Chromosomal Instability Induced by Low-Doses of Ionizing Radiations as a Function of Radiation Quality

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INTRODUCTION

Based on their quality ionizing radiations are classified as low- and high-LET radiations, and the high-LET ones, produce a more severe, complex, less repairable DNA damage. Beside the quality of radiation, another relevant aspect for the biological effects, is represented by the dose. In fact, the harmful effect of ionizing radiations, as for example for cancer induction, have been estimated at high doses. On the other hand at low doses the situation is less clear. A growing number of evidences have been accumulated on the non-linear effects of low-doses of radiations, as the hyper-radiosensitivity (HRS) [1] genomic instability, the bystander effect [2] and the adaptative response [3].

The aim of the present work is to evaluate the induction of chromosome instability in the low-dose range, and as a function of radiation quality. In particular, we report here results aimed to analyse whether in the low-dose range the metabolism of telomere is modulated, as we observed previously for higher doses, and if so, the radiobiological meaning of telomere alterations. For this purpose AG1522 human primary fibroblasts were irradiated with 0.1, 0.25, 0.5 and 1 Gy of X-rays, low-energy protons (0.8 MeV at cell surface; 28.5 keV/μm) and helium-4 ions (8.4 MeV at cell surface; 62 keV/μm). After irradiation cells were detached and reseeded to be harvested 24, 48 and 72 hours later for the analysis of telomere "stickiness". Such telomeric alterations have been measured in anaphases in terms of frequency of chromosome bridges and bridges carrying telomeric signals (FISH analysis of PNA telomeric sequences). Furthermore telomere lengths were measured by quantitative-FISH (Q-FISH) analysis.

MATERIALS AND METHODS

AG1522 human fibroblasts (Coriell Biorepository, USA), were cultured in E-MEM medium supplemented with 15% fetal bovine serum (FBS), 1% Non Essential Aminoacids, 100 units/ml penicillin, 100 mg/ml streptomycin and 2 mM L-glutamine and grown in 5% CO2 atmosphere at 37°C. In these conditions, the cell doubling time, Td, evaluated from the growth curves, was 22.5 ± 1 h.

For X-irradiation, cells seeded in plastic petri dishes were irradiated using a Gilardoni apparatus (250 kV, 6 mA; dose-rate of 0.53 Gy/min).

Proton and helium-4 ion irradiation have been performed at the radiobiological facility of the 7 MV Van de Graaff CN accelerator at the Laboratori Nazionali di Legnaro (LNL) of the Istituto Nazionale di Fisica Nucleare (INFN) [4,5]. For proton and helium-4 irradiations cells were plated on the LNL specially designed stainless steel Petri dishes and grown attached to a Mylar foil (52 μm thick and 6 μm thick for protons and helium-4 irradiation respectively; area 133 mm²). Sham irradiated cells were used in all the experiments as control (unirradiated) cells.

Chromosome bridges induction. After IR exposure, cells were seeded at different densities (from 5×10³ to 30×10³ /cm²) and fixed in 70% ethanol at 24, 48, and 72 hours from exposure. Fixed cells were hybridized with telomeric PNA probes using standard procedures as described in Beradinelli et al. [6]. Anaphase bridge index (ratio of anaphases that display a bridge on total anaphases scored) and frequency of anaphase bridges displaying telomere signals have been evaluated on 200 anaphases in 3 repeated experiments.

Telomere length modulation. To evaluate telomere length, quantitative-FISH experiments were performed on calyculin-A-condensed chromosomes hybridized with Cy3-PNA telomeric probes and chromosome 2 Cy3-PNA centromeric probe used as reference. Images were captured with an Axio Imager M1 (Carl Zeiss, Germany) equipped with a CCD camera, and the telomere size was analysed with ISIS software (MetaSystems, Germany) [6].

RESULTS AND DISCUSSION

Chromosome bridges induction. Samples irradiated with X-rays, protons and helium-4 ions have been processed to score the frequency of chromosome bridges at anaphase at 24,48 and 72 h from exposure (figure 1a,b,c).

The results indicated a dose- and a LET-dependent induction for such endpoint. Irrespectively of the quality of radiation, the highest number of bridges was observed at 24 h compared to the remaining harvesting times.

The frequency of bridges were as high as 2 - 5% for doses as low as 0.1 Gy. Interestingly, also for low-doses of 0.1 and 0.25 Gy, a certain fraction of bridges persisted at 72 h, and this was in function of LET. Furthermore, to assess the contribution of telomere erosion in the formation...
of chromosome bridges, a specific PNA probe directed against telomeres was used to hybridize the samples and quantify the percentage of anaphase bridges carrying the signal (figure 1a,b,c, white portion of the column). We found that neither the dose nor the quality of radiation affected the percentage of anaphase positive to telomere signals (data not shown), thus indicating that telomeres do not play a significant role in the generation of the radiation-induced chromosome bridges, which are rather the product of unstable chromosomal aberrations.

Telomere length modulation. Based on our previous results obtained with 4 Gy of either X-rays or protons (0.8 MeV at cell surface), showing that only high-LET radiations affected telomere length at 24 hours from exposure in human fibroblasts [6-7], we checked whether it could be the same also for low-doses. The analysis for samples collected at 24 hours from irradiation (figure 2) does not show modulation in telomere length as a function of dose or radiation quality. This result is in good agreement with the lack of involvement of telomere in the genesis of anaphase bridges. Samples, collected 48 hours after irradiation will be also processed to investigate a delayed effect on telomere length modulation.

Fig. 1. Frequency of anaphase bridge in fibroblasts exposed to a) X-rays, b) protons and c) helium-4 ions and harvested after 24, 48 and 72 hours. White part of the columns indicates the fraction of anaphase bridges that display telomere signal.

Fig. 2. Telomere length in human fibroblasts exposed to low doses of X-rays, protons and helium-4 ions and analyzed 24 hours after the exposure.

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