The Ion-irradiation Induced Pan-nuclear H2AX Phosphorylation depends on the kinases ATM and DNA-PK*

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Introduction

DNA double-strand breaks (DSBs) elicit the phosphorylation of the histone H2AX around the break to form distinct γH2AX foci detectable by immunofluorescence microscopy. Additionally, heavy ion irradiation provokes H2AX phosphorylation across the whole cell nucleus, also in undamaged chromatin regions distant from the breaks. This pan-nuclear γH2AX is not connected to apoptosis, increases with dose [1] and is fully active only within few hours after irradiation [2]. At DSBs H2AX is phosphorylated by the phosphatidylinositol 3-kinase-related kinases ataxia telangiectasia mutated (ATM) and the DNA-dependent protein kinase (DNA-PK). We investigated the role of both kinases in the ion-induced pan-nuclear H2AX phosphorylation.

Results

Normal human fibroblasts were treated with either specific ATM or specific DNA-PKcs inhibitor, or simultaneously with both inhibitors. Dimethyl sulfoxide (DMSO) treatment served as a control. Cells were irradiated with xenon ions and the pan-nuclear γH2AX signal detected by immunofluorescence staining. In DMSO-treated cells nuclear-wide γH2AX formed and treatment with ATM or DNA-PKcs inhibitor did not eliminate the response (Fig. 1). Only the suppression of both ATM and DNA-PKcs fully impeded the pan-nuclear H2AX phosphorylation.

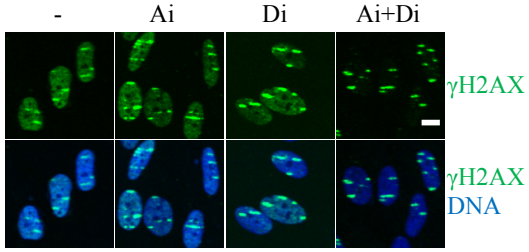

Figure 1: Pan-nuclear H2AX phosphorylation by ATM and DNA-PK. Confluent human skin fibroblasts were incubated with DMSO (-), ATM inhibitor (Ai, KU55933, 10 µM), DNA-PKcs inhibitor (Di, IC86621, 200 µM) or both ATM and DNA-PKcs (Ai+Di) inhibitor from at least 1 h before irradiation until fixation 1 h after irradiation. Cells were irradiated at low angle with xenon ions (8700 keV/µm, $3 \cdot 10^6$ p/cm^2) and γH2AX and the DNA (propidium iodide) detected. Scale bar: 10 µm.

The role of ATM and DNA-PK was further confirmed by the investigation of kinase deficient cell lines. While in ATM deficient human fibroblasts a pan-nuclear γH2AX was induced after ion irradiation, it could be suppressed by additional inhibition of DNA-PKcs (Fig. 2A). In DNA-PKcs deficient mouse embryonic fibroblasts (MEF) an ion irradiation-induced nuclear-wide H2AX phosphorylation was observed but was eliminated by treatment of these cells with ATM inhibitor (Fig. 2B).

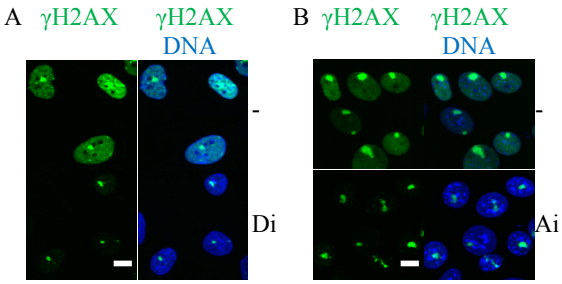

Figure 2: Pan-nuclear H2AX phosphorylation in ATM- and DNA-PKcs-deficient cells. (A) Human ATM-deficient fibroblasts (AT1BR) and (B) DNA-PKcs-deficient MEFs were irradiated at the heavy ion microprobe with 5 nickel ions (3800 keV/µm) targeted to 1 spot within the nucleus. Cells were treated with DMSO (-), DNA-PKcs inhibitor (Di) or ATM inhibitor (Ai) as in Figure 1. 1 h after irradiation γH2AX and the DNA (To-pro-3) were detected. Scale bar: 10 µm.

Conclusion

We show that the ion-induced nuclear-wide phosphorylation of H2AX is clearly dependent on both ATM and DNA-PK that also mediate H2AX phosphorylation at DSBs. The activity of only one of the two kinases is sufficient to induce pan-nuclear γH2AX. Our results suggest that high LET irradiation causes the nuclear-wide activity of ATM and DNA-PK which usually is locally restricted to chromatin areas surrounding DSBs. The impact of the nuclear-wide response on other repair factors is the matter of further studies.

References

[1] B. Meyer et al., "Characterization of the Nuclear-wide γH2AX Response after Ion Irradiation", GSI Scientific Report 2010, 2011, p.440

[2] B. Meyer et al., "Transient Ion Irradiation-induced Pan-nuclear H2AX Phosphorylation", GSI Scientific Report 2011, 2012, p. 492

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