γH2AX STUDY IN PBMCs OF CANCER PATIENTS UNDERGOING RADIOTHERAPY

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Introduction

H2AX protein is a member of H2A histones family and contributes to the transformation of eukaryotic DNA to chromatin. γH2AX constitutes the phosphorylated form of H2Ax on Serine-139.

The radiation causes DNA Double-Strand Breaks (DSB) and these breaks induce the phosphorylation of H2Ax around to the region of the damage. ATM, a member of P13 kinase family, is responsible for this phosphorylation. The cells with high level of H2AX present higher level of genomic instability and higher sensitivity to genotoxic factors.

Previous studies showed that H2AX is crucial for the DNA repair. Moreover, there is a small amount of γH2AX due to DNA damage which is caused by cell senescence and endogenous ROS.

Aim

In this study, we investigated the level of H2AX in lymphocytes from patients with cancer, who are under radiation treatment.

Materials and methods

PBMCs isolation

Whole blood from all patient collected using heparin as an anticoagulant. Pipette 1.5ml Histopaque (Histopaque®-1077; SIGMA) to a falcon tube. Overlay 3ml blood on top of Histopaque and centrifuge tubes at 1x1000g for 30 min at RT. Collect PBMCs from Histopaque interphase, isolated nuclei by centrifuge at 1000g for 15min with lysis nuclei extaction buffer and stored samples in aliquot at -20 °C ,in laemli buffer.

Immunoblotting

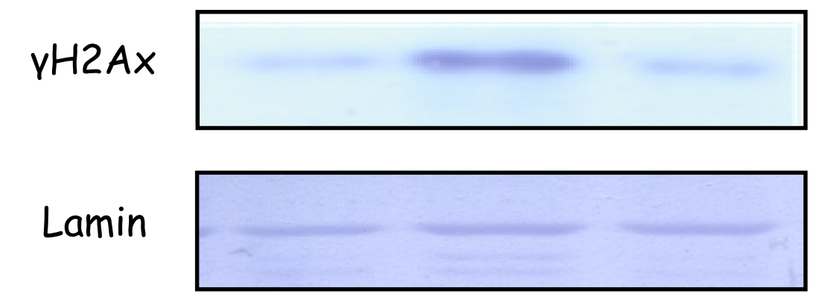
Nuclei fractions were separated on discontinuous SDS gels using 12.5% for γH2AX. Bands on the gel were visualized with Coomassie Blue R-250 and analyzed densitometrically to quantify any changes in the experimental samples relative to the control. Immunoblotting was performed according to Towbin et al. utilizing PVDF-PSQ membranes (0.2 μm pore size, Millipore Corp., ISEQ00010) to detect gammaH2AX. All the experiments were loaded on the same gel and transferred on the same membrane. After being blocked with 5% nonfat dry milk in 150 mM NaCl (Sigma-Aldrich, S5886), 10 mMTris, pH 7.5 (TBS) at room temperature, the membranes were hybridized overnight at 4°C with primary anti-gammaH2AX (1:5,000; Abcam, ab11174) antibody. The membranes were then hybridized for 2 h at room temperature with the secondary antibody, goat polyclonal to rabbit IgG (1:120,000;Abcam,) conjugated to HRP, and developed in TMB solution (KPL Laboratories, 50-77-00). Each of these blots was then stripped [incubated in 2% SDS (w/v) (Sigma-Aldrich, L3771), 62.5 mM TRIS-HCl, pH 6.8, 100 mM β-mercaptoethanol (Sigma-Aldrich, M6250) for 30 min at 60°C, rinsed twice for 10 min each with TBS-T], dried overnight, rehybridized with a polyclonal antibody to Lamin (1:10,000; Abcam, ab16048) and processed as described.

Immunoflorescence

PBMCs cells were plated on gelatin-treated No.1 glass coverslips, fixed in 3.7% formaldehyde/PBS pH 7.4 for 20 min at 37°C and then permeabilized in PBS/0.1% v/v Triton X-100 pH 7.4 for 5 min at room. Cells were blocked in PBS/5% w/v BSA pH 7.4 and stained with Anti-gammaH2A.X (phospho S139) rabbit polyclonal antibody (1:500; Abcam, ab11174)), for 1 h at RT. Cells were washed in PBS ph 7.4, incubated with a CF568 secondary antibody (1:250; Biotium) for 30 min at RT and DNA was counterstained with Hoechst33342 (1 μg/ml; Sigma Aldrich). Following final washes coverslips were mounted on Mowiol mounting medium. Cells were then assessed by confocal microscopy.

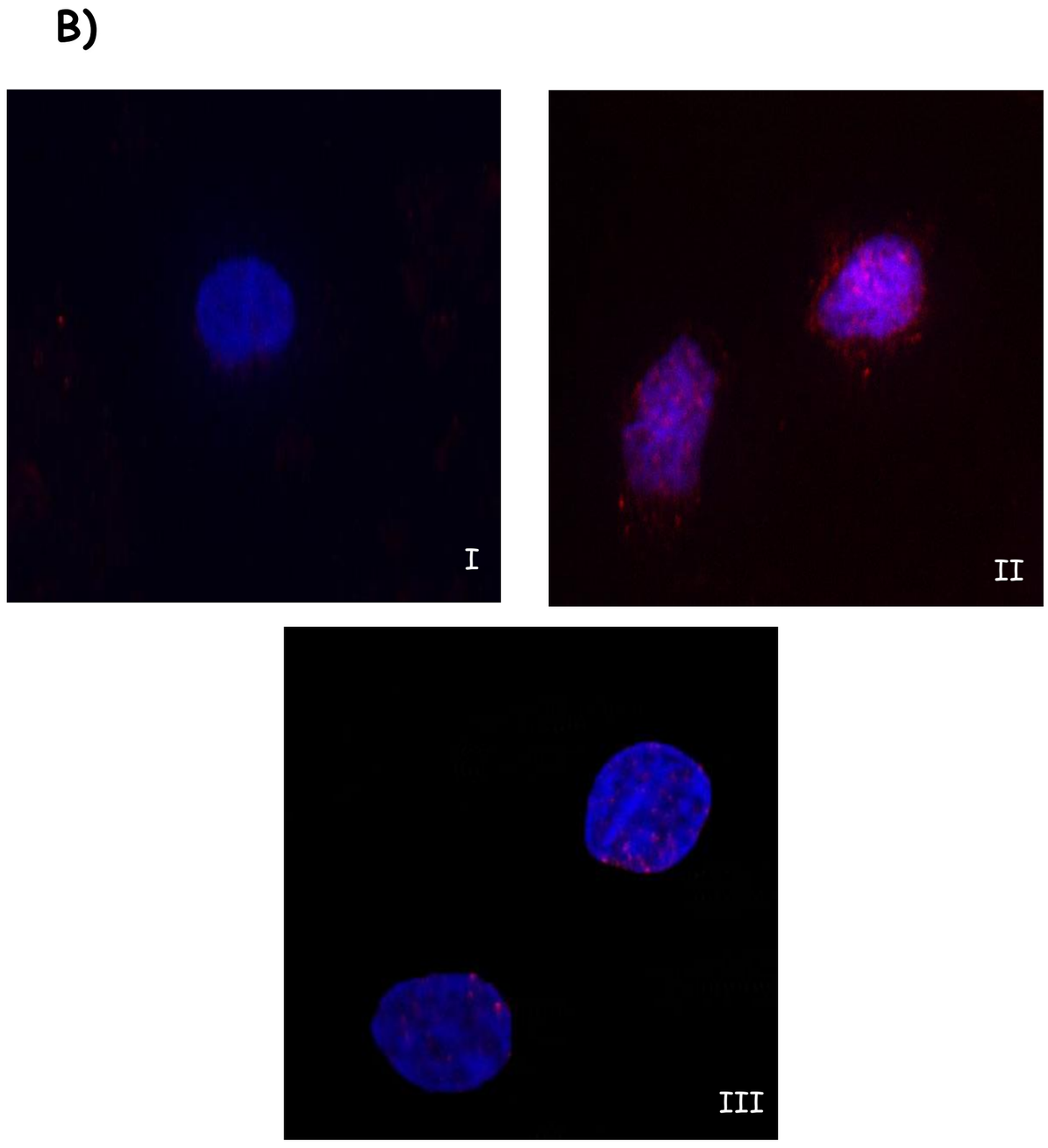
Results

A)



A) Representative immunoblotting images of PBMC cells treated with radiation, 2Gy.First image is for γH2Ax antibody and the second for the loading control antibody Lamin. (I) Control t=0 before radiation (II) t=30 min after radiation (III) t=240 min after radiation.

B)



B) Representative immunofluorescence images of PBMC cells treated with radiation, 2Gy. In the merged images γH2Ax is pseudocolored red, DNA blue. (I) Control t=0 before radiation (II) t=30 min after radiation (III) t=240 min after radiation.

Conclusion

•The level of γH2AX in cells' nuclei, induced 30 minutes after radiation and decreased 4 hours after radiation, reached at the same level with non-irradiated cells.

• A prospective trial is on going to quantify the effects of radiation on γH2AX kinetics in PBMCs of cancer patients undergoing radiotherapy, aiming to assess eventual correlation with radiation induced early and late toxicities.

References

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