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CARBON ION BEAM AS INDUCER OF MELANOMA CELL APOPTOSIS

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Abstract

In vitro effect of carbon ions on apoptosis was studied. The human melanoma HTB140 cells were irradiated with the 62 MeV/u ^{12}C ion beam. Percentage of apoptotic cells was evaluated by flow-cytometry and the corresponding apoptotic indexes were calculated. The expression of apoptosis-associated proteins, p53, Bax and Bcl-2 was estimated by Western blot analyses. A dose dependent increase of apoptosis was revealed, with the maximum value of 17 % after irradiation with 16 Gy, and the apoptotic index of 7.7. Pro-apoptotic effects of carbon ion beams were confirmed by the detected changes of key regulators of the mitochondrial apoptotic pathway, the p53 protein expression and the Bax/Bcl-2 ratio.

Introduction

It is well known that cancer is a major health problem. Approximately 11 million people worldwide are diagnosed with cancer, while each year almost 7 million people die of the disease. About 40 % of cured patients are treated by radiotherapy. Its main aim is to deliver a maximally effective dose of radiation to the tumor site while sparing the surrounding healthy tissues as much as possible [1]. Melanoma, a highly aggressive form of cancer, is known to be radio-resistant with powerful metastatic potential and dissimilar response to conventional radiotherapy [2]. With respect to conventional radiotherapy, carbon ions have a unique advantage due to their physical properties. Thus, due to high linear energy transfer (LET) quite better dose effect on malignant tissue is achieved [3]. In the response to a variety of stresses, the tumor suppressor protein p53 plays a protective role to the genome provoking either cell cycle arrest or programmed cell death - apoptosis [4]. The mechanism by which p53 protein might trigger apoptotic machinery involves transcriptional activation of the pro-apoptotic members of Bcl-2 family and repression of the anti-apoptotic regulators [5]. After an apoptotic stimulus, the ratio of Bax protein, an inducer of apoptosis, to Bcl-2, an inhibitor of apoptosis, determines cell survival or death [6].

With the intent to better understand mechanisms involved in the induction of apoptosis, the effects of accelerated carbon ions on the apoptotic status and the regulatory molecules involved in apoptosis were investigated.

Results and discussion

Analysis of the apoptotic status of the HTB140 human melanoma cells were performed within the Bragg curve of the 62 MeV/u ^{12}C ion beam produced by the superconducting cyclotron at Istituto Nazionale di Fisica Nucleare, Laboratori Nazionaly del Sud (INFN-LNS), in Catania (**Fig.1**). The doses ranged from 2 to 16 Gy, with the average dose rate of 11.45 ± 0.31 Gy/min. At the irradiation position the relative dose was 73.37 ± 3.92 %, while the LET value was ~ 285 keV/μm.

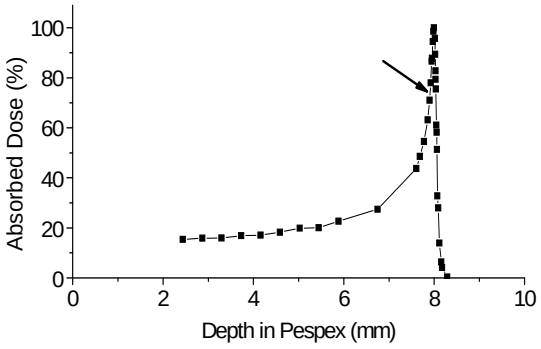

Fig. 1. Depth dose distribution in Perspex of the 62 MeV/u ^{12}C ion beam produced at INFN – LNS. Arrow indicates irradiation position.

Dose (Gy)	Apoptosis (%)	Apoptotic index (AI)
0	2.23±0.06	1
2	5.46±0.14	2.45
4	9.08±0.06	4.07
8	14.2±0.09	6.37
12	13.2±0.01	5.92
16	17.2±0.02	7.71

Table 1. The dose dependent apoptosis in the HTB140 cells 48 h after irradiation with ^{12}C ions.

The percentage of apoptotic cells was evaluated 48 h after irradiation by flow-cytometry. Corresponding apoptotic indexes of HTB140 cells were calculated and the obtained values are given in Table 1.

Carbon ions induced the dose dependent increase of apoptotic cells 48 h after irradiation. Percentage of apoptosis ranged from 5.46 to 17.2 %. Similar level of apoptosis was already reported for the same cell line exposed to protons and γ-rays [7]. All these results point out high radio-resistance of the HTB140 cells. The dose dependent increase of apoptotic cells in this irradiation position could be attributed to the severe damage induced by high LET of carbon ions, leading to the induction of late apoptosis or necrosis.

Apoptotic index also showed a dose dependent increase with maximum value of 7.71 when the cells were irradiated with 16 Gy of ^{12}C ions. At 48 h after irradiation it clearly indicated the ability of ^{12}C ion beam to provoke programmed cell death of the resistant HTB140 cells.

To examine the molecular level of the observed induction of apoptosis, key regulatory molecules of the mitochondrial apoptotic pathway were analyzed under the described experimental conditions. This included the analysis of p53 protein expression (Fig. 2A), as well as the expression of Bax and Bcl-2 regulatory proteins, presented as the ratio of Bax and Bcl-2 (Fig. 2B).

Transcription factor p53 is known to provoke cell cycle arrest or apoptosis in response to cellular stress, such as DNA damage induced by drugs or radiation [8].

The dose dependent increase in the expression of p53 protein confirmed induction of apoptosis. It ranged from 238 to 554 % (Fig. 2A).

Literature data suggest that in certain cell types, after the DNA damage, Bax appears to be transcriptionally induced by p53 [9]. In this study it was shown that with the rise of the radiation dose the level of Bax increased. Moreover, the level of Bcl-2 protein decreased and the calculated Bax/Bcl-2 ratio clearly demonstrated induction of apoptosis. The dose dependent increase of Bax/Bcl-2 from 1.04 to 3.08 was estimated (Fig. 2B**)**.

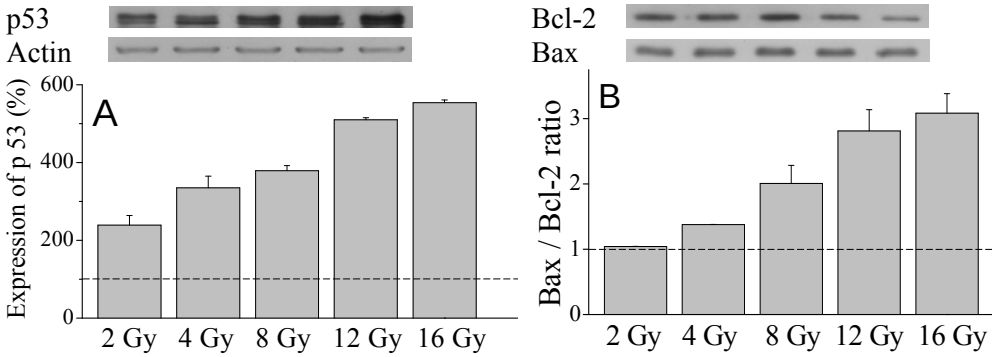

Fig.2. Western blot analysis of p53 protein expression (A) and Bax/Bcl-2 ratio (B) - 48 h after irradiation of HTB140 cells with 62 MeV/u ^{12}C ions.

Conclusions

To better understand biological mechanisms involved in cellular response to irradiation with ^{12}C ions having high-LET, the investigation of apoptotic cell death and corresponding protein expression was undertaken.

The results obtained pointed out a dose dependent increase of apoptosis in HTB140 cells after their irradiation with ^{12}C ions. The induction of apoptotic cell death was associated with the increase of the expression of p53 protein and Bax/Bcl-2 ratio.

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