Low-energy protons and X-ray-induced p21/WAF1 in human primary fibroblasts

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I. INTRODUCTION

Charged particles as low-energy protons have been recognised to show a higher RBE compared to X- and γ-rays for many biological endpoints related to DNA damage, persistence of induced lesions and mutations (1,2,3). On the other hand, the LET dependence for other kinds of cytogenetic and biochemical endpoints such as, the relationship between DNA damage and induction of protein related to DNA-damage and cell cycle modulation have been poorly investigated.

We have previously showed that 28.5 keV/µm protons were far more efficient in inducing micronuclei than 7.7 keV/µm and X-rays. Contrasting to endpoints related to DNA damage cell cycle perturbations as inhibited G1 to S-progression appeared LET independent (4,5,6).

In the present work we have compared the effect of irradiation with low-energy protons (7.7 and 28.5 keV/µm) and X-rays on p21/WAF1 accumulation in human primary fibroblasts. P21/WAF1 is a well known cyclin inhibitor under the transcription control of the oncosuppressor p53. Data on the capability of radiation with different quality are scanty and to our knowledge they are not available for proton treatment.

II. EXPERIMENTAL SET-UP

HFFF2 human primary fibroblasts were grown in D-MEM, supplemented with 10% bovine serum, 2 µm L-Glutamine and antibiotics in a 5% CO2 atmosphere at 37 °C.

Proton irradiation

Proton irradiation, cell killing and MN induction Fortyeight hours before treatment 90,0000 cells were plated in stainless-stell Petri dishes and as monolayer attached to a maylar foil (52 mm thick; 133 mm2 area) on the dish and grown at the Laboratori Nazionali di Legnaro of the Istituto di Fisica Nucleare (LNL INFN). Irradiation was carried out at the radiobiological facility of the 7 MV Van der Graaf accelerator at the LNL. Cells were exposed to 7.7 and 28.5 keV/µm, at a dose rate of 1Gy/min.

X-ray irradiation

Fortyeight hours before treatment fibroblasts were grown in a 30 mm Petri dishes. Cells were treated with 25-200 cGy X-rays delivered from a Gilardoni apparatus operating at a dose rate of 53 cGy/min.

Immunofluorescence staining

Cells irradiated with either 100 and 200 cGy of protons (7.7 and 28.5 keV/µm) or X-rays were collected 4, 8, 24 and 32 hours later by tripinization, washed once in PBS, cytoplasmmed and fixed by immersing the slides in –20 °C cold methanol for 30 minutes. Slides were then air-dried and stored in –20 °C refrigerator until using. Slides were rinsed for 2 min in PBS, then they were incubated with a mouse-primary antibody direct against p21 diluted 1:20 in PBS/2%BSA over-night at +4 °C. The slides were washed five times in PBS/1%BSA and incubated with a secondary anti-mouse FITC conjugated antibody for 1 hour at 37 °C in a humid box. Then cells were washed several times in PBS/1%BSA, once in PBS and mounted with a drop of 1:1 mixture DAPI:antifade solution. For each experimental point the the percentage of nuclei showing a bright nuclear staining were chosen as positive for the iunmunostaining.

III. STATUS OF THE ART

As a measure of the G1/S checkpoint activation the nuclear accumulation of p21, a downstream effector of p53, known to act as a cyclin kinase inhibitor was evaluated by immunofluorescence techniques. The quantitative analysis of p21 indicated that both 7.7 and X-rays were able to induced such protein but to a lower extent than 28.5 keV/µm protons. In samples harvested 32 h after treatment the extent of p21 expression was still higher than in untreated cultures, in particular for the protons (fig.1). Since the G1 arrest was comparable among the radiation types, these results seem to suggest that p21 induction might be more related to differences in the complexity of lesions induced by radiation with different quality than to the inhibition of cells to progress from G1 to S-phase.
FIG. 1: Accumulation of nuclear p21 in irradiated cells after X-rays (a), 7.7 keV/µm (b) and 28.5 keV/µm protons (c) irradiation.