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RADIOBIOLOGICAL STUDIES ON THE 62 MeV THERAPEUTIC PROTON BEAM AT LNS CATANIA: II. FACS ANALYSES OF HTB140 MELANOMA CELLS

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Abstract

The objective of this study was to determine whether apoptosis and cell cycle redistribution were influenced by high-LET irradiation. Exponentially growing HTB140 cells were exposed to an unmodulated 62 MeV proton beam, within the Bragg peak, delivered over the single dose range from 8 Gy to 24 Gy. At 6 h post-irradiation, there was a low level of early apoptosis. At 48 h irradiated cells were more damaged, showing the increase in number of apoptotic nuclei. The dose dependent cell cycle phase distribution was detected at 48 h post-irradiation. The cell population exhibited phase redistribution toward G2/M phase.

Introduction

The basic task of the cell cycle is to provide a faithful DNA replication during S phase and equal distribution to two daughter cells during M phase. The process of malignant transformation includes deregulation of normal cell cycle controls. Most commonly seen cell cycle perturbation, in irradiated tumour cells, is a delay in the transition from G2 to M [1]. The ability of radiation to induce apoptosis is detected in many cell types. However, there are cells that are resistant to radiation induced apoptosis, and they die by radiation induced necrosis or enter a terminal cell cycle arrest [2]. Malignant melanoma has been increasingly analyzed in recent years. Knowing that some forms of melanoma (uveal melanoma) are successfully cured using proton irradiation [3, 4], there is a great interest to investigate the effects of protons on different human melanoma cell lines. The effects of irradiation within the Bragg peak of an unmodulated 62 MeV proton beam on HTB140 cells regarding the induction of apoptosis and cell cycle phase redistribution are given in this study.

Materials and methods

Human HTB140 melanoma cells were maintained and irradiated under the same conditions already described elsewhere [5]. Exponentially growing cells were irradiated within the Bragg peak of an unmodulated 62 MeV proton beam, delivering to the cells doses from 8 to 24 Gy, at the dose rate of 15 Gy/min. To analyse the cell cycle and apoptosis, 1 x 10^6 cells were used. Assays were performed with Annexin-V-Fluos kit (Roche), according to the manufacturer's protocol. Propidium iodide (PI) was added prior to FACS analyzes (Becton Dickenson, Heidelberg, Germany). Apoptotic population was calculated using CellQuest computer program (Becton Dickenson, Heidelberg, Germany). DNA was extracted from irradiated cells at 6 and 48 hours

postirradiation [6] and analyzed electrophoretically on 2 % agarose gel (Gibco BRL) with ethidium bromide (1μg/ml). DNA profiles were analyzed on Gel doc 1000 (Bio-Rad). The Student t-Test was used for statistical analysis. The level of significance was set at $P < 0.05$. Results are presented as the Mean ± S.D.

Results and Discussion

The ability of proton radiation with respect to the induction of apoptosis in the HTB140 cells was examined. The gel electrophoretic analyses of DNA samples, isolated from control and irradiated HTB63 cells, 6 and 48 hours post-irradiation, have shown ladder pattern, a typical DNA fragmentation characteristic for apoptotic cells (data not presented). Table I contains data obtained by FACS analyses for the proportion of cells in apoptosis as a function of dose and time. At 6 hours post-irradiation there was a moderate level of apoptosis, not more than 10% of apoptotic cells for irradiation with 16 Gy. At 48 hours post-irradiation, the highest number of detected apoptosis was 17.71% for the irradiation with 8 Gy, while the number of apoptosis after irradiation with 16 Gy did not exceed 12.11%. Corresponding apoptotic indexes for 6 hours post-irradiation incubation ranged from 2.91 to 3.96 and for 48 hours post-irradiation from 1.76 to 3.15, as shown in Table I.

Table I Radiation-Induced Apoptosis in HTB140 Cells

Post-irradiation time	Dose (Gy)	Apoptosis (%)	Apoptotic index
6 hours	0	2.66 ± 0.85	1
	8	9.77 ± 0.47	3.84 ± 1.04
	12	8.96 ± 1.49	3.37 ± 0.05
	16	10.30 ± 1.98	3.96 ± 0.52
	20	7.47 ± 0.81	2.91 ± 0.62
	24	8.64 ± 3.05	3.23 ± 0.11
48 hours	0	5.98 ± 2.71	1
	8	17.71 ± 3.52	3.15 ± 0.83
	12	15.39 ± 4.09	2.69 ± 0.54
	16	12.11 ± 2.81	2.13 ± 0.49
	20	11.52 ± 2.57	2.04 ± 0.48
	24	10.24 ± 3.45	1.76 ± 0.21

The detected apoptosis, induced in HTB140 cells by proton-beam radiation was almost immediate, appearing at about 6 hours after irradiation. It appears that these cells enter directly into programmed cell death, without a lag typical of those described for intact cell cycle checkpoint processes, which allow cells to repair damage prior to undergoing division [7].

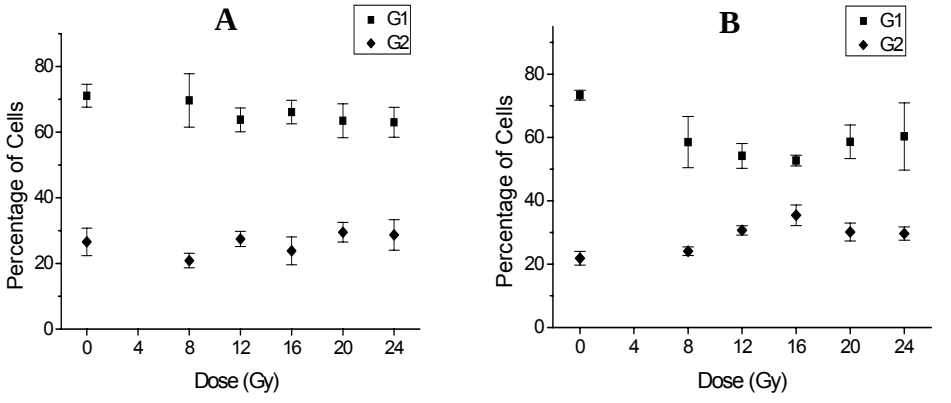

Fig. 1 The effects of proton irradiation on cell cycle phase redistribution in HTB140 cells at 6h post-irradiation (panel A) and 48h post-irradiation (panel B).

The cell cycle phase distribution of control HTB140 cells is approximately 71.09 % in G1 phase, 8.17 % in S phase and 26.56 % in G2/M phase. Cultured cells were exposed to ionizing radiation and sampled at 6 and 48 hours post-irradiation. With the increase of the dose, at 48 hours post-irradiation, the cell population exhibited phase redistribution toward G2/M phase. After single exposure to 12 and 16 Gy, the number of cells in G2/M phase ranged form 30.67 % to 35.44% respectively. Data from two duplicate experiments are represented in Fig. 2. The cell cycle redistribution, evaluated in irradiated cells at 6 (panel A) and 48 hours (panel B) post-irradiation, was not significantly different. These data are in agreement with literature data reported for other cell lines [7].

Conclusions

The results of these experiments indicate that cell cycle phase redistribution in HTB140 cells was dependent on dose but not on the duration of post-irradiation incubation. The position of cells in cell cycle at the time of irradiation did not influence the results obtained.

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