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γH2AX foci formation in the absence of DNA damage: Mitotic H2AX phosphorylation is mediated by the DNA-PKcs/CHK2 pathway

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ABSTRACT

Phosphorylated H2AX is considered to be a biomarker for DNA double-strand breaks (DSB), but recent evidence suggests that γH2AX does not always indicate the presence of DSB. Here we demonstrate the bimodal dynamic of H2AX phosphorylation induced by ionizing radiation, with the second peak appearing when G2/M arrest is induced. An increased level of γH2AX occurred in mitotic cells, and this increase was attenuated by DNA-PKcs inactivation or *Chk2* depletion, but not by ATM inhibition. The phosphorylation-mimic CHK2-T68D abrogated the attenuation of mitotic γH2AX induced by DNA-PKcs inactivation. Thus, the DNA-PKcs/CHK2 pathway mediates the mitotic phosphorylation of H2AX in the absence of DNA damage.

1. Introduction

DNA double-strand breaks (DSBs) are the most dangerous type of DNA damage, as even a single unrepaired DSB can result in cell death [1,2]. DSBs can be repaired through the homologous recombination (HR) or non-homologous end joining (NHEJ) pathways [1,3]. One of the earliest events in the cellular response to DSBs is the phosphorylation of H2AX at Ser139, which is referred to as γH2AX [4]. H2AX is a member of the histone protein H2A family; the other two members are H2A1-H2A2 and H2AZ [5]. γH2AX foci formation at DSB sites occurs rapidly (within minutes) and is highly conserved from yeast to humans [6]. γH2AX can recruit other DSB signaling and repair factors such as MDC1, 53BP1 and the MRN complex to the damage site, forming nuclear ionizing radiation-induced foci (IRIF) [7–9]. Silencing H2AX increases radiosensitivity and interferes with the recruitment of DNA damage response proteins to the damage sites [10].

The phosphorylation of H2AX in response to DSBs is mediated by PIKK family proteins, which are characterized by the SQ/TQ motif. The members of the PIKK family include ataxia telangiectasia mutated (ATM), ataxia telangiectasia and rad3 related (ATR) and DNA dependent protein kinase catalytic subunit (DNA-PKcs). ATM and DNA-PKcs participate in the DSB signaling cascade, and both have been reported to phosphorylate H2AX [11,12]. ATR phosphorylates H2AX during replication fork blockage induced by UV exposure [13]. Thus, all of the PIKK family members contribute to the phosphorylation of H2AX in response to DSBs induced by ionizing radiation [14]. Although ATM is considered to be the major kinase responsible for H2AX phosphorylation during the DSB response, the recruitment and activation of ATM itself requires γH2AX [15], which could partially explain how γH2AX contributes to cell cycle arrest in response to DNA damage [16].

Due to the fact that γH2AX appears early and forms foci at damage sites, it is widely considered to be a sensitive biomarker for IR-induced DSBs [17]. However, γH2AX does not always indicate the presence of DSBs, and there is increasing evidence that calls into question the interpretation of γH2AX involvement in DSBs [18]. For instance, H2AX phosphorylation is also induced by UV radiation [18,19]. Serum starvation, which does not cause DNA damage, can induce the phosphorylation of H2AX through the p38 MAPK signaling pathway, which is closely associated with the induction of apoptosis [20]. Moreover, H2AX can be phosphorylated in mito-

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tic cells in the absence of DNA damage [11,21], and forms nuclear foci that do not recruit DNA damage response proteins [21]. This cell cycle-specific phosphorylation of H2AX suggests that a decrease in γH2AX levels may not accurately reflect DSB repair efficiency, because DSBs induce cell cycle arrest. However, little is known about the regulation of γH2AX in association with cell cycle progression.

The goal of the present study was to increase our understanding of mitotic H2AX phosphorylation. We found that DNA-PKcs, but not ATM, is responsible for the mitotic phosphorylation of H2AX in the absence of DNA damage, and that the cell cycle checkpoint protein 2 (CHK2) directly mediates this process. CHK2 was phosphorylated in a DNA-PKcs-dependent manner and was essential for H2AX phosphorylation. Our results show that the DNA-PKcs/Chk2 pathway is involved in mitotic H2AX phosphorylation.

2. Materials and methods

2.1. Cell culture

HeLa cells were maintained in DMEM (HyClone) supplemented with 10% fetal bovine serum (HyClone). HeLa cell lines overexpressing CHK2 were generated by transfecting with HeLa cells with wild-type and mutated *chk*2-expressing vectors. Meanwhile, a *Chk*2 knock-down HeLa cell line was generated by transfecting HeLa cells with a *chk*2 shRNA-expressing vector and selecting with Hygromycin B. To arrest cells in mitosis, 100 ng/ml nocodazole (M1404-2MG, Sigma) was added to the cell culture medium for 16 h. The cells were treated with 10 μM NU7026 (N1537, Sigma) or 10 μM KU55933 (S1092, Selleck) for 2 h to inhibit the PIKK activity of DNA-PKcs and ATM, respectively.

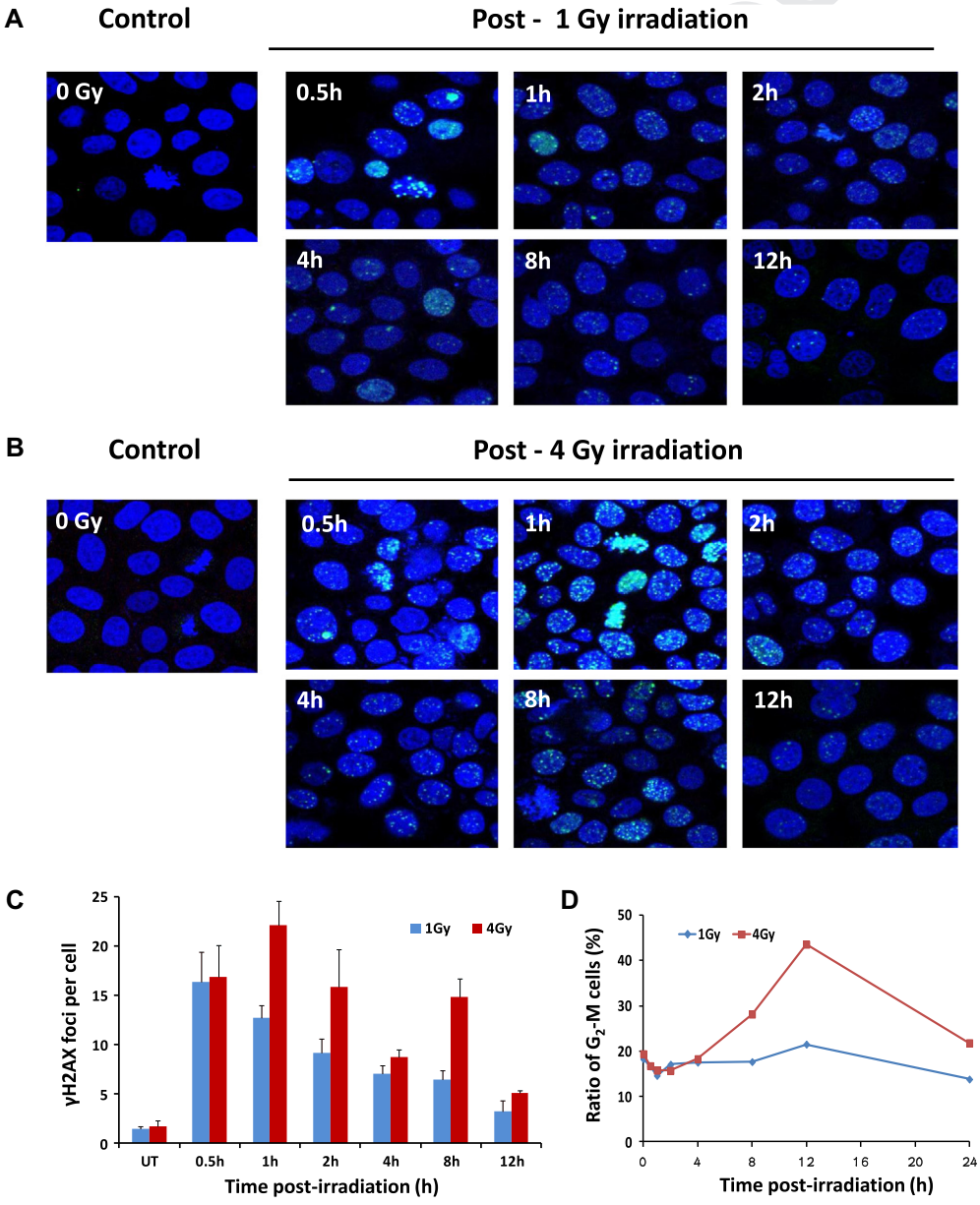

Fig. 1. Bimodal pattern of H2AX phosphorylation after treatment with a high dose of ionizing radiation. (A, B) γH2AX foci in HeLa cells after irradiation with 1 Gy (A) or 4 Gy (B) γ-rays. The cells were collected and fixed at the indicated time points after irradiation. (C) The dynamics of γH2AX foci formation after irradiation as shown in (A) & (B). Data are presented as mean ± S.D. from three independent experiments. (D) The proportion of of G2/M cells after treatment with 1 Gy or 4 Gy of irradiation, as measured by flow cytometric analysis.

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