Mutation Research 756 (2013) 101–107

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Chromosome aberrations in normal human fibroblasts analyzed in G_0/G_1 and G_2/M phases after exposure in G_0 to radiation with different linear energy transfer (LET)

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ARTICLE INFO

Article history:
Received 7 May 2013
Accepted 9 May 2013
Available online 17 May 2013

Keywords:
Chromosome aberration
Premature chromosome condensation (PCC)
Fluorescence in situ hybridization (FISH)
High-LET radiation
Cell cycle arrest

ABSTRACT

We have studied the induction of chromosome aberrations in human fibroblasts exposed in G_0/G_1 to X-rays or heavy ions to study the influence of G_1 cell cycle arrest. Confluent normal fibroblasts were exposed to X-rays or accelerated particles with different LET values and chromosome aberrations were investigated in the first G_0/G_1 and G_2//M phase. The particles used here were 490 MeV/nucleon Si, 500 MeV/nucleon Fe, and 200 MeV/nucleon Fe ions. Cells were subcultured 24 h after exposure and premature chromosome condensation (PCC) was performed by fusion-induced method for analysis of G_0/G_1 cells, and chemically-induced method for analysis of G_2 and metaphase cells. Chromosome damage was assessed in chromosomes 1 and 3 using whole chromosome fluorescence in situ hybridization (FISH). Cell cycle was analyzed by flow cytometry at different incubation times following subculture. After irradiation with 2 Gy of high-LET particles, the yields of chromosome aberrations and fragments were significantly higher in G_0/G_1 phase than in G_2/M phase, whereas similar yields of damage were measured in both phases after exposure to X-rays. In contrast, the yield of misrepair, assessed by the number of color junctions, was similar in the G_0/G_1 and G_2/M phases after exposure to either X-rays or high-LET particles. The yields of chromosome aberrations, fragments, and color junctions in both the G_0/G_1 and the G_2/M phases, increased with LET up to 200 keV/μm, then decreased for 440 keV/μm Fe particles. A good correlation was found between chromosome aberrations in both G_0/G_1 and G_2/M cells and survival fractions after 2 Gy of different LET radiations, although the slopes were steeper for the G_0/G_1 cells. Flow cytometry analysis indicated that high-LET particles induce more non cycling G_0/G_1 cells within 48 h of subculture than X-rays, suggesting that chromosome aberrations scored at the G_2/M phase may not accurately describe the true radiation effect.

1. Introduction

Increased knowledge of the biological effects of charged particles may provide information about health risks to radiology staff and possible late effects of heavy particle cancer therapy [1]. Chromosome aberrations are an ideal tool for studying biological effects because they correlate with both early (e.g. cell killing) and late (e.g. transformation) effects [2]. After lymphocytes or confluent normal fibroblasts are exposed to radiation, chromosomal damage can be studied in the first post-irradiation cell cycle at either G_2/M phase or the G_0/G_1 phase using PCC (prematurely condensed chromosomes) technique [3–11]. However, analysis of chromosome aberrations induced by high-LET (linear energy transfer) radiation is complicated because many cells undergo cell-cycle delay. After heavy particle irradiation, G_2 arrest and/or mitotic delay occur routinely, and analysis of metaphase chromosomes might underestimate the true yield of damage [12–16]. In addition, Lee et al. indicated that analysis of metaphase and G_2 PCC

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http://dx.doi.org/10.1016/j.mrgentox.2013.05.005

collected at one time point after exposure may still underestimate the yield of severe cellular damage induced by high-LET radiation [10].

Using a metaphase assay, G_2 PCC, and the fusion-based G_0/G_1 PCC method in combination with FISH whole chromosome paint probes, Durante et al. studied the association between G_2-phase block and repair of X-rays and carbon radiation-induced chromosome fragments in human lymphocytes. The authors indicated similar yields of fragments in the G_1 and G_2 phases, and that the frequency of chromosome exchanges was similar in G_0, G_2, and metaphase cells after exposure to X-rays, while a lower frequency of exchanges was observed in metaphase cells when lymphocytes were irradiated with high-LET radiation [12].

After irradiation in the G_0/G_1 phase, normal human fibroblasts suffer a prolonged dose-dependent G_1 arrest, while human lymphocytes are predominantly arrested in G_2 phase [5,17–22]. Kovacs et al. exposed confluent normal fibroblasts to 6 Gy of γ-rays, and compared chromosome aberrations in G_0 PCC collected 24 h after irradiation with aberrations at the first post irradiation metaphase, and found fewer aberrations in metaphase cells, particularly un-rejoined breaks [11]. This phenomenon implies loss or delayed mitosis of cells containing aberrations prior to the first division and that the true yield of damage may be underestimated by metaphase analysis even in cells exposed to γ-rays.

Normal human fibroblasts are frequently used for investigating genotoxic effects caused by ionizing radiation, yet little is known about the relationship between G_0/G_1 and G_2/M chromosome aberrations induced in these cells by low- and high-LET irradiation. Specifically, no studies have been conducted comparing G_0/G_1 and G_2/M aberrations after exposure to radiation with different LET values. In the present study, we investigated chromosome aberrations in G_0/G_1 and G_2/M phase cells by exposing non-dividing normal human fibroblasts to X-rays, Si, or Fe ions with different LETs, and allowed the cells to repair for 24 h. One portion of the cells was analyzed by fusion-based PCC to assess chromosome aberrations in G_0/G_1, and the other portion was subcultured and harvested at suitable post-irradiation times using the calyculin-A induced PCC method to obtain G_2 and M cells. As chromosomal aberrations result from mis-repair of DNA double strand breaks, whole chromosome florescence in situ hybridization (FISH) analysis provides useful information concerning mis-rejoined and un-rejoined breaks [4,8,12,23]. FISH with whole chromosome probes specific for human chromosomes 1 and 3 was used to study G_0/G_1 and G_2/M phase chromosome aberrations. In addition to comparing absolute frequencies of chromosome aberrations induced by low- and high-LET radiations, in the present study we focused on mis-rejoining (color junctions) and non-rejoined fragments for assessing the effects of radiation.

2. Materials and methods

2.1. Cells and cell cultures

AG01522 normal human diploid skin fibroblasts were obtained from the NIA Aging Cell Repository. Low-passage AG01522 (12-14) cells were maintained in minimum essential medium (MEM) (Sigma, America) supplemented with 15% fetal bovine serum (HyClone, Canada) and antibiotics (Wako Chemical, Japan) at 37 °C in an atmosphere of 5% CO_2 and 95% air. Cells were plated into T25 flasks at 25% confluence 7 days before being irradiated in the confluent state.

2.2. Irradiations

The confluent AG01522 fibroblasts in T25 plastic flasks (Nunc 152094) were irradiated at room temperature with 2 Gy of X-rays at a dose rate of 2 Gy/min using an MBR-1520R X-ray device (Hitachi Medical: 150 kV and 20 mA with a 1-mm aluminum shielding), or 2 Gy of Si or Fe ions accelerated by the Heavy Ion Medical Accelerator in Chiba (HIMAC) at the National Institute of Radiological Sciences (NIRS), Japan. The initial energy of the Si ions was 490 MeV/μm with an average LET value of 55 keV/μm, and the energies of the Fe ions were 200 MeV/μm and 500 MeV/μm, with LET values of 440 keV/μm and 200 keV/μm, respectively. After irradiation, the cells were incubated at 37 °C for 24 h.

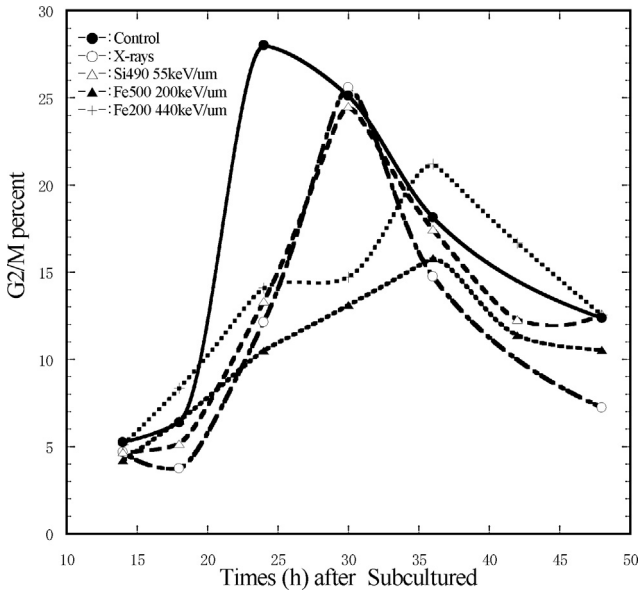

Fig. 1. The percentages of G_2/M cells at different times after being subculture following a 24 h post irradiation incubation. The dose was 0 Gy (control), and 2 Gy for X-rays, 55 keV/μm Si, 200 keV/μm and 440 keV/μm Fe ions.

2.3. Cell survival

Cell survival was assessed by the frequency of colony formation. Cells were trypsinized 24 h after irradiation and the number of cells seeded was adjusted to yield about 100 colonies per 100-mm dish. The cells were incubated for 14 days and then fixed with 20% methanol and stained with 0.2% crystal violet (Wako Chemical, Japan). Survival rates were derived from the number of colonies containing at least 50 cells.

2.4. Induction of premature chromosome condensation (PCC)

2.4.1. Chromosome condensation by the fusion method

PCC was based on previous methods with a few modifications [4,24]. 24 h after irradiation confluent cells were fused with mitotic HeLa cells to induce prematurely condensed chromosomes (PCC). Briefly, 1×10^6 irradiated cells were mixed with an equal number of HeLa mitotic cells (mitotic index > 95%, frozen and thawed) in ice-cold media. The cells were centrifuged at 1500 rpm for 5 min and cell pellets were washed in ice-cold serum-free media, then immediately treated with 2–4 μl of hemagglutinating virus of Japan envelope (HVJ-E; also known as Sendai virus) (Ishihara Sangyo, Japan). The HVJ-E-treated cells were kept on ice for 15 min to allow the virus envelope to attach and were then placed in a water bath at 37 °C for 3 min. The samples were then incubated at 37 °C for 1 h to allow cell fusion and PCC induction to occur. Then cells were fixed as described below.

2.4.2. Chromosome condensation by the chemical method

Calyculin A (Wako Chemicals, Japan) was used to condense chromosomes in G_2 phase [8,12,23]. Briefly, 24 h after exposure cells were trypsinized and transferred from a T-25 flask to a T-75 flask to promote cell division. Colcemid (0.05 μg/ml, Wako Chemical, Japan) was added to the medium 18 h before collecting G_2/M chromosomes to avoid M/G_1 transition during the first post-irradiation cell cycle, and 0.5 h before collecting G_2/M cells, 50 nM calyculin-A was added to the culture medium. The cells were then collected into centrifuge tubes and processed for fixation as described below. The optimal collection time was determined from the peak G_2/M percentages analyzed by flow cytometry (Fig. 1).

2.5. Chromosome fixation and preparation of slides

Cells were centrifuged at 1500 rpm for 5 min and the pellets were carefully resuspended in 8 ml of 75 mM KCl. After a 20-min incubation at 37 °C, 2 ml of freshly