Identification of an upstream regulator of DNMT1-mediated senescence.

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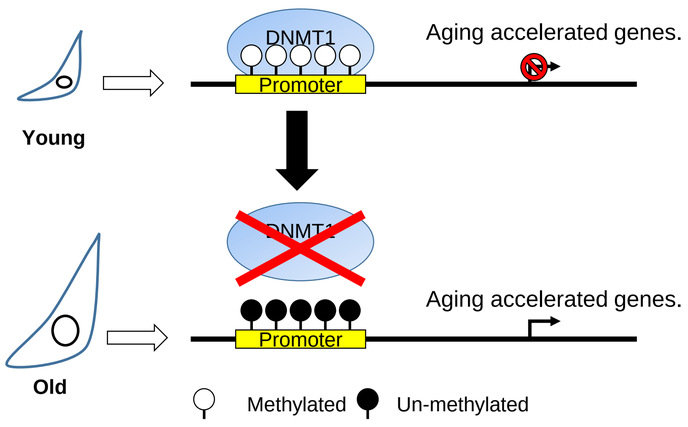
ABSTRACT

Global loss of DNA methylation has been implicated in chronological aging and cell senescence, implying the involvement of overall decreased DNA methyltransferase (DNMT) activities. In this study, we investigated expression profiles of DNMT1-interacting proteins and their effects on DNMT1 expression. In the progress of replicative senescence of human diploid fibroblast (HDF), DNMT1 expression specifically decreased from the earlier stage before gain of SA-β-gal activity, a typical senescence marker, whereas DNMT3 expression did not change. Similar results were obtained in the stress-induced senescence triggered by exogenous subcytotoxic dose of H_2O_2. By analyzing expression patterns of 53 known DNMT1-interacting proteins using cDNA microarray data of the two different cell senescence systems, we identified that 7 genes, such as CBX5, SUV39H1, EZH2, PARP1, UHRF1, CHEK1 and HELLS, were commonly down-expressed at the same time point as DNMT1. Knockdown of UHRF1 regulated DNMT1 expression and effectively induced senescent phenotype of HDF. These results indicated that UHRF1 may be the effective upstream regulator of DNMT1-linked cell senescence.

INTRODUCTION



- Although DNMT1 has the key enzymatic activity for the maintenance methylation, the DNMT1 activity is also delicately controlled by physical interaction with diverse proteins and post-translational modifications.
- Binding of DNMT1 to PCNA increase methylation activity and binding to UHRF1 facilitates recognition of hemi-methylated DNA.
- DNMT1 is acetylated by KAT5 and deacetylated by HDAC1.

⇒ **These observations suggest that overall integrated action of these DNMT1-interacting proteins (DIPs) on DNMT1 exhibit eventual maintenance DNA methylation activity and its associated cellular phenotype.**

Decreased DNMT1-mediated DNA maintenance methylation activity is involved in senescence of HDF.

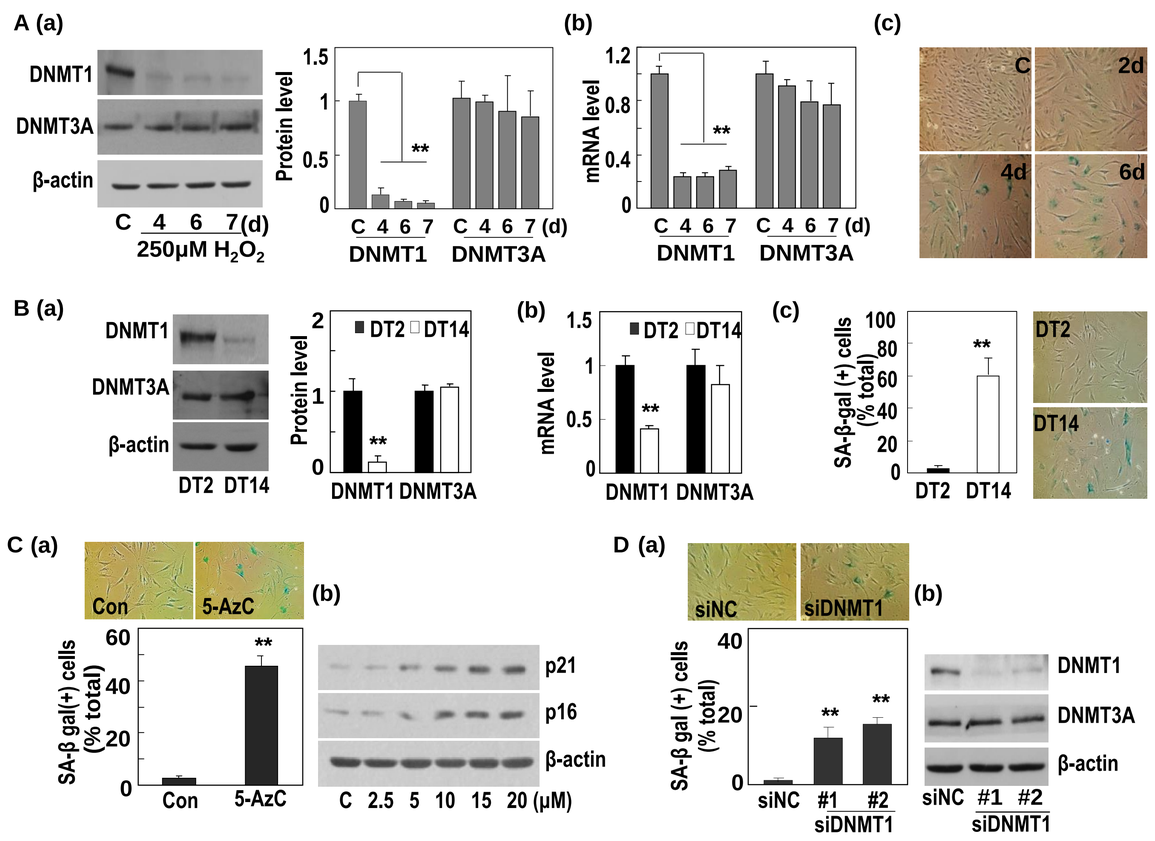


Figure.1

(A) Primary HDFs were treated with 150 μM H_2O_2 twice with 12h interval to stably induce senescence. Western blot analyses for DNMT1 and DNMT3A (left) and their quantification (right) are shown in (a). Messenger RNA levels by qRT-PCR (b) and representative images of SA-β-gal assay (c) are shown. **$p < 0.01$ vs. control HDF by Student ***t*** test.
(B) HDFs were continuously sub-cultured to induce replicative senescence and HDFs of DT2 (black bar) and DT14 (white bar) were used. Western blot analysis (left) and their quantification (right) are shown in (a). Messenger RNA levels by qRT-PCR (b) and SA-β-gal assay (c) are shown. Right panels of (c) show representative images of SA-β-gal assay. **$p < 0.01$ vs. DT2 by Student ***t*** test.
(C) HDF (DT2) was exposed to 5-azacytidine for 5 days. (a) Representative images (upper panel) and quantification data (lower panel) of SA-β-gal assay are shown. (b) Western blot analysis.
(D) HDF (DT2) was transfected with siRNA for DNMT1 for 5 days. (a) Representative images (upper panel) and quantification data (lower panel) of SA-β-gal assay are shown. (b) Western blot analysis. **$p < 0.01$ vs. siNC or control by Student ***t*** test.

Gene expression profiles commonly regulated in both replicative and H_2O_2-induced senescence of HDF.

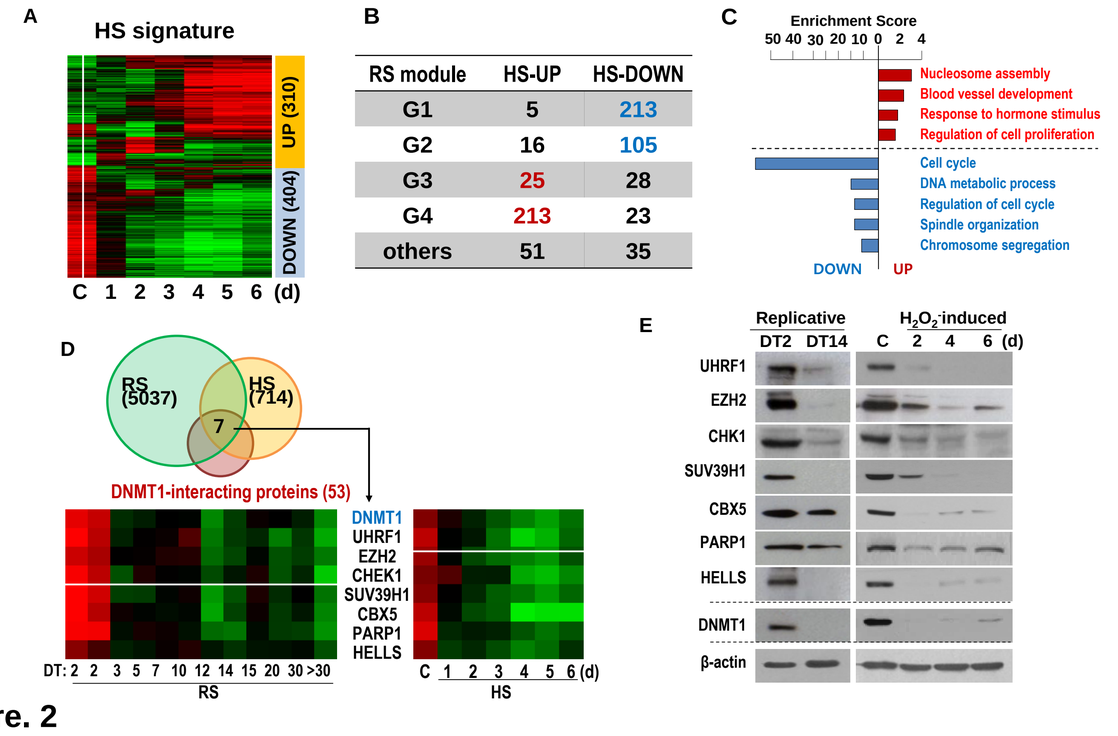


Figure. 2

(A) Primary HDFs were treated with 150 μM H_2O_2 twice with 12h interval to stably induce senescence. Heatmap of time-series gene expression profile was obtained by bioinformatics and cDNA microarray analysis.
(B) Progressively up-regulated (310 genes) and down-regulated genes (404 genes) were matched with four different modular genes obtained from replicative senescence of HDF reported previously.
(C) Enrichment score indicate the –log10-transformed P values which calculated from the gene set enrichment analysis.
(D) By comparing gene expression profiles of the two cell senescence models (RS and HS) and matching them with the 53 DNMT1-interacting proteins reported previously, 7 genes were identified to be commonly regulated in the progress of the two HDF senescence models. Heatmaps of time series gene expression profiles of the 7 genes in the two HDF senescence models are shown in the bottom panels.
(E) Protein expression levels of the 7 genes were validated by Western blot analysis.

UHRF1 is an upstream regulator of DNMT1 expression.

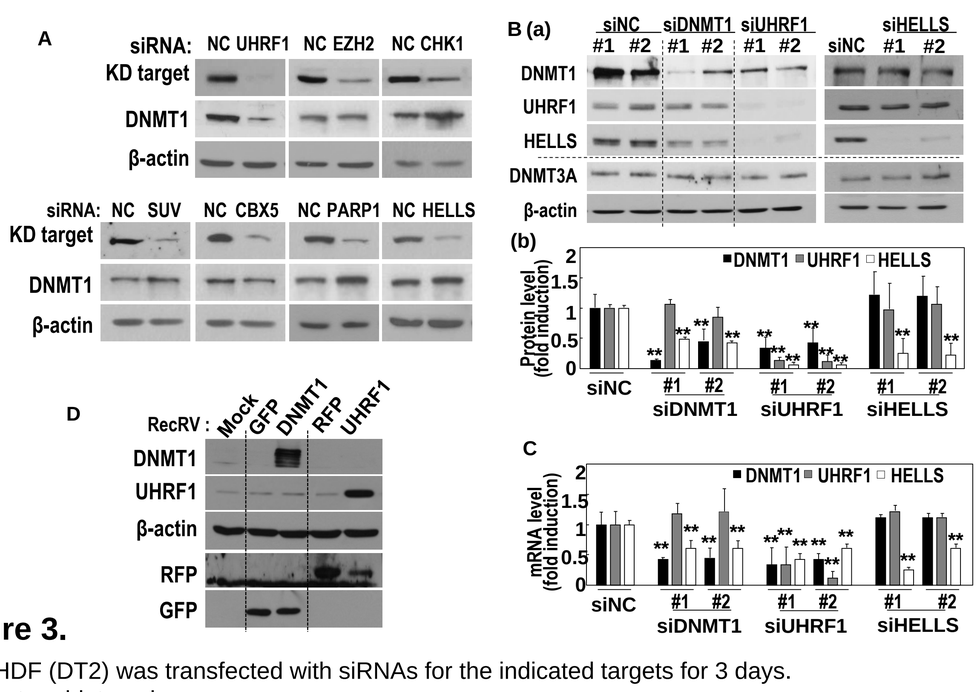


Figure 3.

(A-C) HDF (DT2) was transfected with siRNAs for the indicated targets for 3 days.
(A) Western blot analyses.
(B) Western blot analyses (a) and their quantification (b).
(C) Messenger RNA levels by qRT-PCR. **p < 0.01 vs. siNC.
(D) HDF (DT7) was infected with recombinant retrovirus (RecRV) harboring the indicated target cDNA for 3 days. Western blot analyses were performed.

Knockdown of UHRF1 induces senescence through DNMT1 suppression.

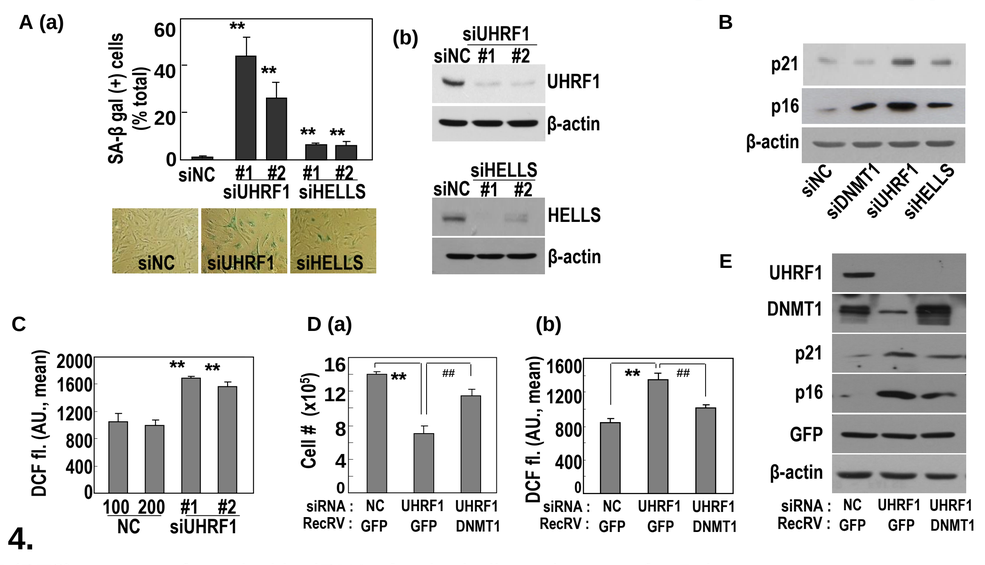
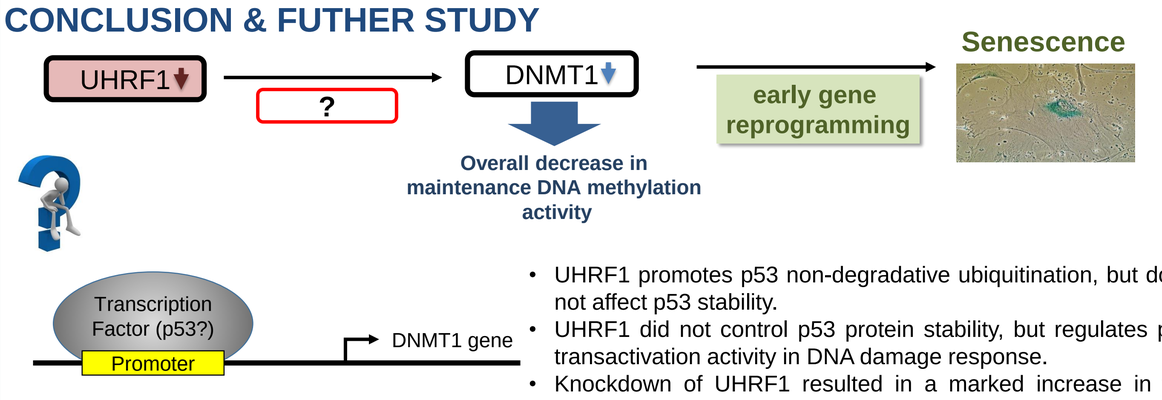


Figure 4.

(A-C) HDF (DT2) was transfected with siRNAs for the indicated targets for 5 days.
(A) Quantification (upper) and representative images (lower) of SA-β-gal assay are shown in (a). Knockdown effect was confirmed by Western blot analyses (b). **p < 0.01 vs. siNC.
(B) Western blot analysis.
(C) Intracellular ROS levels were monitored by flow cytometric analysis after staining cells with DCF-DA fluorescence dye.
(D, E) HDF (DT2) was transfected with siRNAs for the indicated targets and then infected with recRV harboring the indicated target cDNA for 4 days.
(D) Measurement of cell growth rate by counting cell number (a) and intracellular ROS levels using DCF-DA fluorescence dye (b). **p < 0.01 vs. siNC/GFP and ## p < 0.01 vs. siUHRF1/GFP by Student t test.
(E) Western blot analysis.

CONCLUSION & FUTHER STUDY



- UHRF1 promotes p53 non-degradative ubiquitination, but does not affect p53 stability.
- UHRF1 did not control p53 protein stability, but regulates p53 transactivation activity in DNA damage response.
- Knockdown of UHRF1 resulted in a marked increase in the accumulation of p53 transcriptional target p21.

REFFERNCE

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