

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 γ H2AX 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 γ H2AX 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 γ H2AX signal detected by immunofluorescence staining. In DMSO-treated cells nuclear-wide γ H2AX 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.

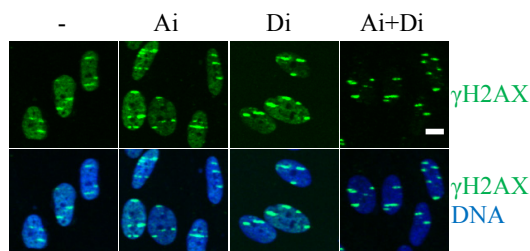


Figure 1: Pan-nuclear H2AX phosphorylation by ATM and DNA-PK. Confluent human skin fibroblasts were incubated with DMSO (-), ATM inhibitor (Ai, KU55933, 10 μ M), DNA-PKcs inhibitor (Di, IC86621, 200 μ M) or both ATM and DNA-PKcs (Ai+Di) inhibitor from at least 1 h before irradiation until fixation 1 h after irradiation. Cells were irradiated at low angle with xenon ions (8700 keV/ μ m, $3 \cdot 10^6$ p/cm²) and γ H2AX and the DNA (propidium iodide) detected. Scale bar: 10 μ m.

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 γ H2AX 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).

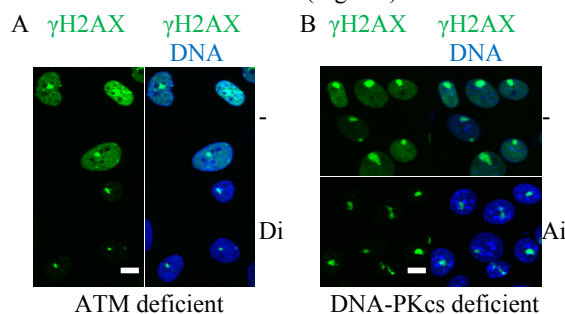


Figure 2: Pan-nuclear H2AX phosphorylation in ATM- and DNA-PKcs-deficient cells. (A) Human ATM-deficient fibroblasts (AT1BR) and (B) DNA-PKcs-deficient MEFs were irradiated at the heavy ion microprobe with 5 nickel ions (3800 keV/ μ m) targeted to 1 spot within the nucleus. Cells were treated with DMSO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

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 γ H2AX. 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 γ H2AX 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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