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Deficiency of Suv39h1 histone methyltransferase caused changes in HP1 protein levels and its nuclear distribution

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ABSTRACT

The epigenetic modifications of histones regulate gene expression by dictating the formation of euchromatin and heterochromatin domains. Here, we studied the relationship between post-translation modifications of histone H3 and a family of HP1 proteins (HP1α, HP1β and HP1γ). We analyzed the role of Suv39h1 methyltransferase, catalyzing methylation of histone H3 on lysine 9 position (H3K9me3). Studies were performed from the view of heterochromatin formation. For analyses we used immunofluorescence, confocal microscopy techniques and Western blotting. In Suv39h1 double null (dn) mouse fibroblasts, we observed that a loss of H3K9me3 results in reduction of HP1α and HP1γ levels. In contrast to wild type cells, no association of HP1β with chromocenters was observed in dn fibroblasts. However, nuclei of these cells were characterized by well distinguishable foci, positive on H3K9 monomethylation (me1) that exactly co-localized with chromocenters. These results suggest that the H3K9me1 may partially substitute the function of H3K9me3 at chromocenters and such histone signature is responsible for nuclear arrangement and compactness of centromeric heterochromatin. In addition, from the view of DNA repair-related events, in irradiated Suv39h1 dn cells by 5 Gy of γ-rays, we observed reduction of 53BP1 foci, which indicate the role of Suv39h1 histone methyltransferase in DNA repair processes, similar as it was observed for HP1 protein isoforms. Thus, aims of our future research will be focused on the studies of HP1β function and nuclear distribution in irradiated cells.

Aims:

1. To study the effect of SUV39h mutation on heterochromatin formation in mouse fibroblasts
2. To study the role of H3K9me3 in DNA repair processes.

Methods:

1. In vitro cultivation of wild type and SUV39h mutant cells. Wild-type mouse embryonic fibroblasts (MEFs, called SUV39h+/+cells) and SUV39h-/- fibroblasts originated from the Laboratory of Prof. Thomas Jenuwein. The cells were cultivated in high glucose DMEM supplemented with 10% fetal calf serum and β-mercaptoethanol (1 ul/500ml).
2. Analysis of expression of nuclear protein by Western blots.
3. Analysis of chromatin arrangement by confocal microscopy.
4. Immunofluorescence detection of nuclear foci after irradiation of cells with gamma rays. Cells were fixed with 4% paraformaldehyde (PFA), permeabilized with 0.2% Triton X-100 for and with 0.1% saponin. We used 1% BSA dissolved in PBS as blocking solution during 1 hour.

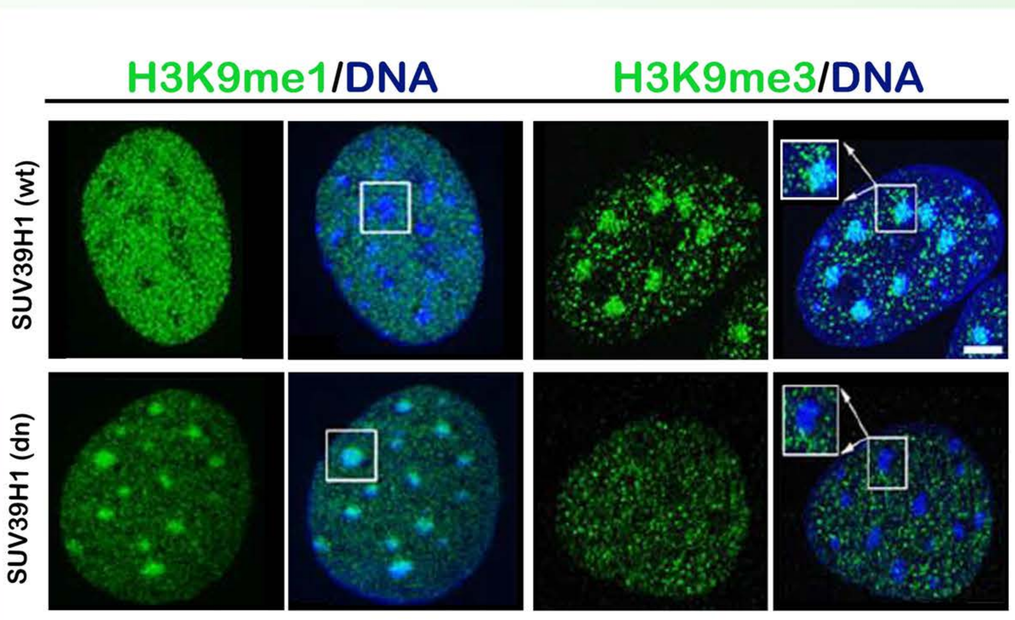


Figure 3. Immunofluorescence detection of selected epigenetic marks in SUV39h1 mutant cells and their wild type counterpart. In SUV39h1 mutant cells, H3K9me1 replaced H3K9me3 at chromocenters (DAPI dense regions is shown in blue fluorescence).

RESULTS

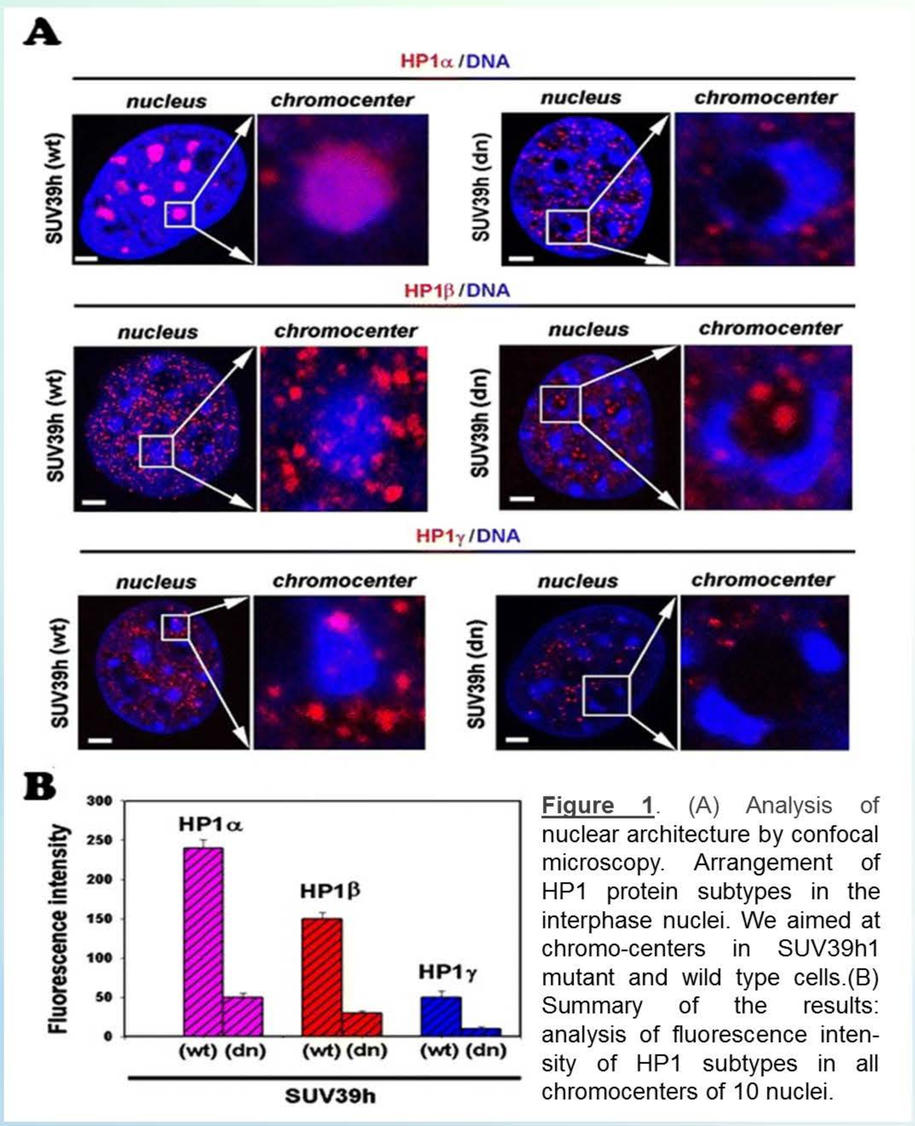
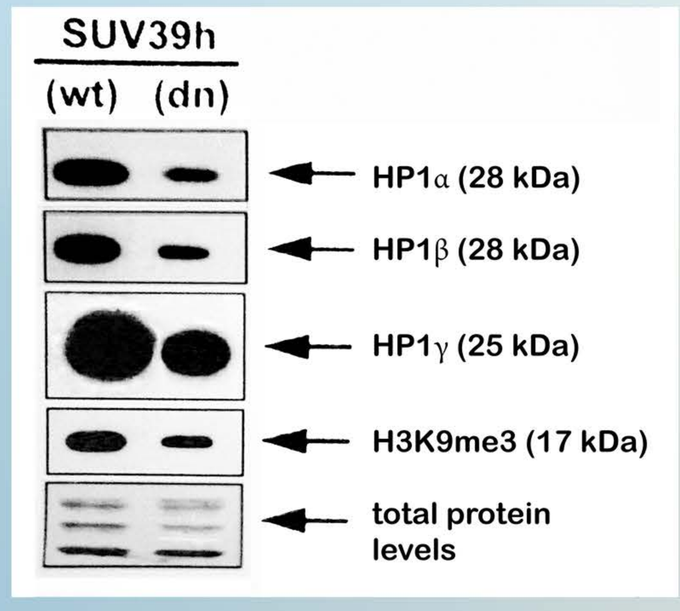


Figure 1. (A) Analysis of nuclear architecture by confocal microscopy. Arrangement of HP1 protein subtypes in the interphase nuclei. We aimed at chromo-centers in SUV39h1 mutant and wild type cells.(B) Summary of the results: analysis of fluorescence intensity of HP1 subtypes in all chromocenters of 10 nuclei.



Figures 2. Western blot analysis of the levels of HP1 protein isoforms. In SUV39h1 deficient cells the following protein levels were decreased: HP1α, HP1β, HP1γ and H3K9me3.

CONCLUSION:

1. The SUV39h1 mutation associates with decreased levels of HP1 protein subtypes (HP1 α, HP1β, HP1γ) (Fig. 2).
2. The SUV39h1 mutation caused a decrease in H3K9me3 levels at chromocenters. This was accompanied by an increased monomethylation of histone H3K9. These results suggest that H3K9me1 may substitute for the reductions of H3K9me3 in centromeric heterochromatin (Fig. 3).
3. The cells containing the SUV39h1 mutation exhibited an altered response to DNA damage caused by ionizing radiation (Fig. 4A, B). An increased number of H3K9me3-positive foci was found in irradiated wt cells. A number of 53BP1-positive foci was lower in SUV39h deficient cells than wt fibroblasts when exposed to gamma-radiation (Fig. 4A).

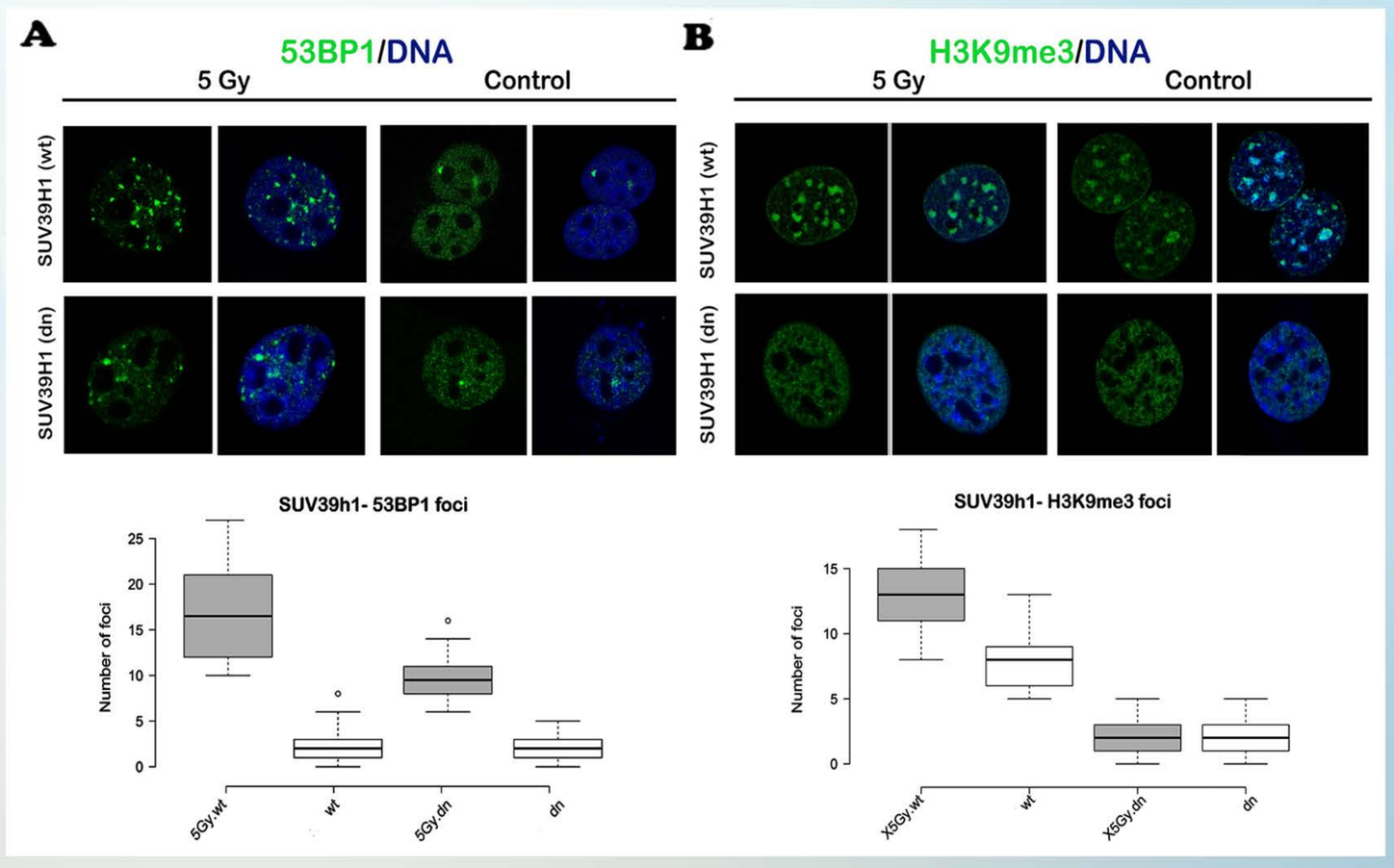


Figure 4. Analysis by confocal microscopy showing nuclear distribution pattern of 53BP1 and H3K9me3. Qquantification of immunofluorescence signals in gamma-irradiated (5Gy) and non-irradiated wt and SUV39h1 dn cells is shown. (A) A number of 53BP1 protein foci was lower in SUV39h1 dn cells when compared with their wt counterpart. (B) A number of H3K9me3-positive foci was increased by irradiation in wt fibroblasts. SUV39h dn cells were absent of H3K9me3 at chromocenters.

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