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Acetylation of H2AX on lysine 36 plays a key role in the DNA double-strand break repair pathway

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ABSTRACT

Phosphorylation of H2AX functions to recruit DNA repair complexes to sites of DNA damage. Here, we report that H2AX is constitutively acetylated on lysine 36 (H2AXK36Ac) by the CBP/p300 acetyltransferases. H2AXK36Ac is required for cells to survive exposure to ionizing radiation; however, H2AXK36Ac levels are not increased by DNA damage. Further, acetylation of H2AX did not affect phosphorylation of H2AX or the formation of DNA damage foci. Finally, cells with a double mutation in both the H2AX acetylation and phosphorylation sites were more radiosensitive than cells containing individual mutations. H2AXK36Ac is therefore a novel, constitutive histone modification located within the histone core region which regulates radiation sensitivity independently of H2AX phosphorylation.

1. Introduction

In response to DNA double-strand breaks (DSBs), serine 139 of H2AX is rapidly phosphorylated by ATM and related kinases [1]. Formation of γH2AX provides binding sites to recruit the mdc1 protein [2], which then functions as a platform to concentrate DNA repair proteins at DSBs [3,4]. Consequently, loss of H2AX is associated with increased genomic instability and sensitivity to ionizing radiation (IR) [5,6]. Histones are also modified on lysine by methylation, ubiquitination and acetylation [7]. Histone acetylation is significantly increased in response to DNA damage, and it is now clear that chromatin acetylation is important for DSB repair. The acetylation of H2A and H2AX on lysine 5 by Tip60 plays a key role in the DNA damage response [8–11]. In drosophila, acetylation of H2Av on lysine 5 is required for removal of H2Av from the chromatin and for its subsequent dephosphorylation [10]. Acetylation of histone H4 [8,9,12] and H2AX [10,11] is required for the formation of open chromatin structures at DSBs, regulating the extent of γH2AX formation and is critical for facilitating access of the DNA repair machinery to DSBs [13]. Increased acetylation of histones at DSBs therefore makes a key contribution to DSB repair by regulating histone exchange, remodeling chromatin structure and facilitating the recruitment of DNA repair proteins to sites of DNA damage. However, whether H2AX contains other sites for lysine acetylation which are important for the DNA damage response is not known. Here, we demonstrate that acetylation of lysine 36 of H2AX is essential for cells to survive exposure to DNA damage, and show that this function of lysine 36 is independent of the phosphorylation of serine 139.

2. Materials and methods

2.1. Cell culture

Flag-HA-H2AX cDNA was inserted into the pIRESpuro3 (Clontech, CA) expression vector. Mutations were created using the QuickChange Site-Directed Mutagenesis Kit (Stratagene, CA). 293T cells, and H2AX$^{-/-}$ MEF cells (provided by A. Nussenzweig [6]) were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum. Transfections were carried out using FuGene6 (Roche, IN) or Lipofectamine 2000 (Invitrogen, CA) and stable cell lines established using puromycin. Cell survival assays, flow cytometry and immunofluorescent staining are described in [14,15]. For cell synchronization, exponentially growing

Abbreviations: DSB, DNA double-strand break; IR, ionizing radiation; HAT, histone acetyltransferase; HDAC, histone deacetylase; H2AXK36Ac, H2AX acetylated on lysine 36; TSA, trichostatin A

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293T cells were incubated twice in the presence of 100 mM thymidine for 18 h, with 10 h in thymidine free medium between exposures. G1/S phase and S phase cells were obtained immediately upon or 2 h after the release from thymidine block. G2/M arrest was achieved with nocodazole (40 ng/ml for 18 h).

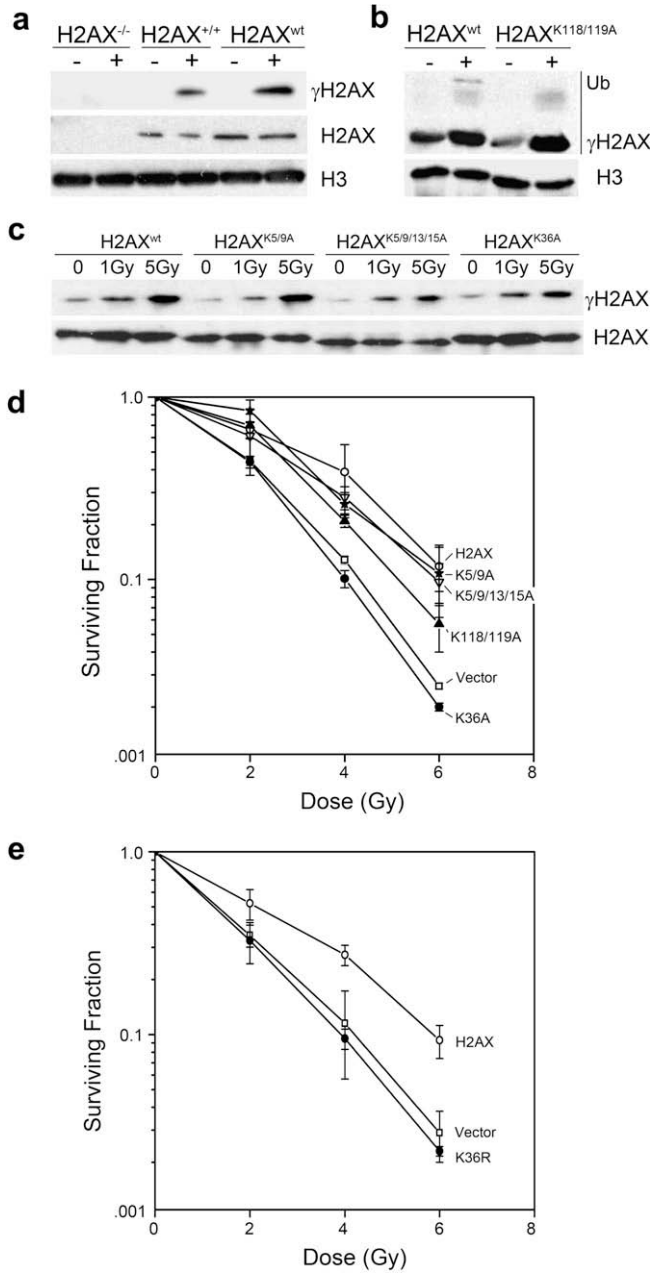

Fig. 1. Mutation of lysine 36 of H2AX confers sensitivity to IR. (a) H2AX$^{-/-}$ MEFs, wild type H2AX MEFs (H2AX$^{+/+}$) or H2AX$^{-/-}$ MEFs reconstituted with H2AX (H2AX^{wt}) were irradiated (5 Gy). H2AX, γH2AX and H3 were measured by western blot. (b) H2AX$^{-/-}$ MEFs reconstituted with H2AX^{wt} or H2AX with alanine mutations at lysines 118 and 119 (H2AX$^{K118/119A}$) were irradiated (IR: 5 Gy). H2AX and γH2AX were measured by western blot analysis. Ubiquitinated γH2AX is shown. (c) H2AX$^{-/-}$ MEF cells reconstituted with H2AX^{wt} or H2AX with alanine mutations at lysines 5 and 9 (H2AX$^{K5/9A}$), lysines 5, 9, 13 and 15 (H2AX$^{K5/9/13/15A}$) or lysine 36 (H2AX^{K36A}) were irradiated. H2AX and γH2AX were measured by western blot. (d) H2AX$^{-/-}$ MEFs expressing vector, wild type H2AX or H2AX with mutations at K5/9A, K5/9/13/15A, K118/119A or K36A were irradiated as indicated. 12 days later, clonogenic cell survival assays were carried out. Results ± S.E. (n = 3). (e) H2AX$^{-/-}$ MEFs expressing H2AX, Vector or H2AX with the mutation K36R were irradiated as indicated. 12 days later, clonogenic cell survival assays were carried out. Results ± S.E. (n = 3).

2.2. Western blotting

Rabbit polyclonal antibody AbK36Ac was raised using the synthetic peptide 29-RVHRLLR(AcK)GHYAER-42. AbK36Ac was purified by sequential affinity chromatography against unacetylated peptide and acetylated peptide. Preparation of cell lysates, antibodies, acid extraction of histones and immunoprecipitation are described in [15,16].

3. Results

To determine the contribution of conserved lysines on H2AX to the cells DNA damage response, lysine residues were individually mutated and expressed in H2AX$^{-/-}$ MEFs. H2AX was cloned into the inducible pIRES vector to allow controlled expression of H2AX. Fig. 1a demonstrates that the expression levels and phosphorylation of exogenous H2AX (H2AX^{wt}) were similar to that of endogenous H2AX in H2AX$^{+/+}$ MEFs. Each of the H2AX constructs

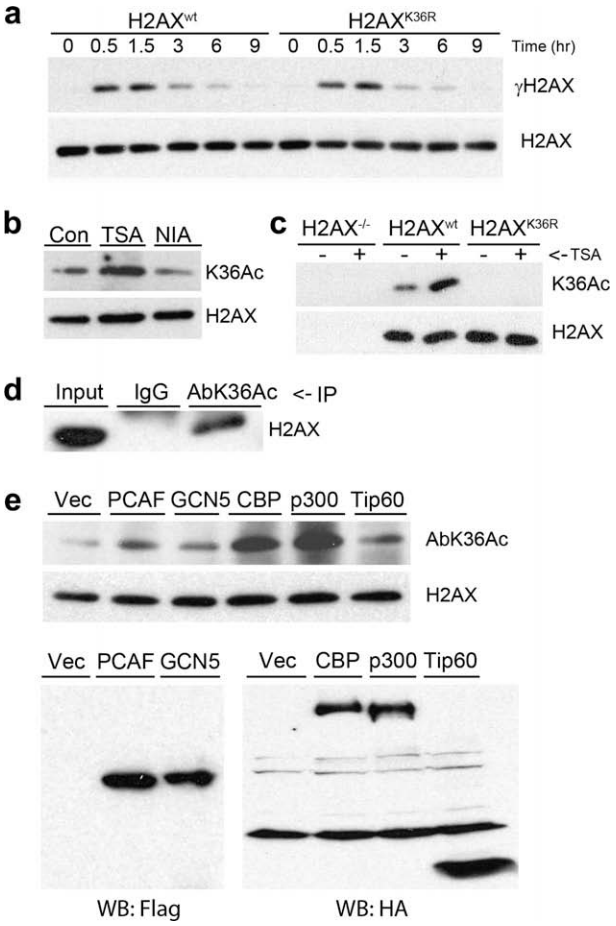

Fig. 2. Lysine 36 of H2AX is acetylated. (a) H2AX$^{-/-}$ MEFs expressing H2AX^{wt} or H2AX^{K36R} were irradiated (5 Gy) and allowed to recover. γH2AX and H2AX were monitored by western blot. (b) 293T cells expressing Flag-H2AX were incubated with TSA (0.4 μM) or nicotinamide (NIA: 10 mM) for 8 h. H2AX and H2AXK36Ac were monitored using antibody AbK36Ac. (c) 293T cells expressing Vector, Flag-H2AX^{wt} or Flag-H2AX^{K36R} were untreated or exposed to TSA (0.4 μM for 8 h) and immunoprecipitated with Flag antibody. H2AXK36Ac was monitored by western blot using AbK36Ac. (d) Histones from H2AX$^{+/+}$ MEF cells were immunoprecipitated with IgG or AbK36Ac. H2AX was detected by western blotting. (e) 293T cells were transiently transfected with Flag-H2AX and either vector, Flag-PCAF, Flag-GCN5, HA-CBP, HA-p300 or HA-Tip60. 72 h later, chromatin associated histones (upper panel) and total proteins (lower panel) were extracted. Acetylation of lysine 36 was detected with AbK36Ac antibody, and expression of HATs was monitored with anti-Flag or anti-HA antibody.