Biochimica et Biophysica Acta 1853 (2015) 2199–2206

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Alpha particles induce pan-nuclear phosphorylation of H2AX in primary human lymphocytes mediated through ATM

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ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form 12 June 2015
Accepted 19 June 2015
Available online 25 June 2015

Keywords:
Alpha particles
Complex damage
DNA double strand break
Pan-nuclear intensity

ABSTRACT

The use of high linear energy transfer radiations in the form of carbon ions in heavy ion beam lines or alpha particles in new radionuclide treatments has increased substantially over the past decade and will continue to do so due to the favourable dose distributions they can offer versus conventional therapies. Previously it has been shown that exposure to heavy ions induces pan-nuclear phosphorylation of several DNA repair proteins such as H2AX and ATM in vitro. Here we describe similar effects of alpha particles on ex vivo irradiated primary human peripheral blood lymphocytes. Following alpha particle irradiation pan-nuclear phosphorylation of H2AX and ATM, but not DNA-PK and 53BP1, was observed throughout the nucleus. Inhibition of ATM, but not DNA-PK, resulted in the loss of pan-nuclear phosphorylation of H2AX in alpha particle irradiated lymphocytes. Pan-nuclear gamma-H2AX signal was rapidly lost over 24 h at a much greater rate than foci loss. Surprisingly, pan-nuclear gamma-H2AX intensity was not dependent on the number of alpha particle induced double strand breaks, rather the number of alpha particles which had traversed the cell nucleus. This distinct fluence dependent damage signature of particle radiation is important in both the fields of radioprotection and clinical oncology in determining radionuclide biological dosimetry and may be indicative of patient response to new radionuclide cancer therapies.

1. Introduction

The phosphorylated histone variant gamma-H2AX is a sensitive and well established biomarker of exposure to ionising radiation [1,2]. Detection of X-ray induced DNA double strand breaks (DSBs) by gamma-H2AX foci/intensity quantification has proved useful for biological dosimetry [3] and for investigating intra-cell and inter-individual responses to low linear energy transfer (LET) radiation [4]. For high LET exposures, such as those induced by heavy ions or alpha particles, slower repair of DSBs has been observed, likely due to the greater complexity and subsequent greater difficulty in resolving the break [5]. This has been demonstrated by the bi-exponential repair of low LET induced DSBs, containing both rapid and slow components [3], whereas high LET induced DSBs are lost at a slower rate with greater numbers of residual DSBs remaining several hours post exposure compared with X-rays. While both low and high LET induced DSBs can be identified using a number of molecular techniques, distinguishing between low and high LET breaks is somewhat more difficult. In biological dosimetry, the use of the dicentric assay has proved useful in identifying high LET radiation exposures from low LET due to the formation of complex aberrations generated by high LET radiation [6,7]. These types of aberrations are more frequent as a result of the formation of densely clustered DSBs caused by high LET particles leading to misrepair events not observed with sparsely induced DSBs from, for example, X-rays [8].

With respects to DSBs, it has been observed that in vitro heavy ion irradiation can induce not only phosphorylation of H2AX at DSBs, but also throughout the nucleus outside of directly induced DSBs [9]. This pan-nuclear phosphorylation of H2AX (and ATM) was not observed after X-ray exposure, suggesting it is a phenomenon associated with high LET radiation only. In clinical oncology, radionuclides are used extensively in the treatment of thyroid cancer (iodine-131), in prostate brachytherapy (iodine-125, palladium-103) and bone metastasis (strontium-90, samarium-153). For these treatments, the radionuclides are exclusively low LET radiation emitters of X- and gamma-rays and beta electrons. More recently the clinical benefit of utilising high LET radionuclides has been demonstrated in a phase III clinical trial [10]. In men with castrate resistant prostate cancer with bone metastases, those treated with the alpha particle emitter radium-223 had a median overall survival of 14.9 months vs. 11.3 in the placebo control group (hazard ratio 0.70). Previously treatment with beta electron emitting radionuclides had shown no overall survival benefit, only pain relief [11–13].

If pan-nuclear phosphorylation of H2AX is present in high LET irradiated primary human tissue, it may expand the paradigm that the biological effectiveness of high LET radiations in regard to cell kill and

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http://dx.doi.org/10.1016/j.bbamcr.2015.06.010
0167-4889/

mutagenesis is due solely to induction of localised complex DNA DSBs. For the therapeutic use of alpha particles and heavy ions, the presence (or absence) of pan-nuclear gamma-H2AX in high LET irradiated tissues will help confirm that the calculated doses to tissues at risk match those actually delivered.

2. Materials and methods

2.1. Blood collection and lymphocyte isolation

After obtaining ethical approval from Queen's University Belfast Medical School Ethics Board (Ref 12.13v2) and informed consent from donors, peripheral blood from healthy donors (no known previous medical radiation exposures, aged 24–31) was collected into EDTA vacutainer tubes. Lymphocytes were isolated from the blood using Histopaque-1077 solution (Sigma-Aldrich) and resuspended in RPMI-1640 (supplemented with 10% foetal calf serum, 1% L-glutamine and 1% penicillin/streptomycin) at 37 °C.

2.2. Alpha particle and X-irradiation of lymphocytes

For alpha particle irradiation, lymphocytes were irradiated under sterile conditions with a 1 μCi americium-241 source, which decays to give ~5.45 MeV alpha particles. Lymphocytes were irradiated in 20 μl of media at a concentration of 2×10^7 cells per ml on top of the source separated by a 0.9 μm Mylar foil. Lymphocytes were irradiated for 8 min, being aspirated every 2 min, giving a non homogenous exposure to the cell population with the majority of the cells containing zero foci, representing the partial body non-homogenous exposures to radium-223 seen clinically. For X-ray exposures lymphocytes were uniformly irradiated with 1 Gy at 225 kVp in flasks. Lymphocytes were also irradiated with X-rays on Mylar and no difference in gamma-H2AX signal (breaks or nuclear intensity) was observed between lymphocytes irradiated in flasks and on Mylar. All controls were sham irradiated and all irradiations took place at 4 °C.

2.3. Alpha particle and X-irradiation of fibroblasts

For alpha particle irradiation, cultured normal human fibroblasts (AGO1522) were grown and irradiated on 0.9 μm Mylar foil using the 1 μCi americium-241 source discussed above. AGO1522 cells were irradiated with 2 Gy of alpha particles to 50% of the cell population (the limit of our alpha irradiation setup) producing a heterogeneous sample of irradiated and unirradiated cells, again mimicking the partial body non-homogenous exposures to radium-223 seen clinically. For X-ray exposures AGO1522 cells were uniformly irradiated with 4 Gy at 225 kVp in flasks. AGO1552 cells were also irradiated with X-rays on Mylar and no difference in gamma-H2AX signal (breaks or nuclear intensity) was observed between cells irradiated in flasks and on Mylar. All controls were sham irradiated and all irradiations took place at 4 °C.

2.4. Inhibitor treatment

ATM inhibitor KU60019 and DNA-PK inhibitor NU7441 (Tocris Bioscience, UK) were used at a concentration of 10 μM. All inhibitors were dissolved in DMSO and controls were treated with DMSO only. Cells treated with inhibitors or DMSO only were treated for 1 h before irradiation.

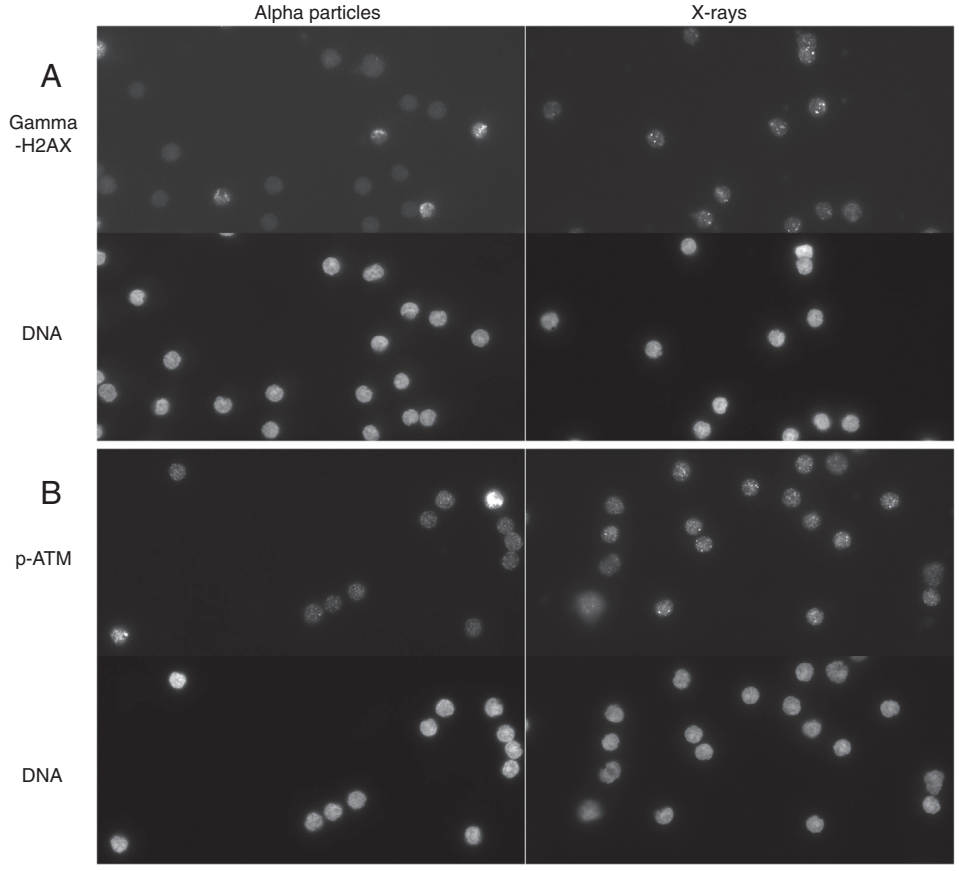

Fig. 1. Grey scale image of gamma-H2AX (A) and phospho-ATM (B) in primary human lymphocytes. The alpha particle irradiated samples contain a heterogeneous population of irradiated and unirradiated cells.