assay, irradiated exponentially growing cells were seeded (7×10^3 cells/ml) and incubated for 6, 24 and 48 h, when cells were fixed with methanol and stained with 10 % Giemsa. Colonies with more than 50 cells were scored as survivors. The Student t-Test was used for statistical analysis (level of significance, $P<0.05$).

Results and Discussion

We have examined early effects of high-energy proton irradiation on human melanoma cells *in vitro*. Figure 1 shows the 62 MeV proton depth-dose distribution measured by inserting thin Perspex plates in front of a plane-parallel PTW 34045 Markus ionization chamber, calibrated according to IAEA TRS 398 [8]. The arrow indicates the irradiation position of the cells, within the Bragg peak, obtained by inserting 25 mm thick Perspex plates just before the Petri dish. This gave the corresponding relative dose of 90.4%±4 %. The level of early cell growth inactivation, estimated at 48 h post-irradiation, has shown a dose dependent increase, reaching values from 55.16% to 58.97% for irradiation with 16 Gy and 20 Gy respectively (MTT assay).

To quantify the dose response in exponentially growing cultures, the surviving fraction of HTB140 cells from an average of three duplicate experiments were fitted to a linear - quadratic equation [3]. For the generation of cell survival curves, possible differences in response were further investigated after giving high single doses similar to those delivered in the radiotherapy of ocular melanoma. The survival curves of HTB140 cells exhibited a shoulder at lower doses and an exponential decrease for doses higher than 8 Gy (Fig. 2). The width of the shoulder and the initial slope of the survival curves generated at 24 and 48 h of post-irradiation time, were not significantly different. At 6 h of post-irradiation incubation mean values for cell-surviving fraction, for all doses applied, were 50.01%±0.11, with no significant difference regarding the increase of the delivered dose. At 24 h post-irradiation, the shape of the fitted curve showed the decrease of the survival fraction with the increase of the dose, having the high S.D. values for the irradiation with 24 Gy. This could be explained by the fact that heterogeneous cell cultures, having melanoma cells in different stages of differentiation, were irradiated in our experiments. The survival curve generated after 48 h post-irradiation, has shown almost the same shape as the survival obtained at 24 h post-irradiation, with significantly lower S.D. values. The present shape of the dose-response curve is consistent with the results already reported [3, 5]. Some differences in the detected cell sensitivity might be a consequence of the nature of cell damage induced by high-LET radiation, cell-repair capacity and other cell parameters involved in the different metabolic processes.
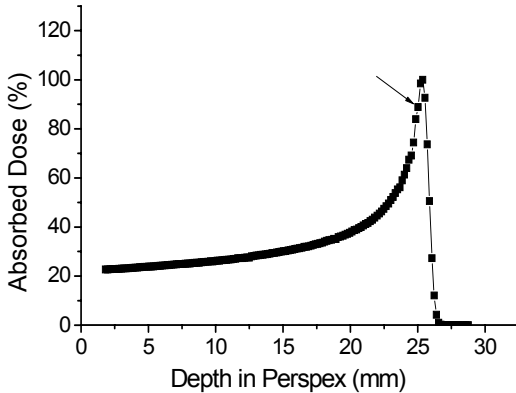
Fig. 1 Measured dose in Perspex *vs.* depth for the 62 MeV proton beam produced at the LNS, INFN, Catania. Arrow indicates position of cell irradiation.
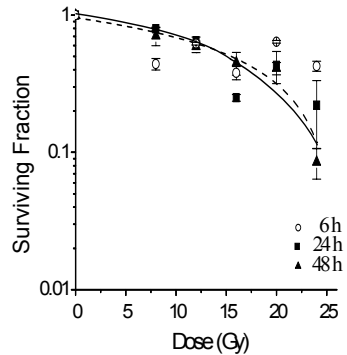
Fig. 2 Survival fraction of HTB140 cells exposed to 62 MeV protons at 25 mm in Perspex.

Conclusions

The data obtained on human melanoma cells irradiated within the Bragg peak of an unmodulated 62 MeV proton beam pointed out a significant dose dependent early cell inactivation. With the increase of the post-irradiation incubation time, a better killing effect, as the consequence of clonogenic survival, was detected.

This study is part of an overall effort to understand the mechanisms involved in cell survival after irradiation with high-LET charged particles of human cells with different radiosensitivity. All the knowledge gained through these investigations is aimed to improve the efficiency of the therapeutic approach to cure different malignant diseases.

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